THE DISPOSITION, METABOLISM, AND PHARMACOKINETICS OF A SELECTIVE METABOTROPIC GLUTAMATE RECEPTOR AGONIST IN RATS AND DOGS

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ABSTRACT:

Compound LY354740 ([+]-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid), an analog of glutamic acid, is a selective group 2 metabotropic glutamate receptor agonist in clinical development for the treatment of anxiety. Studies have been conducted to characterize the absorption, disposition, metabolism, and excretion of LY354740 in rats and dogs after intravenous bolus or oral administration. Plasma concentrations of LY354740 were measured using a validated gas chromatography/mass spectrometry assay. In rats, LY354740 demonstrated linear pharmacokinetics after oral administration from 30 to 1000 mg/kg. The oral bioavailability of LY354740 was approximately 10% in rats and 45% in dogs. In the dog, food decreased the mean area under the plasma concentration-time curve value by approximately 34%, hence, decreasing the oral bioavailability of the compound. Excretion studies in both rats and dogs indicate that the absorbed drug is primarily eliminated via renal excretion. In addition, tissue distribution in rats showed that the highest levels of radioactivity were in the kidney and gastrointestinal tract, which is consistent with the excretion studies. Metabolism of LY354740 was evaluated in vitro using rat and dog liver microsomes and rat liver slices. In addition, urine and fecal samples from rat and dog excretion studies were profiled using HPLC with radio-detection. These evaluations indicated that neither rats nor dogs metabolized LY354740. In summary, LY354740 is poorly absorbed in rats, moderately absorbed in dogs, and rapidly excreted as unchanged drug in the urine.

Compound LY354740 is a potent and selective group 2 metabotropic glutamate (mGlu) receptor agonist currently in clinical development. LY354740 is a conformationally constrained analog of glutamic acid (Fig. 1). Metabotropic glutamate receptors are G protein-coupled receptors involved in the production of second messengers. These receptors are currently divided into three classes based on their mechanism of signal transduction, selectivity, and amino acid sequence homology (Nakanishi, 1992; Nakanishi and Masu, 1994; Pin and Duvoisin, 1995). Group 2 mGlu receptors are negatively coupled to adenylyl cyclase; therefore, activation of the receptor would lead to inhibition of cAMP production. LY354740 has been shown to suppress forskolin-stimulated cAMP production in adult rat brain tissues without affecting other glutamate receptors (Monn et al., 1997; Schoepf et al., 1998). Furthermore, LY354740 was shown to block the veratridine-evoked release of endogenous glutamate in vivo in rats (Battaglia et al., 1997). Oral or intravenous administrations of LY354740 have been shown to potentiate the response of spinal neurons to ionotropic glutamate receptor agonists (Bond et al., 1997). Because glutamate is the principal excitatory amino acid neurotransmitter in the mammalian central nervous system, it is thought that modulation of the activity of this neurotransmitter may be involved in a variety of neurological disease states (Daniysz et al., 1995; Helton et al., 1998b; Cartmell et al., 1999). In vitro studies have shown that LY354740 is a novel and highly selective ligand for group 2 mGlu receptors (Schaffhauser et al., 1997, 1998; Schoepf et al., 1997). Studies with LY354740 have demonstrated potent anxiolytic activity in the fear-potentiated startle and the elevated plus maze models of anxiety in rodents (Monn et al., 1997; Helton et al., 1998b). In addition, LY354740 has also been shown to suppress drug withdrawal induced by benzodiazepines in rats (Helton et al., 1998a) and to attenuate the behavioral signs of morphine withdrawal in morphine-dependent rats (Vandergriff and Rasmussen, 1999). Additional studies have demonstrated neuroprotective effects of LY354740 in rat and gerbil models of ischemia (Bond et al., 1998; Lam et al., 1998). More recent studies have suggested a possible role for group 2 mGlu agonists in the treatment of psychosis (Cartmell et al., 1999).

