Disposition of [14C]Ruboxistaurin in Humans

Jennifer L. Burkey, Kristina M. Campanale, Robert Barbuch, Douglas O’Bannon, James Rash, Charles Benson, and David Small

Lilly Research Laboratories, a Division of Eli Lilly and Company, Lilly Corporate Center, and Lilly Laboratory for Clinical Research, Indiana University Hospital and Outpatient Center, Indianapolis, Indiana

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ABSTRACT:
Ruboxistaurin is a potent and specific inhibitor of the β isoforms of protein kinase C (PKC) that is being developed for the treatment of diabetic microvascular complications. The disposition of [14C]ruboxistaurin was determined in six healthy male subjects who received a single oral dose of 64 mg of [14C]ruboxistaurin in solution. There were no clinically significant adverse events during the study. Whole blood, urine, and feces were collected at frequent intervals after dosing. Metabolites were profiled by high performance liquid chromatography with radiometric detection. The total mean recovery of the radioactive dose was approximately 87%, with the majority of the radioactivity (82.6 ± 1.1%) recovered in the feces. Urine was a minor pathway of elimination (4.1 ± 0.3%). The major route of ruboxistaurin metabolism was to the N-desmethyl ruboxistaurin metabolite (LY338522), which has been shown to be active and equipotent to ruboxistaurin in the inhibition of PKCβ. In addition, multiple hydroxylation metabolites were identified by liquid chromatography-mass spectrometry in all matrices. Pharmacokinetics were conducted for both ruboxistaurin and LY338522 (N-desmethyl ruboxistaurin, 1). These moieties together accounted for approximately 52% of the radiocarbon measured in the plasma. The excreted radioactivity was profiled using radiocromatography, and approximately 31% was structurally characterized as ruboxistaurin or N-desmethyl ruboxistaurin. These data demonstrate that ruboxistaurin is metabolized primarily to N-desmethyl ruboxistaurin (1) and multiple other oxidation products, and is excreted primarily in the feces.

Protein kinase C (PKC) is a group of isozymes important in signal transduction and intracellular signaling through diacylglycerol. In diabetes, hyperglycemia-induced generation of diacylglycerol selectively activates the β2 isofrom of PKC, and increases in diacylglycerol and PKC activity have been measured in the retina, kidneys, aorta, and heart, all organs that are known to develop diabetic complications associated with the vasculature (Craven and DeRubertis, 1989; Craven et al., 1990; Ayo et al., 1991; Inoguchi et al., 1992; Williams and Schrier, 1992, 1993; Shiba et al., 1993; DeRubertis and Craven, 1994; Kikkawa et al., 1994). The selective inhibition of PKCβ has been shown to inhibit diabetes-induced abnormalities in retinal blood flow and intraocular neovascularization caused by retinal ischemia in animals (Dans et al., 1998; Kowluru et al., 1998). These data suggest that hyperglycemia and the resulting activation of PKCβ may contribute to the development of diabetic complications. Furthermore, a selective PKCβ inhibitor may be of benefit in the treatment of diabetic complications by blocking this mechanism.

Ruboxistaurin (Fig. 1), (5S)-13-[(dimethylamino)methyl]-10,11,14,15-tetrahydro-4,9:16,21-dimetheno-1H,13H-dibenzo[e,k]pyrrolo-[3,4-H][1,4,3]oxadiazacyclohexadecene-1,3(2H)-dione, is a potent and specific inhibitor of PKCβ (Jirousek et al., 1996). Previous studies have shown that ruboxistaurin (LY333531) was effective in reversing vascular abnormalities induced in diabetic animals (Ishii et al., 1996) and that diabetes-induced elevations in PKC activity in the retina and kidneys of diabetic animals were normalized (Kowluru et al., 1996). Clinical studies have shown, after administration of ruboxistaurin, an amelioration of abnormal retinal hemodynamics in patients with diabetes (Aiello et al., 1999) and prevention of the reduction in endothelium-dependent vasodilation induced by acute hyperglycemia (Beckman et al., 2002), and have demonstrated promising results in patients with diabetic retinopathy (Aiello et al., 2005; PKC-DRS, 2005) and diabetic nephropathy (Tuttle et al., 2005). Thus, it is thought that ruboxistaurin may be useful in slowing the progression of diabetic complications.

