Citation: Clin Transl Sci (2021) 14, 373–381; doi:10.1111/cts.12888

ARTICLE

Coproporphyrin I Can Serve as an Endogenous Biomarker for OATP1B1 Inhibition: Assessment Using a Glecaprevir/Pibrentasvir Clinical Study

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Organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 are involved in the disposition of a variety of commonly prescribed drugs. The evaluation of OATP1B1/1B3 inhibition potential by investigational drugs is of interest during clinical drug development due to various adverse events associated with increased exposures of their substrates. Regulatory guidance documents on the *in vitro* assessment of OATP1B1/1B3 inhibition potential are conservative with up to a third of predictions resulting in false positives. This work investigated the utility of OATP1B1/1B3 endogenous biomarkers, coproporphyrin (CP)-I and CP-III, to assess clinical inhibition of OATP1B1/1B3 and potentially eliminate the need for prospective clinical drug-drug interaction (DDI) studies. Correlations between CP-I exposures and various OATP1B1 static DDI predictions were also evaluated. Glecaprevir/pibrentasvir (GLE/PIB) 300/120 mg fixed-dose combination is known to cause clinical inhibition of OATP1B1/1B3. In a clinical study evaluating the relative bioavailability of various formulations of GLE/PIB regimen, CP-I peak plasma concentration (C_{max}) ratio and 0–16-hour area under the concentration-time curve ($AUC_{0–16}$) ratio relative to baseline increased with increasing GLE exposures, whereas there was a modest correlation between GLE exposure and CP-III C_{max} ratio but no correlation with CP-III $AUC_{0–16}$ ratio. This suggests that CP-I is superior to CP-III as an endogenous biomarker for evaluation of OATP1B1 inhibition. There was a significant correlation between CP-I and GLE C_{max} (R^2 = 0.65; P < 0.001) across individual subjects. Correlation analysis between GLE OATP1B1 R values and CP-I exposures (C_{max} ratio and $AUC_{0–16}$ ratio) suggests that an R value of > 3 can predict a biologically meaningful inhibition of OATP1B1 when the inhibitor clinical pharmacokinetic parameters are available.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Coproporphyrin (CP)-I and CP-III are potential endogenous biomarkers for hepatic organic anion transporting polypeptide (OATP)1B1/1B3 function. A robust correlation analysis between the exposure of OATP1B1/1B3 inhibitors and those of CP-I and CP-III is not currently available.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Can CP-I or CP-III exposures serve as surrogate markers for clinical inhibition of OATP1B1/1B3 and potentially eliminate the need for prospective clinical drug-drug interaction (DDI) studies?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ CP-I is superior to CP-III for evaluating OATP1B1 inhibition both from a sensitivity and analytical perspectives. R value > 3 can predict a biologically meaningful inhibition of OATP1B1 in a static DDI prediction when the clinical pharmacokinetic parameters of inhibitor are available.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ CP-I change from baseline can be used to assess OATP1B1 inhibition in lieu of a standalone prospective clinical DDI study. An R value of three can be a potential revised cutoff to predict biologically meaningful inhibition of OATP1B1 while minimizing false-positive predictions. Preclinically, consideration of estimated (unbound maximum plasma concentration [$C_{max,u}$])/(OATP1B1 half-maximal inhibitory concentration [IC_{50}]) and R value may improve the OATP1B1 inhibition predictions.

Cumulative evidence demonstrates the importance of organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 in the hepatic disposition of a variety of clinically used drugs, such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), angiotensin-converting enzyme inhibitors, angiotensin receptor II antagonists, and

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Received: May 7, 2020; accepted: July 28, 2020. doi:10.1111/cts.12888

hepatitis C (HCV) protease inhibitors.[1,2] The clinical inhibition of OATP1B1 and OATP1B3 by an investigational drug is of particular concern as it could result in the increased plasma exposure of co-administered OATP1B1/1B3 substrates, potentially leading to increased risk of adverse events (e.g., statin-induced myopathy).[3–5]

To assess and mitigate potential risk of OATP1B1/1B3-mediated drug-drug interactions (DDIs), DDI guidance documents from regulatory agencies (US Food and Drug Administration (FDA), European Medicines Agency (EMA), and Pharmaceuticals and Medical Devices Agency (PMDA)) provide guidelines to characterize the potential of investigational drugs to inhibit OATP1B1 and OATP1B3 during drug development.[6–8] In general, an investigational drug is first characterized for its potential to clinically inhibit OATP1B1 or OATP1B3 in a static model. If a clinical inhibition is predicted, it may be further followed up by a dynamic DDI prediction using a physiologically-based pharmacokinetic model or a prospective clinical DDI study using an OATP1B1/1B3 probe substrate. These DDI predictions, especially the ones with a static model, are conservative. A retrospective analysis of 107 clinical DDI studies pertaining to OATP1B1/1B3 inhibition demonstrated that the cutoff value for the static DDI prediction parameter (*R* value) of > 1.1 as suggested by the FDA and PMDA,[7,8] and *R* > 1.04 as suggested by the EMA[6] resulted in 22% and 33% of false-positive predictions, respectively.[9] Therefore, the evaluation of the effect of investigational drug on endogenous biomarkers for OATP1B1/1B3 early in the clinical development can help guide or potentially eliminate the need for a dedicated clinical DDI study.

