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Further Studies to Support the Use of Coproporphyrin I and III as Novel Clinical Biomarkers for Evaluating the Potential for Organic Anion Transporting Polypeptide 1B1 and OATP1B3 Inhibition

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ABSTRACT

In a recent study, limited to South Asian Indian subjects (*n* = 12), coproporphyrin (CP) I and CPIII demonstrated properties appropriate for an organic anion-transporting polypeptide (OATP) 1B endogenous probe. The current studies were conducted in healthy volunteers of mixed ethnicities, including black, white, and Hispanic subjects, to better understand the utility of these biomarkers in broader populations. After oral administration with 600 mg rifampin, $AUC_{(0–24h)}$ values were 2.8-, 3.7-, and 3.6-fold higher than predose levels for CPI and 2.6-, 3.1-, and 2.4-fold higher for CPIII, for the three populations, respectively. These changes in response to rifampin were consistent with previous results. The sensitivity toward OATP1B inhibition was also investigated by evaluating changes of plasma CP levels in the presence of diltiazem and itraconazole [administered as part of an unrelated drug-drug interaction (DDI) investigation], two compounds that were predicted to have minimal inhibitory effect on OATP1B. Administration of diltiazem and itraconazole did not increase plasma CPI and CPIII concentrations relative to prestudy levels, in agreement with predictions from in vitro parameters. Additionally, the basal CP concentrations in subjects with *SLCO1B1* c.521TT genotype were comparable to those with *SLCO1B1* c.521TC genotype, similar to studies with probe substrates. However, subjects with *SLCO1B1* c.388AG and c.388GG genotypes (i.e., increased OATP1B1 transport activity for certain substrates) had lower concentrations of CPI than those with *SLCO1B1* c.388AA. Collectively, these findings provide further evidence supporting the translational value of CPI and CPIII as suitable endogenous clinical probes to gauge OATP1B activity and potential for OATP1B-mediated DDIs.

Introduction

Organic anion-transporting polypeptide (OATP) 1B1 and OATP1B3 mediate hepatic uptake and govern the elimination of many clinically used drugs such as rosuvastatin, atorvastatin, and repaglinide (Niemi et al., 2011; Shitara et al., 2013). Hence, inhibitory drug-drug interactions (DDIs) with OATP1B have broad implications with respect to drug efficacy, safety, and labeling of a new molecular entity (NME). The high costs incurred when a candidate drugs fail during late clinical trials has prompted interest in having an earlier evaluation of OATP1B liability. Frequently, the initial OATP1B inhibition risk assessment is conducted using in vitro transporter-overexpressing cell models during the preclinical phase. If in vitro studies indicate that an NME is an OATP1B1 or OATP1B3 inhibitor and the estimated ratio of the victim drug *AUC* with and without the investigational drug is greater than the cutoff value [i.e., ratio of victim drug *AUC* in the presence and absence of perpetrator drug (*R*-value) >1.10 and >1.04, respectively], the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) recommend that the sponsor conduct an in vivo DDI study using a substrate drug that is likely to used concomitantly with the investigational drug. Compared with the 2012 FDA Draft Guidance, the current guidance uses a more conservative cutoff to reduce false-negative prediction. The new, lower threshold will result in a greater number of cases in which OATP1B clinical DDI studies are recommended. Moreover, although physiologically based pharmacokinetic modeling and simulation has been used in translating in vitro drug-metabolizing–enzyme inhibition to in vivo DDI, the predictive performance of physiologically based pharmacokinetic modeling for transporter-based DDIs, particularly OATP1B-mediated DDI, has not been well established (Wagner et al., 2015). An endogenous biomarker for detection of in vivo OATP1B inhibition in phase I or early phase II studies can provide further information of potential DDI risk to enable prioritization of clinical DDI studies, or ultimately eliminate the need for such studies. As a result, the investigation of endogenous clinical biomarkers of OATP1B, including identification and validation of these biomarkers, has been exponentially increasing in the last few years (Chu et al., 2017; Mariappan et al., 2017; Muller et al., 2018; Rodrigues et al., 2018).

We have previously proposed coproporphyrin (CP) I and CPIII as endogenous biomarkers for OATP1B inhibition (Shen et al., 2016). A study with 12 South Asian Indian healthy human subjects demonstrated

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ABBREVIATIONS: $AUC_{(0–24h)}$, Area under plasma concentration-time curve from time 0 to 24 hours; C_{max}, maximum plasma concentration; CP, coproporphyrin; DDI, drug-drug interaction; FDA, Food and Drug Administration; IC_{50}, concentration required to inhibit transport by 50%; LC-MS/MS, liquid chromatography–tandem mass spectrometry; OATP, organic anion-transporting polypeptide; *R*-value, ratio of victim drug *AUC* in the presence and absence of perpetrator drug; SNP, single-nucleotide polymorphism.

that treatment with a potent OATP1B1 inhibitor rifampin (600 mg, oral single dose) produced a consistent, marked effect on plasma CP concentrations [3.4- to 4.0-fold increases in area under the plasma concentration-time curve from time 0 to 24 hours ($AUC_{(0–24h)}$)] (Lai et al., 2016; Shen et al., 2017), implying that CPI and CPIII may be ideal probes of OATP1B activity. Although inter-ethnic differences in the activity of OATP1B1, particularly in Asian and Caucasian populations, have been documented by many scientists, the mechanism for these difference is not fully understood (Tomita et al., 2013; Sugiyama et al., 2017; Wu et al., 2017). The variability might be attributed to genetic variation and/or intrinsic inter-ethnic differences in OATP1B1 activity. Concerning genetic factors, a number of mutations have been described for OATP1B1 and OATP1B3 (Rana et al., 2010; Nakanishi and Tamai, 2012). OATP1B1 is polymorphically expressed, and the allelic frequency of the *SLCO1B1* c.521 T > C and c.388 A > G, two known OATP1B1 single-nucleotide polymorphisms (SNPs) with altered transport activity in Europeans, Americans, and East Asians are 8%–20% and 1%–8%, 8%–16% and 30%–45%, and 72%–83% and 59%–86%, respectively (Niemi et al., 2011). However, the influence of ethnicity and OATP1B genetic variation on basal plasma CPI and CPIII concentrations are unknown.

