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CYP-mediated drug-drug interactions with evacetrapib, an investigational CETP inhibitor: in vitro prediction and clinical outcome

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Introduction

Progress has been achieved in reducing cardiovascular disease morbidity and mortality, largely through increased use of lipid-lowering therapies such as low-density lipoprotein-cholesterol (LDL-C)-lowering HMG-CoA reductase inhibitors (statins) [1–4]. However, therapies targeting other lipid-related risk factors are needed to address the residual risk of cardiovascular events that persists in many patients. Substantial epidemiological data indicate high-density lipoprotein-cholesterol (HDL-C) levels are inversely correlated with cardiovascular disease risk [5–7]. Potent inhibition of cholesteryl ester-transfer protein (CETP), a plasma glycoprotein that mediates the transfer of cholesteryl ester from HDL-C to apolipoprotein B-rich lipoproteins in exchange for triglycerides [8], leads to decreases of LDL-C and increases of HDL-C concentrations.

Although two previous CETP inhibitors, torcetrapib and dalcetrapib, were terminated in late-stage clinical development, there is still considerable interest in this mechanism as a therapeutic strategy to decrease atherogenic and increase anti-atherogenic lipoprotein levels. Evacetrapib, an investigational CETP inhibitor, has not demonstrated the off-target liabilities associated with torcetrapib, has been well tolerated and has demonstrated clinically relevant pharmacodynamic (PD) efficacy in early phase development [9]. In patients with dyslipidaemia, evacetrapib by itself or in combination with statins increased HDL-C concentrations and decreased LDL-C concentrations without producing significant changes in blood pressure, aldosterone, cortisol or electrolytes.

CETP inhibitors are likely to be utilized in combination with statins, which are largely metabolised by cytochrome P450 3A (CYP3A) and are substrates for the hepatic organic anion transporting polypeptide 1B1 transporter (OATP1B1) [10]. In addition, the patient population expected to take CETP inhibitors (i.e., those at high risk for vascular disease) are likely to be taking multiple concomitant medications. Consequently, there is a need to understand the benefit-to-risk profile for evacetrapib, including the potential for this drug to affect the safety and/or efficacy of other drugs taken by this patient population. Results from *in vitro* studies, predictions, and two clinical studies conducted to assess the potential for inhibitory and inducing drug-drug interactions (DDI) mediated by evacetrapib are presented.

Methods

Reversible inhibition of cytochrome P450 (CYP) isoforms in human liver microsomes

Experiments were performed at CellzDirect, Inc. (Austin, TX, USA) utilizing evacetrapib as the lysine salt in human hepatic microsomes. Each patient whose tissue was used in this study was fully consented under an institutional review board (IRB) application. Samples were shipped to CellzDirect's facility in Durham, NC, USA for processing under protocols approved during the IRB application process. Briefly, evacetrapib was incubated at 37°C in 100 mM potassium phosphate buffer (pH 7.4) with probe substrates and β-nicotinamide adenine dinucleotide 2′-phosphate (NADPH) under linear conditions with respect to protein content and incubation time. Following the incubation interval, reactions were stopped, processed for analysis and analyzed by LC-MS/MS for metabolite formation. Assay-specific conditions are summarized in Table 1.

Data analysis The apparent K_i value (dissociation constant for the enzyme inhibitor complex) was generated by fit of the appropriate inhibition model using conventional relationships for inhibition to the data using WinNonlin Enterprise Edition version 4.1 (Pharsight Corporation, Mountain View, CA, USA) [11]. To predict the risk of a clinical DDI, basic models of reversible inhibition were used. In accordance with the FDA [12] and EMA [13] guidelines on the investigation of drug interactions, I/K_i and I_u/K_i values were calculated to relate *in vitro* to *in vivo* risk, where I is the predicted maximum total plasma drug concentration (C_{max}) at the highest recommended dose, also taking into consideration the unbound fraction, u, specific to the EMA guideline. Thus, I_u is the unbound mean plasma C_{max} with the highest recommended dose. Since the unbound fraction of evacetrapib in human plasma is very low and prone to uncertainty (<0.1%, data not presented), a surrogate value of 1% is recommended for use in the calculation in accordance with European guidelines. For CYP3A inhibitors, it is also recommended to take into consideration inhibition of the enzyme in the gut, where $R = 1 + [I]_{gut}/K_i$ and $[I]_{gut}$ = molar dose/250 ml in accordance with FDA guidelines. Similarly, using the EMA guidance, this equation is $[I]/K_i$, where [I] is the maximum dose taken at one occasion/250 ml. In addition to the previously described basic model equations, the more recent mechanistic static models of CYP inhibition proposed by Fahmi [14] were also used in accordance with the FDA and EMA guidances for drug interaction studies [12, 13]. The mechanistic static model is described by the following equation:

$$AUCR = \left(1/\left(\left(A_g \times B_g \times C_g\right) \times \left(1 - F_g\right) + F_g\right)\right) \times \left(1/\left(\left(A_h \times B_h \times C_h\right) \times f_m + \left(1 - f_m\right)\right)\right)$$

where A is the reversible inhibition in either the gut (A_g) or liver (A_h), B is the time-dependent inhibition in either the gut (B_g) or liver (B_h), and C is the induction in either the gut (C_g) or liver (C_h). The equations for A, B, and C are further characterized in the FDA and EMA guidances and are not described within the context of this manuscript [12, 13]. Additionally, F_g is described as the fraction of drug available after intestinal metabolism and f_m is the fraction of

Table 1

In vitro assay-specific conditions used in CYP studies in microsomes and hepatocytes