The present studies were conducted to characterize the absorption, disposition, metabolism, and excretion of a novel metabotropic glutamate receptor agonist in rats and dogs. Although the disposition of excitatory amino acid-like compounds has been previously reported (Duncan et al., 1991; Albers et al., 1999; Bullock et al., 1999), nearly all reports describe the disposition of ionotropic receptor agonists or antagonists. By comparison, relatively little information has been...
published on the physiological role (Lang and Ajmal, 1995), metabolism, or disposition of a metabotropic receptor agonist. Therefore, these data will provide important information on the metabolism and disposition properties of an excitatory amino acid-like compound functioning as an mGlu receptor agonist.

### Experimental Procedures

**Materials.** LY354740 (Fig. 1) and internal standard (LSN338771) were synthesized at Eli Lilly and Company (Indianapolis, IN). LY354740 was supplied as the monohydrate. All other chemicals and reagents were of analytical grade. Control rat plasma was purchased from Harlan (Indianapolis, IN), and control dog plasma was from Marshall Farms (North Rose, NY).

**Animal Experiments.** All animal experiments were conducted according to protocols approved by the Eli Lilly Animal Care and Use Committee. Dosing solutions used for all animal studies were prepared by dissolving the required amounts of LY354740 monohydrate in water and adjusting the pH to 6.5 to 7.4 using dilute sodium hydroxide. Concentrations of LY354740 were calculated on the basis of the free acid. Male and female Fischer-344 rats obtained from Taconic Farms (Germantown, NY), ranging in age from 8 to 9 weeks and weighing approximately 200 g, were used for these studies. In the excretion study, dogs were housed in individual metabolism cages during the study period. Rats were given free access to food and water. Male and female beagle dogs obtained from Marshall Farms (North Rose, NY), ranging in age from 3 to 6 years and weighing 12 to 15 kg, were housed in individual cages during these studies. In the excretion study, dogs were fasted for at least 18 h before receiving the compound and fed 4 h after administration.

**Pharmacokinetic Studies in Rats.** The single dose plasma pharmacokinetics of LY354740 were studied in male Fischer-344 rats administered a 5-mg/kg i.v. bolus or 30-mg/kg oral dose of LY354740 monohydrate. Blood samples (three samples/time point) were collected via cardiac puncture at 0 (predose), 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose after i.v. dosing and at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose after oral dosing. In a separate study, single oral doses of 30, 100, 300, and 1000 mg/kg were administered to male and female Fischer-344 rats (20 animals/sex/dosage group). Blood samples (two/time point; one/sex) were collected via the orbital plexus at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose.

**Pharmacokinetic Studies in Dogs.** Before the start of a study, dogs were pretreated with LY354740 (daily oral 15-mg/kg doses) for 3 days to develop tolerance to the drug’s emetic effect. The plasma pharmacokinetics of LY354740 were studied in fed male and female beagles (4/sex) after a single 5-mg/kg i.v. bolus dose and after a single oral 15-mg/kg dose of LY354740 monohydrate. Blood was collected via the jugular or cephalic vein at 0, 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after i.v. dosing and at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after oral dosing. In addition, the effect of food on absorption was studied in a crossover design using four female beagles that were fasted for at least 18 h (feed four h after drug was administered) or fed 15 min before administration of a 15-mg/kg oral dose. Blood was collected via the jugular or cephalic vein at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose. For all studies, blood was collected in heparinized tubes and plasma was obtained by centrifugation (room temperature) and stored at approximately −70°C until subsequent analysis for LY354740.

**Excretion Studies and Radiolabeled Analysis.** The elimination of radiolabeled LY354740 was determined in rats and dogs using [14C]LY354740 monohydrate. Rats were administered a single 30-mg/kg oral or i.v. bolus dose of [14C]LY354740 monohydrate containing 20 μCi/kg radioactivity. Urine and feces were collected in 24-h intervals for up to 96 h postdose. Dogs were pretreated with unlabeled LY354740 monohydrate for 3 days (15-mg/kg daily oral doses) before administration of a 15-mg/kg oral dose of [14C]LY354740 monohydrate containing 5 μCi/kg radioactivity. For dogs, urine and feces were collected at 0 to 12, 12 to 24, 24 to 48, 48 to 72, and 72 to 96 h postdose. Radioactivity in urine was determined by mixing aliquots of urine (0.05−0.5 ml) with approximately 15 ml of Ultima Gold scintillation fluid (Packard Instruments, Meriden, CT) and counting in a liquid scintillation counter (LS5000TD). Radioactivity in fecal homogenates (1:2 dilution) was measured by scintillation counting of trapped 14CO2 after combustion of dried homogenate aliquots. Samples were compounded on a Packard sample oxidizer (model 307; Packard Instruments, Meriden, CT).