In vitro metabolism of ruboxistaurin in dog, human, and rat liver microsomes and fresh liver slices, as well as in vivo metabolism studies in dog, rat, and mouse, have resulted in the identification of multiple metabolites (Barbuch et al., 2006). LY338522 (N-desmethyl ruboxistaurin) was identified in preliminary metabolism studies as a major metabolite of ruboxistaurin, and was found to be equally potent to the parent in the inhibition of PKCβ (Jirousek et al., 1996). N-Desmethyl ruboxistaurin has also been detected in the plasma of animals and humans after oral administration of ruboxistaurin and, therefore, has been considered when estimating the overall exposure to active molecule in clinical studies.

The study described here was designed to assess the disposition of ruboxistaurin after a single oral dose of 64 mg of [14C]ruboxistaurin containing approximately 100 μCi of radioactivity administered to
healthy male subjects, and to examine the metabolites produced in humans.

Materials and Methods

Chemicals. Ruboxistaurin, [14C]ruboxistaurin (Fig. 1), and standards of metabolites 1, 2, 3, 5, and 28 were synthesized or prepared at Eli Lilly and Company (Indianapolis, IN). The structures of these metabolites are described later, in Table 4. All other materials were of HPLC or analytical grade. [14C]Ruboxistaurin was assayed and found to be 99.8% pure with a specific activity of 97.3 μCi/mg.

Dose Formulation. Doses were prepared as a dry blend of both radiolabeled and unlabeled ruboxistaurin. Just before administration, the powder was dissolved in extremely dilute phosphoric acid (3.5% phosphoric acid) to provide approximately 64 mg of ruboxistaurin (free base) with approximately 100 μCi of radioactivity in an oral solution. Assayed samples were found to contain 66 mg of ruboxistaurin and 102 μCi of radioactivity.

Study Design. Six healthy male volunteers between 19 and 49 years old participated in this single-dose, open-label study. Informed consent was obtained according to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practice, and the applicable laws and regulations of the United States, whichever provided the greatest protection of the individual. The drug was administered as a single oral dose of 64 mg of [14C]ruboxistaurin in solution, given in 200 ml of diluent followed by an additional 200 ml of water or diluent used to rinse the container. The dose was administered within 15 min after consumption of a high-calorie meal to maximize bioavailability.

Blood, urine, and feces were collected before and after study drug administration. Heparinized blood (10 ml) was collected at approximately time 0 (predose) and 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72, and 96 h after dosing for determination of radioactivity in blood and plasma and for determination of plasma ruboxistaurin and N-desmethyl ruboxistaurin concentrations. Additional blood samples were collected for determination of potential metabolites in plasma. Plasma was obtained from whole blood using centrifugation.

Urine and fecal samples were collected for measurement of radioactivity, ruboxistaurin, N-desmethyl ruboxistaurin, and other potential metabolites. Urine was collected and samples were pooled for 0- to 6-h, 6- to 12-h, 12- to 24-h, 24- to 48-h, 48- to 72-h, and 72- to 96-h intervals after dosing. Pooled urine collections continued for 24-h intervals until radioactivity study release criteria, described below, were met. Each sample was weighed to determine volume, assuming a specific gravity of 1.0. Fecal samples were collected over 24-h intervals until study release criteria were met. Samples were stored at −20°C or −70°C until analysis. The release criteria stipulated that subjects were released from the study when excreted detectable radioactivity, normalized to a 24-h interval, was equal to or less than 0.2% of the administered dose.

