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Organic Anion-Transporting Polypeptide Genes Are Not Induced by the Pregnane X Receptor Activator Rifampin: Studies in Hepatocytes In Vitro and in Monkeys In Vivo

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ABSTRACT

Induction potentials of the pregnane X receptor (PXR) activator rifampin (RIF) on transporter genes [e.g., organic anion-transporting polypeptides (OATPs)] are still in its infancy or remain controversial in the field. The present investigations characterized changes in transporter gene expression by RIF in sandwich-cultured hepatocytes from multiple donors of human and cynomolgus monkey using real-time quantitative reverse transcription polymerase chain reaction method. Three-day treatment of RIF significantly induced CYP3A4 (~60-fold induction), but not CYP1A2 and CYP2D6 genes. SLC51B was the most highly induced uptake transporter gene (>10-fold) in both human and monkey hepatocytes. A greater induction of CYP2C9 was observed in monkey hepatocytes than that in humans. ATP-binding cassette (ABC)B1 and ABCC2 were induced slightly above 2-fold in human and monkey hepatocytes and appeared to be dose-dependent. The induction of OATP and other transporter genes was generally less than 2-fold and considered not clinically relevant. SLCO2B1 was not detectable in monkey hepatocytes. To investigate in vivo OATP induction, RIF (18 mg/kg per day) was orally dosed to cynomolgus monkeys for 7 days. Pitavastatin and antipyrine were intravenously dosed before and after RIF treatment as exogenous probes of OATP and CYP activities, respectively. Plasma coproporphyrin-I (CP-I) and coproporphyrin-III (CP-III) were measured as OATP endogenous biomarkers. Although a significant increase of antipyrine clearance (CL) was observed after RIF treatment, the plasma exposures of pitavastatin, CP-I, and CP-III remained unchanged, suggesting that OATP function was not significantly altered. The results suggested that OATP transporters were not significantly induced by PXR ligand RIF. The data are consistent with current regulatory guidances that the in vitro characterization of transporter induction during drug development is not required.

SIGNIFICANCE STATEMENT

Organic anion-transporting polypeptide (OATP) genes were not induced by rifampin in sandwich-cultured human and monkey hepatocytes OATP functions measured by OATP probe pitavastatin and endogenous marker coproporphyrins were not altered in monkeys in vivo by 7-day rifampin treatment. The data suggested that OATP transporters are unlikely induced by the pregnane X receptor ligand rifampin, which are consistent with current regulatory guidances that the in vitro characterization of OATP1B induction during drug development is not required.

Introduction

Drug transporters are expressed in organ barriers and play a key role in regulating cellular in- and out-flux of many xenobiotics to affect their disposition and elimination. Alteration of functional activities or gene expression of drug transporters can significantly change the exposure of substrate drugs or endogenous metabolites in plasma and/or organs, subsequently resulting in transporter-mediated drug–drug interactions (DDIs) (Giacomini et al., 2010; Hillgren et al., 2013). Drugs enter the liver via passive diffusion and/or active uptake, and then are directly excreted into the bile and/or biotransformed by metabolizing enzymes (Ishikawa, 1992; Morrow et al., 2000). When passive diffusion into the hepatocytes is low, the active transport mediated by drug transporters on sinusoidal membrane of hepatocytes can be the rate-determining step for drugs that even undergo metabolic clearance (CL) and/or biliary elimination, and therefore affect drug pharmacokinetics (PK) and liver concentration (Hagenbuch and Meier, 2004). It is well-documented that inhibition of transporter function can significantly change the drug CL of coadministrated drugs that are substrates of those transporters. Consequently, regulatory agencies require investigating the role of several major drug transporters in drug disposition and assessing the potential risk of clinical DDIs when there is coadministration with transporter inhibitors http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdfhttp://wwwemaeuropaeu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606pdf; https://wwwfdagov/

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ABBREVIATIONS: ABC, ATP-binding cassette; AUC, area under the curve; CL, clearance; CP, completed plating; CP-I, coproporphyrin-I; CP-III, coproporphyrin-III; DDI, drug–drug interaction; HI, completed incubation; LC-MS/MS, liquid chromatography–tandem mass spectrometry; OATP, organic anion-transporting polypeptide; PK, pharmacokinetics; PXR, pregnane X receptor; q.d., every day; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; RIF, rifampin; SLC, solute carrier; UGT, uridine diphosphate glucuronosyltransferase.

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Various orphan nuclear receptors are associated with gene regulations of metabolizing enzymes and transporters. For example, ligands of constitutive androstane receptor induce phase I cytochrome P450 2B (CYP2B) enzymes, phase II enzymes such as the uridine diphosphate glucuronosyltransferase (UGT) isoforms, and drug transporters such as ATP-binding cassette (ABC)B1 also known as multidrug resistance 1 (MDR1) and ABCC2 ((Multidrug resistance-associated protein 2, MRP2)) genes (Yang and Wang, 2014). Similarly, ligands of pregnane X receptor (PXR) are associated with inductions of CYP3As, CYP2Cs, UGT1A1, sulfotransferases, MDR1, and MRP2. Rifampin (RIF) is an important drug extensively prescribed to treat tuberculosis regardless of its involvement in many DDIs. RIF can induce gene expressions of several cytochrome P450 and phase II enzymes, resulting in accelerating elimination of RIF itself or coadministered drugs that undergo biotransformation through these metabolic pathways (Sugatani et al., 2005). As a result, decreased efficacy of RIF is a concern after repeated doses due to the reduced exposure (Strolin Benedetti et al., 1990). Mechanistically, RIF is known as prototypical activator of PXR, the transcriptional activator involving the regulation of many phase I/II-metabolizing enzymes and several *ABC* transporter gene expressions (Mottino and Catania, 2008; Tolson and Wang, 2010). In fact, gene regulation of several efflux transporters by RIF has been characterized (Stapelbroek et al., 2010; Peters et al., 2011; Vilas-Boas et al., 2013; Weiss and Haefeli, 2013). For example, RIF induces *P-gp* and decreases the area under the curve (AUC) of P-gp substrate efavirenz by 22% (Niemi et al., 2003). However, the induction effects on solute carrier (SLC) uptake transporters remain inconclusive.

Organic anion-transporter polypeptides (OATPs) belong to SLC influx transporters expressed in the liver, intestine, kidney, and brain. Of six subfamilies, three, OATP1B1, 1B3, and 2B1, are the most important players in active hepatic uptake (CL) involved in the hepatic disposition and elimination for many anionic drugs. Literature reports on the induction of hepatic OATP transporters by RIF remain controversial. For example, a statistically significant upregulation of SLCO1B1 mRNA was observed with 10 μM RIF (Williamson et al., 2013) or 50 μM RIF (Jigorel et al., 2006; Sahi et al., 2006), but the induction was not statistically significant at 5 or 0.5 μM (Williamson et al., 2013). In contrast, lack of OATP gene induction by RIF treatment in hepatocytes was also reported (Meyer Zu Schwabedissen et al., 2010; Benson et al., 2016; Han et al., 2017).

