

Absolute bioavailability of evacetrapib in healthy subjects determined by simultaneous administration of oral evacetrapib and intravenous [$^{13}\text{C}_8$]-evacetrapib as a tracer

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This open-label, single-period study in healthy subjects estimated evacetrapib absolute bioavailability following simultaneous administration of a 130-mg evacetrapib oral dose and 4-h intravenous (IV) infusion of 175 μg [$^{13}\text{C}_8$]-evacetrapib as a tracer. Plasma samples collected through 168 h were analyzed for evacetrapib and [$^{13}\text{C}_8$]-evacetrapib using high-performance liquid chromatography/tandem mass spectrometry. Pharmacokinetic parameter estimates following oral and IV doses, including area under the concentration-time curve (AUC) from zero to infinity (AUC[0- ∞]) and to the last measurable concentration (AUC[0- t_{last}]), were calculated. Bioavailability was calculated as the ratio of least-squares geometric mean of dose-normalized AUC (oral : IV) and corresponding 90% confidence interval (CI). Bioavailability of evacetrapib was 44.8% (90% CI: 42.2–47.6%) for AUC[0- ∞] and 44.3% (90% CI: 41.8–46.9%) for AUC[0- t_{last}]. Evacetrapib was well tolerated with no reports of clinically significant safety assessment findings. This is among the first studies to estimate absolute bioavailability using simultaneous administration of an unlabeled oral dose with a ^{13}C -labeled IV microdose tracer at about 1/1000th the oral dose, with measurement in the pg/mL range. This approach is beneficial for poorly soluble drugs, does not require additional toxicology studies, does not change oral dose pharmacokinetics, and ultimately gives researchers another tool to evaluate absolute bioavailability.

Keywords: evacetrapib; cholesteryl ester transfer protein; pharmacokinetics; bioavailability; tracer

Introduction

Aggressive lowering of low-density lipoprotein cholesterol (LDL-C) has been shown to be beneficial in lowering cardiovascular events,¹ but there remains a need for additional therapies targeting other lipid-related risk factors to address residual cardiovascular disease. Considerable efforts have focused on the development of novel therapeutic agents designed to address residual cardiovascular risk. Epidemiological evidence shows that high-density lipoprotein cholesterol (HDL-C) levels are inversely correlated with cardiovascular disease risk,^{2,3} suggesting that agents that raise HDL-C may offer important benefits in treating cardiovascular disease.

A class of compounds that inhibits cholesteryl ester transfer protein (CETP) to promote the exchange and net transfer of triglycerides and cholesterol esters between lipoproteins can increase HDL-C levels and may provide favorable benefits toward lowering cardiovascular risk.^{4–6} Evacetrapib is a potent, selective inhibitor of CETP shown to increase HDL-C and decrease LDL-C.^{7–10} Currently in phase 3 development, evacetrapib is being investigated as a treatment to reduce the risk of major adverse cardiovascular events in patients with high-risk vascular disease.

Studies to assess the absolute bioavailability of new drugs and drug products are often conducted in the course of drug

development and are required by some regulatory authorities. Combined with data from other studies, absolute bioavailability studies provide information helpful in understanding the overall disposition of the drug, such as the fraction of drug absorbed or the fraction of drug that undergoes first pass metabolism.¹¹ Therefore, knowing the fraction of the dose that reaches the

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systemic circulation may assist in the interpretation of other pharmacokinetic data.¹²

To assess absolute oral bioavailability, pharmacokinetic data following intravenous (IV) and oral administration are needed.¹¹ In the past, absolute bioavailability studies have used crossover study designs that dosed the drug by both the oral and IV routes,^{12,13} which, for the IV period, requires the drug to be in solution in a formulation compatible with human usage. Significant formulation and manufacturing work was often required to develop such IV formulations, and nonclinical toxicology studies may have been needed to support human clinical studies.¹⁴ This enabling work is time-consuming and resource-intensive and may not be adequate for poorly soluble drugs, which may be impossible to formulate for IV administration. Thus, researchers have looked to alternative methods to assess absolute bioavailability.^{15–17}