Cytochrome P450	Form-selective catalytic activity assay Substrate	Metabolite	Substrate (μM)	Assay-specific conditions Microsomal protein (mg ml^{-1})	Evacetrapib (μM)	Incubation time (min)
CYP3A4*	Midazolam	1-Hydroxy-midazolam	0.5–15	0.025	1–12.5	4
CYP3A4†	Midazolam	1-Hydroxy-midazolam	100	0.25 (inactivation); 0.0125 (activity)	0.98–31.4	0–10 (inactivation); one (activity)
CYP3A4*	Testosterone	6β-Hydroxy-testosterone	25–200	0.05	2.5–25	7
CYP3A4‡	Testosterone	6β-Hydroxy-testosterone	200	0.375 x 10^6 cells/0.5 mL	0.1, 1, 10	15
CYP2D6*	Bufuralol	1′Hydroxy-bufuralol	5–100	0.05	4.45–44.5	15
CYP2C19*	S-mephenytoin	4′Hydroxy-mephenytoin	10–250	0.1	4–40	30
CYP2C9*	Diclofenac	4′Hydroxy-diclofenac	0.5–25	0.05	0.625–10	4
CYP2C8*	Paclitaxel	6α-Hydroxy-paclitaxel	1–20	0.075	0.25–2.5	10
CYP2B6*	Bupropion	Hydroxybupropion	5–1000	0.25	4–40	20
CYP1A2*	Phenacetin	Paracetamol (acetaminophen)	15–100	0.1	2.5–25	30

*Reversible inhibition in microsomes. †Time-dependent inhibition in microsomes. ‡Effects in hepatocytes.

systemic clearance of the substrate mediated by the CYP enzyme responsible for its metabolism. The complex equation described above is the same in both the FDA and EMA guidances. However, as previously discussed, there are slight differences in the inputs utilized in each. In the EMA, but not FDA, guidance, the unbound fraction is taken into account, with a conservative input of 1% being utilized for highly protein bound drugs to account for uncertainties in the estimates. These equations are described in detail in the regulatory guidances.

Time-dependent inhibition of CYP3A4 in human liver microsomes

In the inactivation assay, 100 mM sodium phosphate buffer (pH 7.4), 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM NADPH and various concentrations of evacetrapib (lysine salt) were pre-warmed for 3 min at 37°C in triplicate. Reactions were initiated with the addition of human liver microsomes. Then, at multiple time points, 5 μl aliquots were diluted 1/20 into pre-warmed (37°C) tubes containing the CYP3A4 residual activity mixture. The residual activity assay mixture contained the CYP3A4 substrate midazolam and NADPH (1 mM). Activity reactions were quenched, samples were processed and then analyzed for 1-hydroxymidazolam by LC-MS/MS. Assay-specific conditions are summarized in Table 1.

Data analysis

Estimates of the following kinetic parameters were determined according to regulatory guidances and Ring *et al.*: k_{inact} (maximal inactivation rate constant), K_I (apparent inactivation constant), CL_{inact} (ratio of k_{inact}/K_I) and K_{obs} (apparent inactivation rate constant) [12, 13, 15]. K_{deg} is the apparent first order degradation rate constant of the affected enzyme. The basic model equation for time-dependent inhibition (TDI), in accordance with both FDA and EMA regulatory guidances [12, 13], utilized was the following:

$$R = \left(K_{obs} + K_{deg}\right)/K_{deg},$$

where $K_{obs} = k_{inact}[I]/(K_I+[I])$, noting that two separate K_{deg} values for CYP3A were utilized, one for intestinal CYP3A and one for hepatic CYP3A. Additionally, in terms of protein binding, the total [I] was utilized according to the FDA guidance, whereas the conservative unbound [I] value of 1% was used for highly protein bound drugs in accordance with the EMA guidance [12, 13].

Effects on CYP3A in human hepatocyte cultures

Experiments and analyses were performed at CellzDirect, Inc. (Durham, NC and Austin, TX, USA). Hepatocytes from three individual donor livers (designated as 'lots') were isolated by a collagenase perfusion method described by LeCluyse and associates [16, 17] and then plated. Cells were also resuspended in 60 mm dishes for microsomal preparation required for immunoblotting. Cells were maintained in media (William's E Medium, 6.25 μg ml^{-1} insulin, 6.25 μg l^{-1} transferrin, 6.25 ng ml^{-1} selenium and extracellular matrix proteins) for 3 days prior to initiating treatment with evacetrapib (in triplicate) for up to 72 h. Cells were also incubated with 0.1% MeOH (vehicle

control) or 10 μM rifampicin (CYP3A induction control). Following incubation, cultures were rinsed twice with ice-cold Hanks' Balanced Salt solution (HBSS). HBSS (0.5 ml) containing the CYP3A-selective substrate, testosterone, was applied to the cells. Following the appropriate incubation period, supernatants were removed and frozen until LC-MS/MS analysis for 6β-OH testosterone. Assay conditions are summarized in Table 1.

mRNA preparation and qRT-PCR determinations

Cells were lyzed and frozen until total RNA was isolated. Approximately 200 ng of total RNA was reverse transcribed using the Applied Biosystems High Capacity cDNA Archive Kit according to the manufacturer's procedures. The resulting cDNA was assayed for relative quantities of CYP3A4 and normalized to the endogenous control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), using Applied Biosystems TaqMan® (Life Technologies, Grand Island, NY, USA).

Western immunoblotting for CYP3A4

Homogenization buffer was added to each dish and cells were harvested by scraping, pooled by treatment group, and lysed. Microsomal pellets were prepared by centrifugation at ~4°C (9000 *g* for 20 min followed by further centrifugation of supernatants at 100 000 *g* for 60 min) and resuspended in 0.25 M sucrose. Samples (20 μg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and proteins were transferred electrophoretically from the polyacrylamide gels to nitrocellulose membranes. Membranes were blocked for ≥1 h at room temperature in casein buffer and incubated ≥2 h at room temperature with primary antibodies diluted in casein buffer. CYP3A4 protein levels were determined with polyclonal antibodies. Immunoreactive bands were visualized on a Fluor-S Chemiluminescence imager with either enhanced chemiluminescence plus (ECL+; Amersham Biosciences, Piscataway, NJ, USA) or SuperSignal detection systems (Thermo Fisher Scientific, Rockford, IL, USA).