**Metabolism.** Hepatic microsomes were prepared by standard differential centrifugation from Fischer-344 rat and beagle dog livers. Liver microsomes (2 mg of protein/ml) were incubated with [14C]LY354740 (40 μM) at 37°C for 1 h in a pH 7.4 phosphate buffer and in the presence of an NADP-regenerating system (1 mM NADP, 10 units of glucose-6 phosphate dehydrogenase, and 20 mM glucose-6-phosphate). The microsomal protein fraction was precipitated with an equal volume of acetone/titrile, and the mixture was centrifuged.

To prepare liver slices, Fischer-344 rats were deeply anesthetized with isoflurane, and their livers were excised through a mid-ventral incision and immediately placed in ice-cold saline. Cylindrical tissue cores were made with a coring press and individual slices (200-μm thickness) were prepared using a Brendel/Vitron tissue slicer. Liver slices were placed in vials (two/vial) containing 1.7 ml of medium with 200 μM [14C]LY354740 and incubated for 24 h in a dynamic roller culture incubator at 37°C under an atmosphere of 95% O2/5% CO2. Slices were sonicated in their medium and centrifuged.

Liver slice and microsomal preparations and urine and fecal samples from excretion studies were profiled by HPLC with radiodetection and analyzed by liquid chromatography with tandem mass spectrometric detection and/or GC/MS to assess metabolism.

**Plasma Protein/Erythrocyte Binding.** Plasma protein binding of LY354740 was investigated using a ultracentrifugation method. Rat and dog plasma were spiked with approximately 500 ng/ml [14C]LY354740 and incubated for 1 h at 37°C. After incubation, three 1-ml aliquots were centrifuged at approximately 400,000 g for 4 h at 37°C. Aliquots of the unbound fraction were diluted with 15 ml of scintillation cocktail and analyzed by liquid scintillation counting.

Erythrocyte binding was determined from blood samples collected from dogs after an oral 15-mg/kg dose of [14C]LY354740 monohydrate. On the day of dosing, blood was collected into tubes from the jugular vein from four female dogs and the blood/plasma ratio and hematocrit value for each dog was measured. After dosing, blood was collected from each dog at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, and 96 h postdose. Triplicate aliquots of blood were analyzed for radioactivity by liquid scintillation spectrometry after combustion using a Packard Sample Oxidizer. Plasma fractions were then obtained from the remaining portions of the blood samples by centrifugation and the radioactivity was determined in triplicate aliquots by direct liquid scintillation counting.

**Tissue Distribution Evaluated by Quantitative Whole-Body Autoradiography (QWBA) in Rats.** Tissue distribution of radioactivity associated with [14C]LY354740 was studied in male Fischer-344 rats at 0.5, 1, 2, 4, 6, 8, 12, and 24 h after a single oral 30-mg/kg dose of [14C]LY354740 monohydrate. Animals were euthanized with isoflurane and exsanguinated via cardiac puncture. Each animal was rapidly frozen in a dry ice-hexane bath and processed for whole-body autoradiographic evaluation as described by Ullberg (1977). The frozen carcasses were embedded in a 2% gel of carboxymethylcellulose (Sigma, St. Louis, MO) which when frozen, supported the carcass for sectioning on a cryomicrotome (Leica, Deerfield, Ill.). Sagittal whole-body sections (approximately 20 μm thick) were then freeze-dried. Sections were...
sampled to include the following tissues and organs for qualitative and/or quantitative evaluations: adrenal gland, blood, bone, bone marrow, brain (cerebellum, cerebrum and medulla), brown fat, cecal wall, epididymis, eye, Harderian gland, intestinal wall, kidney, liver, lung, lymph node, muscle, myocardium, pancreas, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicles, skin, spleen, spinal cord, stomach wall, testis, thymus, thyroid gland, and white fat. In addition, cecal contents, feces, gastrointestinal contents, and urine were qualitatively evaluated. Autoradiographic images were recorded and quantified using phosphor imaging technology as described in detail by Johnston et al. (1990). Quantification of tissue concentrations of radioactivity was conducted using radionuclide commercial standards (American Radiolabeled Chemicals, St. Louis, MO) and a liver homogenate, which was used as an internal standard to correct for section thickness variations.

