PHARMACOKINETICS AND DISPOSITION OF THE OXYTOCIN RECEPTOR ANTAGONIST
L-368,899 IN RATS AND DOGS

KATHRYN L. THOMPSON, STYLIAN H. VINCENT, RANDALL R. MILLER, ADRIA E. COLLETTI, RAUL F. ALVARO, MICHAEL A. WALLACE, WILLIAM P. FEENEY, AND SHUET-HING LEE CHIU


(Received December 24, 1996; accepted June 25, 1997)

ABSTRACT:

L-368,899 is a potent, orally-active oxytocin antagonist that completed phase I clinical trials for the prevention of preterm labor. The pharmacokinetics and disposition of L-368,899 were studied in rats (female and male) and dogs (female), the two species used in the toxicology studies. L-368,899 exhibited similar pharmacokinetics in rats and dogs. After iv dosing at 1, 2.5, and 10 mg/kg, the compound had a t½ of ~2 hr and plasma clearance between 23 and 36 ml/min/kg at all doses and in both species. The exception was female rats at the 10 mg/kg dose where plasma clearance decreased to 18 ml/min/kg. The Vdss was between 2.0 and 2.6 liters/kg for rats and 3.4 to 4.9 liters/kg for dogs. After oral dosing, L-368,899 was rapidly absorbed. Mean Cmax values were achieved at <1 hr at the low doses (25 mg/kg in rats and 5 mg/kg in dogs) and between 1 and 4 hr at the higher doses (100 mg/kg in rats and 33 mg/kg in dogs). In bile duct-cannulated female rats, ~70% of a radioactive 28 mg/kg dose was recovered in bile and urine within 72 hr post dose. Plasma drug concentrations were higher in female than in male rats especially at the 25 mg/kg dose, where mean AUC values were 4.5-fold higher in the females. In both rats and dogs, plasma drug levels increased more than proportionally with increasing oral dose. In female rats, the mean AUC increased by ~8-fold between 25 and 100 mg/kg, while in female dogs, the mean AUC at the 33 mg/kg dose was 12-fold higher than that at 5 mg/kg. Oral bioavailability was estimated at 14% and 18% for the 5 mg/kg dose in female and male rats, respectively, 41% for the 25 mg/kg dose in male rats and 17% and 41%, respectively, for the 5 and 33 mg/kg doses in dogs. Owing to nonlinear kinetics, bioavailability could not be calculated for the other oral doses. L-368,899 was metabolized extensively in both species after iv and oral dosing, with <10% of the dose excreted unchanged. The main route of elimination was via the feces, which contained >70% of the radioactive dose by 48 hr, primarily as metabolites. The gender and dose dependence of the pharmacokinetics of L-368,899 in rats were attributed to gender differences in metabolizing capacity and saturation of hepatic metabolism, respectively. This conclusion was based primarily on results from experiments comparing the rate of in vitro metabolism of L-368,899 in liver microsomes, which showed that the Vmax and Km values for L-368,899 were 4-fold lower in female than in male rat liver microsomes.

Materials and Methods

Chemicals. L-368,899 and L-369,551 (the internal standard used for LC-MS/MS analysis, fig. 1), were synthesized by the Department of Medicinal Chemistry, Merck Research Laboratories (10). L-368,899 was supplied as the tartrate and hemi-sulfate salts. Both salts were crystalline and 99.5–99.9% pure as determined by HPLC. Radiolabeled compound was synthesized with 14C at the terminal carbon of the methionine sulfone side chain and supplied as the tartrate salt of the unlabeled compound for in vivo studies.

HPLC grade acetonitrile, methanol and water, and certified A. C. S. grade chemicals. L-368,899 and L-369,551 (the internal standard used for LC-MS/MS analysis, fig. 1), were synthesized by the Department of Medicinal Chemistry, Merck Research Laboratories (10). L-368,899 was supplied as the tartrate and hemi-sulfate salts. Both salts were crystalline and 99.5–99.9% pure as determined by HPLC. Radiolabeled compound was synthesized with 14C at the terminal carbon of the methionine sulfone side chain and supplied as the tartrate salt of the unlabeled compound for in vivo studies.

HPLC grade acetonitrile, methanol and water, and certified A. C. S. grade ammonium acetate, ammonium hydroxide, and formic acid were purchased from Fisher Scientific Company (Pittsburgh, PA). Photometric grade trifluoroacetic acid (TFA) was purchased from Aldrich Chemical Company (Milwaukee, WI).