In the present studies, induction of transporter genes by RIF was first characterized in three hepatocyte donors in vitro in sandwich culture from human and cynomolgus monkey using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). The comparison of RIF treatment on changes in expression of transporter genes was done in vitro, for the first time, between human and monkey. Monkeys were further dosed with RIF to investigate the induction potential on OATP transporter function using pitavastatin and antipyrine as exogenous probes of OATP and CYP (a positive control) activities, respectively. In addition, the endogenous OATP biomarkers coproporphyrin-I (CP-I) and coproporphyrin-III (CP-III) were also measured in plasma to monitor the OATP functional changes.

Materials and Methods

Chemicals and Reagents. Pitavastatin and RIF were purchased from Sigma-Aldrich (St. Louis, MO). High performance liquid chromatography–grade acetonitrile, water, and methanol were purchased from Burdick & Jackson (Muskegon, MI) and EMD Chemicals (Gibbstown, NJ), respectively. PBS and Hanks balanced salt solution were purchased from Life Technologies (Grand Island, NY). CP-I and CP-III standard were obtained from Sigma-Aldrich and Frontier Scientific (Logan, MO), respectively. CP-I $^{15}N_4$ was obtained from Toronto Research Chemicals (Toronto, ON, Canada). Matrigel (phenol red free) was purchased from Corning (Bedford, MA). Collagen I–coated 96-well plates were obtained from Life Technologies. The hepatocyte-plating medium (InVitro*GRO* CP medium), culture medium [InVitro*GRO* completed incubation (HI) medium], and *Torpedo* antibiotic mix were purchased from BioIVT (Westbury, NY).

Ethics Statement. Cryopreserved primary human and monkey hepatocytes isolated from deceased donor livers were purchased from Life Technologies and BioreclamationIVT (donor demographics in Supplemental Tables 1 and 2). Consent was obtained from the human donor or the donor's legal next of kin for use of these samples and their derivatives for research purposes using institutional review board–approved authorizations.

Sandwich-Cultured Human and Monkey Hepatocytes and RIF Treatment. Cryopreserved hepatocytes were cultured in a sandwich format based on the methods reported previously, with minor modification (Li et al., 2009). Briefly, cryopreserved hepatocytes were quickly thawed at 37°C and then transferred into plating (CP) media. The cells were then seeded at 65,000 cells per well in 96-well collagen-coated plates. After 4-hour attachment, the hepatocytes were washed once with incubation (HI) media and then overlaid with BD Matrigel at a concentration of 0.25 mg/ml in ice-cold HI media for overnight at 37°C in a humidified 5% CO_2 incubator. The HI media were removed, and the cells were treated with RIF at concentrations ranging from 0.1 to 25 μM or DMSO only in HI media. Media containing RIF or DMSO were changed daily for a total of 3-day treatment.

Real-Time qRT-PCR. Total cellular mRNA was isolated from hepatocytes cultured in a 96-well plate using a RNeasy 96 Kit (Qiagen) following the manufacturer's instructions. mRNA was eluted in a total volume of 120 μl. Five microliters was used for qRT-PCR in a final reaction volume of 20 μl. qRT-PCR was performed with TaqMan Fast Virus 1-Step Master Mix (Life Technologies) using a QuantStudio 6 Flex Real-Time PCR System (Life Technologies) following the manufacturer's instructions. β-actin mRNA expression was used to normalize target gene expression. The mRNA levels were expressed as a fold change of the no-treatment control using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), where Ct is the cycle number at which the fluorescence in the reaction crosses the preset arbitrary threshold, ΔCT represents the difference between the CT target and reference, and the $\Delta\Delta$CT is the difference between the ΔCT of the test and the ΔCT of the preassigned control. Gene expression for each condition is expressed as a fold change of mean $\pm$ S.D. from three independent donors (each performed in triplicate). All primer and probe sets were manufactured by Life Technologies and presented in Supplemental Material (Supplemental Table 3). When concentration-dependent induction was observed, the in vitro induction mRNA data were fitted using the sigmoid three-parameter equation of $Y = \text{bottom} + X \times (\text{top-bottom})/(EC_{50} + X)$ that is built in GraphPad Prism (La Jolla, CA) to estimate the EC_{50} (the in vitro concentration of inducer that produced half the maximum induction) values. The statistical analysis was conducted using the paired *t* test for significance.

Effects of Repeated RIF Treatment on PK of Antipyrine and Pitavastatin, and the Plasma Levels of CP-I and CP-III in Monkeys. To assess the effect of repeat doses of RIF on plasma concentration of CP-I and CP-III, a three-arms crossover study was performed in four male cynomolgus monkeys (4–8 kg). The cynomolgus monkeys used in the in vivo pharmacokinetic studies were maintained at a contract research organization in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility. All procedures were approved by an Institutional Animal Care and Use Committee. On day 1, monkeys were dosed with antipyrine (3 mg/kg) and pitavastatin (1 mg/kg) by an approximately 30-minute intravenous infusion via an indwelling catheter in a saphenous vein. Antipyrine and pitavastatin were coformulated in a vehicle containing 5% EtOH, 15% polyethylene glycol 300, and 80% water (pH 3). After dose administration, but before the needle was removed from the animal, the dose apparatus (i.e., the indwelling catheter) was flushed with approximately 1 ml saline. The monkeys were then administered once daily with RIF (18 mg/kg) via oral gavage on study day 3 through day 9. After a 2-day washout period, the monkeys were intravenously infused for 30 minutes with antipyrine and pitavastatin on day 11. Individual doses were calculated based on body weights recorded on study days 1, 7, and 11. Serial blood samples (1 ml) were collected from the saphenous vein on day 1, day 9, and day 11 at predose and 0.25, 0.48, 0.58, 0.75, 1, 2, 4, 6, 8, 12, and 24 hours postdose for antipyrine and pitavastatin,