Sagittal whole-body sections, radionuclide standards, and internal standards were simultaneously exposed for approximately 7 days to phosphor imaging plates (Molecular Dynamics, Sunnyvale, CA). Before exposure, background was erased by exposing the imaging plates to bright visible light using the model 410A Image Eraser (Molecular Dynamics). After exposure, imaging plates were scanned with a helium-neon laser using the model 425E PhosphorImager (Molecular Dynamics). Scanner operations, data display and analysis were performed using ImageQuant (Molecular Dynamics) and Excel (Microsoft, Redmond, WA) software. Quantitative evaluation was done using volume integral phosphor imager signals from tissues which were corrected for section thickness variation using the internal standard as described by Chay and Pohland (1994). Single samples were taken on multiple sections for each tissue from each animal. Standard curves associated with individual scans were fit with a least-squares regression line from which tissue concentrations of radionuclide were interpolated. The reported lower limit of detection was based on the mean standard curve concentrations. Phosphor images were also visually evaluated and representative images were reproduced.

**Analytical Procedure.** LY354740 in plasma was assayed using a validated GC/MS method. To 300 μl of plasma, 100 μl of internal standard (0.5 μg of LSN338771/ml of water) and 1.0 ml of ice-cold ethanol were added. The supernatant was vortexed and allowed to stand for 5 min before centrifuging. The sample was evaporated to a concentrated SAX (5 mg; a 3-ml reservoir) solid phase extraction cartridge (Jones Chromatography, Lakewood, CO). After washing the cartridge with 0.1% acetic acid in acetonitrile (3 ml), the analytes were eluted with a 3% trifluoroacetic acid solution in acetonitrile (3 ml). The compounds of interest were concentrated to dryness and derivatized with 3 N methanolic HCl (0.6 ml) at 90°C for 30 min. The sample was evaporated to dryness under a stream of nitrogen at 60°C. Acetonitrile (0.5 ml) and trifluoroacetic anhydride (0.2 ml) were added and vortexed, and the sample was allowed to stand at room temperature for 30 min. The sample was evaporated to dryness and reconstituted with a 30% methanol in toluene solution (100 μl). The derivatized sample was then analyzed by selected ion monitoring GC/MS using electron impact ionization and positive ion detection (LY354740, M+ mas/250; internal standard, M+ mas/278). The assay was initially validated over the range of 50 to 3000 ng/ml. The assay was later validated over the range of 20 to 3000 ng/ml in both rat and dog plasma. The mean interassay accuracy ranged from 104 to 106% in dog plasma and 96 to 101% in rat plasma. The mean precision of the assay was within 12.1 and 6.7% for rat and dog plasma, respectively. Plasma samples exceeding the upper limit of quantification were diluted with control plasma before extraction.