Clinical study A: drug–drug interaction of evacetrapib with midazolam and tolbutamide in healthy adult subjects

Study design

A phase 1 single-site, placebo-controlled, investigator and subject-blinded, parallel, multiple-ascending dose study evaluated safety, tolerability, pharmacokinetics (PK) and pharmacodynamics of evacetrapib over four planned dose levels [18]. Subjects eligible for the study were between the ages of 18 and 60 years and had a BMI of 18.5 to 35.0 kg m^{-2}. The Clinical Research Unit was located in Baltimore, MD, USA and the study protocol was reviewed by Chesapeake Research Review, Inc (Columbia, MD, USA), which maintains full accreditation with the Association for the Accreditation of Hurman Research Protection Programs. Two cohorts incorporated the evaluation of DDI potential of evacetrapib with two sensitive CYP substrates using an established drug cocktail containing midazolam 200 μg and tolbutamide 500 mg, which are highly selective probe substrates for CYP3A4 and CYP2C9, respectively [19]. Each cohort of 15 healthy adults had subjects randomized to receive either evacetrapib or matched placebo in a ratio of 12 : 3. Subjects received drug cocktail prior to study drug and on day 1. Subjects also received repeated daily doses of evacetrapib 100 mg or 300 mg or placebo for 15 days ($n = 30$ subjects). The pharmacokinetics of midazolam and tolbutamide were evaluated prior to administration of evacetrapib and on day 15 after evacetrapib had reached steady-state concentrations. To maximize enzyme inhibition prior to the probe cocktail administration, evacetrapib was administered ~2 h before the administration of the probes on day 15. Plasma probe concentrations were measured immediately before and by serial collections up to 46 h after giving the drug cocktail (0.25, 0.5, 1, 2, 4, 6, 8, 12, 22 and 46 h).

Bioanalysis

K_2EDTA human plasma samples obtained during this study were analyzed at Bioanalytical Systems, Inc. (BASi; West Lafayette, IN, USA) for concentrations of evacetrapib using a validated LC-MS/MS method (data on file, Eli Lilly and Company). The dynamic range for the assay was 1 to 1000 ng ml^{-1}. Samples above the limit of quantification were diluted and re-analyzed to yield results within the calibrated range. The inter-assay accuracy (% relative error) during validation ranged from −6.0% to 6.2%. The inter-assay precision (% relative standard deviation) during validation ranged from 2.1% to 6.4%. Plasma samples were analyzed for midazolam and 1′-hydroxymidazolam using the validated LC-MS/MS method at Advion BioServices, Inc. (Advion; Ithaca, NY, USA). The dynamic range for both analytes was 0.01 to 10 ng ml^{-1}. The inter-and intra-assay accuracy ranged from −0.56% to 20.0% for midazolam, and from −2.90% to 11.7% for 1′-hydroxymidazolam. The inter- and intra-assay precision was ≤9.64% for midazolam and ≤9.71% for 1′-hydroxymidazolam. Plasma samples were also analyzed for tolbutamide using a validated LC-MS/MS method at Covance Laboratories Inc. (Madison, WI, USA). The dynamic range of the assay was 5 to 2000 ng ml^{-1}. Samples above the limit of quantification were diluted and re-analyzed to yield results within the calibrated range. The intra-assay accuracy for tolbutamide ranged from 94.7% to 106% and the intra-assay precision ranged from 6.2% to 8.3%.

Pharmacokinetic analysis

Pharmacokinetic parameter estimates for evacetrapib, midazolam, 1′-hydroxymidazolam and tolbutamide were

calculated by standard non-compartmental methods of analysis. The primary parameters were maximum concentration (C_{max}) and area under the curve (AUC) of evacetrapib, midazolam, 1′-hydroxymidazolam and tolbutamide. A mixed model repeated measures (MMRM) analysis was conducted to estimate mean AUC(0,∞) and C_{max} of midazolam and tolbutamide in the presence and absence of evacetrapib. The MMRM model used log-transformed AUC(0,∞) and C_{max} as the response and included log-transformed dose, treatment (with or without evacetrapib) and log-transformed dose by treatment interaction as fixed effects, and subject as a random effect. Least squares geometric mean ratios and 95% confidence intervals (CI) were calculated for the inference of exposure ratios of midazolam and tolbutamide in the presence and absence of evacetrapib.

Clinical study B: drug–drug interaction of evacetrapib with simvastatin in dyslipidaemic patients

Study design

A randomized, double-blind, double-dummy, parallel group, placebo- and active-controlled, multi-site phase 2 efficacy and safety study of evacetrapib was conducted in adult patients (≥18 years; $n = 398$) with hypercholesterolaemia or low HDL-C [9]. The trial was designed by the Cleveland Clinic Coordinating Center for Clinical Research (IRB approval # QUI1-09–464) in collaboration with the sponsor and conducted at 70 sites internationally. Patients were randomized to 30, 100 or 500 mg evacetrapib daily as monotherapy, 40 mg simvastatin as monotherapy or in combination with 100 mg evacetrapib, or placebo alone. Treatment duration was 12 weeks. To evaluate CYP-mediated drug interactions, evacetrapib and simvastatin pharmacokinetic sampling occurred at weeks 2, 4, 8 and 12 (visits 4, 5, 6 and 7). Sampling at each visit consisted of a pre-dose and a post-dose blood draw during the treatment to allow for population PK analysis with a maximum allowable window of 4 days. A single sample was also drawn at the follow-up visit (visit 8, week 16–18) or after early discontinuation.