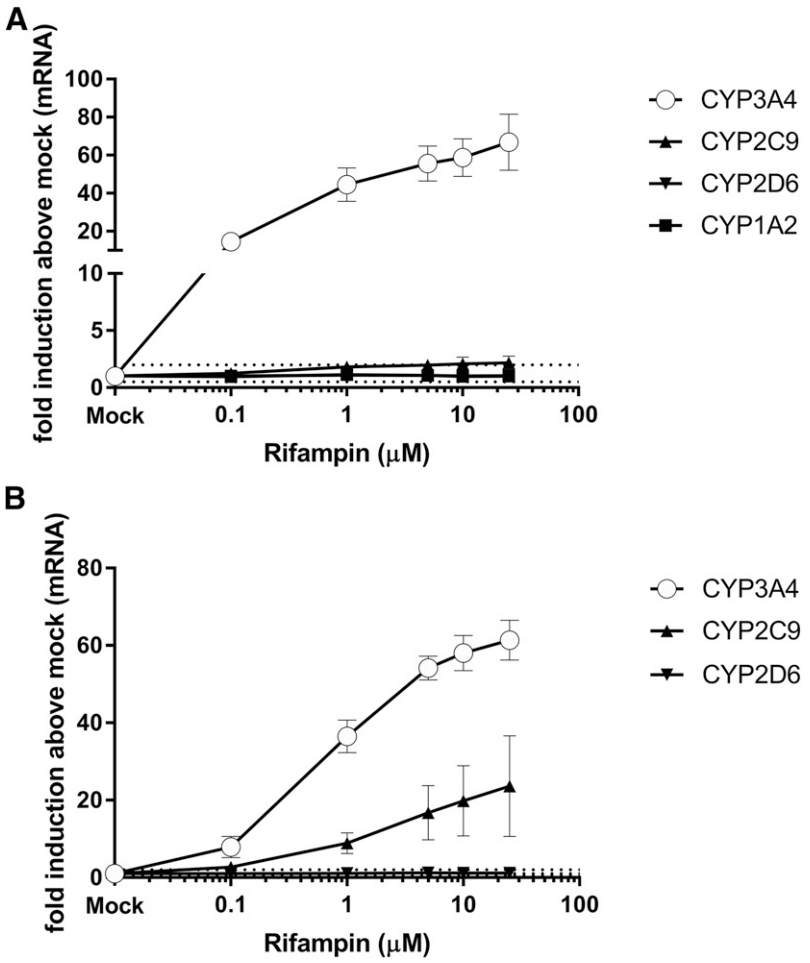

Fig. 1. Rifampin induction of CYP3A4, 2C9, 2D6, and 1A2 mRNA. Gene expression for each condition is expressed as a fold change of mean ± S.D. from three independent donors (each performed in triplicate). (A) Rifampin induction in human hepatocytes. The concentration-dependent induction of CYP3A4 (66.7-fold at 25 μM) and CYP2C9 (2.2-fold at 25 μM) was detected. Induction of CYP1A2 and CYP2D6 was not observed. (B) Rifampin induction in monkey hepatocytes. Concentration-dependent inductions of CYP3A4 (61-fold at 25 μM) and CYP2C9 (23.6-fold at 25 μM) were detected. Induction of CYP2D6 was negative, and CYP1A2 induction was not conducted due to lack of monkey-specific probe. Dot lines indicate the induction of 0.5- or 2-fold.

or at predose and 0.25, 0.5 1, 2, 4, 6, 8, 12, and 24 hours postdose for RIF exposure. The plasma samples were obtained by centrifugation at 3000*g* for 10 minutes and stored at −70°C until PK analysis. Plasma aliquots (0.5 ml) at predose and 2, 4, 8, 12, and 24 hours postdose were placed in separated tubes and stored with light protection until CP analysis.

Liquid Chromatography–Tandem Mass Spectrometry Analysis of Plasma Samples for Antipyrine, Pitavastatin, and RIF. Three hundred microliters 100 ng/ml caffeine, dexamethasone, and chrysin as internal standards in acetonitrile was added to a 50 μl aliquot of each plasma sample, with exception of the matrix blanks. The matrix blank samples received 300 μl acetonitrile only. The precipitated proteins were removed by centrifugation, and supernatant was transferred into two sets of clean 96-well plates. One hundred microliters of supernatant was transferred into clean 96-well plates. One hundred microliters of water was added to each well. An aliquot of 1.5–5.0 μl was injected into an Applied Biosystems API-5500 liquid chromatography–tandem mass spectrometry (LC-MS/MS) system (Applied Biosystems/MDS, Ontario, ON, Canada), coupled with a turbo ion spray interface in positive ion mode, and connected with a Shimadzu LC (SLC-10A) system (WoolDale, IL) and HTS PAL Leap autosampler (Carrboro, NC). Reverse-phase chromatography (mobile phase A, 100:0.1 water:formic acid; mobile phase B, 100:0.1 acetonitrile:formic acid) was used to elute and separate the different substrates with a Waters HSS T3 column (50 × 2.1 mm, 2.5 μm). Separation was achieved using a flow rate of 0.8 ml/min and linear gradient from 10% to 95% over 1.5 minutes. The following transitions were monitored: 189.1 m/z–77.2 m/z for antipyrine, 823.2 m/z–791.3 m/z for RIF, 422.1 m/z–290.1 m/z for pitavastatin, 195.2 m/z–138.0 m/z for caffeine, 255.1 m/z–153.0 m/z for chrysin, and 393.1 m/z–373.1 m/z for dexamethasone. The instrument settings of the API 5500 were as follows: ion spray voltage, 5.5 kV; temperature, 550°C; and collision energy was set at 30 eV for antipyrine, 23 eV for RIF, 38 eV for pitavastatin, 37 eV for caffeine, 43 eV for chrysin, and 15 eV for dexamethasone.

LC-MS/MS Quantification of CP-I and CP-III in Plasma. Sample aliquots were kept from light exposure as much as possible during sample preparation. One hundred microliters of plasma samples was mixed with 500 μl ethyl acetate and 50 μl internal standard solution containing 1.5 nM $^{15}N_4$-CP-I in 12 M formic acid in a 1 ml 96-well plate. After centrifugation at 4000 rpm for 15 minutes, the supernatant was transferred to a new plate and dried using a nitrogen plate dryer. Samples were then reconstituted with 60 μl 1 M formic acid and analyzed on a SCIEX 5500 LC-MS/MS system (Applied Biosystems/MDS SCIEX, Toronto, Canada), coupled to a CTC Analytics UPLC FLUX pump and a CTC Analytics HTS PAL autosampler (CTC Analytics, Switzerland). Samples (10 μl) were injected onto an Ace Excel 2 C18 UPLC, 2.1 × 150 mm (particle size 1.7 μm) (Advanced Chromatography Technologies, Aberdeen, Scotland), and eluted by a gradient program: 20% B to 60% B in 4 minutes, 60% B to 100% B in 0.5 minute, and held 100% B for 0.5 minute with the flow rate of 0.5 ml/min. The mobile phase was 0.1% formic acid in water and 98% acetonitrile in water containing 0.1% formic acid for A and B, respectively. The mass spectrometer was operated in positive, multiple reaction monitoring mode. The multiple reaction monitoring precursor/product ion transitions were as follows: m/z 655.3 > 596.3 for CP-I and CP-III, and m/z 659.3 > 600.3 for the internal standard, $^{15}N_4$-CP-I. The instrument settings on the mass spectrometer were as follows: declustering potential 130 V; collision energy 65 V; dwell time 25 ms. All peak integration and data processing were performed using SCIEX Analyst 1.6.2 (Applied Biosystems/MDS SCIEX).