Several endogenous compounds are substrates for OATP1B1 and/or OATP1B3, including coproporphyrin (CP)-I, CP-III, unconjugated and conjugated bilirubins, bile acids, and their sulfate conjugates.[10] Among them, CP-I and CP-III have been proposed as potential endogenous biomarkers for the function of hepatic OATP1B1/1B3.[11,12] Several endogenous biomarkers, including CP-I, were identified to show good sensitivity and dose-dependent change in exposure ratios in healthy volunteers when oral ascending doses of rifampicin in the range of 150 to 600 mg were administered.[13,14] CP-I and CP-III are porphyrin metabolites from heme synthesis and relatively selective substrates for OATP1B1 and OATP1B3. CP-I and CP-III are metabolically stable and are eliminated in bile and urine as intact forms in humans. The increased urinary excretion of CP-I in patients with Rotor's syndrome, characterized by the complete and simultaneous deficiencies of OATP1B1 and OATP1B3, corroborates the role of OATP1B1 and OATP1B3 in the disposition of CP-I.[15,16]

There have been several reports demonstrating the increase in CP-I or CP-III plasma exposure after administration of OATP1B1/1B3 inhibitors in human.[13,14,17–19] More recent analysis using population pharmacokinetic modeling confirmed the sensitivity of CP-I to identify moderate and weak OATP1B1 inhibitors.[20] However, there has not been any robust correlation analysis between the exposure of OATP1B1/1B3 inhibitors and those of CP-I and III, which is also necessary to establish these endogenous compounds as a biomarker of OATP1B1/1B3. Glecaprevir/pibrentasvir (GLE/PIB) is an all-oral, pan-genotypic, fixed-dose combination regimen approved for the treatment of all major genotypes of HCV infection in many countries worldwide (Mavyret or Maviret).[21] GLE was identified in a collaboration between AbbVie and Enanta and developed by AbbVie. PIB was discovered by and developed by AbbVie. *In vitro* studies demonstrated that GLE is an inhibitor of OATP1B1 and OATP1B3 with half-maximal inhibitory concentration (IC_{50}) values of 0.017 and 0.064 μM, respectively, whereas PIB is an inhibitor of OATP1B1 (IC_{50}:1.3 μM in the presence of 4% bovine serum albumin) but not OATP1B3.[22] The unbound concentration of GLE in the hepatic portal vein was estimated to be sufficient to inhibit both OATP1B1 (*R* value: 5.5) and OATP1B3 (*R* value: 2.2) as per the FDA guidance.[7] A weak inhibition of OATP1B1 by PIB was also predicted (*R* value: 1.4). Consistent with these predictions, there was an increase in the clinical exposure of pravastatin and rosuvastatin (OATP1B1/1B3 substrates) after co-administration with GLE and PIB combination.[22] The aim of this study was to evaluate the correlation between the plasma exposure of GLE or PIB and CP-I or CP-III in humans in order to support their utility as a biomarker of OATP1B1/1B3. In addition, a static DDI prediction was performed and correlated with the change in the CP-I exposure in order to evaluate the predictive accuracy at different cutoff values.

METHODS

Study design

This was a phase I, single dose, complete crossover study, where healthy subjects were randomly assigned to two different test formulations and a reference commercial formulation of GLE/PIB. A signed informed consent approving the use of blood samples collected in this study for exploratory analysis was obtained from all subjects prior to performing any study-related procedures or analyses. This study was conducted in accordance with applicable regulations governing clinical study conduct and ethical principles originating in the Declaration of Helsinki. GLE/PIB was administered under fasting conditions at a 300/120 mg dose in the morning on dosing days of each period. Because the half-life of GLE and PIB is about 6 and 13 hours, respectively,[21] a minimum washout of at least 5 days between treatments was assigned for each participant. Subjects were confined to the study site and supervised for ~ 4 days in each study period and received standardized meals throughout confinement.

Study population

Using standard inclusion and exclusion criteria, healthy male and female subjects between 18 and 55 years of age (inclusive), in general good health with a body mass index of ≥ 18.0 to ≤ 29.9 kg/m^2 were enrolled in the study. Inclusion in the study was based on screening results from medical history, physical examination, clinical laboratory profile, and electrocardiogram evaluations. Female volunteers of child-bearing potential were required to provide negative urine pregnancy test at screening and negative serum tests on day –1 and use a specified method for birth control (if sexually active). Subjects were not eligible for enrollment

into the study if they had a history of gastric surgery or any condition that could interfere with drug absorption or if they screened positive for hepatitis A virus immunoglobulin M, hepatitis B surface antigen, HCV antibody, or HIV antibody, or had a screening alanine aminotransferase or aspartate aminotransferase value above the reference range. Use of the following medications was not allowed during the study: any medications, vitamins, or herbal supplements from 2 weeks prior or within 5-half-lives of the respective medication, whichever was longer, before initial study drug administration through the end of their participation in the study; known inhibitors or inducers of metabolic enzymes, including CYP3A or drug transporters, including breast cancer resistance protein, P-glycoprotein, or OATP1B1 within 30 days before study drug administration, as well as consumption of quinine or tonic water. Other restrictions during the study included consumption of tobacco or nicotine-containing products within the prior 6-month period; or alcohol, grapefruit, star fruit, Seville oranges, or products containing any of these ingredients within 3 days of study drug administration.

Blood sampling

Serial blood samples for measurement of GLE and PIB concentrations as well as CP-I and CP-III levels were obtained by venipuncture before dosing (0 hour) and up to 48 hours after dosing on day 1 in each study period. Blood samples were collected into dipotassium EDTA tubes and stored on ice until centrifugation. Plasma samples were stored at –20°C until analysis.

Bioanalysis of GLE and PIB

GLE and PIB were extracted with standard analytical procedures from human plasma samples using liquid-liquid extraction with ethyl acetate/hexanes assisted with ammonium formate and guanidine hydrochloride salt solutions. The extracts were analyzed utilizing liquid chromatography coupled with tandem mass spectrometry using a Waters XBridge C18 column with isocratic elution using acetonitrile, methanol, water, formic acid, and ammonium formate. Detection was achieved using a Sciex API5500 mass spectrometer in positive ion multiple-reaction monitoring mode. Glecaprevir-D4 and pibrentasivr-D6 were used as the internal standards for the assay of GLE and PIB, respectively. Human plasma with K_2 EDTA was used to prepare calibration standards. A low concentration curve ranging from ~ 0.200 ng/mL to 100 ng/mL for both GLE and PIB was initially utilized. Samples observed to be above the quantitation limit on the low concentration curve range were analyzed in a separate run on a high calibration curve ranging from ~ 85.0 ng/mL to 10,000 ng/mL for GLE and ~ 85.0 ng/mL to 1,050 ng/mL for PIB. Quality control (QC) samples were also prepared in human plasma with K_2 EDTA at concentrations throughout both calibration curve ranges to monitor the performance of each run. The overall precision (coefficient of variation) of the QCs for GLE and PIB were ≤ 7.8% and ≤ 8.9%, respectively. The overall accuracy (expressed as percent bias) of the QCs for GLE and PIB were between –4.5% and 3.5%, and between –10.0% and 3.7%, respectively.