In the current report, we examined the sensitivity of CPI and CPIII to rifampin, diltiazem, and itraconazole within two clinical studies that evaluated DDIs of these compounds with drug candidates. The selection of the well characterized control inhibitors in these studies was made to study impact on other disposition pathways but also allowed an opportunity to collect CPI and CPIII data across a broader subject group. Rifampin served as positive control [OATP1B1 and OATP1B3 concentration required to inhibit transport by 50% (IC_{50}) values of 0.66–0.79 and 0.25–0.27 μM, respectively] (Shen et al., 2017), and diltiazem and itraconazole (both very weak OATP1B inhibitors with IC_{50} values ranging from 30 to 260 μM) served as negative controls (Azie et al., 1998; Yoshida et al., 2012). Additionally, we investigated the impact of race/ethnicity on the basal levels and response to rifampin for CPI and CPIII.

Materials and Methods

Study Design and Subjects. The first study was an open-label, single-sequence, three-treatment, one-way interaction study in healthy male volunteers (n = 16) to investigate the effect of multiple dose of rifampin on the single-dose pharmacokinetics (PK) of Bristol-Myers Squibb (BMS) compound A (BMS-**A**). The subjects received a single oral dose of BMS-**A** on day 1, followed by repeat oral dosing with rifampin (600 mg) once daily on day 7 through day 12. The subjects received a second single dose of BMS-**A** with their dose of rifampin on day 13, following 9 days of continuous administration of rifampin. The subjects were discharged on day 21. To evaluate the effect of rifampin on CPI and CPIII, serial plasma samples were collected at 0 (i.e., predose), 1, 2, 4, 9, 12, and 24 hours on day 7 after a single dose of rifampin.

The second study was an open-label, two single-sequence, two-period crossover study in healthy male volunteers (n = 28) to investigate the effect of steady-state exposure to diltiazem or itraconazole on the single-dose PK of BMS compound B (BMS-**B**). All subjects received a single oral dose of BMS-**B** on day 1, followed by a washout on days 2 and 3. Half of the subjects (n = 14) were assigned to a BMS-**B**-diltiazem sequence and received daily oral doses of diltiazem (240 mg) on day 4 through day 14, whereas the other 14 subjects received daily oral doses of itraconazole (200 mg) on day 4 through day 11 (BMS-**B**-itraconazole). The subjects were discharged on day 15 and day 13 for BMS-**B**-diltiazem and BMS-**B**-itraconazole sequences, respectively. Serial plasma samples for CPI and CPIII PK analyses were collected at 0 (i.e., predose), 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours on day 11 after subjects received diltiazem 240 mg or itraconazole 200 mg daily for 8 days. To potentially explore the effect of the SNP of OTP1B1 on plasma CP levels, the 28 subjects who participated in the second study were genotyped for *SLCO*1B1 c.521T > C and *SLCO1B1* c.388 A > G SNPs using CFX 384 Real-Time PCR Detection System (Bio-Rad, Hercules, CA).

In both studies, subjects were healthy men and women as determined by their lack of clinically significant deviation from normal in medical history, physical examination, ECGs results, or clinical laboratory determinations. Ages were 26–47 years with body mass index (BMI) 21.6–31.2 kg/m^2 (the first study) or 23–51 years with BMI 21.5–29.3 kg/m^2 (the second study). Eligible women who were pregnant or breast-feeding, individuals who had any major surgery within 12 weeks of study drug administration, or individuals who had donated blood to a blood bank or in a clinical study (except a screening or follow up visit) within 4 weeks of study-drug administration were all excluded from the studies. The protocols, any amendments, and the subjects' informed consents were reviewed by Institutional Review Board/Independent Ethics Committee IntegReview IRB (Austin, TX).

Quantification of CPI and CPIII in Plasma by Liquid Chromatography–Tandem Mass Spectrometry. The bioanalytical analyses of CPI and CPIII were performed as described in detail by Kandoussi et al. (Kandoussi et al., 2018). Briefly, a 100-μl plasma sample aliquot was transferred to a 96-well plate and then mixed with 100 μl of internal standard solution (10 nM CPI $^{15}N_4$ and 25 nM CPIII d_8 in 6 M formic acid). After vortex mixing, the total sample was transferred onto a Biotage ISOLUTE SLE+ 96-well plate (Uppsala, Sweden). Following this, three 500-μl aliquots (for a total of 1.5 ml) of ethyl acetate were added to each well and allowed to elute. Samples were evaporated under a nitrogen stream at approximately 55°C. The samples were reconstituted with 100 μl of 0.1:30:70 formic acid/acetonitrile/water, v/v/v, and mixed well for liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. Throughout the entire sample extraction, appropriate precautions were taken to minimize sample exposure to ambient light.

The bioanalysis was conducted on a Triple Quad 6500 mass spectrometer with a TurboIonSpray source from Sciex (Foster City, CA) coupled to a UHPLC (ultra high-performance liquid chromatography) HP 1200 SL pump (Agilent Technologies, Santa Clara, CA) and an LC-PAL DLW autosampler (CTC Analytics, Zwingen, Switzerland). A 20-μl sample was injected onto an Acquity UHPLC HSS T3 column (2.1 × 50 mm, 1.8 μm) and an Acquity guard column (VanGuard BEH C18 column; 2.1 × 10 mm, 1.7 μm) (Waters, Elstree, Herts, UK). The system was held at 60°C and with a constant flow of 0.55 ml/min; a short gradient (7.5 minutes) was used comprising a mobile phase A (0.1:100 formic acid/water, v/v) and mobile phase B (0.1:2:98 formic acid/water/acetonitrile, v/v/v). The UHPLC program starting conditions are 35% B. At 0.9 minutes B was increased to 40% over 3.6 minutes, and then further increased to 95% in 0.15 minutes, maintaining 95% B for 1.35 minutes and then decreasing to starting conditions (35% B) in 0.2 minutes, and this was maintained until 7.5 minutes.