One evolving method for determining absolute bioavailability uses a labeled tracer and a sensitive detection assay. In this study design, a therapeutic oral dose is co-administered with an IV tracer that is isotopically labeled, providing sufficient sensitivity at very low, or microdose, levels. When a ¹⁴C radiolabel is used, the ¹⁴C isotope can be quantified by accelerator mass spectroscopy (AMS).^{18–21} An alternative approach using stable isotopes (e.g., ¹³C) is emerging.^{21–24} Tracer studies in which the stable isotope-labeled IV dose differs from the parent drug's and the internal standard's molecular weights do not require specialized AMS methods. Instead, these tracer studies use standard high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) to quantify both the labeled IV drug and the orally administered drug.¹⁷

This report describes a healthy volunteer study in which a microdose quantity of the tracer [¹³C₈]-evacetrapib was administered intravenously along with a simultaneous oral dose of evacetrapib to determine absolute bioavailability.

Experimental

Labeled compounds

The ¹³C labels were incorporated on a sufficient number of evacetrapib's carbons to distinguish the [¹³C₈]-evacetrapib in the IV dosage form from the unlabeled evacetrapib and the internal standard [¹³C²H₃]-evacetrapib used in the bioanalytical assay (Figure 1). The synthetic methods used to prepare both labeled compounds were based on procedures developed at Eli Lilly and Company.^{25,26} The [¹³C₈]-evacetrapib IV tracer of >98% chemical purity (HPLC column: Waters XBridge Shield RP18 75 × 4.6 mm,

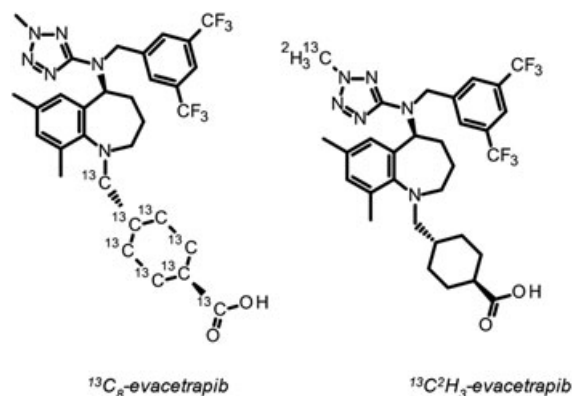


Figure 1. Chemical structures of [¹³C₈]-evacetrapib and [¹³C²H₃]-evacetrapib internal standard.

2.5 μm; mobile phase 0.1% trifluoroacetic acid/water/acetonitrile) was synthesized at Almac (Craigavon, UK). The [¹³C²H₃]-evacetrapib internal standard of >99% chemical purity was synthesized at Syncom (Groningen, Netherlands). Both labeled compounds had no detectable unlabeled isotopomers, as determined by high resolution mass spectroscopy.

Oral dose

Evacetrapib in the form of 130-mg tablets for oral administration was supplied by Eli Lilly and Company. This is the same tablet formulation being used in the phase 3 study ACCELERATE.²⁷

Intravenous formulation development and sterility testing

Evacetrapib is poorly soluble in aqueous media,²⁸ and having sufficient evacetrapib in an IV formulation equivalent to the oral dose of 130 mg would require a solvent or infusion volume incompatible with human usage. To overcome solubility issues, a stock Investigation Medicinal Product (IMP) solution was manufactured at a concentration of 24.0 μg/mL [¹³C₈]-evacetrapib and contained 50% (v/v) ethanol and 0.1 M sodium hydroxide. The infusion solution was made by diluting 25 mL of the 24.0 μg/mL [¹³C₈]-evacetrapib stock solution to 1000 mL with sterile 5% glucose solution. Because of nonspecific binding of evacetrapib to the infusion line, both the infusion line and in-line filter were pretreated by allowing the infusion solution to flow through them for 3-h predose at a rate of 80 mL/h in order to saturate the inner wall of the tubing. The time required to stabilize the evacetrapib concentration in the solution exiting the infusion line was determined in preliminary experiments.

The 24.0 μg/mL [¹³C₈]-evacetrapib stock solution for injection was sterilized by double filtration with two 0.22 μm Millex-GV polyvinylidene fluoride filters (EMD Millipore, Billerica, MA) in series into 50 mL sterile vials. The vials were stored in sterile bags in the dark at room temperature for up to 72 h from the time of the start of sterilization. These activities were performed in a Grade A positive pressure isolator in a Grade C area. Validation of the aseptic procedure was performed by simulating the process of manufacture of [¹³C₈]-evacetrapib solution for injection in addition to three process simulation batches of nutrient media consisting of tryptone soya broth solution in sterile water.