Assay of Total Radioactivity. All radioactivity calculations were based on the actual dose of 102 μCi and 66 mg of ruboxistaurin. Fecal samples were collected in polyethylene bags and homogenized on a Seward Manufacturing Lab Systems model 3500 Stomacher (Seward Limited, Norfolk, UK), with a volume of tap water approximately equal to the fecal sample weight. If there were two or more samples within a 24-h period, each sample was weighed and processed separately. The samples were processed either fresh or after thawing after storage at −20°C. Three aliquots (approximately 0.3–1.0 g/ aliquot) of each homogenate were oxidized using a Packard model 307 sample oxidizer (PerkinElmer Life and Analytical Sciences, Boston, MA) using Carbosorb (Varian, Inc., Palo Alto, CA) to absorb 14C and Permafluor E (PerkinElmer Life and Analytical Sciences) as the liquid scintillant. Trapped 14CO2 was subsequently determined by liquid scintillation counting. Urine samples for the specified time periods were collected, weighed, kept on ice, and processed either fresh or after thawing after −20°C storage. The radioactivity in 1-ml aliquots of urine was determined by liquid scintillation counting. Selected blood samples (approximately 0.2-g aliquots in duplicate or triplicate) were oxidized, and trapped 14CO2 was determined by liquid scintillation counting as for feces. The radioactivity in 1-ml aliquots of plasma was also determined directly by liquid scintillation counting techniques.

Radiolabeled Compound Quantification and Profiling in Plasma, Urine, and Feces. Plasma. Plasma samples were frozen and stored at −20°C. After thawing, each plasma sample (3 ml) was divided in half, processed in duplicate, and combined. For every 1.5 ml of plasma, 2 ml of acetonitrile were added and the mixture was vortexed for approximately 30 s. The resulting sample was centrifuged at 3000 rpm for 5 min. The supernatant was removed to a clean, labeled tube and the remaining plasma solids were vortexed with an additional 2 ml of acetonitrile and centrifuged, and the supernatant was removed. The remaining plasma solids were again vortexed with 2 ml of acetonitrile and centrifuged, and supernatant was removed for a third time. The supernatants were all combined and evaporated to dryness under nitrogen using a TurboVap (Zymark Corporation, Hopkinton, MA) at 37°C. The sample was reconstituted with 75 μl of acetonitrile + 175 μl of 10 mM ammonium acetate. Recovery of radioactivity after extraction from the profiled plasma samples (from 4 h to 24 h) was 89 ± 18% (mean ± S.D., n = 7), and the total percentage of each metabolite in plasma was adjusted accordingly. Radioactivity in plasma was profiled by HPLC with 96-well fraction collection, using a Waters (Milford, MA) 2690 Alliance module system with a Foxy 200 fraction collector and Deepwell LumaPlate-96 solid scintillation-coated plates (PerkinElmer Life and Analytical Sciences). The plasma samples were applied to a YMC Basic (5 μm particle size, 4.6 × 150 mm) column (YMC, Inc., Wilmington, NC) at ambient temperature and with a mobile phase flow rate of 1.0 ml/min. The mobile phase solvents were 10 mM ammonium acetate buffer (A) and acetonitrile (B) with the following gradient profile: (min% B): 0/10, 50/60, 50/180, 52/80. The column effluent was collected into Deepwell LumaPlate-96 solid scintillation-coated plates. The recovery off the HPLC column was 115%. The LumaPlates were dried under centrifugal vacuum and counted on a Packard microplate scintillation and luminescence counter (Top-Count NXT; PerkinElmer Life and Analytical Sciences). The cpms were plotted against time using ProFSA Plus version 3.1 software (PerkinElmer Life and Analytical Sciences) to obtain radio profiles. The percentage of each metabolite in plasma was calculated from the percentage area of the radioactive peak of interest versus the total area of radioactivity above background in the sample radiochrochromatogram.

Urine. For each sample, 2 ml of urine were mixed with 5 ml of 0.4% formic acid solution. Waters Oasis HLB (60 mg/3 ml) SPE columns were prepared by rinsing with 3 ml of methanol and then 3 ml of Mill-Q water. The urine mixture was then loaded onto the SPE columns and the radioactivity eluted with 1 ml of methanol followed by 1 ml of 0.2% (v/v) ammonium hydroxide in methanol. This 2-ml elution solution was evaporated to dryness under nitrogen using a TurboVac at 37°C. The urine concentrate was reconstituted with 33.3 μl of acetonitrile + 66.7 μl of 10 mM ammonium acetate for injection into the HPLC system. The radioactivity in urine was profiled using the same HPLC conditions, and the percentage of each metabolite in urine was calculated as described for plasma. The percentage of each metabolite was then multiplied by the percentage dose excreted in that sample to calculate the percentage dose comprised by that metabolite. The recovery after extraction from profiled urine samples (from 6 h to 24 h) from the SPE column was 113 ± 14% (mean ± S.D., n = 3) and the recovery off the HPLC column was 102%.