Bioanalysis

Plasma samples were analyzed for evacetrapib as described above. Plasma samples were also analyzed for simvastatin (parent = simvastatin lactone) and its metabolite (simvastatin acid) at Advion BioServices, Inc. (Advion; Ithaca, NY, USA) utilizing a validated LC-MS/MS assay. For simvastatin and its metabolite, the detection range was 0.10 to 30 ng ml^{-1} for both analytes. The inter-assay accuracy ranged from −1.0% to 13.2% for simvastatin and from −8.0% to 8.5% for simvastatin acid. The inter-assay precision was ≤7.9% for simvastatin and ≤8.9% for simvastatin acid. For all analytes, when samples had concentrations above the limit of quantitation, samples were diluted to yield results within the calibrated range.

Pharmacokinetic analysis

Analyses were performed using a non-linear mixed effect modelling approach as implemented in NONMEM version 7. The *post hoc* Bayesian estimates of CL/*F* from the population PK models were used to calculate the ratio of the steady-state AUC of the statin when co-administered with evacetrapib relative to when the statin was administered alone, resulting in a mean ratio and the corresponding 95% CI. The geometric mean ratio of the plasma concentration of simvastatin to the corresponding metabolite at trough and C_{max} was also calculated for the statin alone and when co-administered with evacetrapib.

Additional assessments for both clinical drug–drug interaction studies

Safety assessments in the drug–drug interaction studies

Safety measurements included recording adverse events and evaluation of clinical laboratory data in both of the clinical studies.

Ethical review of clinical studies

Ethical Review Boards for the two clinical studies approved their respective protocols. Healthy subjects and patients in the clinical studies provided written informed consent prior to participation in study procedures.

Results

Reversible inhibition of CYP isoforms in human liver microsomes

Based on *in vitro* inhibition of the metabolism of form-selective substrates, evacetrapib inhibited several major CYP isoforms (CYPs 3A4, 2C19, 2C9, 2D6, 2C8, 2B6 and 1A2). Inhibition constant (K_i) values for evacetrapib ranged from 0.57 μM (CYP2C9) to 7.6 μM (CYP2C19) and are presented in Table 2. To predict the risk of a clinical DDI, I/K_i values were calculated to relate *in vitro* to *in vivo* risk, where I is the predicted maximum total drug concentration (C_{max}) value (1.9 μM) for the phase 3 dose of 130 mg. The I/K_i values ranged from 0.25 (CYP2C19) to 3.30 (CYP2C9), also shown in Table 2.

In accordance with regulatory agency guidelines, the FDA and EMA basic models were applied as a first line assessment of DDI potential [12, 13]. Using the FDA guidance equation, the resulting R value was 2.57 or 4.30 for

Table 2

in vitro inhibition constant (K_i, I/K_i, and I_u/K_i) values for evacetrapib

Cytochrome P450 (Form-selective catalytic activity)	Model best describing the data	Apparent K_i (μM)	†I/K_i	‡I_u/K_i
CYP3A4 (Midazolam 1′-hydroxylation)	Non-competitive	1.2 ± 0.15	1.57	0.02
CYP3A4 (Testosterone 6β-hydroxylation)	Competitive	3.6 ± 0.9	0.52	0.005
CYP2D6 (Bufuralol 1′-hydroxylation)	Competitive	2.1 ± 0.5	0.89	0.009
CYP2C19 (S)-(Mephenytoin 4′-hydroxylation)	Mixed competitive/non-competitive	7.6 ± 1.1 α = 4.5 ± 1.2	0.25	0.003
CYP2C9 (Diclofenac 4′-hydroxylation)	Competitive	0.57 ± 0.05	3.30	0.03
CYP2C8 (Paclitaxel 6α-hydroxylation)	Non-competitive	1.3 ± 0.1	1.45	0.01
CYP2B6 (Bupropion hydroxylation)	Non-competitive	6.3 ± 1.0	0.30	0.003
CYP1A2 (Phenacetin metabolism to paracetamol (acetaminophen))	Competitive	7.1 ± 2.1	0.26	0.003

†I = predicted C_{max} of 1.879 μM at 130 mg dose. ‡unbound fraction = 1% in accordance with EMA guidance[13]

CYP3A or CYP2C9, respectively, also shown in Table 3. Using the similar EMA equation, with different inputs described previously and an I_u value of 0.019 μM for the predicted phase 3 dose of 130 mg evacetrapib the $I_u/K_i = 0.02$ for CYP3A and $I_u/K_i = 0.03$ for CYP2C9 (Table 3), with the lowest I_u/K_i value being 0.003 for CYP2C19 (Table 2). A clinical interaction study with a sensitive probe substrate is recommended if the R value >1.1, using the FDA basic model, or if the ratio of $I_u/K_i \geq 0.02$ using the EMA basic model. In both instances, the clinical DDI potential could not be excluded for evacetrapib and clinical studies were warranted.

Additionally, the potential for inhibition of CYP3A in the gastrointestinal tract by evacetrapib similarly could not be excluded. A clinical drug interaction study with a sensitive probe substrate is recommended when the R value ≥ 11. For a 130 mg maximum recommended dose of evacetrapib, the R value was 679.46 (Table 3).

In a second line assessment, the mechanistic static models recommended in the FDA and EMA regulatory guidances [12, 13] were used to predict evacetrapib interactions with CYP3A and CYP2C9. The evacetrapib AUC ratio for CYP3A was predicted to be 1.76X or 2.95X using the mechanistic static models utilized by the FDA and EMA, respectively. The differences in the predicted AUC ratios derived by each method are attributed to the different inputs used in the respective equations as described previously. Nonetheless, since the AUC ratio falls outside of the 0.8 to 1.25 range, the modelled values are considered a positive effect regardless of which model is used, and clinical evaluation is warranted (Table 3). The FDA mechanistic static model predicted the evacetrapib AUC ratio for CYP2C9 to be 1.0, whereas using the more conservative unbound fraction term in the EMA mechanistic static model predicted slightly different AUC ratios of 1.13X or 1.12X, using either warfarin or tolbutamide as substrate, respectively (Table 3).