Bioanalysis of CP-I and CP-III

CP-I and CP-III were extracted from human plasma samples using liquid-liquid extraction with ethyl acetate. The extracts were analyzed utilizing liquid chromatography coupled with tandem mass spectrometry using an Advanced Materials Technology Halo C18 column with isocratic elution using acetonitrile, water, ammonium acetate, and trifluoroacetic acid. Detection was achieved using a Sciex API6500 mass spectrometer in positive ion multiple-reaction monitoring mode. CP-I-$^{15}N_4$ and CP-III-$^{15}N_4$ were used as the internal standards for the assay of CP-I and CP-III, respectively. A 4× charcoal stripped human plasma was used to prepare the calibration standards with a curve range from 0.0500 to 5.00 ng/mL for both CP-I and CP-III. QC samples were also prepared in 4× charcoal stripped human plasma at concentrations throughout the calibration curve range to monitor the performance of each run. The overall precision (coefficient of variation) of the QCs for CP-I and CP-III were ≤ 4.9% and ≤ 6.8%, respectively. The overall accuracy (expressed as percent bias) of the QCs for CP-I and CP-III were between –3.8% and –3.2%, and between –2.0% and 2.4%, respectively.

Pharmacokinetic assessment and statistical analysis

Pharmacokinetic parameters for GLE, PIB, CP-I, and CP-III were estimated from individual plasma concentration-time profiles using noncompartmental methods with Phoenix WinNonlin version 6.4 (Certara USA, Princeton, NJ). The primary pharmacokinetic parameters of interest for GLE and PIB included the peak concentration (C_{max}), the area under the concentration-time curve (AUC) from time 0 to 16 hours postdose ($AUC_{0–16}$), and AUC from time 0 to 48 hours postdose ($AUC_{0–48}$). AUC was estimated using the linear trapezoidal method. Absorption rate constant (K_a) for GLE was estimated by method of residuals. Concentrations below the lower limit of quantitation were treated as zero for computational purposes. Concentration of CP-I and CP-III prior to dosing (0 hour) on dosing day was considered to be the baseline concentrations. Baseline AUC for each endogenous analyte was estimated by taking the product of baseline concentration and the duration for which AUC was being estimated. The primary pharmacokinetic parameters of interest for CP-I and CP-III included C_{max}, $AUC_{0–16}$, and $AUC_{0–48}$, as well as ratios for change from the baseline for these parameters. Exposure parameter $AUC_{0–16}$ was considered along with $AUC_{0–48}$ as 16 hours postadministration represented majority of GLE exposure. Within the mixed modeling framework, a comparison was performed for C_{max}, $AUC_{0–16}$, and $AUC_{0–48}$ of CP-I and CP-III for formulation A or B vs. reference formulation C. Pearson correlation analysis was performed to evaluate the strength of correlation between each GLE or PIB exposure parameters with corresponding CP-I and CP-III exposure parameters (significance, $P < 0.05$).

Prediction of transporter inhibition

Prediction of clinical OATP1B1 inhibition by GLE and PIB was made in accordance with the FDA's guidance[7] by calculating OATP1B1 *R* values for each subject and for each administered formulation using the following equations:

$$\text{GLE OATP1B1 } R = 1 + \frac{f_{u,p} \times I_{in,max}}{IC_{50}}$$

$$\text{PIB OATP1B1 } R = 1 + \frac{I_{in,max}}{IC_{50}}$$

$$I_{in,max} = C_{max} + \frac{F_a \times F_g \times K_a \times \text{Dose}}{Q_h \times R_B}$$

where $f_{u,p}$ is the fraction of unbound inhibitor in plasma, $I_{in,max}$ is the estimated maximum plasma inhibitor concentration at the inlet to the liver, C_{max} is the maximum systemic plasma concentration of inhibitor, $F_a \times F_g$ is the fraction of the dose of inhibitor which is absorbed, K_a is the absorption rate constant of the inhibitor, dose is the inhibitor dose, Q_h is the hepatic blood flow (97 L/hour/70 kg), and R_B is the blood-to-plasma concentration ratio. The observed C_{max}, K_a, and estimated $F_a \times F_g$ of GLE and PIB for each subject and formulation were used in the calculation (**Table S1**). The $F_a \times F_g$ values of GLE and PIB were estimated relative to the geometric mean of formulation C, which was assumed to represent the complete absorption ($F_a \times F_g = 1$). Additionally, GLE unbound maximum plasma concentration to OATP1B1 IC_{50} ratios ($[C_{max,u}]$/[OATP1B1 IC_{50}]) and PIB total maximum plasma concentration to OATP1B1 IC_{50} ratios ($[C_{max}]$/[OATP1B1 IC_{50}]) were calculated for each individual subject for each formulation to compare against CP-I C_{max} ratio and CP-I AUC_{0-16} ratio. GLE parameters used for R value calculation are as follows: OATP1B1 IC_{50}, 0.017 μM; $f_{u,p}$, 0.025; R_B, 0.57; and dose, 300 mg.[22] PIB parameters used for R value calculation are as follows: OATP1B1 IC_{50}, 1.3 μM (determined in the presence of 4% bovine serum albumin); R_B, 0.62; and dose, 120 mg.[22] Furthermore, the net OATP1B1 R value of the combination regimen (GLE/PIB) was calculated assuming that the two inhibitors are mutually exclusive using the following equation:

$$\text{Net OATP1B1 } R = 1 + \frac{f_{u,p,GLE} \times I_{in,max,GLE}}{IC_{50,GLE}} + \frac{I_{in,max,PIB}}{IC_{50,PIB}}$$

which was derived from the simple generalized equation for the analysis of simultaneous inhibition by multiple inhibitors.[23] The same approach is also described elsewhere.[24,25] Because the OATP1B1 IC_{50} value of PIB was determined in the presence of physiological concentration of albumin (4% bovine serum albumin), the total C_{max} of PIB was compared with its IC_{50} value ($[C_{max}]$/[OATP1B1 IC_{50}]), and the estimated total plasma exposure of PIB in the hepatic inlet was compared with IC_{50} in the calculation of PIB OATP1B1 R value and the net OATP1B1 R value (total IC_{50} method).[26]

