Transporter expression in liver tissue from subjects with alcoholic or hepatitis C cirrhosis quantified by targeted quantitative proteomics

Li Wang, Carol Collins, Edward J. Kelly, Xiaoyan Chu, Adrian S. Ray, Laurent Salphati Guangqing Xiao, Caroline Lee, Yurong Lai, Mingxiang Liao, Anita Mathias, Raymond Evers, William Humphreys, Cornelis E.C.A. Hop, Sean C. Kumer, and Jashvant D. Unadkat

Department of Pharmaceutics, University of Washington, Seattle, P.O. Box 357610, WA 98195, USA (L.W., C.C., E.J.K., J.D.U.); Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck & Co., Rahway, NJ 07065, USA (X.C.); Departments of Clinical Pharmacology and Drug Metabolism and Pharmacokinetics, Gilead Sciences, Inc., Foster City, CA 94404, USA (A.S.R., A.M.); Drug Metabolism and Pharmacokinetics, Genentech, South San Francisco, CA, 94080, USA (L.S., C.E.C.A.H.); Preclinical PK and In Vitro ADME, Biogen, 125 Broadway, Cambridge, MA 02142, USA (G.X.); Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Ardea Biosciences, Inc., 9390 Towne Centre Drive, San Diego, CA 92121, USA (C.L.); Pharmaceutical Candidate Optimization, Bristol-Myers Squibb Company, 3551 Lawrenceville Road, Princeton, NJ 08540, USA (Y.L.,W.H.); Takeda Pharmaceuticals International Co. 40 Landsdowne St. Cambridge, MA 02139, USA (M.L.); Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck & Co., Kenilworth, NJ 07033, USA (R.E.); Department of Surgery, University of Kansas Medical Center, Kansas City, KS 66160, USA (S.C.K.).

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Corresponding author: *Department of Pharmaceutics, University of Washington, Seattle, P.O. Box 357610, WA 98195, USA. Phone: +1-206-685-2869. Fax: +1-206-543-3204. Email: jash@uw.edu

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ABBREVIATIONS: AUC, area under the curve; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; FDA, the US Food and Drug Administration; LC-MS/MS, liquid chromatography tandem mass spectrometry; MATE1, multidrug and toxin extrusion protein 1; P-gp, P-glycoprotein; MRP, multidrug resistance-associated protein; NTCP, sodium-taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; OCT1, organic cation transporter 1; PBPK, physiologically based pharmacokinetics; QC, quality control.

Abstract

While data are available on the change of expression/activity of drug metabolizing enzymes in liver cirrhosis patients, corresponding data on transporter protein expression are not available. Therefore, using quantitative targeted proteomics, we compared the protein expression of major hepatobiliary transporters, BCRP, BSEP, MATE1, MRP2, MRP3, MRP4, NTCP, OATP1B1, 1B3, 2B1, OCT1, and P-gp in alcoholic (n=27) and hepatitis C cirrhosis (n=30) livers with our previous data on non-cirrhotic control livers (n=36). Compared to control livers, the yield of membrane protein from alcoholic and hepatitis C cirrhosis livers was significantly reduced by 56% and 67%, respectively. The impact of liver cirrhosis on transporter protein expression was transporter-dependent. Generally, reduced protein expression (per gram of liver) was found in alcoholic cirrhosis livers vs. control livers, except that the expression of MRP3 was increased while no change was observed for MATE1, MRP2, OATP2B1, and P-gp. In contrast, the impact of hepatitis C cirrhosis on protein expression of transporters (per gram of liver) was diverse, showing an increase (MATE1), decrease (BSEP, MRP2, NTCP, OATP1B3, OCT1, and P-gp), or no change (BCRP, MRP3, OATP1B1, and 2B1). The expression of hepatobiliary transporter protein differed in different diseases (alcoholic vs. hepatitis C cirrhosis). Finally, incorporation of protein expression of OATP1B1 in alcoholic cirrhosis into the Simcyp PBPK cirrhosis module improved prediction of the disposition of repaglinide in liver cirrhosis patients. These transporter expression data will be useful in the future to predict transporter-mediated drug disposition in liver cirrhosis patients.