Time-dependent inhibition of CYP3A4 in human liver microsomes

Time-dependent inhibition (TDI) of CYP3A4-mediated midazolam 1-hydroxylation was observed for evacetrapib, yielding a k_{inact} value of 0.144 min^{-1} and K_I value of 34.0 μM. The corresponding inactivation clearance (CL_{inact}) was 4.2 min^{-1} mM^{-1}. Using the FDA and EMA TDI basic models, the predicted R values were 24.4 and 1.25, respectively. The difference in predicted R values is attributed to the unbound fraction inputs into the models, as previously described. However, predictions based on both FDA and EMA models warranted further clinical investigation (Table 3).

Effects on CYP3A in human hepatocytes

Primary cultures of fresh human hepatocytes responded to the positive control, rifampicin, appropriately, and the assay was deemed acceptable. Figure 1 shows the time-course of effects of evacetrapib on CYP3A4 mRNA (Figure 1A) and CYP3A-mediated testosterone 6β-hydroxylation activity (Figure 1B). The effects of evacetrapib were concentration- and time-dependent. There was little effect of 0.1 μM evacetrapib on mRNA and activity. However, in hepatocyte cultures treated with 1 and 10 μM evacetrapib, CYP3A4 mRNA levels increased by 8 h and remained elevated at 72 h. The average effect (treatment/vehicle) of 10 μM evacetrapib in the three lots at 72 h was 11.5 (51% of rifampicin, data on file). Decreases in CYP3A-mediated activity were observed for 1 and 10 μM evacetrapib over the shorter treatment periods (2, 4 and 8 h). By 72 h, however, CYP3A-mediated activity had returned to baseline (1 μM) or had rebounded to approximately 50% of baseline (10 μM) (Figure 1). Representative semi-quantitative immunoblots showing CYP3A4 protein levels (Figure 2) show evacetrapib concentration-related decreases in CYP3A4 protein at 8 h and slight concentration-related increases in CYP3A4 protein at 72 h.

Table 3

Predictions of clinically relevant drug–drug interactions using 130 mg dose of evacetrapib based on reversible basic, TDI basic and mechanistic static models proposed by FDA or EMA regulations

Model	CYP3A	Clinical study warranted?	CYP2C9	Clinical study warranted?
Reversible basic				
- FDA*,††	R = 2.57	Yes	R = 4.30	Yes
- EMA†,§§	I_u/K_i = 0.02	Yes¶¶	I_u/K_i = 0.03	Yes
Reversible basic - Gut				
- FDA‡	R = 679.46	Yes	NA	NA
- EMA‡	R = 679.46	Yes	NA	NA
TDI/MBI basic				
- FDA§,†	R = 24.4	Yes	NA	NA
- EMA¶,§§	R = 1.25	Yes¶¶	NA	NA
Mechanistic static				
- FDA**,‡‡	AUCR =1.76	Yes	AUCR =1.0	No
- EMA**,§§	AUCR =2.95	Yes	AUCR =1.13 (warfarin)	No
			AUCR =1.12 (tolbutamide)	No

*Clinical study warranted when R ≥ 1.1. †Clinical study warranted when $I_u/K_i \geq 0.02$. ‡Clinical study warranted when R ≥ 11. §Clinical study warranted when R ≥ 1.1. ¶Clinical study warranted when R ≥ 1.25. **Clinical study warranted when AUCR >1.25. ††Total drug concentrations used in calculation ‡‡Unbound drug concentrations used in calculation. §§Conservative estimate of unbound drug concentrations (1%) used in calculation for highly protein bound drugs. ¶¶Using full precision, these values fell slightly below the threshold to conduct a clinical study. However, values were rounded to account for inaccuracies, leading to the recommendation to conduct a clinical study.
NA = not applicable. TDI = time-dependent inhibition. MBI = mechanism-based inhibition.

Clinical study A: Drug–drug interaction study of evacetrapib with midazolam and tolbutamide in healthy human subjects

Pharmacokinetics of midazolam and tolbutamide Administration of evacetrapib slightly increased midazolam exposure, whereas the effect on tolbutamide exposure was minimal (Figure 3A and B). Geometric mean ratios of plasma midazolam AUC from time 0 to infinity (AUC(0,∞)) in the presence on day 1 and absence on day 15 of evacetrapib were 1.19 (95% CI 1.06, 1.33) ($P = 0.004$) and 1.44 (95% CI 1.28, 1.62) ($P < 0.001$) for evacetrapib 100 mg and 300 mg, respectively (Table 4). Geometric mean ratios of C_{max} were 1.29 (95% CI 1.07, 1.54) ($P = 0.009$) and 1.26 (95% CI 1.04, 1.52) ($P = 0.020$), respectively. The exposures of the midazolam metabolite, 1′-hydroxymidazolam, were not as strongly affected by evacetrapib as was midazolam itself. The AUC and C_{max} of 1′-hydroxymidazolam were not significantly affected by 14 daily doses of 100 mg of evacetrapib. Following 14 daily doses of 300 mg of evacetrapib, the AUC(0,∞) of 1′-hydroxymidazolam increased by a factor of 1.18. The C_{max} of 1′-hydroxymidazolam was not significantly affected by 300 mg of evacetrapib (Table 4). The geometric mean ratios of plasma tolbutamide AUC(0,∞) in the presence and absence of evacetrapib were 0.85 (95% CI 0.77, 0.94) ($P = 0.002$) and 1.06 (95% CI 0.95, 1.18) ($P = 0.262$) following 14 daily doses of 100 mg or 300 mg, respectively; for C_{max} the ratios were 1.02 (95% CI 0.92, 1.14) ($P = 0.675$) and 0.99 (95% CI 0.89, 1.12) ($P = 0.917$), respectively.