Safety

The following safety evaluations were performed during the study: adverse event monitoring and vital signs, physical examination, electrocardiogram, and laboratory tests assessments. The Institutional Review Board at Vista Medical Center East (Waukegan, IL) reviewed and approved the study protocol and informed consent form.

RESULTS

Subject demographics

Study subjects ($N = 10$) had a mean age of 38.7 years (range 24–55), weight of 72.1 kg (range 55.9–89.8), and height of 167 cm (range 154–183). Seven of the subjects were women. Three of the subjects were African American and seven of them were white with four among them identifying themselves as Hispanic or Latino.

Pharmacokinetic assessment of GLE and PIB

The mean ± SD concentration-time profiles and the pharmacokinetic parameters (C_{max}, AUC_{0-16}, and AUC_{0-48}) of GLE and PIB following the administration of formulations

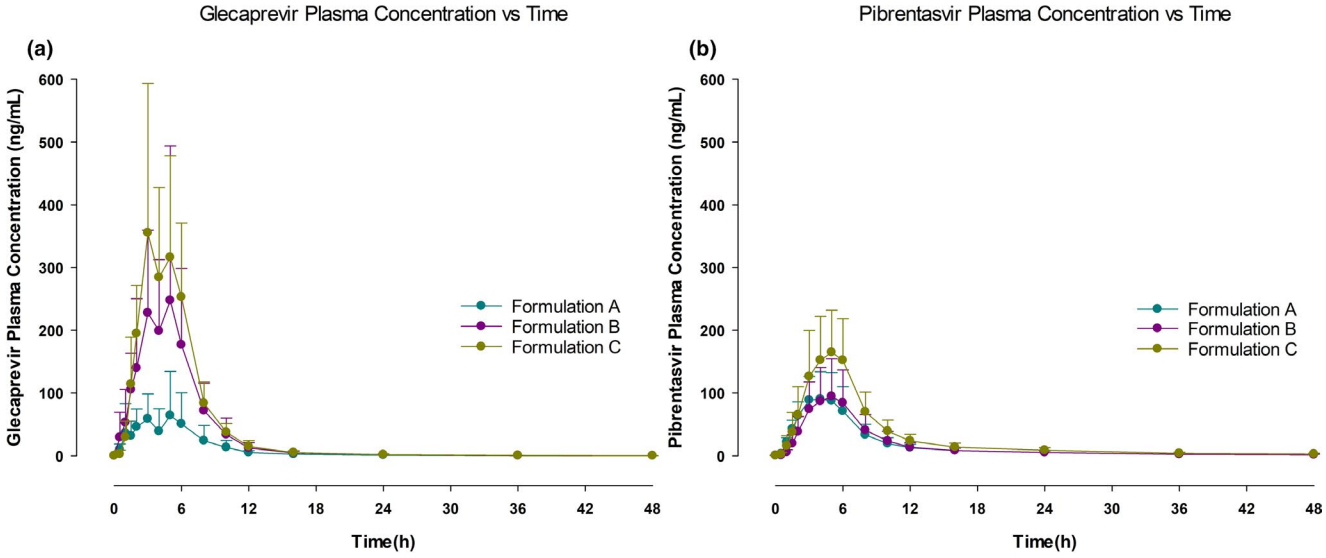

Figure 1 Mean ± SD plasma concentrations-time profile in linear scale following administration of a single dose of GLE/PIB 300/120 mg formulation A, B, or C under fasting conditions to healthy volunteers. Plasma concentration vs time profiles for (**a**) Glecaprevir (GLE) and (**b**) Pibrentasvir (PIB).

Table 1 Geometric mean (mean, %CV) GLE, PIB, CP-I and CP-III exposures following the administration of single dose GLE/PIB 300/120 mg

Analyte	PK Parameter	Formulation A	Formulation B	Formulation C
GLE	C_{max} (ng/mL)	88.5 (104, 62)	330 (370, 51)	444 (471, 36)
	AUC_{0-16} (ng·h/mL)	344 (407, 65)	1,330 (1,400, 32)	1800 (1,850, 25)
	AUC_{0-48} (ng·h/mL)	367 (429, 65)	1,370 (1,430, 31)	1,850 (1,900, 25)
PIB	C_{max} (ng/mL)	86.8 (97.4, 61)	83.7 (101, 80)	163 (177, 47)
	AUC_{0-16} (ng·h/mL)	546 (615, 60)	514 (611, 77)	977 (889, 47)
	AUC_{0-48} (ng·h/mL)	658 (739, 60)	622 (735, 76)	1,170 (1,270, 47)
CP-I	C_{max} (ng/mL)	0.726 (0.740, 21)	1.19 (1.24, 26)	1.44 (1.48, 23)
	AUC_{0-16} (ng·h/mL)	8.98 (9.10, 16)	11.7 (11.9, 23)	13.2 (13.3, 16)
	AUC_{0-48} (ng·h/mL)	25.4 (25.6, 12)	28.2 (28.5, 18)	30.3 (30.6, 13)
CP-III	C_{max} (ng/mL)	0.104 (0.105, 16)	0.113 (0.116, 25)	0.115 (0.119, 26)
	AUC_{0-16} (ng·h/mL)	1.20 (1.22, 19)	1.07 (1.24, 73)	1.23 (1.30, 36)
	AUC_{0-48} (ng·h/mL)	3.32 (3.44, 23)	2.77 (3.44, 41)	2.87 (3.23, 41)

%CV, percent coefficient of variation; AUC_{0-16}, 0–16 hours area under the concentration-time curve; AUC_{0-48}, 0–48 hours area under the concentration-time curve; C_{max}, peak plasma concentration; CP, coproporphyrin; GLE, glecaprevir; PIB, pibrentasvir; PK, pharmacokinetic.