Introduction

Liver cirrhosis resulting from chronic liver injury (e.g. chronic infection with hepatitis virus or excess alcohol use), characterized by loss of functional hepatocytes and formation of scar tissue (fibrosis), can lead to significant morphological changes in the architecture of the liver (Schuppan and Afdhal, 2008). At the end-stage of chronic liver disease, liver cirrhosis may impair hepatic and renal function, reduce plasma protein concentrations, elevate intrahepatic resistance (resulting in portal hypertension), and increase cardiac output. These outcomes can have a profound impact on the pharmacokinetics of drugs administered to patients with liver cirrhosis which may result in unexpected adverse effects (Bosch and Garcia-Pagan, 2000; Dincer et al., 2005; Schuppan and Afdhal, 2008) and/or potential change of drug efficacy. For example, compared to healthy subjects, the plasma AUC of chlormethiazole, used in the treatment of alcoholism, is increased by 17-fold in liver cirrhosis patients (Pentikainen et al., 1980). Indeed, according to the FDA Guidance on pharmacokinetics in patients with impaired hepatic function, dose adjustment is recommended when at least a two-fold change in plasma AUC is observed in patients with hepatic impairment (FDA, 2003).

There is considerable amount of data showing that drug metabolizing enzymes (e.g. CYP1A2, 2C9, 2E1, and 3A4) expression is decreased in cirrhotic human livers (George et al., 1995). Clinical studies confirm that the urinary metabolic ratio of caffeine, mephenytoin, debrisoquine, and chlorzoxazone, which are *in vivo* probes of CYP1A2, 2C9, 2D6, and 2E1 respectively, is reduced (Ohnishi et al., 2005; Frye et al., 2006). However, there is a paucity of data on the effect of cirrhosis on expression and activity of hepatic drug transporters (Ogasawara et al., 2010; More et al., 2013). Therefore, using quantitative proteomics, we quantified the protein expression of the major hepatobiliary transporters in liver tissue obtained from patients with alcoholic and

hepatitis C cirrhosis. Then, the results obtained were compared with our previous data on the expression of these transporters in non-cirrhotic human livers (controls) determined using the same methodology (Prasad et al., 2016). Finally, we used these data to predict changes in the disposition of a drug, repaglinide, in patients with liver cirrhosis.

Materials and Methods

Chemicals and Reagents

Synthetic signature peptides for quantified transporters (Table 1) were obtained from New England Peptides (Boston, MA). The corresponding stable isotope-labeled internal standards (AQUA QuantPro, +/- 25% precision) were purchased from Thermo Fisher Scientific (Rockford, IL). These peptides were identical to those used previously to quantify hepatobiliary transporters in control livers (Prasad et al., 2013; Prasad et al., 2014; Wang et al., 2015). The ProteoExtract native membrane protein extraction kit was obtained from Calbiochem (Temecula, CA). Sodium deoxycholate (98% purity) was purchased from MP Biomedicals (Santa Ana, CA). Iodoacetamide, dithiothreitol, ammonium bicarbonate (98% purity), BCA protein assay kit, and in-solution trypsin digestion kit were obtained from Pierce Biotechnology (Rockford, IL). HPLC-grade acetonitrile (99.9% purity), methanol (99.9% purity), and formic acid (≥99.5% purity) were purchased from Fischer Scientific (Fair Lawn, NJ). Deionized water was obtained from a Q-Gard 2 Purification Pack water purifying system (Millipore, Bedford, MA).

Human Liver Tissue

Twenty seven frozen alcoholic cirrhosis human liver tissues were obtained from the Liver Tissue Cell Distribution System, University of Minnesota. In addition, 30 frozen hepatitis C cirrhosis liver tissues were obtained from the Liver Center Tissue Bank of University of Kansas Medical Center. These tissues were obtained from patients undergoing liver transplant. The donor demographics and clinical characteristics of the liver donors are shown in Table 2. Most clinical pharmacokinetic studies for liver cirrhosis patients use the Child-Pugh score to indicate the level of liver impairment (Rodighiero, 1999; Hatorp et al., 2000; Stangier et al., 2000; Hui et

al., 2005). However, the Child-Pugh scores of the donors of liver tissues were not available. As these tissues were obtained from donors who received a liver transplant, it is highly likely that they belong to the Child-Pugh score C category, which represents end-stage liver disease. These livers were obtained under protocols approved by the appropriate committees for the conduct of human research. All liver tissues were stored at –80 °C until analysis. Demographics, procurement and storage of the control (non-cirrhotic) livers have been published before (Paine et al., 1997). In brief, most of these livers were obtained from vehicular accident victims who were otherwise healthy.