Study subjects and clinical study design

The study protocol²⁹ was approved by an Ethics Committee and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided written informed consent prior to participating. Eligible subjects included healthy men and women not of childbearing potential, 18 to 65 years of age with a body mass index less than 32 kg/m². Use of over-the-counter or prescription medication was prohibited within 14 days prior to dosing and during the study.

This was an open-label, single-period study in healthy men and women, at a single Clinical Research Unit (CRU). Subjects were admitted to the CRU on Day-1, remained resident until after the Day 3 assessments had been completed, and returned for a follow-up visit at least 14 days after dosing. On Day 1, subjects received a single oral dose of 130 mg evacetrapib at the same time that an IV infusion was started to deliver an approximate total dose of 175 μg [¹³C₈]-evacetrapib. The IV infusion was administered at a constant rate of around 80 mL/h over 4 h so that the end of the IV dose occurred at the anticipated *t*_{max} of the evacetrapib oral dose. Samples of the infusate exiting the infusion line were collected immediately before and immediately after the 4-hour infusion to each subject, and the concentrations of [¹³C₈]-evacetrapib in the effluent were used to calculate the dose of [¹³C₈]-evacetrapib administered (Table 2).

Blood samples were collected for pharmacokinetic analysis predose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 (immediately prior to the end of IV infusion), 4.25, 4.5, 5, 5.5, 6, 6.5, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144, and 168-h postdose. During the infusion and for at least 4-h post-infusion, blood samples were taken from the arm contralateral to the infusion site.

High-performance liquid chromatography/tandem mass spectrometry analysis of evacetrapib and [¹³C₈]-evacetrapib plasma concentrations

An HPLC-MS/MS method for the concurrent measurement of evacetrapib and [¹³C₈]-evacetrapib as a single injection of plasma extract was developed, validated, and executed by Covance Bioanalytical Services, LLC (Indianapolis, IN). Analytes were isolated from plasma by solvent-induced protein precipitation by mixing 100 µL of each sample with 50 µL of acetonitrile/water (1:1; v/v) containing 2 ng/mL of [¹³C₂H₃]-evacetrapib internal standard, adding 0.65 mL of 0.1% formic acid/acetonitrile, mixing, and then centrifuging the samples to pellet the denatured proteins. A 0.6 mL aliquot of the supernatant was then transferred to a clean vial, evaporated to dryness, reconstituted in 0.2 mL acetonitrile/water/formic acid (50:50:0.1, v/v) and analyzed by HPLC-MS/MS.

The HPLC-MS/MS detection system included a Shimadzu Prominence HPLC system (Shimadzu America, Columbia, MD) and an AB Sciex API 5000 triple quadrupole mass spectrometer (Sciex, Framingham, MA) equipped with a TurbolonSpray interface and operated in positive ion mode. Chromatography was performed on a 2.1 × 50 mm, 5 µm XBridge C18 column (Waters, Milford, MA) maintained at 30 °C with a gradient elution system consisting of mobile phase A (5 mM ammonium bicarbonate in water) and mobile phase B (acetonitrile/methanol [1:1, v/v]) at a flow rate of 1.0 mL/min. Sciex Analyst Version 1.4.2 software (Sciex, Framingham, MA) was used to collect and process the data. Detection was accomplished by selected reaction monitoring (SRM) of the precursor-to-product ion pairs m/z 641.4 > 316.2 for evacetrapib, m/z 647.4 > 322.2 for [¹³C₈]-evacetrapib, and m/z 643.4 > 314.2 for the [¹³C₂H₃]-evacetrapib internal standard. The evacetrapib +2 SRM was used to reduce the signal intensity and allow simultaneous detection of evacetrapib and [¹³C₈]-evacetrapib.