A, B, and C are presented in **Figure 1** and **Table 1**, respectively. Under fasting conditions, GLE exposures (C_{max}, AUC_{0-16}, and AUC_{0-48}) following a single administration of formulations A and B were ~ 80% and 26% lower relative to the reference formulation C, whereas PIB exposures were similar between formulations A and B, and 28–49% lower relative to the reference formulation C.

Pharmacokinetic assessment of CP-I and CP-III

The mean ± SD concentration-time profiles and the pharmacokinetics parameters (C_{max}, AUC_{0-16}, and AUC_{0-48}) of CP-I and CP-III are provided in **Figure 2**, **Figure S1**, and **Table 1**, respectively. The concentrations of CP-I were higher compared with CP-III. Most CP-III concentrations were close to and often below the lower limit of quantitation (0.05 ng/mL) for the analytical method used in the present study. The baseline plasma concentration of CP-I was comparable among individuals in all study periods (mean and SD: 0.59 ± 0.09 ng/mL or 0.90 ± 0.14 nM).

Point estimate of CP-I and CP-III exposure comparing to the baseline

The point estimates and 90% confidence intervals for the CP-I and CP-III exposures relative to baseline are shown in **Figure 3** and **Table 2**. CP-I C_{max} ratio and AUC_{0-16} ratio

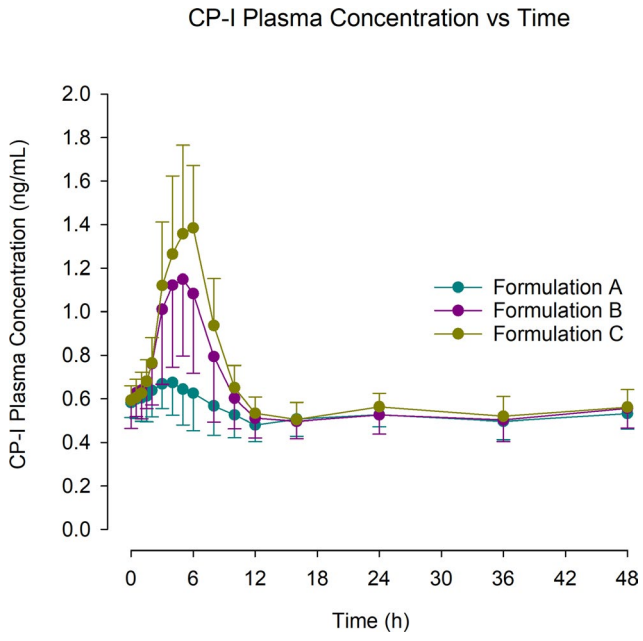

Figure 2 Mean ± SD coproporphyrin (CP)-I plasma concentrations-time profile in linear scale following administration of a single dose of glecaprevir/pibrentasvir (GLE/PIB) 300/120 mg formulation A, B, or C under fasting conditions to healthy volunteers.

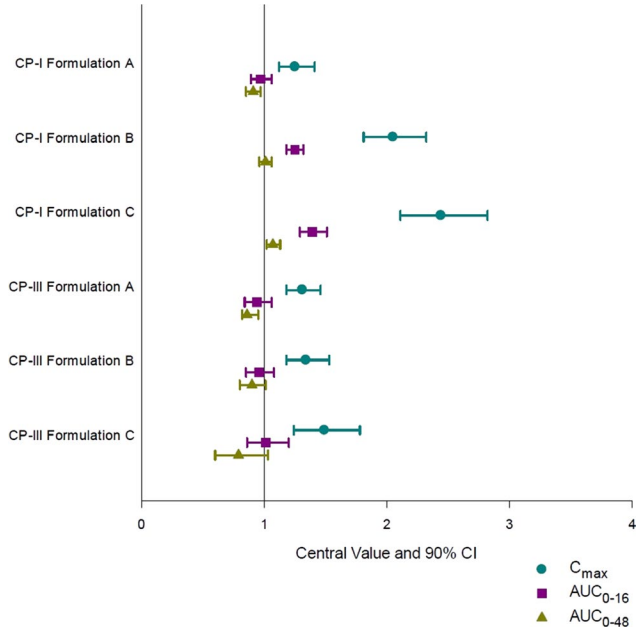

Figure 3 Point estimates and 90% confidence intervals (CIs) of coproporphyrin (CP)-I and CP-III exposures (peak plasma concentration (C_{max}), 0–16 hours area under the concentration-time curve (AUC_{0-16}), and 0–48 hours AUC (AUC_{0-48})) relative to corresponding baseline exposures following administration of a single dose of glecaprevir/pibrentasvir (GLE/PIB) 300/120 mg formulation A, B, or C to healthy volunteers.