Animals. Male and female Sprague-Dawley rats were used for the studies...
indwelling catheter in the cephalic vein. Oral dosing was by gavage. Blood was collected from the jugular vein at 0 (pre-dose), 5 (iv only), 15, 30 min, 1, 2, 4, 6, 8, and 24 hr into heparinized tubes. Plasma was obtained by centrifugation and stored at −20°C until analyzed by LC-MS/MS.

Excretion in dogs. Excretion of [14C]L-368,899 dose was monitored after iv dosing at 2.4 mg/kg (~12 µCi/mg). Urine and feces were collected over 24-hr intervals up to 96 hr and a blood sample was withdrawn at 1 hr post dose for a radioactivity profile.

Radioactivity Profiles. Plasma, fecal homogenates (~0.5 ml), and microsomal incubation mixtures were treated with 4 volumes of methanol followed by a methanol wash of the resulting precipitate (2 volumes). The mixture was centrifuged (500g, 5 min), and the supernatant was concentrated to dryness under nitrogen at 37°C. The residue was dissolved in 95:5 water:acetonitrile, containing 0.1% TFA (0.25 to 1 ml) and centrifuged (500g, 5 min). Using this method, >85% of the radioactivity in the sample was recovered from both plasma and feces. Bile and urine were prepared for HPLC analysis by diluting with 1 to 8 volumes of 95:5 water:acetonitrile containing 0.1% TFA, centrifuged (500g, 5 min), and the supernatant was analyzed. The samples were analyzed using HPLC Method 1. The radioactivity profiles were obtained using a Raytest Romana-5-LS fitted with a glass-TSX scintillator flow cell (200 µl volume, 4 mm diameter). The eluent was monitored at either 210 or 240 nm for the UV profiles.

Metabolism in Rat Hepatic Microsomes. The rate of the metabolism of L-368,899 was determined using microsomal preparations from male and female rats (N=40). L-368,899 was incubated at 37°C in 0.05 M phosphate buffer in a NADPH-regenerating system. Preliminary experiments were conducted to determine the linear kinetic region in regard to incubation time and protein concentration. Based on these experiments, the metabolism rate studies were conducted using 0.3 mg/ml protein, incubation time of 10 min, and substrate concentrations ranging from ~0.5 to 25 µM with 2 to 5 samples/condition. L-368,899 was preincubated with the microsomes at 37°C for 5 min without NADP; at t = 0, an aliquot was withdrawn, NADP was added, and the incubations were continued. Aliquots (0.4 or 0.8 ml) were withdrawn at 10 min and quenched with acetonitrile (3 ml) containing the internal standard L-369,551. After centrifugation (500g, 5 min), the supernatant was decanted and concentrated to dryness under nitrogen at 37°C. The residue was reconstituted in a mixture of 1:1:1 of methanol:acetonitrile:water containing 0.1% TFA (300 µl). Using this method, >85% of L-368,899 and the internal standard was recovered. HPLC Method 2 was used for sample analysis. The amount of L-368,899 in the samples was determined by comparing the peak area ratio of L-368,899:internal standard with ratios determined in standard curves. The rate of L-368,899 metabolism was calculated based on the change in concentration of L-368,899 at t = 0 vs. intact substrate levels after incubation. The maximal rate of conversion (Vmax) and substrate concentration at half-maximal rate of conversion (K0.5) were determined by hyperbolic fit of the rate of metabolism of L-368,899 vs. L-368,899 initial concentration. Intrinsic clearance was calculated as the Vmax/K0.5 ratio.

Analytical Methods. Radioactivity measurements. Radioactivity in dosing solutions, plasma, bile, urine, and HPLC fractions was determined by direct liquid scintillation counting. Aliquots (0.05 to 1 ml) were mixed with 5 to 20

---

**Pharmacokinetics in rats.** Male and female rats (3 to 5 animals/sex/dose) were implanted with cannulas in the right femoral vein and artery for iv administration and blood sampling, respectively. The surgical preparation was performed under nembutal anesthesia (25–50 mg/kg ip) 2 days before dosing. L-368,899 was administered intravenously at 1, 2.5, and 10 mg/kg, respectively. Blood samples (0.4 ml) were obtained from the cannulas at 0, 5, 15, 30 min, 1, 2, 4, 6, and 8 hr (except 5 mg/kg oral dose) using heparinized syringes. At 24 hr post dose, the animals were anesthetized, blood was obtained by heart puncture, and the rats were euthanized. Plasma was obtained by centrifugation at room temperature and stored at −20°C until analyzed by LC-MS/MS.