Peptide Selection, Membrane Protein Extraction, and Trypsin Digestion

Signature peptides were selected for quantification of each transporter (Table 1) based on previously reported criteria (Kamiie et al., 2008; Prasad and Unadkat, 2014). Isolation of total membrane protein (in triplicate), methanol-chloroform precipitation, and trypsin digestion were performed as described previously (Wang et al., 2015). After total membrane protein isolation, 100 µL of 2.0 mg/mL (or lower concentration) of membrane protein was used for further treatment. Finally, 5 µL of the processed sample was injected onto the LC-MS/MS system.

Liquid Chromatography-tandem Mass Spectroscopy Analyses

Waters® Xevo TQS tandem mass spectrometer coupled to Waters® Acquity™ UPLC system (Waters, Hertfordshire, UK) operated in electrospray positive ionization mode was used for analyses of the signature peptides. The mass spectrometry conditions were as follows: capillary 3.5 kV, source offset 30 V, source temperature 500 °C. Analyte-specific parameters are listed in Table 1. The transitions from doubly charged parent ion to singly charged product ions for the analyte peptides and their respective stable isotope-labeled peptides were monitored (Table 1). These peptides were separated on a UPLC column (Acquity UPLC® HSS T3 1.8 µm, 2.1 x 100

mm, Waters) with a Security Guard column (C18, 4 mm × 2.0 mm) from Phenomenex (Torrance, CA, USA). Mobile phases (0.3 mL/min) consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The linear gradient was: 0-3 min: 3% B; 3-10 min: 3%-13% B; 10-20 min: 13%-25% B; 20-24 min: 25-50% B; 24-24.1 min: 50%-80% B; 24.1-25min: 80% B; 25-25.1 min: 80%-3% B; and 25.1-29 min: 3% B. Intraday accuracy and precision was determined using quality control (QC) samples. A coefficient of variation of ≤15% and ≤25% for these QC samples, prepared in extraction buffer II and pooled liver membrane, respectively, was considered acceptable. The signal from the endogenous expression of the transporter was subtracted when calculating the signal in the QC samples prepared in pooled liver membrane. The stability (freeze and thaw, bench-top, and autosampler conditions) of the selected peptides has been reported in previous publications (Prasad et al., 2013; Prasad et al., 2014; Wang et al., 2015).

Genotyping Methods and Genotype Dependent Changes in OATP1B1 Protein Expression

The SLCO1B1 single nucleotide polymorphisms (SNPs) rs4149015, rs2306283, rs4149056, rs4149057 and rs2291075 define the variants -11187G>A, 388A>G, 521T>C, 571T>C and 597C>T, respectively. These SNPs were genotyped using validated TaqMan® assays from Applied Biosystems (Foster City, CA). The cycling conditions for PCR amplification were one cycle at 95°C for 10 min followed by 50 cycles of 95°C for 15 sec and 60°C for 90 sec in a reaction volume of 10 µL containing 1 µL of genomic DNA (~25-100 ng) and 1X final concentrations of TaqMan® universal PCR master mix and SNP assay primers and probes. The allelic discrimination was determined in a post-PCR analysis on a Bio-Rad (Hercules, CA) CFX Connect Real-Time System running software CFM Manager V 3.1 with allele specific probes for the common and variant SNPs (FAM and VIC). The SLCO1B1 SNP rs11045819 (463C>A) was