PK Analysis. The PK parameters of antipyrine, RIF, and pitavastatin were estimated by a noncompartmental analysis using Phoenix 8.0 software (Pharsight, Mountain View, CA). Area under the concentration–time profiles (AUC) was calculated by the linear trapezoidal rule from zero to the last measured concentration point (AUC_{last}). C_{max} was the highest concentration observed from the time–concentration curve. The plasma CL was estimated by the equation of CL = dose/AUC. Exposure of CP-I and CP-III in the plasma was assessed by the

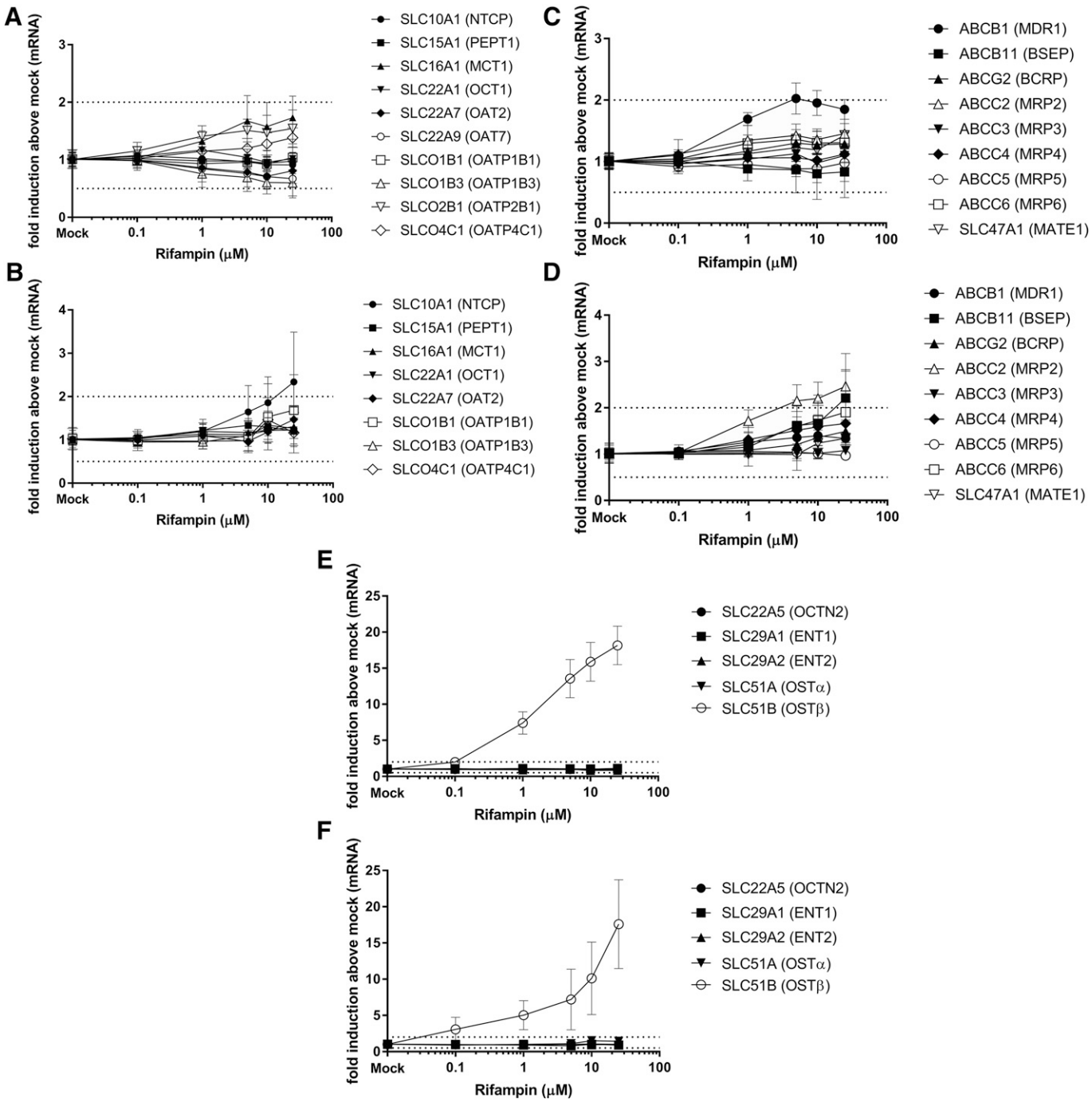

Fig. 2. Rifampin induction of transporter gene transcripts. Gene expression for each condition is expressed as a fold change of mean ± S.D. from three independent donors (each performed in triplicate). (A, C, and E) Rifampin induction of transporter mRNAs in human hepatocytes. (B, D, and F) Rifampin induction of transporter mRNAs in monkey hepatocytes. Dot lines indicate the induction of 0.5- or 2-fold.

AUC calculated from 0 to 24 hours measured concentration point. Data are expressed as mean ± standard deviation (S.D) Statistical analysis of parameters before and after RIF treatment was performed by the GraphPad Prism 7 (GraphPad Software, San Diego, CA). The statistical analysis was conducted using one-way ANOVA, followed by Tukey's multiple comparison test in the Prism, and $P < 0.05$ was considered as statistically significant.

Results

In Vitro Assessment of RIF Induction of Transporter and Metabolizing Enzyme Genes. The potential of transporter gene induction by the PXR ligand RIF was investigated in three lots of cryopreserved human or monkey hepatocytes. Sandwich-cultured human or monkey hepatocytes were incubated with RIF at concentrations ranging from 0.1 to 25 μM for 72 hours. Cell death or loss of adhesion of hepatocytes during or after incubation was examined by microscopy and by measuring β-actin mRNA levels to confirm that RIF treatments did not show significant cytotoxicity. As expected, RIF demonstrated concentration-dependent CYP3A4 mRNA induction in both human and monkey hepatocytes to maximal changes of 66-fold and 61-fold at 25 μM (Fig. 1), respectively. The estimated EC_{50} of RIF

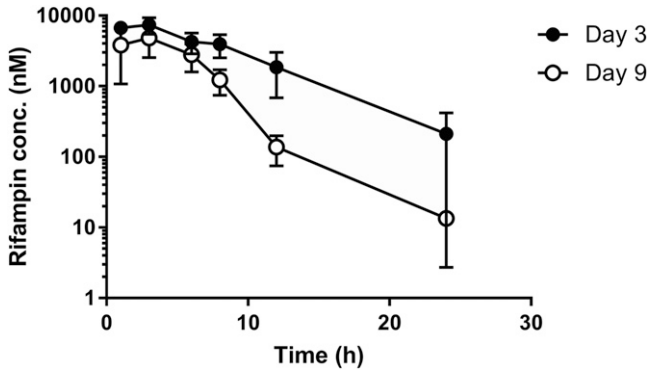

Fig. 3. Rifampin plasma concentration–time profiles on day 3 (or day 1 of RIF administration) and day 9 (or day 7 of RIF administration). Data represent mean ± S.D. from four monkeys.

induction of CYP3A4 was 0.4 and 0.72 μM for human and monkey CYP3A4, respectively. The EC_{50} values were consistent with literature data (Vermet et al., 2016). The induction of human and monkey CYP2D6 and human CYP1A2 mRNA by RIF was generally less than 2-fold (Fig. 1). CYP1A2 mRNA was not measured in monkey hepatocytes due to lack of monkey-specific primers and probe. Concentration-dependent induction of human and monkey CYP2C9 mRNA was also observed. The induction of human CYP2C9 mRNA by RIF was about 2-fold at 25 μM, whereas monkey CYP2C9 mRNA induction was about 24-fold at 25 μM, resulting in an EC_{50} of 2.5 μM for monkey CYP2C9 induction (Fig. 1B).