Feces. After thawing, an aliquot of the fecal homogenate (0.3–0.5 g) was removed and shaken with 1.5 ml of acetonitrile on a Mistral multi-mixer for approximately 45 min. The fecal mixture was centrifuged at 3000 rpm for 5 min on a Beckman Coulter (Fullerton, CA) GPKR centrifuge. The supernatant was removed to a clean labeled tube and the remaining solids were shaken with 1.0 ml of 50:50 (v/v) acetonitrile/water for an additional 45 min. The fecal mixture was centrifuged at 3000 rpm for 5 min, and the second supernatant was removed and added to the corresponding first supernatant. The combined supernatants for each sample were evaporated to dryness under nitrogen using a TurboVac at 37°C. Each sample was reconstituted with 100 μl of acetonitrile.
followed by 0.2% ammonium hydroxide in methanol, evaporated to dryness
method described for radioprofiling. For the 50 positive ion mode. The isocratic mobile phase consisted of 20% mobile phase
r-desmethyl ruboxistaurin. The validation intra-assay and interassay relative
ranged from 0.87 to 17.46% for ruboxistaurin and from 1.26 to 12.93% for
determine the precision and accuracy of the human plasma assay. Validation
concentrations of 0.5, 80, and 150 ng/ml (six replicates per concentration) to
samples for ruboxistaurin and
Polar-RP column (3.0
10AXL autosampler and a Surveyor PDA detector. Chromatographic separa-
phase A) and acetonitrile (mobile phase B), and the analytes were eluted using a gradient profile (min/% B): 0/10, 5/10, 50/75, 50.1/90, 52/90. Mass spectro-
interface (Applied Biosystems/MDS Sciex (Foster City, CA), Shimadzu HPLC
ionization triple quadrupole mass spectrometer equipped with TurboIonSpray
desmethyl ruboxistaurin were determined using a validated turbo ion spray
Analyte for Quantification of Ruboxistaurin and N-Desmethyl Ruboxistaurin in Plasma. Plasma concentrations of ruboxistaurin and N-desmethyl ruboxistaurin were determined using a validated turbo ion spray LC/MS/MS analytical method using a SCIEX API 3000 atmospheric pressure ionization triple quadrupole mass spectrometer equipped with TurboIonSpray interface (Applied Biosystems/MDS Sciex (Foster City, CA), Shimadzu HPLC pumps (LC-10AD vp), and a PerkinElmer autosampler (PerkinElmer Series 200). The assay demonstrated a lower limit of quantitation of 0.5 ng/ml. The calibration curves were fit by a weighted (1/x^2) quadratic equation from 0.5 to 150 ng/ml. The coefficients of determination (r^2) of the calibration curves were 0.9952 for ruboxistaurin and 0.9973 for N-desmethyl ruboxistaurin. Validation samples for ruboxistaurin and N-desmethyl ruboxistaurin were prepared at concentrations of 0.5, 80, and 150 ng/ml (six replicates per concentration) to determine the precision and accuracy of the plasma assay. Validation intra-assay and interassay relative standard deviation for the validation samples ranged from 0.87 to 17.46% for ruboxistaurin and from 1.26 to 12.93% for N-desmethyl ruboxistaurin. The validation intra-assay and interassay relative error for the validation samples ranged from -2.67 to 6.92% (97-107% accuracy) for ruboxistaurin and from -8.52 to 15.80% (91-116% accuracy) for N-desmethyl ruboxistaurin.