The mass spectrometer was operated in positive multiple-reaction monitoring (MRM) mode. The MRM precursor/product ion transitions were as follows: m/z 655.4 → 596.3 for CPI and CPIII, and m/z 659.3 → 600.3 and m/z 663.3 → 602.3 for the internal standards CPI $^{15}N_4$ and CPIII d_8, respectively. The instrument settings on the mass spectrometer were as follows: curtain gas, 30 psi; CAD gas, 8 psi; gas 1, 75 units; gas 2, 60 units; turbo-ion-spray voltage 4000 V and turbo probe temperature at 650°C. The chromatographic peak integration and data processing were performed using Analyst software (version 1.6.2; SCIEX, Framingham, MA) and the peak areas were exported to Assist LIMS (version 6.06 a validated LIMS system). The calculation of peak area ratios, regression of calibration standards, calculations of quality controls and study samples were performed using Assist LIMS.

Pharmacokinetic and Statistical Analyses. CPI and CPIII PK analyses were performed using validated software Kinetica v. 5.0 (Thermo Fisher Scientific, Waltham, MA). Maximum plasma concentration (C_{max}) and area under the concentration-time curve from time zero to 24 hours ($AUC_{(0–24h)}$) were derived from plasma concentrations versus data by a noncompartmental analysis by using mixed log-linear trapezoidal summations. The baseline control values of C_{max} and $AUC_{(0–24h)}$ were calculated using predose levels.

The results are expressed as mean ± S.D. in the text, tables, and figures. To test for statistically significant differences in C_{max} and $AUC_{(0–24h)}$ among three ethnic groups during the treatments (rifampin and baseline control) in the first study, and among multiple treatments (diltiazem, itraconazole, and baseline control) in the two 14-subject groups of the second study, two-way analysis of variance (ANOVA) was carried out with drug treatment as one factor and group as the other. In addition, to compare the basal plasma CP concentrations among ethnic

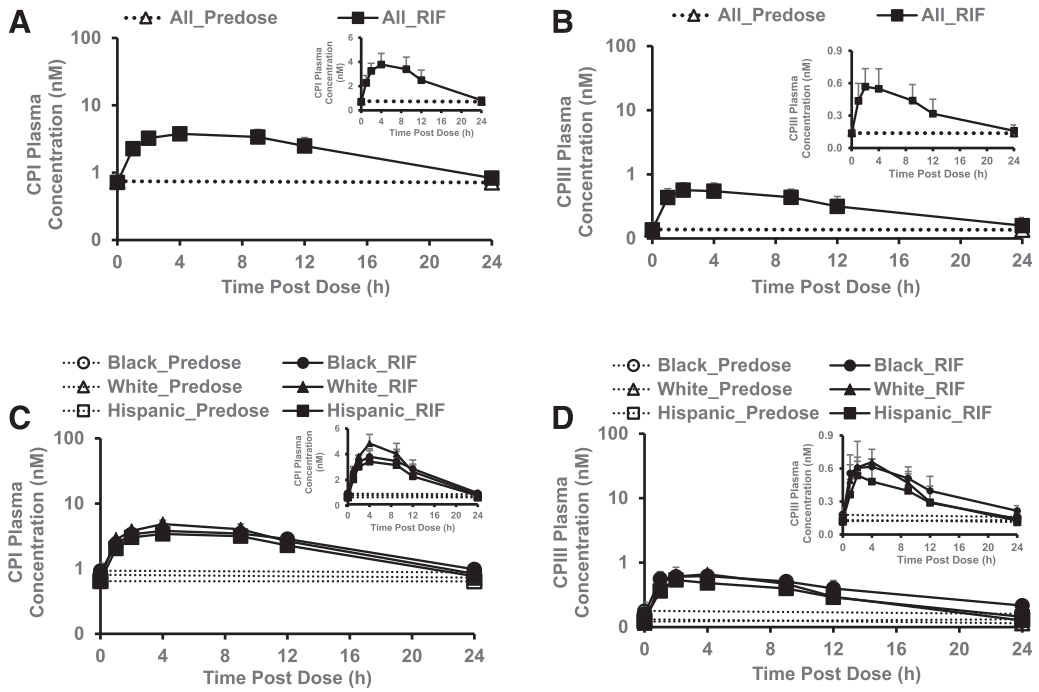

Fig. 1. Effect of 600 mg rifampin (RIF) doses on plasma CP concentrations. The plasma concentration-time profiles of CPI (A and C) and CPIII (B and D) are shown as the mean and S.D. values obtained from four black (circles), three white (triangles), and nine Hispanic subjects (squares), before dosing (open labels) and following a single oral dose of RIF (closed labels).

groups and genotypes, one-way ANOVA was carried out. When the F ratio showed that there were significant differences among days, the Tukey method of multiple comparisons was used to determine which treatments differ. All statistical analyses were carried out using GraphPad Prism version 7.03 (GraphPad Software, Inc., San Diego, CA), and a P-value of less than 0.05 was considered statistically significant.

Results

Interaction between Rifampin and CP. The systemic exposures of CPI and CPIII for different ethnic groups after treatment with rifampin are shown in Fig. 1 and Table 1. The table also presents the statistical comparisons for each rifampin group relative to baseline and for each ethnic group relative to the other ethnic groups.