**Biliary excretion in rats.** Female rats were implanted with cannulas in the proximal duodenum and femoral vein (for iv administration only) under nembutal anesthesia (25 mg/kg ip), one day prior to dosing. The rats were infused via the duodenal cannula with a solution of 10 mM sodium taurocholate/5% dextrose/0.9% NaCl/0.05% KCl at 2 ml/hr to replace bile salts lost during the study. Rats (three animals/dose) were dosed with [14C]L-368,899 tartrate salt intravenously at 2.5 mg/kg (~9 µCi/mg) or orally at 28 mg/kg (~2 µCi/mg). Bile was collected at 1-hr intervals for the first 8 hr and from 8 to 24, 24 to 48, and 48 to 72 hr. Urine and feces were collected at 24-hr intervals for 3 days.

**Excretion in rats.** Rats (three animals/sex/dose) were dosed with [14C]L-368,899 tartrate salt intravenously at 2.5 mg/kg (~9 µCi/mg) or orally at 22 mg/kg (~4 µCi/mg). Intravenous dosing was via the femoral vein under light ether anesthesia and oral dosing was by gavage. Urine and feces were collected at 24-hr intervals for 3 days. Blood was collected via the tail vein at 1 hr for the determination of a plasma radioactivity profile.

**Pharmacokinetics in dogs.** Three female beagle dogs were dosed with L-368,899 tartrate salt intravenously at 1, 2, 4, and 10 mg/kg and orally with the sulfate salt at 5 and 33 mg/kg. There was a 3–4-week washout period between each dosing period. The iv dose was administered by a 2-min infusion via an

---

**Fig. 1.** Chemical structures of [14C]L-368,899 and L-369,551.

**Fig. 2.** Mean (± SD) concentrations of L-368,899 in plasma of female and male rats dosed intravenously at 1.0, 2.5, and 10 mg/kg.
DISPOSITION OF AN OXYTOCIN ANTAGONIST IN RATS AND DOGS

**TABLE 1**

Pharmacokinetic parameters of L-368,899 in intravenously dosed male and female rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>AUC_{0-\infty} (µg hr/ml)</th>
<th>Cl_{p} (ml/min/kg)</th>
<th>Vd_{ss} (liters/kg)</th>
<th>t_{1/2} (hr)</th>
<th>MRT (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>4</td>
<td>0.52 ± 0.15</td>
<td>35 ± 12</td>
<td>2.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>0.56 ± 0.03</td>
<td>29 ± 2</td>
<td>2.0 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.0</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>1.7 ± 0.6</td>
<td>26 ± 7</td>
<td>2.1 ± 0.4</td>
<td>1.9 ± 0.5</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>1.2 ± 0.5</td>
<td>36 ± 12</td>
<td>2.6 ± 0.5</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>9.5 ± 1.5</td>
<td>18 ± 3</td>
<td>2.0 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>5.3 ± 1.3</td>
<td>33 ± 8</td>
<td>2.1 ± 0.4</td>
<td>1.6 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

a Mean ± SD values listed.

**Fig. 3.** Mean (± SD) concentrations of L-368,899 in plasma following oral dosing in female and male rats at 5, 25, and 100 mg/kg.

ml of Insta-Gel XF (Packard Instruments, Meridan, CT) and counted in a liquid scintillation spectrometer (Beckman LS 5000TD or LS 3801, Beckman Instruments, Columbia, MD). Radioactivity in fecal homogenates (1:8 dilution) was measured by scintillation counting of trapped 14CO2 after combustion of aliquots of the homogenate. Samples were combusted in a Packard Tri-Carb Sample Oxidizer, Model B306 or 301 (Packard). The trapped 14CO2 was counted for total radioactivity in a Packard Tri-Carb Liquid Scintillation Spectrometer B3255 or a Packard Tri-Carb 1900 TR Liquid Scintillation Analyzer. Quench correction was carried out by the external ratio method.