The HPLC-MS/MS method was fully validated to quantify evacetrapib and [¹³C₈]-evacetrapib in accordance with current regulatory guidances,^{30,31} with lower and upper limits of quantification of 500 to 200 000 pg/mL and 2.5 to 2000 pg/mL, respectively. A 100-fold dilution was validated to accommodate any samples above the upper limit of these ranges. Overall, the assay had good accuracy (−7.3–0.4% relative error for evacetrapib; −10.4–0.4% relative error for [¹³C₈]-evacetrapib) and precision (6.3–11.0% relative standard deviation for evacetrapib; 7.0–13.2% relative standard deviation for [¹³C₈]-evacetrapib). Both compounds were stable in human plasma during the time required for storage and analysis of the samples and for up to 4 freeze-thaw cycles (freezing at −20 °C and −80 °C and thawing at room temperature). The concentrations for selected samples subjected to re-analysis aligned with initial results as specified for incurred sample re-analysis.³²

Pharmacokinetic assessments

Pharmacokinetic parameter estimates for evacetrapib were calculated using standard noncompartmental methods of analysis using Phoenix WinNonlin Version 6.2.1 (Certara, Princeton, NJ). The primary parameter for analysis was area under the concentration-time curve (AUC) from zero to infinity (AUC[0–∞]) following oral and IV doses. Other reported noncompartmental parameters included the following: AUC from time zero to the last measureable concentration (AUC[0–t_{last}]); percentage of AUC(0–∞) derived by extrapolation (%AUC[t_{last}–∞]); maximum observed drug concentration (C_{max}); half-life (t_{1/2}); time of C_{max} (t_{max}); clearance following the IV dose (CL); volume of distribution during the apparent terminal phase following the IV dose (V_z); apparent clearance following the oral dose (CL/F); and apparent volume of distribution during the terminal phase following the oral dose (V_z/F). Dose-normalized values for AUC(0–t_{last}), AUC(0–∞), and C_{max} were determined by dividing the original parameter estimate by the dose. The absolute bioavailability in each subject was calculated as the dose-normalized AUC(0–t_{last}) or AUC(0–∞) after the oral dose divided by the dose-normalized AUC(0–t_{last}) or AUC(0–∞) after the IV tracer dose.

Safety assessments

Safety and tolerability were assessed during the study by physical examinations, clinical laboratory evaluations, vital sign measurements, and safety electrocardiograms (ECGs) as well as monitoring of treatment emergent adverse events (AEs). Assessment of clinical laboratory safety parameters included hematology, urinalysis, and biochemistry panels.

Statistical analysis

A mixed-effect analysis of variance model was applied to the log-transformed, dose-normalized AUC(0–∞), and AUC(0–t_{last}) of evacetrapib obtained after oral dosing, and that of the IV administered [¹³C₈]-evacetrapib. The model contained formulation (oral or IV) as a fixed effect and subject as a random effect. The absolute bioavailability was expressed as the ratio of the least-squares geometric means of the formulations (oral/IV) along with its corresponding 90% confidence interval (CI).

Eight subjects were enrolled so that at least six subjects would have evaluable pharmacokinetic data for both treatments. The sample size was considered adequate for phase 1 studies evaluating absolute bioavailability and was not intended to meet any a priori statistical requirement.

Results

Demographics and disposition

Seven healthy males and one healthy female participated in and completed the study (Table 1).

Pharmacokinetics

Infusate concentrations of [¹³C₈]-evacetrapib immediately before and immediately after the 4-h infusion did not differ within or between subjects (Table 2). Based on the predose and postdose infusate concentrations, the individual IV doses of [¹³C₈]-evacetrapib ranged from 173.7 to 181.1 µg (mean = 178.1 µg).

After oral administration, the evacetrapib plasma concentration versus time profile was characterized by a steady absorption phase with a median t_{max} of about 4 h (Figure 2; Table 3), which corresponded with the end of the IV infusion. After t_{max}, evacetrapib concentrations for both oral and IV administration declined in a biphasic manner (Figure 2) with a mean apparent terminal-phase t_{1/2} of 42 h (Table 3). Concentrations of evacetrapib and [¹³C₈]-evacetrapib were within their limits of quantitation out to 168-h postdose.

The geometric mean bioavailability of evacetrapib was about 45% for AUC(0–∞) and AUC(0–t_{last}) (Table 4). For individual subjects, the bioavailability based on AUC(0–∞) ranged from 39% to 51%.