Twenty-four hepatic drug transporters were tested for the potential of changes in expression of mRNA following treatment with RIF in vitro using human- or monkey-specific primers and probe. As expected, all 24 transporter genes were found to be expressed in all three donors of human hepatocytes. Of those, SLCO2B1 mRNA was not detectable and SLC22A9 mRNA was not measured due to lack of specific primers and probe in monkey hepatocyte donors (Fig. 2B). Concentration-dependent gene induction by RIF of SLC16A1 (two of three donors), SLCO2B1 (two of three donors), SLCO4C1, ABCC2, ABCB1, and SLC51B was observed in human hepatocytes (Fig. 2, A, C, and E). The average fold induction for the transporter genes in human hepatocytes was generally less than 2-fold, except for SLC51B (approximately 16-fold at 25 μM RIF) (Fig. 2E). In contrast, mRNA levels of SLC10A1 (two of three donors), SLC51B, ABCC2, ABCC4 (two of three donors), and ABCC6 (two of three donors) were induced by RIF in a dose-dependent manner in monkey hepatocyte (Fig. 2, B, D, and F). The highest induced transporter gene in monkey hepatocytes was also SLC51B (about 10-fold at 25 μM RIF) (Fig. 2F). The fold induction for other transporter genes in monkey hepatocytes was less than 2-fold as well. The donor difference in gene regulation by RIF was generally less than 2-fold in both human (Supplemental Fig. 1, A, C, and E) and monkey (Supplemental Fig. 1, B, D, and F) hepatocytes.

PK of RIF in Cynomolgus Monkeys. The potential of treatment with the PXR activator RIF (18 mg/kg) to enzyme and transporter function was assessed in monkeys in vivo. The plasma PK of RIF was determined on day 3 (or day 1 of RIF administration) and day 9 (or day 7 post-RIF administration). As shown in Fig. 3, PK parameters of RIF on day 9 differed from those on day 3. The C_{max} and AUC_{last} values of RIF were 5.55 ± 1.27 μM and 26.8 ± 4.90 μM'hour on day 9, as comparison with 8.19 ±1.27 μM and 61.2 ± 20.01 μM'hour on day 3, respectively. The exposure on day 9 was significantly lower than that on day 3 ($P < 0.05$). The clearance over bioavailability (CL/F) values of RIF were 0.38 ± 0.13 and 0.84 ± 0.17 l/h per kilogram on days 3 and 9, respectively. The reduction of RIF exposure after 7-day RIF-repeated doses demonstrated the increases of its own metabolism in cynomolgus monkeys, suggesting the autoinduction of metabolic enzymes by RIF treatment.

PK of Pitavastatin and Antipyrine in Cynomolgus Monkeys. Pitavastatin and antipyrine were dosed via intravenous infusion before and after RIF treatment. The plasma concentration–time profiles of pitavastatin and antipyrine were determined to assess the RIF induction of OATP transporters and CYP enzymes, respectively. To determine whether pitavastatin is a sensitive probe for OATP function in monkey, a pre-experiment of pitavastatin intravenous infusion (1 mg/kg) was conducted in monkeys with single oral dose RIF (18 mg/kg). RIF increased pitavastatin AUC and C_{max} about 4.3- and 3.2-fold, respectively, suggesting that pitavastatin is a sensitive probe for OATP function (Supplemental Fig. 2). As shown in Fig. 4, the plasma exposure AUC_{last} of antipyrine was significantly reduced by the repeat treatment of RIF (Fig. 4A). Antipyrine AUC_{last} on day 11, after 7-day treatment of RIF, was 8970 ± 1480 nM'hour, about 36% of the AUC_{last} on day 1 (25,100 ± 3640 nM'hour) (Table 1). The plasma CL on day 11 increased greater than 2-fold from 0.65 ± 0.09 to 1.65 ± 0.270 (l/h per kilogram). The changes of AUC_{last}, C_{max}, and plasma CL were statistically significant ($P < 0.05$) (Table 1). The in vivo findings were consistent with in vitro inductions of CYP genes. In contrast, the plasma exposure of pitavastatin was not significantly altered by the treatment of RIF (Fig. 4B). As shown in Table 1, the AUC_{last} and C_{max} of pitavastatin on day 11 was about 95% and 94% of the corresponding values on day 1, respectively (676 ± 92.7 nM'hour and 1280 ± 166 nM vs. 713 ± 196 nM'hour and 1360 ± 328 nM, respectively). The changes of pitavastatin AUC_{last} and plasma C_{max} were not statistically significant ($P > 0.05$). It is worth noting that the plasma concentrations of pitavastatin at 6, 8, and 12 hours postdose on day 11 were lower than that on day 1. Although the overall CL was not significantly altered, the lower exposures of pitavastatin at elimination phase following repeated RIF doses suggested

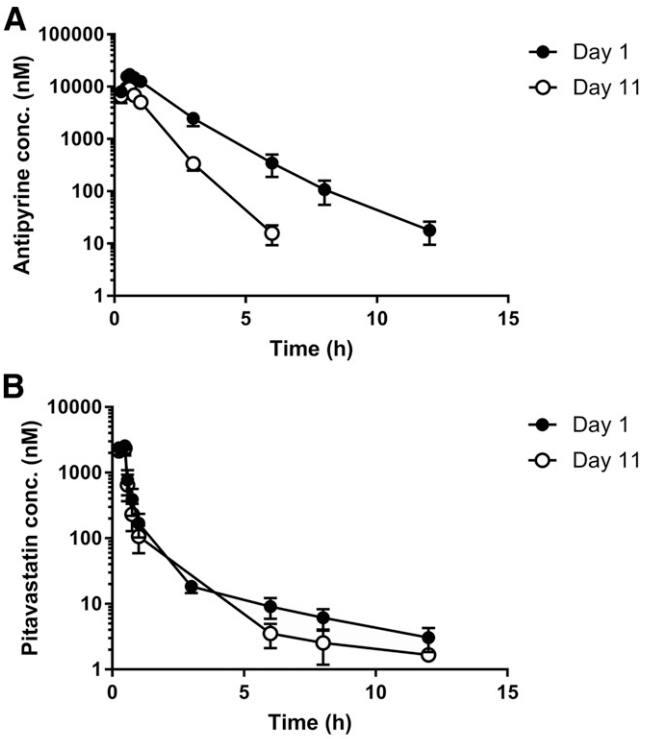

Fig. 4. Antipyrine (A) and pitavastatin (B) plasma concentration–time profiles on day 1 (prior to RIF induction) and day 11 (post-RIF induction). Data represent mean ± S.D. from four monkeys.

TABLE 1

Pharmacokinetics parameters of antipyrine and pitavastatin before (day 1) and after (day 11) RIF treatment

The parameters of antipyrine and pitavastatin were estimated by a noncompartmental analysis using Phoenix 8.0 software (Pharsight).