Quantitation of L-368,899 in plasma from rats and dogs by LC-MS/MS. Concentrations of L-368,899 in rat and dog plasma were determined by LC-MS/MS on a SCIEX API III tandem mass spectrometer interfaced via a SCIEX heated nebulizer to an HPLC system. The HPLC system consisted of Shimadzu LC-600 pumps, SCI-6B controller, and a SIL-6B autoinjector (Shimadzu Scientific Instruments, Inc., Columbia, MD). Plasma (0.1 to 1 ml) was prepared for analysis by adding 40 µl of an acetonitrile solution containing 10 mg of L-369,551 (internal standard) and the internal standard (0.02 µg/ml plasma). The diluted plasma was applied to a 3 ml ion-exchange solid phase extraction cartridge (Varian Bond Elut Certify, Varian Harbor City, CA) which was preconditioned with methanol (2 ml) and 0.1% TFA (2 ml). After a wash with 0.1% TFA (2 ml), the column was eluted with 2% NH4OH in methanol (3 ml). The dried extract was reconstituted in 200 µl of mobile phase (70% acetonitrile/30% 10 mM ammonium acetate/0.1% formic acid (v/v/v)) and chromatographed using a Spherisorb C8 column at 1 ml/min (analysis time 2.5 min).

Quantitation of L-368,899 and the internal standard was achieved using selected reaction monitoring of the precursor/product combinations of m/z 555/136 and 583/164, respectively. Concentrations were calculated using established methods (11) with log-linear interpolation from t_{1/2} and to extrapolate AUC from the last measurable plasma levels to infinity, determined by linear regression from the terminal phase of the log plasma concentration vs. time curve.

Plasma clearance of L-368,899 was calculated as the iv dose divided by the plasma AUC_{0-\infty}. The volume of distribution at steady state (Vd_{ss}) was calculated using established methods (12). The mean residence time (MRT) was calculated from the ratio of AUMC (total area under the first moment of the plasma concentration-time curve) to AUC.

The bioavailability values of the 5 and 25 mg/kg oral doses in rats were calculated from the dose-adjusted ratio of the AUC to that of the 1 and 10 mg/kg iv doses, respectively. The bioavailability values of the 5 and 33 mg/kg oral doses in dogs were calculated using the AUC of the 1 and 10 mg/kg iv doses, respectively.

**Results**

Pharmacokinetics in Rats. Mean plasma concentration-time profiles of L-368,899 after iv dosing in female and male rats at 1, 2.5, and 10 mg/kg are shown in fig. 2 and the pharmacokinetic parameters are summarized in table 1. In both male and female rats, L-368,899 was eliminated from plasma in a biphasic fashion with a t_{1/2} of 1.0 to 1.9 hr. The value of Vd_{ss} varied from 2 to 2.6 liters/kg over the dosing range for both genders. In male rats, there was a proportional increase in AUC with dose, and plasma clearance varied from 29 to 36 ml/min/kg. In female rats, AUC increased proportionally from 1 to 2.5 mg/kg, but more than proportionally between 2.5 and 10 mg/kg. Plasma clearance in female rats decreased from 35 ml/min/kg at 1 mg/kg to 18 ml/min/kg at 10 mg/kg.

The mean plasma concentration vs. time curves in orally dosed rats are shown in fig. 3, and the absorption pharmacokinetic parameters are summarized in table 2. There was considerable individual variation in the plasma levels of L-368,899 in both male and female rats.
increases in AUC in males were 5.6-fold between 1 and 10 mg/kg dose. At the 25 and 100 mg/kg doses, the AUC in females was 4.5- and 2.5-fold greater than the AUC in male rats, respectively. There was a disproportional increase in AUC with oral dose in both genders. In female rats, AUC increased 65-fold between 5 and 25 mg/kg dose and 8-fold between 25 and 100 mg/kg; the respective increases in AUC in males were 10- and 15-fold. The oral bioavailability of L-368,899 was estimated at 14% and 18% for the 5 mg/kg dose in female and male rats, respectively, and 41% for the 25 mg/kg dose in males. Bioavailability was not calculated for the 25 mg/kg dose in female rats and the 100 mg/kg dose in both male and female rats because AUC values at these higher doses were outside the range of those obtained from the iv doses.

Excretion of radioactivity in bile, urine and feces from female rats dosed with [14C]L-368,899 intravenously at 2.5 mg/kg or orally at 28 mg/kg.

Absorption kinetics of L-368,899 in orally dosed female and male rats.