The analytes were extracted from plasma samples (0.2 ml) prepared using an Isolute CBA (100 mg) (Biotage AB, Uppsala, Sweden) 96-well extraction procedure. The extraction block was prepared by washing with 400 μl of methanol. Plasma samples, with [3H]ruboxistaurin added as the internal standard, were mixed with 500 µl of 10 mM ammonium acetate and then loaded onto the prepared extraction block. Using a Tomtec Quadra 96-well sample processor (Tomtec, Orange, CT), the cartridge bed was washed with 1 ml of water and then 400 µl of methanol. The bed was dried by applying full vacuum for 15 s, after which the analytes were eluted off with 1 ml of 1% (v/v) formic acid in methanol. Each effluent was evaporated to dryness under nitrogen using a TurboVac at 45°C. Each dried extract was reconstituted in 100 μl of 75:25 (v/v) methanol/10 mM ammonium formate, pH 3.6, before analysis on a Shimadzu HPLC system. Extracted samples were analyzed using a Genesis CN 2.1 × 50 mm (4 μm) HPLC column (FK596SE; Jones Chromatography USA Inc., Lakewood, CO) and turbo ion spray LC/MS/MS in the positive ion mode. The isotopic mobile phase consisted of 20% mobile phase

\(1911\) Ruboxistaurin was well tolerated in all six subjects, all of whom completed the study according to the protocol. All calculations were based upon the 66 mg (102 μCi) that was actually dosed, but is referred to as 64 mg (100 μCi) targeted dose.

Mass Balance of Total Radioactivity. Mass balance determination included urine and feces through the sample collection period of 21 days. The majority of the [14C]ruboxistaurin dose was excreted in feces with only a small amount excreted in urine as shown in Fig. 2 and Table 1. Total recovery of radioactivity from the six subjects in this study was approximately 87.0%, with approximately 82.6% ± 1.1% of the radiocarbon dose recovered in the feces and approximately 4.1% ± 0.3% of

FIG. 2. Cumulative elimination (mean ± S.D.) of total radioactivity in feces and urine after a single oral 64-mg (100-μCi) dose of [14C]ruboxistaurin to healthy male subjects (n = 6).
the radiocarbon dose recovered in the urine. Excretion of fecal radioactivity appeared to plateau after 6 days, whereas the urinary excretion of radioactivity reached a plateau earlier.

**Pharmacokinetics.** Plasma concentration curves are shown in Fig. 3, and the key median pharmacokinetic parameters for plasma concentrations of total radiocarbon, ruboxistaurin, N-desmethyl ruboxistaurin (LY338522), and combined concentrations of ruboxistaurin and N-desmethyl ruboxistaurin are shown in Table 2. The mean estimate of $\text{AUC}_{\text{0-126 h}}$ for combined ruboxistaurin and N-desmethyl ruboxistaurin is about half that for plasma total radiocarbon, suggesting that about half the plasma radioactivity consisted of these two analytes. Ruboxistaurin and N-desmethyl ruboxistaurin comprised comparable fractions of the total radiocarbon in plasma, with means of 23% and 28%, respectively. Individual contributions ranged from 16% to 36%. The mean combined AUCs of ruboxistaurin and N-desmethyl ruboxistaurin accounted for approximately 52% of the total radiocarbon, whereas combined AUCs of ruboxistaurin and N-desmethyl ruboxistaurin in individual subjects ranged from 39% to 64%.

Ruboxistaurin concentrations in all plasma samples collected after 24 h postdose were below the quantitation limit for ruboxistaurin, whereas N-desmethyl ruboxistaurin concentrations were quantifiable up to approximately 120 h postdose. Plasma concentrations of total radiocarbon were detectable through at least 72 h in all subjects and, in three subjects, were detectable up to the last sampling time of 216 h.

The mean terminal half-life of total radiocarbon in plasma was longer than that of ruboxistaurin or N-desmethyl ruboxistaurin individually. The half-life of $^{14}$C reflects the composite half-life of ruboxistaurin and all of its metabolites and, therefore, represents a complex mixture of compounds with potentially widely varying individual half-lives. As such, it should be interpreted cautiously. The longer mean half-life of $^{14}$C could reflect the presence of a metabolite with a longer half-life than that of N-desmethyl ruboxistaurin, or it could be attributable to variability. Given the historically high variability in ruboxistaurin and N-desmethyl ruboxistaurin pharmacokinetics and the very wide range of 11.3 to 106 h in half-life estimates for $^{14}$C (Table 2), variability is a plausible explanation for the relatively small difference between half-lives of N-desmethyl ruboxistaurin and total radiocarbon.