Safety and tolerability

The evacetrapib doses were well-tolerated. Of the eight subjects who received evacetrapib, four reported a total of five treatment-emergent AEs. All treatment-emergent AEs were mild in severity, none required treatment with concomitant medications, and none were considered by the investigator to be related to evacetrapib. Headache was the only AE reported by more than one subject (two subjects reported one event each) and all AEs resolved prior to the follow-up visit. A single event of catheter site pain was considered by the investigator to be related to a study procedure; all other AEs were considered to be due to other contemporaneous medical illnesses.[‡] There

[‡]Correction added on 14 December 2015, after first online publication: sentence has been corrected.

Table 1. Subject demographics

Overall (N = 8)		
Age (years)	Mean	42.9
	SD	14.1
	Range	25–58
Sex	Male	7 (87.5%)
	Female	1 (12.5%)
Race	White	8 (100.0%)
Weight (kg)	Mean	74.6
	SD	12.8
	Range	60.2–101.7
Body mass index (kg/m ²)	Mean	24.9
	SD	3.84
	Range	20.6–30.8

N, number of subjects; SD, standard deviation.

were no clinically significant findings in the panel of safety assessments from clinical laboratory evaluations, vital signs, and 12-lead ECGs for individual subjects during the study.

Discussion

This study estimated the bioavailability of evacetrapib dosed as the oral tablet formulation used in the phase 3 study ACCELERATE,²⁷ compared with IV administered evacetrapib, which is 100% bioavailable. Following simultaneous administration of a single oral dose of 130 mg evacetrapib and a 4-h IV infusion of about 175 µg [¹³C₈]-evacetrapib as a microdose tracer, data from eight healthy subjects demonstrated a geometric mean bioavailability of about 45% for both AUC(0–∞) and AUC(0–t_{last}). The 90% CIs for the absolute bioavailability estimates for this study would be considered very narrow in any clinical study, but are especially narrow considering that

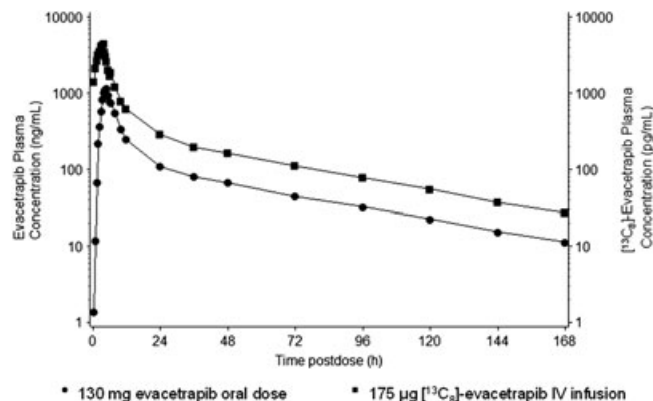


Figure 2. Arithmetic mean plasma concentration-time profiles of evacetrapib following a single oral dose of 130 mg evacetrapib or a single intravenous dose of 175 µg [¹³C₈]-evacetrapib.

they were derived from only eight subjects. The range of absolute bioavailability for individual subjects based on AUC(0–∞) was also tight at 39–51%. The high precision of the absolute bioavailability estimates may stem in part from giving the oral and IV doses simultaneously to ensure that each subject's clearance was the same for both treatments.

Traditional studies of absolute oral bioavailability typically use a crossover design in which an IV dose and an oral dose are given to a subject during different visits separated by enough time to allow complete washout of the drug dosed at the previous visit. An inherent assumption of such studies is that the clearance within each subject does not change between visits. In fact, intrasubject variability in AUC and C_{max} is never zero, so, while such a design minimizes variability in pharmacokinetic parameters between doses, it does not eliminate it. The simultaneous administration of the oral and IV doses using the tracer approach provides an advantage over traditional studies because the drug from the IV dose is being cleared by the body in the same manner and at the same time

Table 2. [¹³C₈]-evacetrapib infusate concentrations before and after infusion

Subject	Collection timepoint	Concentration ^a (µg/mL)	Ratio of predose: postdose concentration	Actual dose (µg)
1	Predose	0.554	0.991	177.9
	Postdose	0.559		
2	Predose	0.553	0.972	179.5
	Postdose	0.569		
3	Predose	0.541	1.00	173.7
	Postdose	0.541		
4	Predose	0.555	1.00	177.3
	Postdose	0.553		
5	Predose	0.561	0.982	180.6
	Postdose	0.571		
6	Predose	0.575	1.01	180.4
	Postdose	0.567		
7	Predose	0.557	1.04	174.4
	Postdose	0.534		
8	Predose	0.564	0.991	181.1
	Postdose	0.569		

^aConcentrations were measured in infusate exiting the infusion line.