Table 2 Ratio for change in CP-I and CP-III exposures (90% CI) relative to baseline

Analyte	PK Parameter	Formulation A	Formulation B	Formulation C
CP-I	C_{max} ratio	1.25 (1.12 to 1.41)	2.05 (1.81 to 2.32)	2.44 (2.11 to 2.82)
	AUC_{0-16} ratio	0.97 (0.89 to 1.06)	1.25 (1.18 to 1.32)	1.39 (1.29 to 1.51)
	AUC_{0-48} ratio	0.91 (0.85 to 0.97)	1.01 (0.96 to 1.06)	1.07 (1.02 to 1.13)
CP-III	C_{max} ratio	1.31 (1.18 to 1.46)	1.34 (1.18 to 1.53)	1.49 (1.24 to 1.78)
	AUC_{0-16} ratio	0.94 (0.84 to 1.06)	0.96 (0.85 to 1.08)	1.01 (0.86 to 1.20)
	AUC_{0-48} ratio	0.86 (0.82 to 0.95)	0.90 (0.80 to 1.01)	0.79 (0.60 to 1.03)

AUC_{0-16}, 0–16 hours area under the concentration-time curve; AUC_{0-48}, 0–48 hours area under the concentration-time curve; CI, confidence interval; C_{max}, peak plasma concentration.

increased with increasing GLE exposure (formulations A < B < C). There was a modest correlation between GLE exposure and CP-III C_{max} ratio but no correlation with CP-III AUC_{0-16} ratio. Single dose administration of GLE/PIB 300/120 mg had negligible effect on the AUC_{0-48} ratio of CP-I and CP-III regardless of the formulations evaluated in this study.

Correlation between GLE or PIB and CP-I exposures

A correlation analysis was performed between the C_{max} of GLE or PIB and that of CP-I in individual subjects following a single dose administration using either formulations A, B, or C (**Figure 4**, **Table 1**). A significant correlation was observed between C_{max} of GLE and CP-I (R^2 = 0.65; P < 0.001). A weaker but significant correlation was also observed between C_{max} of PIB and CP-I (R^2 = 0.14; P = 0.043). Subsequently, [$C_{max,u}$]/[OATP1B1 IC_{50}] and OATP1B1 R values of GLE, and [C_{max}]/[OATP1B1 IC_{50}] and OATP1B1 R value of PIB were calculated in individual subjects and compared with C_{max} ratio or AUC_{0-16} ratio of CP-I (**Figure 5**, **Figure S2, Table 2**, **Table S1**). A biologically meaningful increase in CP-I C_{max} ratio (> 1.25) was associated with GLE [$C_{max,u}$]/[OATP1B1 IC_{50}] of > 0.2 (**Figure 5a**) and GLE OATP1B1 R value of > 3 (**Figure 5b**) in most cases. CP-I AUC_{0-16} ratio was less sensitive to the increase in these parameters (**Figure 5c,d**). There was no clear association between the PIB [C_{max}]/[OATP1B1 IC_{50}] or PIB OATP1B1 R-value and CP-I C_{max} ratio (**Figure S2a,b**). On the other hand, the correlation between the net OATP1B1 R value and CP-I C_{max} ratio was similar to what was observed with GLE OATP1B1 R value (**Figure S2c**).

Safety

The formulations tested were generally well-tolerated by the subjects. No clinically significant vital signs or laboratory measurements were observed during the course of the study. There was no pattern to the adverse events reported, and no new safety issues were identified from this study. Adverse events were infrequent, and all observed adverse events were grade 1 (mild) in severity.

DISCUSSION

The prediction of clinical OATP1B1/1B3 inhibition by an investigational drug is an integral part of drug development as it relates to patient safety and possibly efficacy of co-administered drugs. CP-I and CP-III have been proposed as potential endogenous biomarkers to evaluate the clinical inhibition of OATP1B1 and OATP1B3. Use of endogenous biomarkers in early clinical studies is a promising approach to identify false-positive predictions based on the preclinical data and to avoid conduct of dedicated clinical DDI studies using a probe substrate. In the present study, a robust correlation analysis was performed using known clinical OATP1B1/1B3 inhibitors (GLE and PIB) to further establish the utility of CP-I and CP-III to delineate the potential inhibition of these transporters by an investigational drug. OATP1B1 static DDI predictions for GLE and PIB, and the net effect of the combination regimen (GLE/PIB) were calculated and compared with CP-I exposures (C_{max} and AUC) in an attempt to identify cutoff values resulting in a reduce false-positive rate.

The clinical bioavailability study of GLE/PIB evaluating three different formulations yielded a range of exposures of GLE between subjects and formulations (**Figure 1**, **Table 1**).

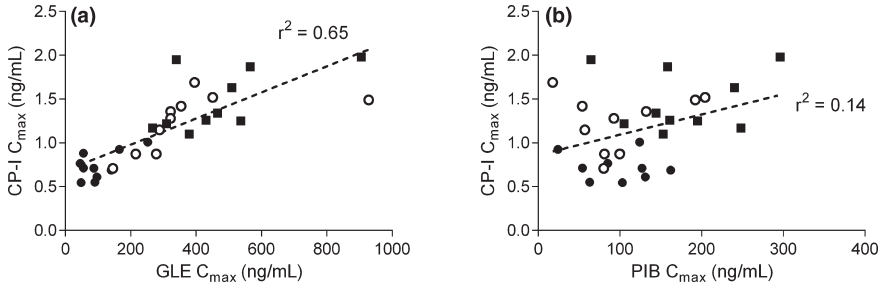

Figure 4 Correlation between glecaprevir (GLE) or pibrentasvir (PIB) peak plasma concentration (C_{max}) and coproporphyrin (CP)-I C_{max} following administration of a single dose of GLE/PIB 300/120 mg formulations A, B, or C to healthy volunteers. (**a**) CP-I C_{max} vs. GLE C_{max}. (**b**) CP-I C_{max} vs. PIB C_{max}. Each dot represents the data in individual subjects following administration of different formulations. Solid circles, open circles, and solid squares represent the subjects receiving formulation A, B, and C, respectively.