After administration of a single oral dose of 600 mg rifampin, a strong in vitro and in vivo inhibitor of OATP1B1 and OATP1B3 (in vitro IC_{50} values were 1.1 and 0.49 μM, and R values of 6.5 and 13.3, respectively, Table 3), C_{max} of CPI and CPIII were approximately 4.4- to 5.8-fold higher than basal control in black, white, and Hispanic subjects (C_{max} ratios of 4.46 ± 0.22, 6.70 ± 1.17, and 5.73 ± 0.98, and 4.66 ± 1.01, 5.84 ± 0.47, and 4.42 ± 1.22 for CPI and CPIII, respectively). In agreement, the $AUC_{(0–24h)}$ of CPI and CPIII increased approximately 2.4- to 3.7-fold higher than prestudy control in black, white, and Hispanic subjects ($AUC_{(0–24h)}$ ratios of 2.84 ± 0.15, 3.72 ± 0.64, and 3.36 ± 0.66, and 2.55 ± 0.15, 3.07 ± 0.28, and 2.42 ± 0.60 for CPI and CPIII, respectively) (Table 1). The increases were statistically significant ($P < 0.001$). The magnitude of changes in CPI and CPIII $AUC_{(0–24h)}$ in this study were slightly less than those in Asian Indian subjects previously reported (2.4- to 3.7-fold vs. 3.4- to 4.0-fold) (Lai et al., 2016). Prestudy $AUC_{(0–24h)}$ values for CPI and CPIII were similar between the three ethnic groups in the current study ($P > 0.05$) (Table 1). In contrast, exposure to CPI in whites and exposure to CPIII in blacks during rifampin treatment were higher compared with Hispanic subjects ($P < 0.05$).

Effects of Administration of Diltiazem and Itraconazole on Plasma CP Levels. In vitro, diltiazem and itraconazole are weak and/or noninhibitor of OATP1B1 and OATP1B3 (IC_{50} of 260 and >100 μM, and 170 and >30 μM, respectively) (Yoshida et al., 2012; Nakakariya et al., 2016). However, hydroxyitraconazole, a metabolite of itraconazole, is a strong in vitro inhibitor of OATP1B1 and OATP1B3 (IC_{50} of 0.23 and 0.10 μM, respectively) (Vermeer et al., 2016). The predicted changes in systemic exposure in vivo in the presence of diltiazem and itraconazole are 1.05-fold and less than 1.17-fold, respectively (Table 3).

Figure 2 shows the arithmetic mean plasma concentrations ± S.D. of CPI and CPIII during the three treatments and Table 2 shows the arithmetic mean ± S.D. of C_{max}, $AUC_{(0–24h)}$, and the ratios of CPI and CPIII during the three periods. Administration of 240 mg diltiazem and 200 mg itraconazole at steady state significantly increased C_{max} of CPI and CPIII compared with the predose baseline (1.11- to 1.32-fold) (Table 2). In contrast, the $AUC_{(0–24h)}$ values were not significantly higher than the basal levels after administration of diltiazem and itraconazole ($P > 0.05$) (Fig. 2).

Effects of OATP1B1 Genetic Variation on Basal Plasma Concentrations of CPI and CPIII. The individual basal plasma CPI and CPIII concentrations for black ($n = 12$), white ($n = 13$), Hispanic ($n = 18$), and American Indian subjects ($n = 1$) from these two studies and Asian Indian subjects ($n = 12$) from the previous study (Lai et al., 2016) are presented in Fig. 3A. Both the CPI and CPIII levels seemed to be broadly similar across ethnic groups, and there were no significant differences in plasma CPI or CPIII concentrations between ethnic groups ($P > 0.05$).

In total, 28 subjects completed the second study and gave consent for pharmacogenetic analysis. Several genetic variants known to influence the activity of OATP1B1 were examined (Niemi et al., 2011; Nakanishi and Tamai, 2012). For *SLCO1B1* c.521 T > C SNP, an SNP associated with reduced OATP1B1 transport activity, no subject was homozygous for the c.521CC allele, and there were 3 and 25 subjects with c.521 TC and c.521TT genotypes (10.7% and 89.3%, respectively). Few subjects

TABLE 1

Comparison of C_{max} and $AUC_{(0–24h)}$ of CPI and CPIII in black (n = 4), white (n = 3), and Hispanic subjects (n = 9) following a single oral dose of rifampin (600 mg)

Data are expressed as mean ± S.D.

Analyte	Ethnicity	Parameter	Rifampin	Basal[a]	Fold-Change
CPI	Black	C_{max} (nM)	3.93 ± 0.69***	0.88 ± 0.14	4.46 ± 0.22
	(n = 4)	$AUC_{(0–24h)}$ (nM*h)	60.29 ± 11.98***	21.08 ± 3.31	2.84 ± 0.15
	White	C_{max} (nM)	4.84 ± 0.70***, ###	0.73 ± 0.09	6.70 ± 1.17
	(n = 3)	$AUC_{(0–24h)}$ (nM*h)	72.58 ± 8.92***, #	17.49 ± 2.09	3.72 ± 0.64
	Hispanic	C_{max} (nM)	3.60 ± 0.82***	0.64 ± 0.16	5.73 ± 0.98
	(n = 9)	$AUC_{(0–24h)}$ (nM*h)	59.62 ± 17.39***	15.32 ± 3.92	3.36 ± 0.66
	All	C_{max} (nM)	3.91 ± 0.94***	0.72 ± 0.15	5.60 ± 1.07
	(n = 16)	$AUC_{(0–24h)}$ (nM*h)	56.01 ± 15.11***	17.17 ± 3.58	3.30 ± 0.65
CPIII	Black	C_{max} (nM)	0.74 ± 0.11***, ##	0.16 ± 0.02	4.66 ± 1.01
	(n = 4)	$AUC_{(0–24h)}$ (nM*h)	9.87 ± 2.17***, #	3.85 ± 0.45	2.55 ± 0.15
	White	C_{max} (nM)	0.67 ± 0.03***	0.12 ± 0.01	5.84 ± 0.47
	(n = 3)	$AUC_{(0–24h)}$ (nM*h)	8.42 ± 0.33***	2.75 ± 0.22	3.07 ± 0.28
	Hispanic	C_{max} (nM)	0.56 ± 0.19***	0.13 ± 0.03	4.42 ± 1.22
	(n = 9)	$AUC_{(0–24h)}$ (nM*h)	7.71 ± 3.00***	3.04 ± 0.76	2.42 ± 0.60
	All	C_{max} (nM)	0.62 ± 0.17***	0.14 ± 0.03	4.75 ± 1.24
	(n = 16)	$AUC_{(0–24h)}$ (nM*h)	8.21 ± 2.60***	3.19 ± 0.66	2.57 ± 0.60

[a]Baseline control values of C_{max} and $AUC_{(0–24h)}$ were calculated using predose levels.
***$P < 0.001$, statistically significant difference compared with the control; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, statistically significant difference compared with Hispanic subjects.