Adverse events Among cohorts that received DDI probes, one subject receiving 100 mg evacetrapib showed an elevation in ALT and AST. The elevation in hepatic transaminases in this subject occurred after the first administration of probe substrates and was less than three times the upper limit of normal range. Total bilirubin was in the normal range.

Clinical study B: Drug–drug interaction study of evacetrapib with simvastatin in dyslipidaemic patients

Pharmacokinetics of simvastatin Co-administration of evacetrapib with simvastatin resulted in a mean AUC($0,\tau_{ss}$) ratio of 1.25 (95% CI 1.03, 1.53), relative to administering the statin alone (Table 5). The effect of evacetrapib on simvastatin exposure was statistically significant ($P < 0.025$). The impact of evacetrapib on the geometric mean ratios between parent (simvastatin lactone) and metabolite (simvastatin acid) plasma concentrations at trough and C_{max} of simvastatin was minimal. At trough, the parent to metabolite ratios were 1.09 in both the absence and presence of evacetrapib, resulting in an overall ratio (statin + evacetrapib/statin alone) of 1.00. At C_{max}, the parent to metabolite ratios were 4.15 and 4.77 in the absence and presence of evacetrapib, respectively, resulting in an overall ratio of 1.15.

Adverse events In patients receiving simvastatin alone or in combination with evacetrapib, no clinically important differences were found regarding clinical laboratories or treatment emergent adverse events.

Discussion

Evacetrapib is a reversible inhibitor of multiple cytochromes P450 (3A4, 2D6, 2C19, 2C9, 2C8, 1A2) *in vitro*. A risk assessment using the basic and conservative I/K_i methods was performed and suggested that evacetrapib had the potential to inhibit the metabolism of co-administered drugs metabolized by CYP3A and CYP2C9 because estimated I_u/K_i ratios exceeded the EMA-recommended threshold of 0.02 [13]. Based on the more recent FDA and EMA guidances, the reversible basic models of inhibition predicted clinically relevant DDI for both CYP3A and CYP2C9, while clinically relevant inhibition of CYP3A, but not CYP2C9, was predicted using the mechanistic static models [12, 13]. Furthermore,

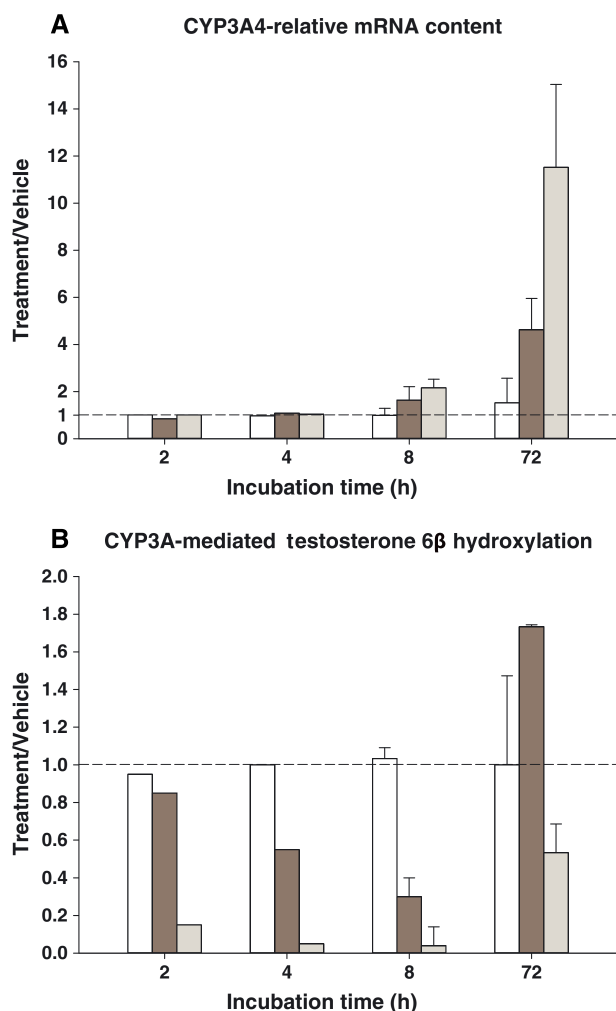

Figure 1

Time course (2–72 h) of mean effects (treatment/vehicle) of evacetrapib on CYP3A4 relative mRNA content (A) and CYP3A-mediated testosterone 6β-hydroxylation (B) on multiple lots ($n = 3$) of primary human hepatocytes. Error bars indicate standard deviation at 8 and 72 h timepoints. At 2 and 4 h timepoints, error bars are not shown because only two lots were incubated. Treatment/vehicle = 1 indicates no effect. (☐ 0.1 μM, ■ 1 μM, ☐ 10 μM)

inhibition of CYP3A in the gut by evacetrapib was also predicted.

In vitro, evacetrapib was also identified as a time-dependent inhibitor of CYP3A4 activity in microsomes and cultured hepatocytes. In the hepatocyte experiments, we investigated the time course of the effects of evacetrapib on CYP3A4 mRNA, total protein and activity in addition to the standard 48–72 h terminal time point. The effects of evacetrapib were dependent on the CYP3A end point being measured, treatment time, and evacetrapib concentration. At concentrations similar to those achieved in the clinic (1.9 μM for the phase 3 dose of 130 mg), mRNA levels increased within 8 h while inhibition of CYP3A activity in hepatocyte preparations appeared to manifest over short treatment intervals (2–8 h). At 72 h, the inhibitory effects on activity were no longer evident in preparations treated with 1 μM evacetrapib, but continued to dominate in preparations treated with 10 μM evacetrapib even though protein levels had rebounded. It was concluded that CYP3A4 was likely inactivated during the *in vitro* metabolism of evacetrapib, with the inactivated enzyme efficiently being removed from the pool of CYP3A4 recognizable by the immunoblotting antibody. Clinically relevant inhibition of CYP3A could not be ruled out, based on predictions utilizing the FDA and EMA basic models for time-dependent/mechanism-based inhibition.