determined by PCR amplification and DNA sequencing using forward and reverse oligonucleotides flanking the variant. In brief, DNA (~25-100 ng) was subjected to an amplification profile: 95˚C for 3 min, 40 cycles of PCR cycling (95˚C for 30 sec, 57.6˚C for 30 sec and 72˚C for 1 min), 72˚C for 10 min and a final 4˚C hold. Each PCR reaction was composed of: 1.0 μL genomic DNA (~25-100 ng), 2.0 μL Pfu 10X buffer (Agilent Corp., La Jolla, CA), 200 μM dNTPs, 300 nM forward (TTTCTAGGAAAAGTGAAAATATTCAGTAGATAAGC) and reverse (TTGCTAATGAATATCACAACAATTTTTAGAGATGT) primers and 0.2 μL (0.5 U) Pfu HotStart Turbo DNA polymerase (Agilent Corp.) in a total volume of 20 μL. The 274 bp amplicon was purified using Qiagen QIAquick® spin column (Qiagen Inc, Valencia, CA) and DNA sequencing determined using an internal forward primer (CAGTGATGTTCTTACAGTTACAG) and BigDye V3.1 chemistry (Applied Biosystems, Foster City, CA); allele calls were based on visual inspection of resultant electropherograms.

Statistical analyses

Data are reported as mean ± S.D. Statistical difference ($p<0.05$) in the expression of transporters among different sample types (non-cirrhotic control livers, alcoholic cirrhosis livers, and hepatitis C cirrhosis livers) or OATP1B1 genotype/haplotype was assessed using the Kruskal-Wallis test followed by Dunn's multiple comparison correction.

PBPK simulations

Using the Simcyp cirrhosis population module (version 14 release 1, SimCYP Ltd, Sheffield, UK), the effect of alcoholic liver cirrhosis on the pharmacokinetics of OATP1B1 substrate, repaglinide, was predicted. This module incorporates the physiological changes in liver cirrhosis

(Child-Pugh score: B and C), including the impact on the expression/activity of CYP enzymes (Johnson et al., 2010). Liver weight was assumed not to be altered in cirrhotic patients. To conduct these simulations, repaglinide parameters, shown in Supplement Table 1, were obtained from a previous publication (Varma et al., 2013) and used without any modifications. The mean expression of OATP1B1 in alcoholic cirrhotic livers vs. control livers, obtained from the present study, was used and assumed to be the same for Child-Pugh score B and C. Data for 120 subjects (ratio of Child-Pugh B vs. C: 3:1; age 42-62 years; proportion of females: 0), given a single dose of repaglinide (4 mg, po), were simulated (10 trials × 12 subjects). The simulated pharmacokinetic profiles and parameters were compared to the observed data from a previous clinical study of repaglinide in 12 healthy subjects and 12 liver cirrhosis patients (n=9 and 3 for Child-Pugh score B and C, respectively; the authors did not specify the cause of cirrhosis) (Hatorp et al., 2000).

Results

Analytical

The calibration curves for the signature peptide was linear (r^2>0.995) over two orders of magnitude and the lower limit of quantification for all signature peptides were in the low femtomole range (Table 1). The accuracy and precision (% CV) of the assay, based on QC samples, was 87-115% and <20% respectively.

Comparison of Membrane Protein Yield between Control and Cirrhotic Human Livers

We previously quantified the expression of hepatobiliary transporters in 36 control (non-cirrhotic) human livers from the human liver bank of the School of Pharmacy, University of Washington (Prasad et al., 2016). The yield of membrane protein from these control livers was 37.7±7.0 mg /gm of liver (Fig. 1). The yield of membrane protein from alcoholic and hepatitis C cirrhosis human livers was significantly lower, i.e. 21.1±7.9 and 25.1±8.3 mg /gm of liver, respectively (Fig. 1).

Comparison of Expression of Hepatobiliary Transporters between Control and Cirrhotic Human Livers

All the investigated transporters could be quantified except for MRP4 which was below the lower limit of quantification (on column LLOQ=6.41 femtomole) for all livers. When the expression of the transporters was normalized to milligram of membrane protein, a number of transporters demonstrated altered expression in cirrhotic livers compared with control livers (Fig. 2-3). Specifically, in alcoholic cirrhosis livers, the expression of OATP1B1, OATP1B3, and OCT1 was significantly reduced by 40%, 56%, and 49%, respectively, whilst the expression of MATE1, MRP3, and P-gp was significantly increased by 67%, 110% and 75%, respectively. In contrast, in hepatitis C cirrhosis livers, the expression of only NTCP and OCT1 was decreased