(n = 5; 17.9%) were homozygous for the c.388GG allele, and 9 and 14 subjects were homozygous and heterozygous for the c.388 A > G allele (32.1% and 50.0% for c.388AA and c.388AG, respectively), an SNP associated with increased in vitro OATP1B1 activity. Two out of the three subjects who carried a copy of the *SLCO1B1* c.521TC also carried at least one copy of the c.388G allele. Owing to the small number of subjects studied, it was not possible to determine the effects of *SLCO1B1* haplotypes (combined c.521 T > C and c.388 A > G SNPs) on CPI and CPIII. As shown in Fig. 3B, the basal plasma CPI and CPIII concentrations, on average, were not significantly different in subjects carrying the *SLCO1B1* c.521 T > C polymorphism (i.e., c.521TC genotype) compared with those not carrying this allele (c.521TT genotype) (0.777 ± 0.209 nM vs. 0.771 ± 0.107 nM and 0.129 ± 0.034 nM vs. 0.113 ± 0.032 nM for CPI and CPIII, respectively).

The basal CPI levels were 16%–19% lower in subjects carrying the *SLCO1B1* c.388 A > G allele (c.388AG and c.388GG genotypes) compared with those who did not carry the SNP (c.388AA genotype) (0.885 ± 0.204, 0.718 ± 0.203, and 0.739 ± 0.074 nM, respectively) (Fig. 3C), suggesting a potential allele-exposure relationship. The difference in CPI level between c.388AA and c.388AG genotypes is slightly but statistically significant ($P < 0.05$). However, there is no significant difference in CPIII level across the genotypes (0.129 ± 0.031, 0.129 ± 0.038, and 0.125 ± 0.031 nM for c.388AA, c.388AG and c.388GG, respectively) (Fig. 3C).

Discussion

We have previously shown that CPI and CPIII are taken up from the blood circulation into the liver by OATP1B1 and OATP1B3 (Shen et al.,

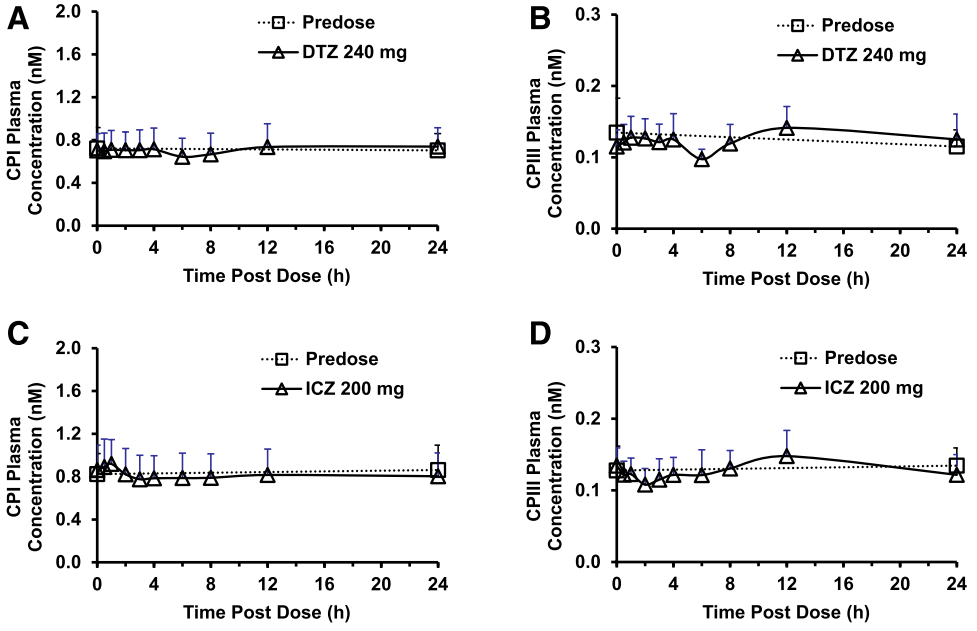

Fig. 2. Effect of 240 mg diltiazem (DTZ) and 200 mg itraconazole (ICZ) doses on plasma CP concentrations. The plasma concentration-time profiles of CPI (A and C) and CPIII (B and D) are shown as the mean and S.D. values obtained from 14 healthy subjects before (open squares) and following administration of DTZ or ICZ (open triangles).

TABLE 2

Comparison of C_{max} and $AUC_{(0–24h)}$ of CPI and CPIII in healthy subjects (n = 14) following administration of diltiazem (240 mg) or itraconazole (200 mg)

Data are expressed as mean ± S.D.

Treatment		CPI		CPIII	
		C_{max}	$AUC_{(0–24h)}$	C_{max}	$AUC_{(0–24h)}$
		nM	*nM*h*	*nM*	*nM*h*
Diltiazem group (n = 14)	Diltiazem	0.79 ± 0.21*	15.91 ± 6.27	0.15 ± 0.04***	2.80 ± 1.01
	Basal[a]	0.70 ± 0.17	16.88 ± 3.78	0.12 ± 0.02	2.71 ± 0.58
	Fold-change	1.11 ± 0.10	1.00 ± 0.10	1.32 ± 0.22	1.17 ± 0.44
Itraconazole group (n = 14)	Itraconazole	0.96 ± 0.23**	19.42 ± 5.33	0.16 ± 0.03**	2.95 ± 0.83
	Basal[a]	0.86 ± 0.23	20.69 ± 5.57	0.13 ± 0.03	3.23 ± 0.59
	Fold-change	1.12 ± 0.10	0.94 ± 0.10	1.17 ± 0.17	0.95 ± 0.13

[a]Baseline control values of C_{max} and $AUC_{(0–24h)}$ were calculated using predose levels.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, statistically significant difference compared with the control.