**Data Analysis.** Plasma concentrations were calculated using the ChemStation computer software (Hewlett Packard, Palo Alto, CA) and transferred to a laboratory information management system database. Pharmacokinetic parameters were determined using standard noncompartmental methods via the proprietary ADME WINPTK computer software package (Eli Lilly and Company). AUC was calculated by the linear trapezoidal rule. Plasma clearance of LY354740 was calculated as the i.v. bolus dose divided by the plasma AUC0–∞. Values for Cmax and Tmax were obtained from observed data. The elimination rate constant, ke, was determined by linear regression of the terminal log-linear phase of the concentration versus time curve; 1/τ1/2 was calculated as 0.693/ke. The mean residence time (MRT) was calculated from the ratio of total area under the first moment of the drug concentration curve to AUC. The volume of distribution at steady state (Vdss) was calculated as clearance/MRT. The apparent volume of distribution (Vd0) was calculated as clearance/k. Oral bioavailability in rats and dogs was estimated from the dose-adjusted ratio of the AUC0–∞ relative to that of the 5-μg/kg i.v. bolus dose. Samples below the 20 ng/ml limit of quantitation were assigned a value of zero for calculating kinetic parameters. Radiation absorbed dose of radiocarbon associated with LY354740 was calculated as follows: absorbed dose (mRad) = % dose/organ × S factor × exposure, where S factor is the factor for the given organ and exposure is the equivalent of a microcurie dose in humans. Percent protein binding of LY354740 was calculated as follows: % protein binding = (1 – C′/C) × 100, where C′ is the amount of radioactivity in protein-free fraction of plasma sample and C is the amount of radioactivity in plasma sample.

**Results**

**Pharmacokinetics. Rats.** The mean plasma concentration versus time profiles for LY354740 after single intravenous bolus and oral doses of LY354740 monohydrate are presented in Fig. 2, and the pharmacokinetic parameters are summarized in Table 1. After an i.v. dose, LY354740 was eliminated from plasma with a 1/2 of approximately 3 h. The mean plasma clearance of LY354740 was 11.1 ml/min/kg. After an oral 30-μg/kg dose of LY354740 monohydrate, peak plasma concentrations generally occurred at 2 h postdose (Table 1), and the bioavailability of LY354740 was determined to be approximately 10%. In a separate study, single oral doses of 30, 100, 300, and 1000 mg/kg to rats indicated linear pharmacokinetics (AUC0–∞ versus dose; r2 = 0.992) from 30 to 1000 mg/kg. The pharmacokinetic parameters used for this evaluation are summarized in Table 2.

**Dogs.** There were no gender-specific differences in plasma drug concentrations or pharmacokinetics. The mean plasma concentration versus time profiles for LY354740 after single intravenous bolus or oral doses of LY354740 monohydrate to eight dogs (4/sex) are shown in Fig. 3, and the mean pharmacokinetic parameters are summarized in Table 3. After i.v. administration, LY354740 was eliminated from...
LY354740 was poorly distributed. For those tissues that showed
concentrations in the kidney and liver of 316.41 and 4.28 g/g,
respectively. In a simple fed/fasted crossover study, fasted dogs
at 45%; however, the absorption of LY354740 may be affected by the
dose, mean peak plasma concentrations were reached at 2.5 h
ml/min/kg; and 0.2 and 0.3 l/kg, respectively. After a 15-mg/kg oral
dose, mean peak plasma concentrations were reached at 2.5 ± 0.3 h
of dosing. Oral bioavailability of LY354740 in fed dogs was estimated
at 45%; however, the absorption of LY354740 may be affected by the
presence of food. In a simple fed/fasted crossover study, fasted dogs
(n = 4) showed higher mean C_{max} (5.96 ± 1.14 versus 3.72 ± 0.30
µg/ml) and mean AUC_{0–∞} values (31.67 ± 4.54 versus 20.97 ± 3.23
µg/ml), and a slightly longer T_{max} (3.0 ± 1.0 versus 2.3 ± 0.6) than
dogs after an oral 15-mg/kg dose. In addition, three of the fasted
dogs had increased bioavailability of LY354740, whereas the fourth
dog showed virtually no change.