Overall, the combined data from the *in vitro* studies of evacetrapib in liver microsomes and hepatocyte preparations suggest a complex interplay of reversible inhibition, time-dependent inhibition, induction of CYP3A and concentration-dependent effects. Thus, a clinical CYP3A DDI study was warranted and midazolam was chosen as the sensitive substrate to probe this potential interaction. The *in vitro* risk assessment also suggested that evacetrapib had the potential to inhibit the metabolism of co-administered drugs metabolized by CYP2C9, although the more recent mechanistic static model predictions suggested that there would not be a clinically significant interaction. However, a conservative approach was taken and the clinical DDI study was conducted utilizing tolbutamide as the CYP2C9 probe substrate.

In the phase 1 study in healthy subjects, which utilized midazolam and tolbutamide probes, the AUC ratio data at steady-state indicated that evacetrapib is a weak inhibitor of CYP3A but had no statistically significant effect on CYP2C9-mediated metabolism. These conclusions are drawn based on the FDA and EMA classification criteria whereby weak inhibitors result in ≥1.25-fold increase to <2-fold increase in AUC values. Our data indicate that a weak interaction with CYP3A4 occurred at both 100 and 300 mg doses of evacetrapib, whereas the AUC ratios for CYP2C9 ranged from 0.85 to 1.06, indicating minimal interaction with CYP2C9. Thus, these studies suggested a low risk of clinically relevant DDIs between evacetrapib and CYP3A4 and CYP2C9 substrates that may be taken as concomitant medications. Furthermore, since the most potent observed *in vitro* K_i value was associated with CYP2C9, and given that the DDI study indicated that evacetrapib is unlikely to cause clinically significant interactions requiring dose adjustments of co-medications metabolized by CYP3A4 or CYP2C9, it can be concluded that clinically significant inhibition of the other CYP enzymes with less potent K_i values (up to 7.6 μM for CYP2C19) is unlikely.

The potential for CYP3A-mediated DDIs was also investigated in the anticipated dyslipidaemic patient population for evacetrapib, which includes patients with high-risk vascular disease taking concomitant therapies such as statins. The subsequent phase 2 study in patients demonstrated that evacetrapib had minimal impact on the steady-state AUC of simvastatin. Co-administration of simvastatin with evacetrapib resulted in a mean statin

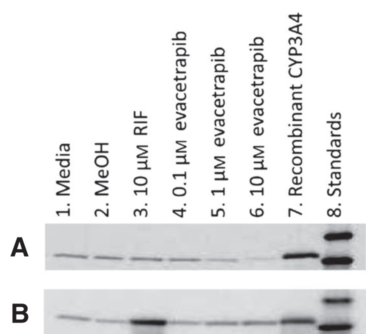

Figure 2

Representative Western immunoblots following treatment with evacetrapib and controls. Panel A: 8 h; Panel B: 72 h

AUC ratio of 1.25, relative to administering the statin alone. Evacetrapib also had only a very limited effect on the parent : metabolite concentration ratio.

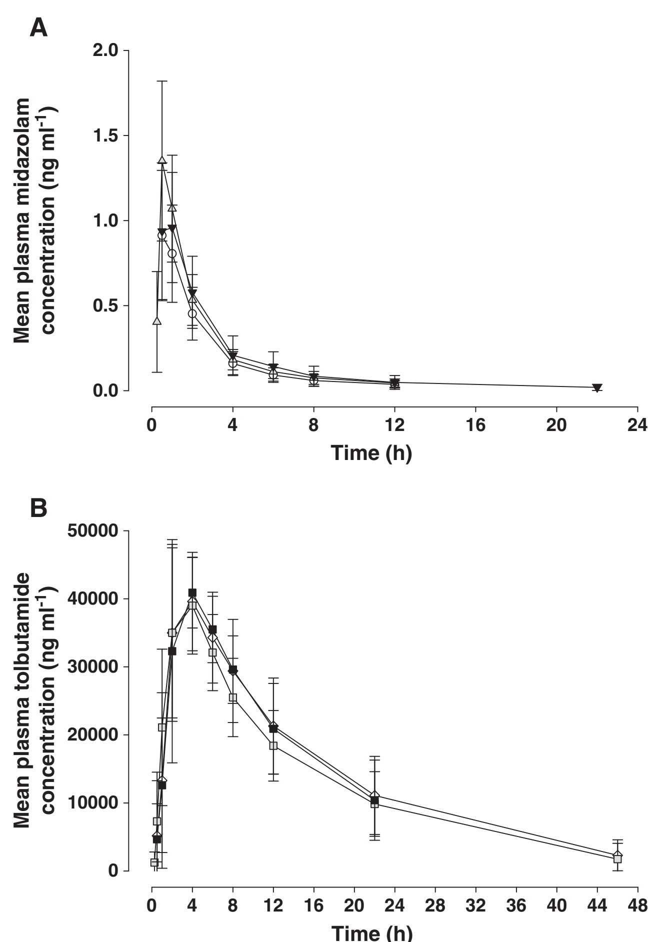

Figure 3

Plasma midazolam (Panel A, —○— Midazolam only (Day −4), —△— Midazolam + 100 mg evacetrapib (Day 15), —▼— Midazolam + 300 mg evacetrapib (Day 15)) and tolbutamide (Panel B, —◇— Tolbutamide only (Day −4), —□— Tolbutamide + 100 mg evacetrapib (Day 15), —■— Tolbutamide + 300 mg evacetrapib (Day 15)) concentrations over time in Phase 1 study

Phase 2 safety data showed that 12-week therapy with daily doses of evacetrapib up to 500 mg as monotherapy or evacetrapib 100 mg in combination with simvastatin was well tolerated. No evidence of any adverse effect of evacetrapib on hepatic or muscle safety emerged during the active treatment phase. In their analysis of the phase 2 study database, Nicholls *et al*. reported that evacetrapib was well tolerated [9]. Although their analysis was underpowered to rule out uncommon adverse effects, these investigators did not detect any clinically significant side effects or laboratory abnormalities related to liver, kidney or muscle toxicity.