2016), and studies in healthy Asian Indian subjects treated with rifampin indicated that CPI and CPIII have potential as endogenous biomarkers for OATP1B transporter activity (Lai et al., 2016; Shen et al., 2017). The studies presented herein aimed to further verify their utility and sensitivity to inform OATP1B-mediated DDIs. The utility of CPI and CPIII as clinical OATP1B probes was further confirmed by the

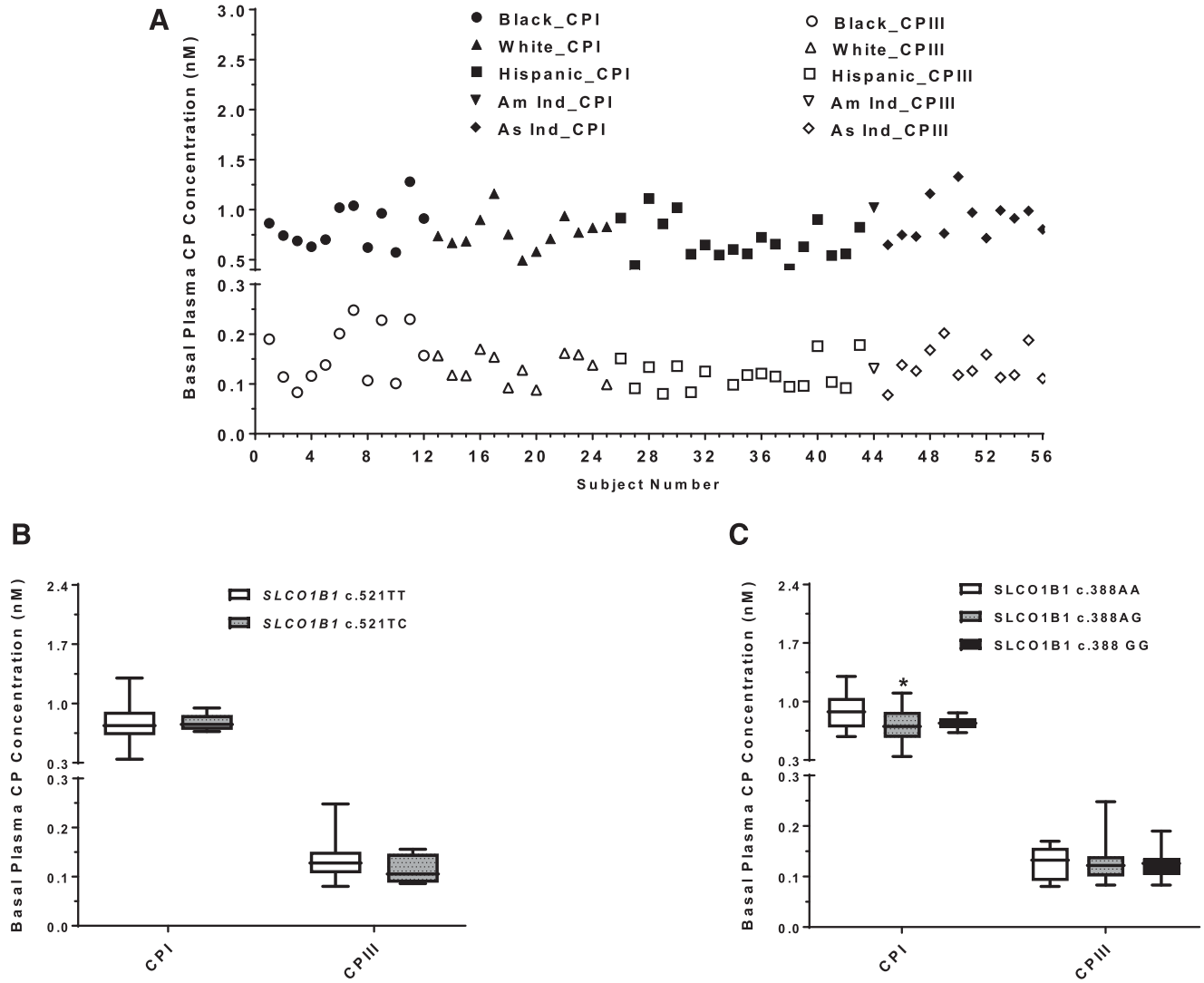

Fig. 3. Comparison of basal plasma CP levels in different ethnic and genotype groups. (A) Basal plasma concentrations of CPI (closed labels) and CPIII (open labels) were measured in healthy black (n = 12), white (n = 13), Hispanic (n = 18), American Indian (Am Ind) (n = 1), and Asian Indian (As Ind) (n = 12) from the two studies and previous study (Lai et al., 2016) by LC-MS/MS. (B) The basal plasma CPI and CPIII concentrations were analyzed in healthy subjects with the *SLCO1B1* c.521 TT (n = 25) and *SLCO1B1* c.521 TC genotypes (n = 3) by LC-MS/MS. (C) The basal plasma CPI and CPIII concentrations were analyzed in healthy subjects with the *SLCO1B1* c.388AA (n = 9), *SLCO1B1* c.388AG (n = 14), and *SLCO1B1* c.388GG genotypes (n = 5) by LC-MS/MS. *$P < 0.05$, statistically significant difference compared with subjects with the *SLCO1B1* c.388AA.

TABLE 3

Prediction of OATP1B-mediated DDIs for rifampin, diltiazem and itraconazole using *R*-value and endogenous biomarker methods

Data are expressed as mean ± S.D.