by 36% and 38%, respectively, whilst the expression of BCRP, MATE1, MRP3, and OATP2B1 was increased by 36%, 87%, 88%, and 50%, respectively. However, to utilize these findings in PBPK predictions of in vivo transporter-mediated drug disposition, these data need to be expressed per gram of tissue which can then be scaled up to the whole liver (assuming that the weight of the liver does not change due to cirrhosis). When this was done for each individual, the pattern of differences between cirrhotic and control livers changed. All investigated transporters showed reduced protein expression (37-73%) in alcoholic cirrhosis livers vs. control livers, except that the expression of MATE1, MRP2, OATP2B1, and P-gp was not affected, while MRP3 (32%) was increased (Fig. 2- 3). In contrast, the expression of six transporters, including BSEP, NTCP, OATP1B3, OCT1, MRP2, and P-gp exhibited significant decrease (32-56%) in hepatitis C cirrhosis livers vs. control livers while the expression of BCRP, OATP 1B1, and 2B1 was not affected and the expression of MATE1 (46%) was increased (Fig. 2- 3) . The expression of none of the transporters was significantly ($p<0.05$) correlated with clinical markers of liver function listed in Table 2.

Protein-protein expression correlation of transporters in cirrhotic livers

The protein expression (normalized to gram of liver) of the following transporters was correlated with $r^2 > 0.5$ in both alcoholic and hepatitis C cirrhosis livers: BSEP vs. NTCP, BSEP vs. OATP1B1, BSEP vs. OATP2B1, and NTCP vs. OATP1B1. In addition, the expression of MRP2 vs. NTCP and MRP2 vs.OATP2B1 in hepatitis C cirrhosis livers, as well as BCRP vs. MRP2, BSEP vs. OCT1 in alcoholic cirrhosis livers showed a correlation of $r^2 > 0.5$. Protein-protein expression correlation of the remaining transporters in cirrhotic human livers was poor or nonexistent ($r^2 < 0.5$).

Effect of OATP1B1 genotype on hepatic transporter protein expression in cirrhotic livers

The protein expression of OATP1B1 in cirrhotic livers was not dependent (data not shown) on the observed SNPs (Table 3). More importantly, the protein expression of OATP1B1 in these diseased human livers was not dependent on *SLCO1B1* haplotypes of the three previously described key variants (c.388A>G, c.463C>A and c.521T>C) (data not shown). The sample size of 1a/*1a, *1b/*1a, *14/*1a, and *15/*1a haplotypes was 11, 4, 2, and 4 for alcoholic cirrhosis livers, and 6, 4, 6, and 4 for hepatitis C cirrhosis livers.

Quantitative impact of liver cirrhosis on the pharmacokinetics of OATP1B1 substrate, repaglinide.

Utilizing the protein expression data (Fig. 3 and Supplement Table 1), we predicted the impact of liver cirrhosis on the human pharmacokinetics of an OATP1B1 substrate, repaglinide. To do so, we used the Simcyp cirrhosis population module which incorporates changes in physiology (e.g. hepatic blood flow) and CYP expression/activity due to cirrhosis, with the assumption that liver weight is not altered in cirrhotic liver. When our transporter data were not incorporated in this PBPK model, the simulated mean plasma concentrations of repaglinide underestimated the observed plasma concentrations, with ratio of observed vs. simulated AUC of 2.5 (Fig. 4A). Next, assuming that the subjects in the reported study were alcoholic cirrhosis patients and that repaglinide is predominately transported by OATP1B1, the relative mean expression of OATP1B1 in alcoholic cirrhotic livers vs. control livers was incorporated into the PBPK model. Clearly, incorporation of change in OATP1B1 expression improved the ability to predict the observed repaglinide plasma concentrations vs.time profile, with the ratio of the observed vs. simulated AUC of repaglinide improved from 2.5 to 1.5 (Fig. 4B).