**Metabolite Profiles in Plasma, Feces, and Urine.** For plasma, the recovery of radioactivity from the extraction steps was found to be 89%, and recovery from the HPLC column was determined to be approximately 100%, demonstrating that no major loss of radioactivity occurred from the plasma during sample preparation or analysis. Using LC/MS and LC/MS/MS evaluation described in previous work (Barbich et al., 2006), ruboxistaurin and four metabolites were detected in plasma (Table 3). The four metabolites were 1, the N-desmethyl of ruboxistaurin (LY338522); 2, the N,N-didesmethyl of ruboxistaurin; 3, produced by hydroxylation at the 7-position of indole

![Graph of individual plasma concentration-time profiles for total radiocarbon (as quantitated by scintillation counting of plasma samples) (top), ruboxistaurin (as quantitated by LC/MS (middle), and N-desmethyl ruboxistaurin (as quantitated by LC/MS) (LY338522; bottom) after oral administration of 64 mg of [14C]ruboxistaurin in six healthy males. Note different axis scales.](image.png)
ring A (see Fig. 1); and 6, the N-oxide of ruboxistaurin (Table 4). Ruboxistaurin itself accounted for the majority of the total radioactivity at 4 and 8 h (Table 3; Fig. 4). The most abundant metabolite in plasma is 1 (N-desmethyl ruboxistaurin), representing approximately 16% and 11% of the mean percentage of total radioactivity at 4 h and 8 h, respectively. Ruboxistaurin and N-desmethyl ruboxistaurin combined comprised 39 to 64% of the radiocarbon AUC in plasma, indicating that N-desmethyl ruboxistaurin is the major metabolite. The other observed metabolites (3, 2, and 6) were present at much lower amounts as indicated in Table 3. The structures of the plasma metabolites were determined using MS/MS and confirmed using either standards (1 and 2) or NMR data (3 and 6) (Barbuch et al., 2006). Lower overall concentrations at 8, 12, and 24 h led to fewer distinct radioactive peaks for identification, and all compounds were below the quantification limit (BQL) for radioactivity by 12 h and 24 h. Approximately 68% of the mean percentage of the total radioactivity was identified as parent or a metabolite at 4 h, dropping to approximately 45% at 8 h, consistent with the pharmacokinetic results.

Ruboxistaurin and a total of 16 metabolites were detected in urine using LC/MS/MS (Table 4), with unchanged ruboxistaurin representing less than 1% of the radioactive dose in urine. The major urinary metabolite based on the radiochromatograms was 1 (N-desmethyl ruboxistaurin), representing approximately 1% of the radioactive dose. Additional metabolites observed in human urine included 3 (7-hydroxy ruboxistaurin), 5, and 27 (ruboxistaurin + O2), each representing less than 1% of the radioactive dose, and 2, 14, 6, 18, 10, 12, 15, 8, 17, 28, 11, and 9, each representing less than 0.1% of the radioactive dose (Fig. 5). Table 5 shows the urinary metabolites and the corresponding percentage of the radioactive dose they comprise.

Based on LC/MS/MS analysis, ruboxistaurin and 14 metabolites were detected in feces (Table 4). Unchanged ruboxistaurin represented approximately 1% of the radioactive dose in feces. The major fecal metabolite based on the radiochromatograms was 1 (N-desmethyl ruboxistaurin), which represented approximately 28% of the radioactive dose eliminated in the feces. Additional metabolites observed in feces included 5, 28, 3, 17, 14, and 2. These six metabolites represent approximately 9, 4, 4, 3, 3, and 2% of the radioactive dose eliminated in the feces, respectively. The definitive structures of both 5 and 3 have been confirmed using MS/MS and NMR. Metabolites representing 1% or less of the dose were 10, 12, 16, 15, 8, 11, and 29. These metabolites are primarily hydroxylated products of either ruboxistaurin or 1, N-desmethyl ruboxistaurin (Fig. 6). Table 5 shows the fecal metabolites and the corresponding percentage of the radioactive dose they comprise. A detailed description, with an explanation of the MS product ion spectra and, when available, NMR data, for ruboxistaurin and its metabolites is found in earlier work describing [14C]ruboxistaurin metabolism in animals (Barbuch et al., 2006).