Drugs	Dose Regimen	C_{max}[a]	f_u[b]	OATP1B1		OATP1B3		Observed CPI and CPIII $AUC_{(0-24h)}$ Changes[d]	Reported Rosuvastatin and Atorvastatin *AUC* Changes[e]
				IC_{50}[c]	*R*-Value	IC_{50}[c]	*R*-Value		
		μM		μM		μM			
Rifampin	600 mg SD	30.6	0.15	1.1	6.50	0.49	13.34	2.84- to 4.11-fold and 2.42-to 3.61-fold, respectively	3.03- to 4.67-fold and 4.62- to 12-fold, respectively
Diltiazem	240 mg QD for 8 days	0.44	0.22	260	1.03	170	1.05	1.00 ± 0.10-fold and 1.17 ± 0.44-fold, respectively	No data
Itraconazole	200 mg QD for 8 days	2.79	0.036	>100	<1.01	>30	<1.03	0.94 ± 0.10-fold and 0.95 ± 0.13-fold, respectively	1.78-fold and 5.58-fold, respectively; no increase of pitavastatin *AUC*
Hydroxylitraconazole		2.85	0.005	0.23	1.06	0.10	1.14		
Itraconazole + Hydroxylitraconazole					<1.07		<1.17		

f_u, Unbound fraction; IC_{50}, concentration required to inhibit transport by 50%; R-value was calculated using the method recommended by FDA, and estimated maximal unbound concentration in hepatic inlet is used for *R*-value calculation.

[a]Maximum inhibitor plasma concentration following administration of the proposed dose (Barone et al., 1998; Sista et al., 2003; Lai et al., 2016).

[b]Plasma unbound fraction of inhibitors (Templeton et al., 2008; Prueksaritanont et al., 2014, 2017) and Hardman et al., 2001.

[c]Concentrations required to inhibit OATP1B-mediated transport by 50% using statins and estradiol 17β-glucuronide (Yoshida et al., 2012; Shen et al., 2013; Nakakariya et al., 2016; Vermeer et al., 2016).

[d]Data obtained from the present studies and previously reported (Lai et al., 2016).

[e]Data obtained from University of Washington Metabolism and Transport Drug Interaction Database.

following findings: 1) statistically significant systemic exposure changes of CPI and CPIII in response to strong OATP1B inhibitor rifampin in black, white, and Hispanic subjects; 2) marginally increased C_{max} but not $AUC_{(0-24h)}$ by weak or non-OATP1B inhibitors diltiazem and itraconazole; 3) no effect of ethnicity on basal plasma CPI and CPIII levels; and 4) slightly lower basal CPI levels in individuals carrying the *SLCO1B1* c.388 A > G allele compared with those with c.388AA genotype.

A single oral dose of 600-mg rifampin greatly increased the *AUC*s of pitavastatin (Chen et al., 2013; Prueksaritanont et al., 2014, 2017; Takehara et al., 2018), rosuvastatin (Prueksaritanont et al., 2014, 2017; Lai et al., 2016; Wu et al., 2017; Takehara et al., 2018), and atorvastatin (Lau et al., 2007; He et al., 2009; Maeda et al., 2011; Prueksaritanont et al., 2017; Takehara et al., 2018) by 2.8- to 6.7-fold, 3.0- to 4.7-fold, and 4.6- to 12.0-fold, respectively, in multiple clinical DDI studies. In agreement, the administration of 600-mg rifampin in this study resulted in a 2.8- to 3.7-fold elevation in CPI $AUC_{(0-24h)}$ and a 2.4- to 3.1-fold elevation in CPIII $AUC_{(0-24h)}$ in black, white, and Hispanic subjects. Previous studies already indicated that rifampin administration caused 3.4- to 4.0-fold increases in CPI and CPIII $AUC_{(0-24h)}$ in Asian subjects (Lai et al., 2016). Therefore, in the presence of 600 mg rifampin, the CPI and CPIII exposure changes were of a magnitude similar to the changes in statin exposure, supporting the use of CPI and CPIII as suitable endogenous probes to detect OATP inhibition.

In contrast to rifampin, the multiple-dose regimens of 240-mg diltiazem and 200-mg itraconazole were not expected to cause in vivo inhibition of OATP1B activity. The in vitro IC_{50} values of these compounds relative to their anticipated unbound maximal portal concentration resulted in *R*-values of less than 1.10, the FDA *R*-value cutoff (Table 3). Diltiazem has been determined to be a weak in vitro inhibitor of OATP1B1 and OATP1B3 (260 and 170 μM, respectively) (Nakakariya et al., 2016). Coadministration of pravastatin with diltiazem (120 mg twice a day) did not affect the oral *AUC* and C_{max} of pravastatin, indicating no clinical OATP1B inhibition potential (Azie et al., 1998). Consistently, in this study, oral administration of 240 mg diltiazem once daily for 8 days in 14 healthy subjects did not influence the $AUC_{(0-24h)}$ of CPI and CPIII, although it significantly ($P < 0.05$) increased the C_{max} of CPI and CPIII by 1.11- and 1.32-fold, respectively, compared with prestudy values. The impact of itraconazole on CPI and CPIII plasma concentrations from healthy volunteers was also investigated. In vitro studies demonstrated that itraconazole did not substantially inhibit OATP1B1 and OATP1B3 activity at concentration up to 100 and 30 μM, respectively (IC_{50} of > 100 and > 30 μM, respectively) (Yoshida et al., 2012). However, hydroxyitraconazole, a prominent metabolite of itraconazole, is an OATP1B1 and OATP1B3 inhibitor (IC_{50} of 0.23 and 0.10 μM, respectively) (Table 3) (Vermeer et al., 2016). The mechanistic static model approach would predict no pharmacokinetic interaction at the liver inlet because the overall estimated *R*-value is low (<1.17) (Table 3). In addition, in clinical DDI studies in healthy subjects, 200 mg itraconazole has been shown to have no influence on *AUC* of pitavastatin (Nakagawa et al., 2013; Prueksaritanont et al., 2017). Pitavastatin is a more sensitive and selective OATP1B clinical probe than rosuvastatin owing to lack of complications from drug-metabolizing enzymes and gut-efflux transporters (Prueksaritanont et al., 2014). It is worth noting that the administration of itraconazole resulted in a 1.8- and 5.6-fold increases in *AUC* of rosuvastatin and atorvastatin, respectively, in the same subjects. The magnitude of this interaction is probably attributable to the impact of itraconazole on CYP3A metabolism of these statins (Prueksaritanont et al., 2017). As anticipated, on the basis of in vitro data and previous clinical data, following administration of 200 mg itraconazole in 14 healthy subjects, CPI and CPIII $AUC_{(0-24h)}$ levels were not altered significantly (Tables 2 and 3). Single doses of BMS-**A** and BMS-**B** also resulted in slight increases in the C_{max} of CPI and CPIII (1.05 to 1.24-fold for BMS-**A**; 1.13 to 1.24-fold for BMS-**B**), but not $AUC_{(0-24h)}$, compared with baseline levels. These data were in agreement with *R*-values calculated for OATP1B1/1B3 for the two compounds (between 1.00 and 1.01 for BMS-**A** and ≤1.06 for BMS-**B**) (unpublished data). The suitability of CPs as endogenous biomarkers to predict low to intermediate clinical OATP1B inhibition signals have been investigated recently. Barnett et al. recently developed a semi-mechanistic model to assess CPI as an endogenous biomarker of OATP1B by evaluating its synthesis, elimination pathways, sensitivity, and selectivity (Barnett et al., 2017). The model was able to predict the change in CPI plasma concentrations in response to the strong inhibitor rifampin. Additionally, simulation of a weaker inhibitory interaction suggested that changes in CPI concentrations could be observed in an adequately powered clinical study. Furthermore, clinical studies indicated that change in CPI *AUC* was predictive for a mild OATP1B-mediated drug interaction (1.4-fold CPI *AUC* increases and 1.5-fold