	Parameters	Day 1	Day 11
Antipyrine	C_{max} (nM)	13,221 ± 917	6786 ± 1002*
	AUC_{last} (nM•hour)	25,100 ± 3640	8970 ± 1480*
	CL (l/h per kilogram)	0.65 ± 0.09	1.65 ± 0.27*
	Vss (l/kg)	0.82 ± 0.06	1.16 ± 0.20
	$T_{1/2}$ (h)	1.37 ± 0.04	0.72 ± 0.14
Pitavastatin	C_{max} (nM)	615.8 ± 39.0	644 ± 120.8
	AUC_{last} (nM•hour)	754.8 ± 196.0	676.3 ± 92.7
	CL (l/h per kilogram)	3.27 ± 0.93	3.53 ± 0.45
	Vss (l/kg)	2.02 ± 0.91	1.16 ± 0.17
	$T_{1/2}$	2.73 ± 0.73	2.30 ± 0.56

$T_{1/2}$, half-life; Vss, steady-state volume of distribution.
*$P < 0.05$ as comparison with day 1.

the potential inductions of metabolic enzymes and/or hepatobiliary efflux transporters. Species difference in pitavastatin metabolism exists, and CYP2C76 is a major enzyme that is responsible for pitavastatin metabolism in cynomolgus monkey. However, due to lack of monkey-specific primers and probe for the enzyme, the induction of CYP2C76 was not assessed in the current experiments. Therefore, further investigation is warranted for the explanation.

Plasma Exposure of CP-I and CP-III in Cynomolgus Monkeys. The plasma levels of CP-I and CP-III were determined on days 1, 3, 9, and 11, which represented the values of baseline, in presence of RIF (OATP inhibition on days 1 and 7) and post-RIF induction. As shown in Fig. 5, the plasma exposures of both CP-I and CP-III measured by AUC_{0-24} increased significantly with single dose of RIF (on day 3) and the last day that RIF (on day 9) was dosed, suggesting the inhibition of OATP1B activities. The AUC increases of CP-I and CP-III on day 9 were significantly lower than that with single dose of RIF (24.70 ± 2.16 and 8.24 ± 1.31 vs. 29.23 ± 4.99 and 17.9 ± 3.40 nM'hour for CP-1 and CP-III, respectively) (Fig. 5). In contrast, the plasma exposures of CP-I and CP-III were similar between pre- (on day 1) and post-RIF treatment (on day 11) (AUC_{0-24}: 9.12 ± 1.85 nM'hour and 2.31 ± 0.43 nM'hour vs. 9.29 ± 1.27 nM'hour and 1.89 ± 0.34 nM'hour for CP-I and CP-III, respectively). RIF treatment had no effect on OATP1B biomarkers CP-I and CP-III, suggesting that SLCO1B genes were not induced by RIF treatment in monkeys.

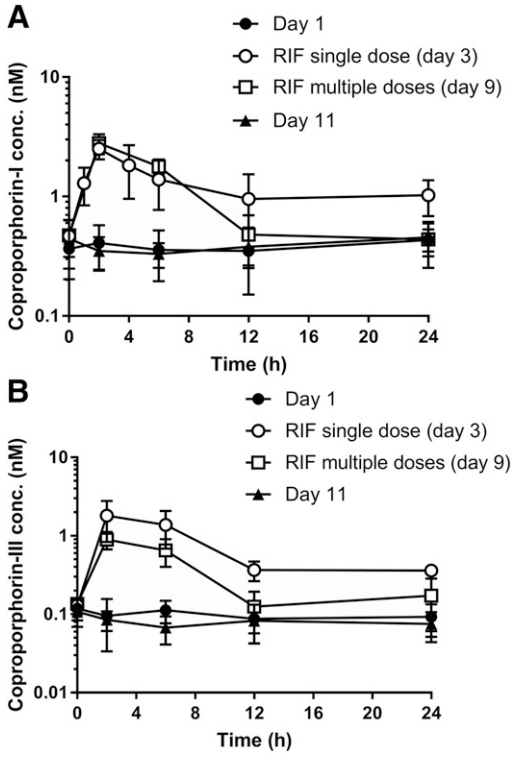

Fig. 5. Plasma concentration of CP-I (A) and CP-III (B) on day 1 (prior to RIF induction), day 3 (first dose of RIF treatment) day 9 (last dose of RIF treatment), and day 11 (post-RIF induction). Data represent mean ± S.D. from four monkeys.

Discussion

Understanding transporter gene regulation in the liver is of great importance both from a clinical DDI and drug liver exposure point of view, owing to the central role of drug transporters in drug disposition and elimination. OATPs expressed on the basolateral membrane of hepatocytes play critical roles in disposition, elimination, and liver exposure of a wide range of drugs that are actively taken up into hepatocytes (Kalliokoski and Niemi, 2009; Morse et al., 2015; Varma et al., 2015, 2017). Accumulative evidences indicate that inhibition of OATP transporter functions by comedications can cause significant clinical DDIs, leading to unexpected adverse effects or limitations of use in clinic for the substrate drugs. Thus, assessment of potential transporter DDIs of a new chemical entity with commonly administered comedications is required as part of the regulatory approval process for drugs (http://wwwemaeuropaeu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606pdf; https://wwwfdagov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM581965pdf; Lai and Hsiao, 2014; Chu et al., 2018). In contrast, induction of metabolizing enzyme and/or transporter genes in the liver can increase the hepatic CL of drugs and subsequently reduce systemic exposure. Previous studies have reported that oral pretreatment with 600 mg RIF for 7 days decreased the AUC values of simvastatin and atorvastatin by 87% and 80% in healthy volunteers, respectively (Kyrklund et al., 2000; Backman et al., 2005). RIF greatly increased hydroxylated metabolites of atorvastatin (acid or lactone), followed by the decrease of atorvastatin AUC, which indicated the strong induction of the CYP3A4-mediated hydroxylation of atorvastatin (Backman et al., 2005). The mechanism of CYP enzyme induction by RIF is caused by the pregnane X receptor (PXR) activation. This regulation pathway is also showed as a shared mechanism for induction of both CYP3A4 (Amacher, 2010) and efflux transporters, such as P-gp and MRP2 (Greiner et al., 1999; Fromm et al., 2000). Because CYP3A and P-gp induction share the same mechanism, preclinical tests of CYP3A induction can aid

TABLE 2

Impact of RIF treatment on statins that are stable in metabolizing enzymes

Statin	Clinical Probe Recommended by ITC[a]	Dose	Rifampin Pretreatment	Change in AUC (%)	Change in C_{max} (%)	Washout (h)	Reference
Rosuvastatin	Yes	20 mg	450 mg QD, 7 days	No change		NA	Drug label (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021366s037lbl.pdf)
Rosuvastatin	Yes	20 mg	450 mg QD, 6 days	No change		12	Zhang et al., 2008
Pitavastatin	Yes	4 mg	600 mg QD, 5 days	↑ 29	↑ 100	NA	Drug label (https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022363s000lbl.pdf)
Pravast	No	40 mg	600 mg BID, 5 days	↓ 31	↓ 30	17	Kyrklund et al., 2004
Rosuvastatin	Yes	10 mg	2 mg QD, 10 days	↓ 12	↓ 3	48	Lutz et al., 2018
			10 mg QD, 10 days	↓ 21	↓ 15		
			75 mg QD, 10 days	↓ 54	↓ 41		
			600 mg QD, 10 days	↓ 63	↓ 30		
Pravastatin	No	20 mg	2 mg QD, 10 days	↓ 17	↓ 14		
			10 mg QD, 10 days	↓ 19	↓ 21		
			75 mg QD, 10 days	↓ 58	↓ 59		
			600 mg QD, 10 days	↓ 58	↓ 53		