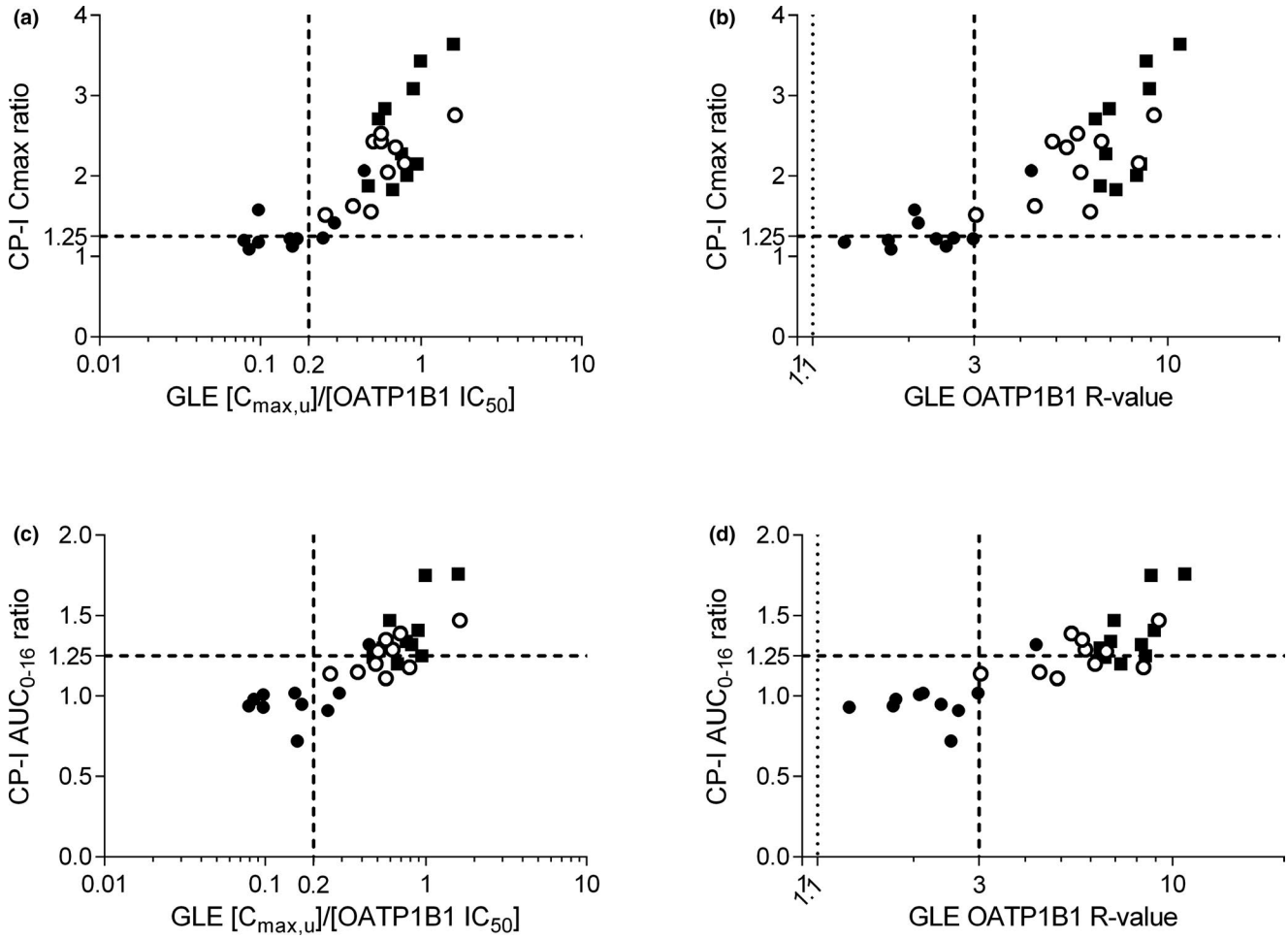

Figure 5 Correlation between glecaprevir (GLE) [unbound maximum plasma concentration ($C_{max,u}$]/[organic anion transporting polypeptide (OATP)1B1 half-maximal inhibitory concentration (IC_{50})] or OATP1B1 *R*-value and coproporphyrin (CP)-I C_{max} or 0–16 hours area under the concentration-time curve (AUC_{0-16}) ratios following administration of a single dose of GLE/PIB 300/120 mg formulation A, B, or C to healthy volunteers. (**a**) CP-I C_{max} ratio vs. GLE [$C_{max,u}$]/[OATP1B1 IC_{50}]. (**b**) CP-I C_{max} ratio vs. GLE OATP1B1 R-value. (**c**) CP-I AUC_{0-16} ratio vs. GLE [$C_{max,u}$]/[OATP1B1 IC_{50}]. (**d**) CP-I AUC_{0-16} ratio vs. GLE OATP1B1 R-value. Each dot represents the data in individual subjects following administration of different formulations. Solid circles, open circles, and solid squares represent the subjects receiving formulations A, B, and C, respectively.

This allowed the evaluation of the sensitivity of CP-I and CP-III as an endogenous biomarker of OATP1B1 and OATP1B3. There was an increase in CP-I exposure (C_{max} ratio and AUC_{0-16} ratio) associated with increasing GLE exposure. On the other hand, the effect on CP-III exposure was modest for C_{max} ratio and negligible for AUC_{0-16} ratio (**Figure 3**, **Table 2**). When comparing the three GLE/PIB formulations, changes in CP-I AUC_{0-16} ratio were more sensitive to increasing GLE exposures than CP-I AUC_{0-48} ratio. This is likely because 94–97% of GLE exposures were observed in the first 16 hours postdose (**Table 1**) and because both GLE and CP-I have similar exposure-time profiles (**Figures 1** and **2**).

In line with previous reports,[17–19] CP-I exposure was also higher than CP-III in all samples analyzed in the present study (**Table 1**). These observations corroborated the superiority of CP-I over CP-III as an endogenous biomarker for evaluation of hepatic OATP1B1 and OATP1B3 inhibition both from a sensitivity and analytical perspective. It is also worthwhile to note that the baseline CP-I concentrations in this study (0.90 ± 0.14 nM) fell within the previously reported range (0.5–1.5 nM, n = 56; across different ethnicities).[27] This supports the use of CP-I levels normalized to predose baseline levels for the evaluation of OATP1B1 or OATP1B3 inhibition potential in clinical pharmacokinetic studies. Furthermore, these observations make a case to negate the need for a separate period to determine the plasma exposure-time profile of CP-I in the absence of an inhibitor and simplify clinical pharmacokinetic study design.