Table 3. Summary of evacetrapib pharmacokinetic parameter estimates⁵

Parameter	130 mg evacetrapib oral	175 µg [¹³ C ₈]-evacetrapib IV
	Geometric mean (CV%) (N = 8)	Geometric mean (CV%) (N = 8)
AUC(0–t _{last}) (ng·h/mL/mg) [norm]	105 (18)	237 (14)
AUC(0–∞) (ng·h/mL/mg) [norm]	111 (21)	248 (16)
%AUC(t _{last} –∞) (%)	3.57 (142)	2.99 (132)
C _{max} (ng/mL/mg) [norm]	10.3 (22)	24.9 (12)
t _{max} ^a (h)	4.38 (3.00–8.00)	3.98 ^c (3.50–4.02)
t _{1/2} ^b (h)	42.3 (27.4–65.5)	42.3 (27.9–63.7)
CL/F (L/h)	8.98 (21)	NA
V _z /F (L)	548 (32)	NA
CL (L/h)	NA	4.03 (16)
V _z (L)	NA	246 (33)

AUC(0–∞), area under the concentration–time curve from zero to infinity; AUC(0–t_{last}), area under the concentration–time curve from zero to the last measurable concentration; %AUC(t_{last}–∞), percentage of AUC(0–∞) derived by extrapolation; CL, clearance following IV dose; CL/F, apparent clearance following oral dose; C_{max}, maximum observed drug concentration; CV, coefficient of variation; IV, intravenous; N, number of subjects; NA, not applicable; [norm], dose-normalized; t_{1/2} = apparent terminal elimination half-life; t_{max}, time of C_{max}; V_z, volume of distribution during the terminal phase following IV dose; V_z/F, apparent volume of distribution during the terminal phase following oral dose.

^aMedian (range).

^bGeometric mean (range).

^cThe t_{max} listed for IV infusion occurred at the end of the infusion.

Table 4. Statistical analysis of dose-normalized AUC(0–t_{last}) and AUC(0–∞)⁵

Parameter	Formulation	N	Geometric LS mean	Ratio of geometric LS means
				[Oral:IV] (90% CI)
AUC(0–t _{last}) (ng·h/mL/mg) [norm]	130 mg evacetrapib oral dose	8	105	0.443 (0.418, 0.469)
	175 µg [¹³ C ₈]-evacetrapib IV infusion	8	237	
AUC(0–∞) (ng·h/mL/mg) [norm]	130 mg evacetrapib oral dose	8	111	0.448 (0.422, 0.476)
	175 µg [¹³ C ₈]-evacetrapib IV infusion	8	248	

AUC(0–∞), area under the concentration versus time curve (AUC) from zero to infinity; AUC(0–t_{last}), AUC from time zero to the last time point with a measurable concentration; CI, confidence interval; IV, intravenous; LS, least squares; N, number of subjects; [norm, dose-normalized.

Model: Log(PK) = Formulation + Subject + Random Error, where Subject was fitted as a random effect.

as the oral dose, which eliminates the time between doses as a source of variability. Ultimately, the tracer study design contributes to less variability and higher data integrity.¹⁶

The IV tracer dose used in this study targeted 1/1000th the oral dose in order to be low enough to not affect the pharmacokinetics of the oral dose, yet high enough to produce plasma concentrations that could be quantified by the HPLC-MS/MS method used to measure [¹³C₈]-evacetrapib. The mean IV actual dose of 178 µg [¹³C₈]-evacetrapib was 0.14% of the 130-mg oral dose and, when adjusted for the 45% bioavailability of the oral dose, was just 0.3% of the orally dosed evacetrapib that made it to the systemic blood. The pharmacokinetics of the 130-mg oral dose in this study were similar to that previously reported for evacetrapib.^{9,33} The mean apparent terminal-phase

evacetrapib t_{1/2} was 42.3 h for both oral and IV administration, indicating that the tracer did not affect the pharmacokinetics of the oral dose and that absorption after oral dosing is not the rate-limiting step for evacetrapib elimination in the terminal phase. If absorption were the rate-limiting step, then the terminal-phase t_{1/2} after oral dosing would be longer than the half-life after IV infusion. Given how small the IV dose was compared with the mass of evacetrapib appearing in the blood from the oral dose, it is also reasonable to assume that the clearance of the unlabeled evacetrapib in the oral dose and the [¹³C₈]-evacetrapib in the IV dose was the same.