BID, twice daily; NA, not applicable; QD, once a day.
[a]ITC, International Transporter Consortium; ITC white paper [Clinical Probes and Endogenous Biomarkers as Substrates for Transporter Drug–Drug Interaction Evaluation: Perspectives from the International Transporter Consortium 2018 (Chu et al., 2018)].

decisions about the clinical assessment of P-gp induction (https://wwwfdagov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM581965pdf; Zhang et al., 2009).

Although OATP-mediated hepatic uptake is often the rate-determining mechanism for atorvastatin CL (König et al., 2013), the increase of hydroxylated metabolite exposure clearly demonstrated that the AUC decrease of atorvastatin is mainly attributed by the induction of CYP metabolic enzymes. Similarly, the reduction of RIF exposure observed in the current investigation was due to the induction of CYP enzymes, although RIF is also an OATP substrate. In vitro studies demonstrated that OATP1B1 mRNA is induced by the oxysterol-sensing liver x receptorα and the bile acid sensor farnesoid x receptor, but not PXR or constitutive androstane receptor agonists (Meyer Zu Schwabedissen et al., 2010). As summarized in Table 2, rosuvastatin exposures are not altered by RIF dosed orally at 450 mg once a day (q.d.) for 6 or 7 days in two separate clinical trials (Zhang et al., 2008; https://

TABLE 3

Summary of literature data of in vitro rifampin induction in human hepatocytes (fold changes)

References	PXR Ligand	Cell Model	Incubation Time (h)	Concentration (μM)	CYP3A4	OATP1B1	OATP1B3	OATP2B1	Other Transporter Genes
Nishimura et al., 2002	Rifampin	Plated hepatocytes	48	50	5	1.1	NA	NA	ABCC2, 1.27 ABCC1, 1.1 ABCB1, 1.58
Jigorel et al., 2006	Rifampin	Plated hepatocytes	72	50	37	2.4[a]	0.8	1.1	ABCC2, 2.5 ABCB1, 2.9
Sahi et al., 2006	Rifampin	SCHH	72	50	17	2.3[a]	NA	NA	ABCC2, 8.2 ABCB1, 1.7
	CI-1034	SCHH	72	5	15	1.5	NA	NA	ABCC2, 5.1 ABCB1, 1.6
Dixit et al., 2007	Rifampin	Plated hepatocytes	72	10	23	2.7[b]	2.6[b]	NA	ABCC2, 2.4 ABCB1, 4.2
				25	41	1.3[b]	5.5[b]	NA	ABCC, 2, 7 ABCB1, 10.5
Meyer Zu Schwabedissen et al., 2010	Rifampin	Plated hepatocyte				1			
	Rifampin	Plated hepatocytes	24	10	80	1.9[a]	1.09	NA	ABCC2, 2.4 ABCC1, 1.8 ABCB1, 2.2
Williamson et al., 2013	Rifabutin	Plated hepatocytes	24	10	24	0.81	3.06	NA	ABCC2, 1.9 ABCC1, 1.4 ABCB1, 1.8
	Rifapentine	Plated hepatocytes	24	10	20	1.3	1.3	NA	ABCC2, 1.9 ABCC1, 2.2 ABCB1, 3.3
Benson et al., 2016	Rifampin	Plated hepatocytes	24	10	22	1.04	0.63	1.73	ABCC2, 1.85 ABCB1, 1.72
Han et al., 2017	Rifampin	Plated hepatocytes	48	50	28	0.81	0.73	0.72	
Moscovitz et al., 2018	Rifampin	SCHH	48	10	75	3.14[b]	0.47	2.86[b]	ABCB1, 2.50

NA, Not assayed.
[a]Statistically significant.
[b]Statistically not significant.

TABLE 4

Changes in gene expression of CYP enzymes and drug transporters in sandwich-cultured human and monkey hepatocytes treated with RIF

Sandwich-cultured human (SCHH) or monkey hepatocytes (SCMH) were treated with 10 μM rifampin or vehicle for 72 hours. Fold change is the ratio of gene expression in rifampin treatment over control cells. Data are presented as mean ± S.D. from triplicate measurements of each donor in three human or monkey donors. A fold change equal 1 is defined as no change, and a fold change of 2 is defined as 2× increase of the expression.

Common Name	Gene Name	Fold Change		
		This Study in SCHH	This Study in SCMH	Benson et al. (2016) [a]
CYP3A4	CYP3A4	58.8 ± 9.9	58.1 ± 4.6	22
CYP2C9	CYP2C9	2.1 ± 0.6	19.9 ± 9.1	
CYP1A2	CYP1A2	1.0 ± 0.1	NA	
CYP2D6	CYP2D6	1.0 ± 0.2	1.2 ± 0.2	
OSTβ	SLC51B	15.9 ± 2.7	10.1 ± 5.0	12.67
MRP2	ABCC2	1.4 ± 0.1	2.2 ± 0.4	1.85
P-gp	ABCB1	2.0 ± 0.2	1.4 ± 0.1	1.72
OATP2B1	SLCO2B1	1.5 ± 0.3	ND	1.73
MRP6	ABCC6	1.3 ± 0.2	1.7 ± 0.6	1.65
OATB1B3	SLCO1B3	0.6 ± 0.2	1.3 ± 0.3	−1.57
OATP4C1	SLCO4C1	1.3 ± 0.2	1.5 ± 0.5	1.46
MATE1	SLC47A1	1.2 ± 0.3	1.2 ± 0.3	1.37
ENT1	SLC29A1	1.0 ± 0.1	1.0 ± 0.5	1.38
OAT7	SLC22A9	0.8 ± 0.2	NA	−1.34
PEPT1	SLC15A1	0.9 ± 0.1	1.3 ± 0.2	−1.27
MRP3	ABCC3	1.0 ± 0.14	1.0 ± 0.1	1.18
OSTα	SLC51A	1.0 ± 0.2	1.5 ± 0.4	1.21
BCRP	ABCG2	1.3 ± 0.1	1.4 ± 0.3	1.18
OCT1	SLC22A1	1.0 ± 0.1	1.2 ± 0.3	−1.16
OAT2	SLC22A7	0.7 ± 0.2	1.2 ± 0.2	−1.15
MRP5	ABCC5	0.9 ± 0.2	1.0 ± 0.1	1.17
MRP4	ABCC4	1.0 ± 0.1	1.6 ± 0.5	1.17
NTCP	SLC10A1	0.9 ± 0.1	1.9 ± 0.6	−1.08
OCTN2	SLC22A5	0.9 ± 0.1	1.0 ± 0.2	−1.08
BSEP	ABCB11	0.8 ± 0.4	1.7 ± 0.5	−1.07
OATP1B1	SLCO1B1	0.9 ± 0.1	1.5 ± 0.8	1.04
MCT1	SLC16A1	1.6 ± 0.4	1.2 ± 0.3	1.03
ENT2	SLC29A2	0.8 ± 0.2	0.9 ± 0.2	−1.03