Although CP-I is a substrate of both OATP1B1 and OATP1B3, it shows a higher affinity for OATP1B1 (K_m 0.13 μM) than OATP1B3 (K_m 3.95 μM).[28] Meta-analysis of the transporter expression data demonstrated that the mean expression of OATP1B1 in human liver is threefold > OATP1B3.[29] In addition, the plasma concentration of CP-I is higher in subjects with OATP1B1 *15/*15 genotype compared with *1b/*1b or *1b/*15 genotype,[30] which is consistent with the reduced transport activity of OATP1B1 *15 allele.[31] Collectively, these observations suggest a

larger contribution of OATP1B1 than OATP1B3 to the hepatic uptake of CP-I. There was a significant correlation in the maximum plasma exposure between GLE and CP-I and to a much lesser extent between PIB and CP-I (**Figure 4**), suggesting that the increase in CP-I exposure is mainly ascribed to the OATP1B1 inhibition by GLE but not PIB. This is in line with the higher OATP1B1 *R* value of GLE (5.5) than PIB (1.4).[22] The lack of association between PIB [C_{max}]/[OATP1B1 IC_{50}] or OATP1B1 *R* value and CP-I C_{max} ratio (**Figure S2a,b**) also indicates that the contribution of PIB to the increase in the CP-I exposure is modest if any.

It has been demonstrated that the *R* value approach for OATP1B1 and OATP1B3 inhibition can yield false-positive predictions with varying degrees depending on the cutoff values.[9] It is likely that the conservative assumptions in the model contribute to these false-positive predictions, where the absorption of inhibitor is assumed to be complete ($F_a \times F_g = 1$) and the absorption rate constant (K_a) is set to the theoretically maximum value (6 $hour^{-1}$) when the human pharmacokinetics parameters are not available. In this study, there was an association between CP-I C_{max} ratio (> 1.25) and GLE [$C_{max,u}$]/[OATP1B1 IC_{50}] (> 0.2) (**Figure 5a**). With a reasonable pharmacological and absorption, distribution, metabolism, and excretion/pharmacokinetic characterization at preclinical stage, [$C_{max,u}$]/[OATP1B1 IC_{50}] of an investigational drug can be estimated using an in vitro parameters ($f_{u,p}$ and OATP1B1 IC_{50}) together with the predicted maximum plasma concentration in human (C_{max}). Use of this new criteria ([$C_{max,u}$]/[OATP1B1 IC_{50}] > 0.2) in addition to the *R* value estimation recommended by regulatory agencies may improve the predictive accuracy of OATP1B1 inhibition from preclinical data. Furthermore, the correlation analysis between the OATP1B1 *R* values of GLE or the net OATP1B1 *R* values and increase in CP-I exposure in individual subjects indicates that *R* values of > 3 can appropriately predict a biologically meaningful inhibition of OATP1B1 while minimizing the number of false-positive predictions (**Figure 5b**, **Figure S2c**), which is substantially higher than the *R* value cutoff values recommended by regulatory agencies (EMA, 1.04[6]; FDA/PMDA, 1.1[7,8]). This suggests that a higher cutoff value of 3 can be more appropriate to minimize the probability of false-positive prediction of OATP1B1 inhibition potential when additional clinical pharmacokinetics parameters (e.g., $F_a \times F_g$, K_a,) for the investigational drug are available. Further analysis using different inhibitors is warranted to refine the cutoff values in the static prediction of OATP1B1 inhibition. In addition, a caution is warranted in the interpretation of endogenous biomarker data in early clinical studies and resulting DDI predictions because the translation to drug substrates has yet to be established.

In conclusion, the clinical pharmacokinetic study for known OATP1B1/1B3 inhibitors, GLE/PIB, was utilized for the measurement of CP-I and CP-III as a biomarker of these transporters in order to further establish their utility in early clinical studies. CP-I exposures (C_{max} and AUC) increased with increasing GLE exposure whereas there was only a modest correlation between C_{max} of CP-I and PIB, indicating that the clinical inhibition of OATP1B1 by GLE/PIB regimen is likely ascribed to GLE. The correlation analysis between GLE OATP1B1 *R* values or the net OATP1B1 *R* values and CP-I exposures (C_{max} ratio and AUC_{0-16} ratio) suggests that *R* value of > 3 can appropriately predict a biologically meaningful inhibition of OATP1B1 while minimizing the number of false-positive predictions. At preclinical stage, consideration of estimated [$C_{max,u}$]/[OATP1B1 IC_{50}] in addition to the *R* value may improve the OATP1B1 inhibition predictions.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Acknowledgments. Medical writing support was provided by Wesley Wayman, an employee of AbbVie. The authors thank the AbbVie Clinical Pharmacology Research Unit staff for conducting this study and Rachael K. Bridgman, Kenneth Ruterbories, Lance J. Heinle, and Gary J. Jenkins for their contribution to the initial bioanalysis method development of CP-I and CP-III.

Funding. This study was funded by AbbVie. AbbVie contributed to the study design, research, and interpretation of data, writing, reviewing, and approving the publication.

Conflicts of Interest. H.V.K., R.K., S.C., J.S., M.F.M., D.A.J.B., and A.H.S. are AbbVie employees and may hold AbbVie stocks or options.

Author Contributions. H.K., R.K., S.C., J.S., M.M., D.A.J.B., and A.H.S. wrote the manuscript. H.K., R.K., S.C., M.M., D.A.J.B., and A.H.S. designed the research. H.K., R.K., S.C., and J.S. performed the research. H.K., R.K., S.C., and J.S. analyzed the data.

Data Sharing Statement. AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual and trial-level data (analysis data sets), as well as other information (e.g., protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html

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