Discussion

We present here, the protein expression of major hepatobiliary transporters in alcoholic and hepatitis C cirrhosis livers. The impact of liver cirrhosis on transporter protein expression exhibited transporter-dependent pattern irrespective of whether it was normalized to milligram of membrane protein or gram of liver. For example, when normalized to the former, the expression of OCT1 was significantly reduced and the expression of MATE1 and MRP3 was significantly increased in both alcoholic and hepatitis C cirrhosis livers. In contrast, the expression of OATP1B1and OATP1B3 was significantly decreased while that of P-gp was significantly increased in only the alcoholic cirrhosis livers. Likewise, the expression of BCRP and OATP2B1 was significantly increased in only the hepatitis C cirrhosis livers. The expression of other transporters was not affected by cirrhosis. These results differ from previous studies where the mRNA and protein expression of some of these transporters was determined (Ogasawara et al., 2010; More et al., 2013). Ogasawara et al. reported that the mRNA expression of BCRP, MATE1, MRP2, MRP3, OATP1B1, 1B3, 2B1, and OCT1 was significantly reduced in hepatitis C cirrhosis livers vs. control livers (Ogasawara et al., 2010). Another study, in a limited number of alcoholic cirrhosis livers (n=10), found that BCRP, MRP1, and MRP3-5 protein expression (but not that of MRP2 or MRP6) was elevated (More et al., 2013). The reasons for the discrepancies between these data and our data are not known but could be differences in ethnicity (Japanese vs. Caucasian liver tissues), endpoints (mRNA vs. protein expression), and severity of the disease or sample size.

To predict transporter-mediated drug disposition in vivo through PBPK simulation, protein expression in vitro (in liver tissue) needs to be scaled to that in vivo. To do so, the yield of the membrane protein needs to be taken into consideration. In cirrhotic livers, scar tissue is diffused

throughout the liver and therefore could lead to reduced yield of membrane protein. Indeed, in the current study, the yield of membrane protein from cirrhotic livers was 56-67% lower than from the control livers. This finding is consistent with other studies in which the functional hepatocyte volume (measured by ultrasound or binding of a highly selective radioligand to a specific hepatocyte surface protein) in cirrhotic livers with Child-Pugh score B and C was found to be 56-67% lower than the functional hepatocyte volume of control livers (Johnson et al., 2010).

To take into consideration the above lower membrane protein yield in cirrhotic livers, we expressed our transporter expression data per gram of liver tissue. In doing so, the pattern of liver cirrhosis on transporter expression was altered. For example, the expression of BCRP and NTCP in alcoholic cirrhosis was not significantly different from that in control livers when normalized to milligram of membrane protein, but it was when normalized to gram of liver. These differences highlight the importance of normalizing transporter expression data to gram of tissue for the purpose of predicting in vivo transporter-mediated drug disposition. In doing so, the effect of cirrhosis on transporter protein expression was also transporter-dependent. For example, while OATP1B1 and 1B3 expression was reduced in alcoholic cirrhosis vs. control livers, the same was not true for OATP2B1. These data suggest differential regulation of transporters in liver cirrhosis. We also found that the expression of hepatic transporters was cirrhosis type-dependent. For example, the expression of BCRP and OATP1B1 (normalized to gram of liver tissue) was reduced only in alcoholic cirrhosis livers and not in hepatitis C cirrhosis livers, while MATE1 and MRP2 expression was significantly altered only in hepatitis C cirrhosis livers. This difference could be due to different mechanisms of regulation of transporters in alcohol vs. hepatitis C virus cirrhosis. These findings suggest that the pharmacokinetics of

drugs, cleared by hepatobiliary transporters, will differ between alcoholic and hepatitis C cirrhosis patients. Therefore, we recommend that patients be classified into these two diseases when conducting pharmacokinetic studies in the clinic. Unfortunately, the current classification of Child-Pugh score does not distinguish between these two diseases.

The above interpretation of OATP1B1 protein expression data in the cirrhotic livers was not confounded by genotype. We found that neither the six OATP1B1 SNPs (rs4149015, rs2306283, rs4149056, rs4149057, rs2291075, and rs11045819) nor *SLCO1B1* haplotypes (1a/*1a, *1b/*1a, *14/*1a, and *15/*1a) significantly affected OATP1B1 protein expression in the cirrhotic livers. These findings differ from our previously published data in a larger number of control livers (Prasad et al., 2014), likely due to the smaller sample size of the cirrhotic livers.