**Tissue Distribution Evaluated by QWBA in Rats.** The mean
radiocarbon tissue concentrations in male rats after a single oral
30-mg/kg dose of [14C]LY354740 monohydrate as determined by
QWBA are shown in Table 4. Additional tissues that were evaluated
but not listed contained radiocarbon concentrations that were at back-
ground levels or below the lower limit of quantification (mean level of
1.45 µg/g and range of 0.77 to 2.15 µg/g). The kidney showed a
heterogeneous distribution of radioactivity, and both the high and low
regions were reported. At 0.5 and 1 h postdose, the highest levels of
radioactivity were observed in the gastrointestinal tract, kidney, and
urine (Fig. 4). At 2 h postdose, high levels of radiocarbon associated
with [14C]LY354740 were present in the cecal contents with increasing
concentrations in the kidney and liver of 316.41 and 4.28 µg/g,
respectively. Peak tissue concentrations of radiocarbon occurred at 4 h
postdose in Harderian gland, intestinal wall, kidney, and liver (Fig. 5).
At 12 and 24 h postdose, the highest levels of radiocarbon were
observed in intestinal and fecal contents, and only kidney and thymus
contained quantifiable concentrations of radiocarbon; 2.43 and 3.17
µg/g, respectively. Radiocarbon tissue concentrations indicated that
LY354740 was poorly distributed. For those tissues that showed
measurable radioactivity, the estimated pharmacokinetic parameters are
summarized in Table 5. The AUC value was greatest in the
kidney; approximately 3670 µg h/g. The distribution half-lives ranged
from approximately 2 h in the kidney to 16 h in the thymus, although
the half-life for thymus is estimated as the sampling interval that is
similar to the reported half-life (Table 5). Overall, the QWBA results
showed no significant organ or tissue accumulation after a single oral
dose of [14C]LY354740 monohydrate.

**Excretion.** Rats. The excretion of radioactivity was studied in male
rats administered single 30-mg/kg i.v. bolus or oral doses of
[14C]LY354740 monohydrate. After i.v. administration, approximately
78% of the radioactivity was eliminated in the urine, whereas
only 10% was eliminated in the feces (Table 6). The amount of
radioactivity in the carcass and cage wash was approximately 2 and
8%, respectively. Thus the total recovery after i.v. administration

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**Table 1**

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>i.v.</th>
<th>p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (µg/ml)</td>
<td>18.1±0.8</td>
<td>0.51±0.03</td>
</tr>
<tr>
<td>AUC_{0–12} (µg · h/ml)</td>
<td>7.5±0.4</td>
<td>4.7±0.6</td>
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<tr>
<td>AUC_{0–∞} (µg · h/ml)</td>
<td>7.6±0.4</td>
<td>4.8±0.6</td>
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<td>t_{1/2} (h)</td>
<td>3.2±0.2</td>
<td>5.1±0.6</td>
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<tr>
<td>CL /F (ml/min/kg)</td>
<td>11.1±0.6</td>
<td>11.2±1.3</td>
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<tr>
<td>MRT (h)</td>
<td>1.9±0.1</td>
<td>9.0±1.7</td>
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<tr>
<td>Vd_B (l/kg)</td>
<td>1.2±0.1</td>
<td>—</td>
</tr>
<tr>
<td>Vd_d (l/kg)</td>
<td>3.2±0.4</td>
<td>—</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>—</td>
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<tr>
<td>F (%)</td>
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**Table 2**

<table>
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<td>AUC_{0–∞} (µg · h/ml)</td>
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</tr>
<tr>
<td>C_{max} (µg/ml)</td>
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</tr>
<tr>
<td>T_{max} (h)</td>
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</tr>
<tr>
<td>t_{1/2} (h)</td>
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</tr>
</tbody>
</table>

*Not determined.*

**Table 3**

<table>
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<tr>
<th>Pharmacokinetic Parameters</th>
<th>i.v.*</th>
<th>p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (µg/ml)</td>
<td>25.9±1.6</td>
<td>6.3±0.9</td>
</tr>
<tr>
<td>AUC_{0–12} (µg · h/ml)</td>
<td>24.5±2.2</td>
<td>33.9±4.0</td>
</tr>
<tr>
<td>AUC_{0–∞} (µg · h/ml)</td>
<td>24.7±2.2</td>
<td>34.6±4.1</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>5.3±1.0</td>
<td>3.9±0.8</td>
</tr>
<tr>
<td>CL /F (ml/min/kg)</td>
<td>3.4±0.3</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.6±0.3</td>
<td>5.5±0.4</td>
</tr>
<tr>
<td>Vd_B (l/kg)</td>
<td>0.3±0.0</td>
<td>—</td>
</tr>
<tr>
<td>Vd_d (l/kg)</td>
<td>1.2±0.3</td>
<td>—</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>—</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>F (%)</td>
<td>—</td>
<td>45.0±4.2</td>
</tr>
</tbody>
</table>