### Discussion

Ruboxistaurin and N-desmethyl metabolite (LY338522 or 1) are potent and specific inhibitors of PKCζ. The concentrations of N-desmethyl ruboxistaurin and ruboxistaurin were comparable in subjects given ruboxistaurin. Renal elimination of ruboxistaurin as a percentage of dose is very minor, with the majority of the compound’s clearance from the body appearing to be biliary and via the feces, consistent with preclinical models (Burkey et al., 2002; Barbuch et al., 2006). Mean cumulative total recovery of the administered radioactivity was 87%, and metabolites definitively identified in feces comprised 56% of that administered dose, with a total of 14 metabolites and unchanged ruboxistaurin identified in the feces. The majority of the metabolites in feces are primarily hydroxylated products of either ruboxistaurin or 1, N-desmethyl ruboxistaurin. In general, this pattern of multiple hydroxylated metabolites holds true of the urinary metabolites as well.

Ruboxistaurin itself comprised only 1.3% of the excreted dose, indicating that it was extensively metabolized in this study. The largest proportion of the dose was eliminated as the N-desmethyl...
metabolite 1 (29.2%). Other more minor metabolites included 5 (9.4%), a hydroxylated N-desmethyl metabolite, and 3 (4.2%), a hydroxylated ruboxistaurin metabolite. These moieties represent the primary metabolic biotransformations for ruboxistaurin, inasmuch as all other metabolites comprised less than 4% of the overall dose. The proposed primary metabolic pathways for ruboxistaurin biotransfor-
ruboxistaurin (alcohol); in previous studies, the primary metabolite is \( N \)-desmethyl ruboxistaurin \( \text{N-oxide of ruboxistaurin} \); \( 6 \) = \( N \)-oxide of ruboxistaurin; \( 27 \) = ruboxistaurin + \( O_2 \); \( 18 \) = ruboxistaurin + \( O_2 \) + glucuronide.

Other metabolites include \( 2 \) and numerous hydroxy ruboxistaurin and hydroxy \( N \)-desmethyl ruboxistaurin metabolites. The majority of the metabolites were confirmed as being identical to the metabolites identified in dogs, mice, and rats. Metabolite 27 was identified as a dioxygenated product of ruboxistaurin, but the absolute structure of this metabolite could not be discerned from the MS product ion spectrum. This metabolite was seen in dog feces but not previously reported because of very low concentrations. Metabolite 28 gives the same MS/MS data as the animal metabolite 7, and the two metabolites are either identical to each other or may be diastereomers of one another. Metabolite 29 is an alcohol that is derived from the same metabolic pathway (of oxidative deamination to an aldehyde) as the acid 20 seen in animal species. All three metabolites are minor in humans and animals, and do not substantially contribute to the overall metabolism of the compound. Thus, all metabolites identified in human were also identified in preclinical animal species or were generated in the same pathway as was seen in an animal species, and all metabolites circulating in humans were found circulating in animal species.

In conclusion, ruboxistaurin is absorbed upon oral administration, and both ruboxistaurin and its metabolites are eliminated primarily in the feces secondary to metabolism and biliary elimination. The majority of the excretion in both urine and feces occurred within 1 week.
of dosing. Ruboxistaurin and N-desmethyl ruboxistaurin are the major moieties in plasma, feces, and urine, comprising 52% of the circulating metabolites. Multiple additional metabolites are present in all three matrices, but at significantly lower concentrations. Because these metabolites exist at such low concentrations, it is doubtful that they are of pharmacologic significance.

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Address correspondence to: Dr. Jennifer Burkey, Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285. E-mail: Burkey_jennifer_l@lilly.com