NA, not applicable; ND, underdetermined.
[a]Decrease of gene expression is defined as negative value, and a fold change of −2 is defined as 2× decrease of the expression.

www.accessdata.fda.gov/drugsatfda_docs/label/2017/021366s037lbl.pdf). Pitavastatin AUC and C_{max} were increased by 29% and 100%, respectively by RIF pretreatment at 600 mg q.d. for 5 days (https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022363s000lbl.pdf). Pravastatin AUC and C_{max} were decreased by 31% and 65%, respectively, with considerable interindividual variations by RIF dosed at 600 mg twice daily for 5 days (Kyrklund et al., 2004). As only 7 of 10 human subjects showed decreased pravastatin exposure by RIF treatment that is in line with the RIF induction on intestinal expression of MRP2 (Fromm et al., 2000), and also the renal CL and elimination half-life of pravastatin were unaffected, the authors deliberated that the interaction occurs primarily during the absorption rather than the OATP-mediated hepatic uptake (Kyrklund et al., 2004). As such, accumulative literature data, at least prior to 2017, suggested that OATP induction via PXR agonism appears to be negative for other statins in which CYP metabolism plays a minor role on drug CL. In 2018, Lutz et al. (2018) conducted clinical trials to elucidate relative induction relationships of RIF between CYP3A and transporters, including P-gp and OATP using cassette doses containing dabigatran etexilate, pravastatin, rosuvastatin, and a midazolam/tolbutamide/caffeine cocktail received before and after dosed RIF 2, 10, 75, or 600 mg q.d. The authors found that RIF treatment decreased AUC of both pravastatin and rosuvastatin with no changes of the renal CL and no induction of CYP2C9 as measured by tolbutamide PK, and concluded that RIF can moderately induce hepatic OATP transporters in comparison with the strong induction of CYP3A measured by midazolam PK (Lutz et al., 2018). Although the induction of intestinal transporters can also be as complicated as the reduction of rosuvastatin and pravastatin exposure observed above, this recent clinical observation raised the questions on whether hepatic OATP genes are indeed induced by RIF treatment.

Induction potential of transporter genes by RIF is commonly conducted in vitro in various laboratories using either plated hepatocyte or sandwich-cultured hepatocyte format (Table 3). Regardless of the culture formats being used, CYP3A4 gene is greatly induced in all reports. The induction of OATP1B1 and OATP1B3 ranged from 0.81 to 2.7 and 0.63 to 5.5, respectively. According to the guidance of regulatory agencies, a 2-fold increase in mRNA is considered the threshold for a positive in vitro induction signal (https://wwwfdagov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM581965pdf). As a result, only three of seven experiments are considered as positive in in vitro OATP1B1 induction. To date, only one report investigated OATP1B1 gene regulation in liver biopsy samples from human subjects following 7 days of RIF treatment (600 mg q.d.) (Marschall et al., 2005). The report showed the enhanced expressions of CYP3A4, UGT1A1, and MRP2 and described the lack of OATP1B1 induction in both mRNA and protein level (Marschall et al., 2005). Nevertheless, taking together in vitro results and recent clinical observations, skepticism remains on the induction of OATP genes. With that in mind, investigation was conducted to elucidate the induction potential of RIF on transporter genes in both sandwich-cultured monkey and human hepatocytes. In general, the transporter gene inductions in the current investigation agreed with the literature reports (Table 4), and the similar induction patterns in sandwich-cultured cynomolgus monkey hepatocytes, except for the greater induction of CYP2C9 (~20-fold vs. 2.1-fold in monkeys and humans, respectively), were observed. As expected, significant OATP gene induction by RIF in both sandwich-cultured human and monkey hepatocytes was not observed. The most

highly induced uptake transporter gene in both human and monkey hepatocytes was the SLC51B (OSTβ >10 fold). The induction of SLC51B gene is consistent with literature data observed in plated human hepatocytes treated with 10 μM RIF for 24 hours (Benson et al., 2016). The clinical outcome of the gene induction remains unclear, and further investigation on OSTβ transporter induction by PXR agonism was warranted.

Potential OATP induction was further assessed in cynomolgus monkey using pitavastatin as the OATP exogenous probe, and plasma CP-I and CP-III as the OATP endogenous biomarkers. The use of cynomolgus monkey as the in vivo model to investigate OATP induction is considered from three aspects. First, the patterns of transporter gene regulation between human and monkey hepatocyte are similar. Second, intravenous administration of OATP probe drugs eliminates the complications attributed by intestinal efflux transporters. Third, there are similar orthologs and functional similarity of OATP1B1 and 1B3 between humans and monkeys in in vitro and in vivo DDI studies with specific substrates and inhibitors (Shen et al., 2013). In addition, the magnitude of hepatic transporter inhibition DDI was comparable between cynomolgus monkeys and humans when using pitavastatin as a probe in combination with RIF (Supplemental Fig. 2) or other OATP inhibitors (Takahashi et al., 2013). CP-I and CP-III in plasma were recommended, as suitable endogenous biomarkers for OATP function as the plasma levels of CP-I and CP-III increased to similar extent compared with rosuvastatin by RIF and cyclosporine A in cynomolgus monkeys and humans (Lai et al., 2016; Shen et al., 2016). Although, to our knowledge, there are no data describing the plasma concentration of CP-I and CP-III is truly sensitive to OATP transporter induction, the plasma CP-I levels are sensitively associated gene polymorphisms in humans (Lai et al., 2016; Yee et al., 2019). As such, in contrast to the AUC reduction of CYP3A substrates antipyrine and RIF observed by repeated treatment of RIF, no change in plasma exposures of CPs and pitavastatin suggested that the OATP induction is unlikely in monkeys in vivo.

Collectively, RIF treatment showed similar induction patterns between human and monkey hepatocytes for transporter genes. The data from in vitro assessment in human and monkey hepatocytes demonstrated that OATP transporter genes were not significantly induced by RIF treatment up to 25 μM. The overall OATPs' functional activities in monkey measured by a sensitive substrate and endogenous biomarkers were not affected by RIF in vivo. Our data suggest that OATP transporters are unlikely induced by RIF treatment, and the conflicting clinical results observed in literatures are likely complicated by the gastrointestinal absorption due to the gene regulations of efflux transporters or through indirect regulation mechanisms. The current findings are consistent with regulatory guidance that the in vitro characterization of OATP1B induction during drug development is not required.

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Authorship Contributions

Participated in research design: Lai, Niu, Wang.

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