Evacetrapib has a propensity for nonspecific binding to glass and plastics, and preliminary experiments showed that evacetrapib bound to the infusion apparatus during infusion.⁴

⁵ Correction added on 14 December 2015, after first online publication: 'ng/h/mL/mg' corrected to 'ng·h/mL/mg'

⁴ Correction added on 14 December 2015, after first online publication: sentence has been corrected.

This led to declining evacetrapib concentrations exiting the infusion line during the infusion. To overcome this property of the molecule, an IMP solution was developed consisting of a 50% (v/v) ethanol and 0.1 M sodium hydroxide mixture. This solution was chemically stable, resistant to nonspecific binding effects, and was able to be sterilized by double filtration using 0.2 µm filters. However, upon 1:40 dilution of the IMP solution with 5% glucose solution, some binding occurred in the infusion bottle and/or infusion line and in-line filter.

Over the course of subsequent experiments, it was determined that not allowing the drug solution to remain static in the infusion line for more than 60 s, accompanied by running the infusion solution through the infusion line and in-line filter for 3 h just prior to starting the infusion in subjects, would control the nonspecific binding to the apparatus and ultimately prevent or minimize loss of the drug during the subsequent 4-h infusion to the subjects. After optimizing the flushing process, the evacetrapib concentration in the infusate exiting the infusion line after 3 h of pretreatment was 93% of the theoretical concentration of 0.600 µg/mL, with the final concentration of evacetrapib being about 0.558 µg/mL. The lines were pretreated as described before infusing the study subjects, and concentrations of the [¹³C₈]-evacetrapib infusion solution exiting the infusion line were measured immediately before and immediately after the 4-h infusion to the subjects to confirm the concentration of the IV dose solution and the actual dose that the subjects received (Table 2). The pre-infusion and post-infusion concentrations were nearly identical within each subject, showing that the pretreatment strategy enabled consistent delivery of [¹³C₈]-evacetrapib during the 4-h infusion and accurate estimation of absolute bioavailability. Averaged across all eight subjects, the mean ratio of [¹³C₈]-evacetrapib exiting the infusion line pre-infusion and post-infusion was close to unity.

Use of a stable isotope ¹³C label, as opposed to a radioisotope ¹⁴C label, offers several advantages. Because the IV tracer was not radioactive, volunteers and study staff were not exposed to additional radioactivity. There was no need to monitor radioactivity, and the study did not require a special site qualified to handle radioactivity. There were also no special handling and safety concerns for the material and samples at the CRU or the bioanalytical lab. Lastly, a standard HPLC-MS/MS bioanalysis method could be used, which is less expensive and more widely available than AMS.

This is among the first studies to estimate absolute bioavailability using simultaneous administration of an unlabeled oral dose with a ¹³C-labeled IV microdose tracer at about 1/1000th the oral dose, with measurement in the pg/mL detection range. Although stable isotope labeling of simultaneous IV and oral dosing has been used to determine absolute bioavailability for over 40 years,^{34,35} past studies used similar doses for the oral and IV administration requiring additional formulation, manufacturing, and nonclinical toxicology work. Use of a microdose tracer for IV administration is beneficial for poorly soluble drugs, does not require additional toxicology studies, and does not change the pharmacokinetics of the oral dose. Ultimately the use of an unlabeled oral dose with a ¹³C-labeled IV microdose tracer can save time and resources, and it gives researchers another tool to evaluate absolute bioavailability.

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Conflict of interest

The authors declared a potential conflict of interest (e.g., a financial relationship with the commercial organizations or products discussed in this article) as follows: E. A. C., A. A., B. C., KR, J. G. S., D. O., B. W. P., W. F. A., Q. L., and D. S. S. are employees of, and may hold stock in, Eli Lilly and Company. C. W., C. H., J. R., and S. L. B. are employees of Covance Inc.

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