pitavastatin *AUC* increase) (Kunze et al., 2018). Biomarker sensitivity validations are especially important in the light of the current draft guidance by FDA, which uses an *R*-value of 1.10 as new cutoff for clinical DDI study.

Although CPI and CPIII have been suggested as putative endogenous markers of OATP1B activity, very little is known about plasma concentrations of CPs in humans, particularly regarding inter-ethnic difference. In the first study described here, the prestudy $AUC_{(0–24)}$ and C_{max} levels of CPI and CPIII in black, white, and Hispanic subjects appeared to be, on average, similar (Fig. 1; Table 1). Rifampin increased the CPI and CPIII $AUC_{(0–24h)}$ by 2.4- to 3.7-fold compared with the basal levels. The changes in CPI and CPIII $AUC_{(0–24h)}$ were slightly less than those in Asian Indian subjects reported by us previously (2.4- to 3.7-fold vs. 3.4- to 4.0-fold) (Lai et al., 2016). In addition, the CPI $AUC_{(0–24h)}$ in whites and CPIII $AUC_{(0–24h)}$ in blacks in the presence of rifampin were significantly higher compared with Hispanic subjects ($P < 0.05$). We further compared the basal plasma levels from all subjects in the current two studies to those in the previous study of Asian Indian subjects, and there was no effect of ethnicity on basal plasma CPI and CPIII levels (Fig. 3A). In the second study, we found that *SLCO1B1* c.388 A > G SNP, which has a higher frequency of expression in blacks and East Asians compared with Caucasians (Niemi et al., 2011; Nakanishi and Tamai, 2012), was associated with 16%–19% lower basal CPI but not CPIII plasma levels (higher OATP1B1-mediated CPI transport capacity) than c.388AA genotype in 28 subjects (Fig. 3C). Previous studies have shown that *SLCO1B1* c.388 A > G SNP is strongly linked to elevated activity of OATP1B1, resulting in 10.4%–31.7% lower *AUC* of many OATP1B1 substrate drugs (Mwinyi et al., 2004; Lee et al., 2005; Maeda et al., 2006; Yamada et al., 2011; Birmingham et al., 2015a; Choi et al., 2015; Zhao et al., 2017). It is worth noting that these findings have not been confirmed in additional studies examining changes in the transport of other OATP substrates (Lee et al., 2005; Choi et al., 2008; Birmingham et al., 2015b). These contradictory findings about the effects of that *SLCO1B1* c.388 A > G SNP on drug disposition may be explained in part by a substrate-dependent effect of the genetic variant. Although no change was observed for the heterozygous carriers of *SLCO1B1* c.521TC compared with those of c.521TT (Fig. 3B), the low number of TC genotype subjects and lack of any CC allele subjects makes it difficult to make conclusions about the impact of this polymorphism. Unfortunately, subjects in the first study were not genotyped for OATP1B polymorphisms.

Interestingly, only CPIII, but not CPI, was actively secreted into urine in healthy volunteers, suggesting involvement of renal transporter(s) in the elimination of CPIII but not CPI (Lai et al., 2016). In addition, only CPIII but not CPI was transported by OATP2B1 (Bednarczyk and Boiselle, 2016; Shen et al., 2017). Although CPI was recently identified as a substrate for MRP3 and MRP2, localized on the sinusoidal and canalicular membranes of hepatocytes, respectively (Gilibili et al., 2017; Kunze et al., 2018), the OATP1B-mediated uptake is considered to be the rate-limiting step of the hepatic clearance of these endogenous substrates; the magnitude of MRP-mediated DDI is generally small (less than 2-fold). On the basis of these findings and the higher plasma levels, CPI may be a more reliable endogenous biomarker for OATP1B transporters than CPIII.

In conclusion, we have provided further evidence to support use of CPI and CPIII as endogenous probes to detect and guide assessment of clinical OATP1B-based DDIs. Subjects treated with rifampin, known to strongly inhibit OATP1B, had highly elevated plasma levels of CPI and CPIII across three ethnic groups. Diltiazem and itraconazole, which were predicted not to inhibit OATP1B in vivo, did not significantly increase plasma CP exposures when given to healthy subjects. Basal plasma CP concentrations were not greatly influenced by ethnicity or affected differentially by *SLCO1B1* polymorphisms. Additional study of these biomarkers, especially regarding polymorphic effects on basal CP levels, is warranted.

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Authorship Contributions

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