*Not determined.*
90%) was eliminated within 48 h, and the total recovery after 96 h in the urine and approximately 56% in feces over a 96-h period. Less than 2% was recovered in the carcass, and none was apparent in the cage wash. The total recovery first 24 h after dosing (Table 7). Less than 2% was recovered in the liver, stomach wall, thymus, and Harderian gland. These tissues accounted for approximately 98% of the radioactivity being eliminated within the first 24 h after dosing (Table 7). Less than 2% was recovered in the carcass, and none was apparent in the cage wash. The total recovery after oral administration was >100%.

Dogs. The excretion of radioactivity was studied in four female dogs orally administered [14C]LY354740 monohydrate at 15-mg/kg (Table 8). Approximately 40% of the radioactivity was eliminated in the feces, whereas 15% was excreted into the urine over a 96-h period with approximately 98% of the radiocarbon being eliminated within the first 24 h after dosing (Table 7). Less than 2% was recovered in the carcass, and none was apparent in the cage wash. The total recovery after oral administration was >100%.

**Discussion**

These studies were conducted to characterize the absorption, disposition, metabolism, and excretion of LY354740 in rats and dogs. LY354740 is a highly water-soluble, low molecular weight drug, which does not bind to plasma proteins nor does it partition into erythrocytes. Furthermore, both in vitro liver slice evaluations and in vivo profiles of excreted radioactivity indicate that LY354740 was not metabolized by either species. The administration of LY354740 showed no adverse effects in rats (after a single oral dose 30 mg/kg), and dogs exhibited tolerance to the emetic effect of this compound after receiving a second or third daily dose. Thus, to allow the dogs to become tolerant to the drug, pretreatment was necessary to pretreat dogs for 3 days (single daily 15-mg/kg oral doses) before the start of the studies to ensure the integrity of a study, it was necessary to pretreat dogs for 3 days (single daily 15-mg/kg oral doses) before the start of the studies to allow the dogs to become tolerant to the drug. After pretreatment, residual plasma concentrations of LY354740 in all dogs were below 0.3 µg/ml, and thus these residual concentrations would have negligible contribution to the reported pharmacokinetic parameters. In addition, because the majority of radioactivity after oral or i.v. administration was excreted in urine within 24 h, this supports the assumption that the pretreatment regimen would be unlikely to alter the pharmacokinetic profile after administration of a single oral dose. After intravenous administration of LY354740 to rats and dogs, the extracts subjected to radioprofiling, only one radiolabeled component was apparent in the radiochromatograms. This component eluted at the same retention time as LY354740, indicating that the entire recovered radioactivity from either species was unchanged parent compound. In addition, liquid chromatography with tandem mass spectrometric detection and/or GC/MS analysis was conducted on the in vivo and in vitro samples, which confirmed that the radioactive component detected in the HPLC radioprofiles was parent compound. Thus, metabolism of LY354740 was not observed in either species.

**Plasma Protein/Erythrocyte Binding.** The extent of in vitro binding of [14C]LY354740 to plasma proteins was evaluated by ultracentrifugation in dog and rat plasma. LY354740 showed low affinity (<1%) for plasma proteins in both rat and dog plasma after incubation at 37°C for 60 min at a concentration of 533 ng/ml of [14C]LY354740. The binding of LY354740 to red blood cells was determined in blood samples collected from dogs during the excretion study. Similarly to the low binding to plasma proteins, LY354740 showed virtually no binding to erythrocytes at concentrations ranging from 32 to 2400 ng/ml.