**TABLE 2**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>AUC&lt;sub&gt;0-ss&lt;/sub&gt; (µg/hr/ml)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hr)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (hr)</th>
<th>t&lt;sub&gt;ss&lt;/sub&gt; (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4</td>
<td>0.38 ± 0.10</td>
<td>0.22 ± 0.13</td>
<td>0.6 ± 0.9</td>
<td>1.2 ± 0.5</td>
<td>14</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>0.52 ± 0.14</td>
<td>0.58 ± 0.05</td>
<td>0.2 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>18</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>25 ± 7</td>
<td>8.7 ± 2.6</td>
<td>0.6 ± 0.3</td>
<td>2.4 ± 0.7</td>
<td>—c</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>5.5 ± 1.3</td>
<td>2.7 ± 1.1</td>
<td>0.5 ± 0</td>
<td>1.6 ± 0.7</td>
<td>41</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>208 ± 14</td>
<td>26 ± 17</td>
<td>4.0 ± 2.0</td>
<td>4.1 ± 3.1</td>
<td>—c</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>84 ± 36</td>
<td>17 ± 3</td>
<td>2.5 ± 1.0</td>
<td>2.3 ± 0.8</td>
<td>—c</td>
</tr>
</tbody>
</table>

a Mean ± SD values are listed.  
b The bioavailability of the 5 mg/kg dose in male and female rats and of the 25 mg/kg dose in male rats was calculated relative to the dose adjusted-ratio AUC of the 1 and 10 mg/kg intravenous doses, respectively.  
c Bioavailability was not calculated because plasma AUC values were outside the range observed after intravenous dosing.

**TABLE 3**

<table>
<thead>
<tr>
<th>Dosing Route</th>
<th>Time (hr)</th>
<th>% Dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Bile</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>0–24</td>
<td>80.4 ± 3.4</td>
<td>6.1 ± 1.8</td>
<td>0.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24–48</td>
<td>0.2 ± 0.2</td>
<td>0.8 ± 0.8</td>
<td>0.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–72</td>
<td>80.6 ± 3.3</td>
<td>7.0 ± 2.8</td>
<td>1.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>PO</td>
<td>0–24</td>
<td>59.9 ± 5.6</td>
<td>9.4 ± 5.0</td>
<td>0.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24–48</td>
<td>1.3 ± 0.4</td>
<td>2.8 ± 0.7</td>
<td>9.0 ± 11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–72</td>
<td>61.5 ± 5.4</td>
<td>12.7 ± 4.8</td>
<td>17.2 ± 11.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD from three rats/dose.

Absorption of the 5 and 25 mg/kg po doses was rapid in both sexes with mean peak plasma concentrations reached between 0.2 and 0.6 hr. Absorption was somewhat prolonged at the highest dose (100 mg/kg) with a mean T<sub>max</sub> of 2 to 4 hr.

Plasma AUC was dose- and gender-dependent in rats after oral dosing, except that there was no gender difference in the AUC at the 5 mg/kg dose. At the 25 and 100 mg/kg doses, the AUC in females was 4.5- and 2.5-fold greater than the AUC in male rats, respectively. There was a disproportional increase in AUC with oral dose in both genders. In female rats, AUC increased ~65-fold between 5 and 25 mg/kg dose and 8-fold between 25 and 100 mg/kg; the respective increases in AUC in males were ~10- and ~15-fold. The oral absorption kinetics of L-368,899 in orally dosed female and male rats a

**FIG. 4.** Mean (± SD) concentrations of L-368,899 in plasma of female dogs dosed intravenously at 1.0, 2.4, and 10 mg/kg and orally at 5 and 33 mg/kg.

Regardless of species, sex, or dosing route, unchanged L-368,899 accounted for most of the radioactivity in the plasma samples (63–82% at 1 hr post-dose), while in the bile, feces, and urine about 5 to 10% of the radioactivity was parent compound, with the majority of the radioactivity attributed to four to six metab-
The bioavailability of 17–18% at the 5 mg/kg dose and 41% at the higher oral doses, and clearance of 23–36 ml/min/kg over the 1 to 10 mg/kg iv dose range. Both species exhibited nonlinear pharmacokinetics, especially after oral dosing.

