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

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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 t\textsubscript{1/2} 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 V\textsubscript{dss} 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 C\textsubscript{max} 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 V\textsubscript{max} 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 \(^{14}\)C at the terminal carbon of the methionine sulfone side chain and supplied as the tartrate salt (fig. 1). The radiochemical purity was >99% based on TLC and HPLC analysis. \(^{14}\)C-L-368,899 was diluted to a specific activity of ~2.5 to 12 \(\mu\)Ci/mg with 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 wash of the resulting precipitate (2 volumes). The mixture was centrifuged (500 g, 5 min), and the supernatant was discarded. The remainder was acidified to pH 3 with 1 N NaOH and centrifuged (500 g, 5 min), and the supernatant was discarded. The remainder was extracted with ether and the aqueous phase was acidified to pH 2 with 1 N HCl. The mixture was centrifuged (500 g, 5 min), and the supernatant was discarded. The remaining acidified sample was lyophilized.

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 = 10). 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 per concentration. 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 (500 g, 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 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 (500 g, 5 min), and the supernatant was analyzed. The samples were analyzed using HPLC Method 1. The radioactivity profiles were obtained using a Raytest Romana-S-LS fitted with a glass-TSS 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 Dogs. The metabolism of L-368,899 was determined using microsomal preparations from male and female rats (N = 10). 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 per concentration. 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 (500 g, 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 (Km) 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/Km ratio.
AUC \(_{0-\infty}\) (\(\mu g\text{-}hr/ml\)) | \(\text{Cl}_p\) (mL/min/kg) | \(V_{dss}\) (liters/kg) | \(t_1/2\) (hr) | MRT (hr)
---|---|---|---|---
1.0 | Female | 4 | 0.52 ± 0.15 | 35 ± 12 | 2.0 ± 0.2 | 1.0 ± 0.2 | 1.0 ± 0.3
 | Male | 4 | 0.56 ± 0.03 | 29 ± 2 | 2.0 ± 0.2 | 1.1 ± 0.1 | 1.1 ± 0.0
2.5 | Female | 3 | 1.7 ± 0.6 | 26 ± 7 | 2.1 ± 0.4 | 1.9 ± 0.5 | 1.4 ± 0.3
 | Male | 4 | 1.2 ± 0.5 | 36 ± 12 | 2.6 ± 0.5 | 1.3 ± 0.4 | 1.3 ± 0.4
10 | Female | 3 | 9.5 ± 1.5 | 18 ± 3 | 2.0 ± 0.1 | 1.8 ± 0.3 | 1.9 ± 0.4
 | Male | 4 | 5.3 ± 1.3 | 33 ± 8 | 2.1 ± 0.4 | 1.6 ± 0.2 | 1.1 ± 0.1

*Mean ± SD values listed.

**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 \(V_{dss}\) 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, but more than proportionally between 2.5 and 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.
Absorption kinetics of L-368,899 in orally dosed female and male rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>AUC0-∞ (µg*h/ml)</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (hr)</th>
<th>t1/2 (hr)</th>
<th>bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</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></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</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></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</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 2

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

<table>
<thead>
<tr>
<th>Dosing Route Time (hr)</th>
<th>% Dose</th>
<th>Bile</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–24</td>
<td>80.4 ± 3.4</td>
<td>6.1 ± 1.8</td>
<td>0.2 ± 0.2</td>
<td></td>
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<tr>
<td>24–48</td>
<td>0.2 ± 0.8</td>
<td>0.8 ± 0.8</td>
<td>0.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<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></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<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>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>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>

a 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_max 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 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 in Rats. The biliary excretion of [14C]L-368,899 was studied in female rats with cannulated bile ducts after dosing orally at 28 mg/kg and intravenously at 2.4 mg/kg. About 74% of the oral dose was recovered in the bile and urine by 72 hr, as compared to 88% of the iv dose, indicating that the radiolabeled dose was well-absorbed (table 3). Excretion into the bile was rapid, with 40% of the radioactivity recovered within 1 hr after iv dosing and within 4 hr after oral dosing (data not shown). The excretion of L-368,899 also was studied in four female and four male rats dosed with [14C]L-368,899 orally at 22 mg/kg and intravenously at 2.5 mg/kg. After both routes of administration in both genders, about 80% of the radioactivity was excreted in the feces within 48 hr and about 4–10% in the urine (data not shown).

Pharmacokinetics in Dogs. Plasma concentrations of L-368,899 in three female beagle dogs after iv dosing at 1, 2.4, and 10 mg/kg and oral dosing at 5 and 33 mg/kg are shown in fig. 4, and the pharmacokinetic parameters are presented in table 4. In general, L-368,899 concentrations after iv dosing were comparable with those in male rats. The t_{1/2} was 1.7 to 2.7 hr. Plasma clearance varied from 23 to 36 ml/min/kg and Vdss varied from 3.4 to 4.9 liters/kg over the dosing range. After oral dosing, absorption was rapid with a mean T_max of 0.3 hr at the 5 mg/kg dose. Absorption was somewhat prolonged at the 33 mg/kg dose with a mean T_max of 1.2 hr.

As in rats, plasma AUC increased more than proportionally with the increase in the oral dose, namely ~12-fold between 5 and 33 mg/kg (table 4). Oral bioavailability was estimated at 17% and 41%, respectively, for the 5- and 33-mg/kg doses.

Excretion in Dogs. The excretion of [14C]L-368,899 was studied in three female dogs dosed intravenously at 2.4 mg/kg. About 72% of the dose was recovered in feces and another 4% in urine within the first 2 days. After 6 days, a total of 79% and 6% of the dose were recovered in the feces and urine, respectively (data not shown).
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), indicating metabolism was mediated by CYP3A 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 isozymes, which are preferentially expressed in the male rat (16–19).

Discussion

The pharmacokinetics and disposition of L-368,899 were studied in female and male rats and female dogs. For all animals the disposition of the dose recovered in the urine. L-368,899 was metabolized extensively in both species, with <10% of the dose excreted unchanged. The pharmacokinetics were similar in rats and dogs, particularly between the male rat and female dog, with L-368,899 exhibiting a gender difference being ~4 fold less in females than in males.

The resulting $V_{\text{max}}/K_M$ of L-368,899 was not significantly different between male and female rats. The lack of gender differences in microsomal intrinsic clearance is consistent with the comparable plasma clearance observed at the lower iv doses and similar AUC at the lowest oral dose (5 mg/kg) for male and female rats. The lower maximal rate of turnover of L-368,899 in microsomal preparations from female rats is consistent with the slower 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-368,899 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.4

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.

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References