Although our *in vitro* data suggested that evacetrapib has the potential to affect multiple CYPs, several factors may account for the mild interactions observed in clinical studies. First, when one considers the high plasma protein binding of evacetrapib (≥99.9%) [20] and low unbound fraction in the circulation, the likelihood of a clinically significant DDI is reduced. Secondly, the *in vitro* data suggested that evacetrapib is potentially only a weak time-dependent inhibitor of CYP3A4 *in vivo*. Evacetrapib is also an inducer of CYP3A and thus possesses potentially offsetting inductive and inhibitory properties. Although evacetrapib showed the potential to induce CYP3A *in vitro*, the potential to produce clinically relevant *in vivo* induction of CYP3A catalytic activity over the concentration range examined was low due to the apparent dual inducing and inhibiting effects of evacetrapib on CYP3A mRNA and enzyme activity. Such normalization appears to translate into a low risk of clinically meaningful DDIs. Other examples of marketed drugs thought to have similar mechanisms of dual inhibition and induction of CYP3A observed *in vitro*, which presumably offsets the clinical significance, are tadalafil [15] and ritonavir [21].

In conclusion, the initial *in vitro* data predicted that evacetrapib could be an inhibitor of multiple CYP enzymes and could be an inducer of CYP3A. The CYP3A4 inhibition potential was further supported by using updated models to predict the likelihood of DDI. However, well designed clinical studies with probe substrates selected on the basis of *in vitro* risk assessment tools and modelling approaches suggested that there was no evidence of any clinically relevant DDI with evacetrapib as the perpetrator drug. Furthermore, there was no evidence of adverse effects of evacetrapib on liver or muscle safety when given as monotherapy or in combination with simvastatin, nor were there any statistically or clinically significant effects of evacetrapib on mean change from baseline in liver or muscle-related laboratory parameters. Evacetrapib minimally impacted exposure of simvastatin, which suggests that dose adjustments of this concomitant medication, or other CYP3A substrates, will not be warranted and that interactions with drugs cleared through this pathway are unlikely. Furthermore, atorvastatin was included in the phase 2 study, as a

Table 4

AUC(0,∞) and C_{max} of midazolam, 1′-hydroxymidazolam and tolbutamide before and after 14 daily doses of 100 mg or 300 mg evacetrapib in the phase 1 study

Parameter	Dose (mg)	With evacetrapib (95% CI)	Without evacetrapib (95% CI)	Ratio (95% CI)	*P* value
			Midazolam		
AUC(0,∞) (ng ml^{-1} h)	100	3.1 (2.5, 4.0)	2.6 (2.1, 3.4)	1.19 (1.06, 1.33)	0.004
	300	3.1 (2.4, 4.0)	2.3 (1.8, 2.9)	1.44 (1.28, 1.62)	<0.001
C_{max} (ng ml^{-1})	100	1.3 (1.1, 1.6)	1.0 (0.9, 1.2)	1.29 (1.07, 1.54)	0.009
	300	1.0 (0.8, 1.3)	0.8 (0.6, 1.1)	1.26 (1.04, 1.52)	0.020
			1′-Hydroxymidazolam		
AUC(0,∞) (ng ml^{-1} h)	100	1.0 (0.9, 1.1)	0.9 (0.7, 1.0)	1.11 (0.99, 1.26)	0.07
	300	0.8 (0.6, 1.0)	0.7 (0.6, 0.8)	1.18 (1.04, 1.33)	0.01
C_{max} (ng ml^{-1})	100	0.4 (0.3, 0.5)	0.3 (0.3, 0.4)	1.16 (0.94, 1.43)	0.16
	300	0.3 (0.2, 0.3)	0.3 (0.2, 0.3)	0.96 (0.78, 1.20)	0.73
			Tolbutamide		
AUC(0,∞) (μg ml^{-1} h)	100	581 (466, 725)	678 (548, 839)	0.85 (0.77, 0.94)	0.002
	300	559 (492, 635)	579 (460, 728)	1.06 (0.95, 1.18)	0.26
C_{max} (μg ml^{-1})	100	42 (38, 47)	41 (37, 46)	1.02 (0.92, 1.14)	0.68
	300	43 (39, 48)	44 (38, 50)	0.99 (0.89, 1.12)	0.92

Note: except for doses and *P* values, the numbers presented in each cell are the geometric mean (95% CI). AUC(0,∞), plasma area under the concentration *vs*. time curve from time 0 to infinity; C_{max}, maximum plasma concentration.

Table 5

$AUC\tau_{ss}$ and $AUC\tau_{ss}$ ratio of evacetrapib plus simvastatin *vs*. simvastatin alone in the Phase 2 study

	Geometric mean AUC (0,τ_{ss}) (ng ml^{-1} h)	AUC(0,τ_{ss}) ratio (evacetrapib and statin/statin alone) (95% CI)
Evacetrapib/ Simvastatin	79.8*	1.25 (1.03, 1.53)
Simvastatin alone	63.7	

AUC(0,τ_{ss}), area under the concentration *vs*. time curve (dosing interval, steady-state), calculated using the statin dose and post-hoc individual estimate of statin clearance from the population PK analysis (Dose/CL). *$P < 0.025$ based on two-sided hypothesis test at the 0.025 level (95% CI).

comparator drug, in which evacetrapib also had minimal effect on its exposure (data not shown). Overall, the present studies support the conclusion that the risk of clinically relevant CYP-mediated DDIs is low with evacetrapib at the likely prescribed dose level in the intended patient population.

Competing Interests

This work was supported by Eli Lilly and Company.

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