**TABLE 4**

Mean tissue concentrations of radiocarbon (micrograms per gram) in rats following a single oral 30-mg/kg dose of [14C]LY354740 monohydrate.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
<th>12 h</th>
<th>24 h</th>
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<tr>
<td>Harderian gland</td>
<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
<td>3.59</td>
<td>2.69</td>
<td>3.16</td>
<td>BQL</td>
<td>BQL</td>
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<tr>
<td>Intestinal wall</td>
<td>BQL</td>
<td>BQL</td>
<td>—</td>
<td>40.95</td>
<td>30.79</td>
<td>23.51</td>
<td>10.19</td>
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<tr>
<td>Kidney (high)</td>
<td>61.29</td>
<td>151.13</td>
<td>316.41</td>
<td>706.34</td>
<td>441.56</td>
<td>183.47</td>
<td>25.40</td>
<td>2.43</td>
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<tr>
<td>Kidney (low)</td>
<td>3.58</td>
<td>4.93</td>
<td>10.61</td>
<td>17.33</td>
<td>8.55</td>
<td>4.26</td>
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<tr>
<td>Liver</td>
<td>BQL</td>
<td>2.07</td>
<td>4.28</td>
<td>8.08</td>
<td>5.22</td>
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<td>2.02</td>
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<tr>
<td>Stomach wall</td>
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<td>3.16</td>
<td>BQL</td>
<td>4.25</td>
<td>4.71</td>
<td>6.30</td>
<td>5.31</td>
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<tr>
<td>Thymus</td>
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<td>BQL</td>
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<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
</tr>
</tbody>
</table>

* Tissue not sampled due to quality or size.

Thymus

**FIG. 4.** Phosphor image of a whole-body section from a rat 1 h after receiving a single oral 30-mg/kg dose of [14C]LY354740 monohydrate.

The image has been magnified 1×.

**FIG. 5.** Phosphor image of a whole-body section from a rat 4 h after receiving a single oral 30-mg/kg dose of [14C]LY354740 monohydrate.

The image has been magnified 1×.
plasma concentration-time profiles were biphasic and were composed of a rapid distribution phase (1/2 distribution of 0.5 h for dogs and 0.2 h for rats) followed by a slower elimination phase (1/2 elimination of 4.2 h for dogs and 3.2 h for rats). The systemic clearance rate in rats was considerably slower, which may suggest some reabsorption of the compound.

Pharmacokinetic studies demonstrated that the oral bioavailability was poor in rats (approximately 10%) and moderate in dogs (approximately 45%). A marked food effect was observed in the dog, as the mean oral bioavailability was approximately 34% lower in fed dogs than in fasted dogs; however, the degree of reduction in oral bioavailability varied widely between individual dogs. Data from the QWBA study in rats indicate that there are no significant liver concentrations of LY354740 at any given time (Tables 4 and 5). Therefore, it appears that the compound is not taken up by the liver and rapidly excreted into the bile. Thus, the data from these studies suggest that the transfer across the intestinal epithelial membrane limited the oral bioavailability of this compound. The absorption of LY354740 appeared to be fairly slow in both species as evident by the large difference in MRT values obtained after oral and intravenous dosing (approximately a 7 h difference for rats and a 4 h difference for dogs). Radiolabeled excretion studies show that, after oral administration of LY354740, the majority of the absorbed dose was eliminated predominantly in the urine in both rats and dogs. Approximately 78 and 15% of the administered radioactivity was excreted in the urine of rats after intravenous or oral administration, respectively. After oral administration to dogs, approximately 40% of the administered radioactivity was excreted in the urine. The urinary excretion data supports the absorption results because the percentage of urinary excretion in both species was consistent with the observed oral bioavailability of LY354740. Profiles of radioactivity excreted in the urine demonstrated that the radioactivity was unchanged LY354740, thereby confirming the in vitro prediction that LY354740 was not metabolized.

In conclusion, we have investigated the disposition, metabolism, and single-dose pharmacokinetics of LY354740 in rats and dogs. These studies show that LY354740 is an orally available compound that is excreted as unchanged drug in the urine.

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References