The pharmacokinetics of L-368,899 in the female rat were comparable with the male at the 1 and 2.5 mg/kg iv dose and the low oral dose. However, gender differences were observed at the higher doses. At the 10 mg/kg iv dose the clearance in females dropped from 26–35 ml/min/kg to 18 ml/min/kg, while linear pharmacokinetics were observed in males. The most notable gender difference was observed after oral dosing, with AUC values in females higher than in males by 4.5-fold at the 25 mg/kg dose and 2.5-fold at the 100 mg/kg dose. The occurrence of gender-dependent pharmacokinetics and pharmacodynamics are not uncommon in rats (13, 14). One factor commonly known to contribute to gender differences for compounds cleared primarily by oxidative metabolism is differential expression of hepatic cytochrome P-450 isoforms (14–16). This is considered a probable explanation in this case because L-368,899 is cleared primarily by metabolism. Furthermore, metabolism of L-368,899 by both male and female rat hepatic microsomes was inhibited by TAO (80% inhibition at 100 μM TAO), 3 indicating metabolism was mediated by CYP3A4 isozymes, which are preferentially expressed in the male rat (16–19). When the rate of total metabolism of L-368,899 was compared in liver microsomal preparations from male and female rats, a substantial gender difference was observed in both microsomal preparations from female rats is consistent with the slower plasma clearance observed at the lower iv doses and comparable plasma clearance observed at the 100 mg/kg iv dose range. Both species exhibited nonlinear pharmacokinetics, especially after oral dosing.

The pharmacokinetics of L-368,899 in the female rat were comparable with the male at the 1 and 2.5 mg/kg iv dose and the low oral dose. However, gender differences were observed at the higher doses. At the 10 mg/kg iv dose the clearance in females dropped from 26–35 ml/min/kg to 18 ml/min/kg, while linear pharmacokinetics were observed in males. The most notable gender difference was observed after oral dosing, with AUC values in females higher than in males by 4.5-fold at the 25 mg/kg dose and 2.5-fold at the 100 mg/kg dose. The occurrence of gender-dependent pharmacokinetics and pharmacodynamics are not uncommon in rats (13, 14). One factor commonly known to contribute to gender differences for compounds cleared primarily by oxidative metabolism is differential expression of hepatic cytochrome P-450 isoforms (14–16). This is considered a probable explanation in this case because L-368,899 is cleared primarily by metabolism. Furthermore, metabolism of L-368,899 by both male and female rat hepatic microsomes was inhibited by TAO (80% inhibition at 100 μM TAO), 3 indicating metabolism was mediated by CYP3A4 isozymes, which are preferentially expressed in the male rat (16–19). When the rate of total metabolism of L-368,899 was compared in liver microsomal preparations from male and female rats, a substantial gender difference was observed in both microsomal preparations from female rats is consistent with the slower plasma clearance observed at the lower iv doses and comparable plasma clearance observed in females at the 10 mg/kg iv dose of 18 vs. 33 ml/min/kg in male. Assuming there is no sex difference in absorption, this result also is consistent with the 4.5-fold higher AUC observed in female compared with male rats at the 25 mg/kg oral dose. At the high oral dose in rats (100 mg/kg) the gender difference in plasma AUC decreased, with the AUC in females only 2.5-fold higher than in males. The smaller difference in AUC at the 100 vs. 25 mg/kg dose in
male and female rats, may result from extensive saturation of the liver clearance process now occurring in both genders. Therefore, the relatively low $K_m$ of L-368,889 in both male and female rat liver microsomes may have contributed to the nonlinear pharmacokinetics in this species, as the result of L-368,899 concentrations in the liver at the higher doses exceeding $K_m$. Another possible explanation for gender-dependent pharmacokinetics of L-368,899 is gender differences in plasma protein binding (20). However, this is unlikely to be a major contributing factor in the case of L-368,899 since this compound is bound extensively to plasma protein, 88–95% in both male and female rats.  

In conclusion, L-368,899 is rapidly absorbed in rats and dogs. The compound exhibits linear pharmacokinetics at iv doses between 1 and 10 mg/kg in male rats and female dogs and between 1 and 2.5 mg/kg in female rats. The pharmacokinetics of L-368,899 are dose- and gender-dependent in rats and dose-dependent in dogs. Based on in vitro studies, it is postulated that gender differences in hepatic metabolic capacity and saturation of metabolism may contribute to the gender- and dose-dependent pharmacokinetics of L-368,899 in the rat. The main pathway of elimination in both species is metabolism followed by excretion into the feces.

Acknowledgments. We wish to thank the following for their assistance: Mr. P. Cunningham and Mr. D. Hora with the dog studies, Ms. E. McGowan and Dr. P. Krieter with the rat studies, Ms S. Painter with plasma protein binding studies, Dr. D. Dean for the preparation of the radiolabeled L-368,899, Dr. P. Williams for the supply of L-368,899 and L-369,551, and Drs. J. Lin, X. Xu, R. Stearns, and M. Chiba for helpful discussions.


References