Repaglinide is used for the treatment of type 2 diabetes. After oral administration, repaglinide is absorbed rapidly and eliminated predominantly by CYP3A4- and CYP2C8-mediated hepatic metabolism (Hatorp, 2002). Clinical studies have shown that OATP1B1 polymorphism significantly impacts the AUC of repaglinide (60-190% increase or 30% decrease, depending upon OATP1B1 genotype), indicating that OATP1B1-mediated transport is the rate-determining step for the hepatic uptake of repaglinide (Niemi et al., 2005; Zhang et al., 2006; Kalliokoski et al., 2008; Kalliokoski et al., 2010). The plasma AUC of repaglinide is increased by 4-fold in patients with cirrhosis (Hatorp et al., 2000). This increase may be due to a decrease in the expression of CYP3A4, 2C8, OATP1B1 or all three (Johnson et al., 2010). Therefore, in addition to changes in CYP expression due to cirrhosis, we investigated whether incorporation of cirrhosis-induced changes in OATP1B1 expression in the PBPK model improved the prediction of plasma concentration of repaglinide. Indeed it did (Fig. 4B). This demonstrates the potential

of incorporating transporter expression data in PBPK models to improve prediction of drug disposition in patients with cirrhosis.

We found significant correlation between protein expression of some of the hepatobiliary transporters (e.g. NTCP vs. OATP1B1; $r^2 > 0.5$) in both alcoholic and hepatitis C cirrhosis livers. This is in contrast to our findings in non-cirrhotic livers where this correlation was poor ($r^2 < 0.3$) or nonexistent (Prasad et al., 2014; Wang et al., 2015). These data suggest that cirrhosis may down-regulate the expression of multiple transporters resulting in a correlation when none existed in non-cirrhotic livers. Such correlation data are important when simulating the inter-individual variability in disposition of drugs within a population via PBPK modeling and simulation.

In summary, this is the first report to quantify the abundance of major hepatobiliary transporters in liver cirrhosis patients using quantitative proteomics. These transporter expression data should facilitate prediction of transporter-mediated drug disposition in the liver cirrhosis patients through PBPK modeling and simulation.

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Authorship contributions

Participated in research: Wang, Collins, Kelly, Chu, Ray, Salphati, Xiao, Lee, Lai, Liao, Mathias, Evers, Humphreys, Hop, and Unadkat

Conducted experiments: Wang and Kelly

Performed data analysis: Wang

Wrote the manuscript: Wang, Collins, Kelly, Chu, Ray, Salphati, Xiao, Lee, Lai, Liao, Mathias, Evers, Humphreys, Hop, and Unadkat

Contributed new reagents: Kumer

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Footnotes:

Chu, Ray, Salphati, Xiao, Lee, Lai, Liao, Mathias, Evers, Humphreys, and Hop contributed equally to the research. Li Wang's current address: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA. This work was supported by UWRAPT (University of Washington Research Affiliate Program on Transporters sponsored by Ardea Biosciences, Biogen, Bristol-Myers Squibb, Genentech, Gilead Sciences, Merck & Co., and Takeda (https://sop.washington.edu/department-of-pharmaceutics/research-affiliate-program-on-transporters-uwrapt). Cirrhotic human liver tissues were obtained through the Liver Tissue Cell Distribution System, Minneapolis, Minnesota, which was funded by NIH Contract [Grant HHSN276201200017C]

Legends for Figures

Figure 1. The yield of membrane protein from alcoholic cirrhosis (AL-C) and hepatitis C cirrhosis (HCV-C) livers was significantly lower than that from control livers. Horizontal line: median; +: mean; boxes: 25th-75th percentiles; whiskers: non-outlier range. ***: $p < 0.001$.

Figure 2. Hepatic uptake transporter protein expression in alcoholic cirrhosis (AL-C), and hepatitis C cirrhosis (HCV-C) livers differed from that in control livers. This difference was transporter- and disease-dependent and also depended on whether the data were normalized to milligram of membrane protein (upper panel) or gram of liver (lower panel). Horizontal line: median; +: mean; boxes: 25th-75th percentiles; whiskers: non-outlier range. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. MP: Membrane protein.

Figure 3. Hepatic efflux transporter protein expression in alcoholic cirrhosis (AL-C), and hepatitis C cirrhosis (HCV-C) livers differed from that in control livers. This difference was transporter- and disease-dependent and also depended on whether the data were normalized to milligram of membrane protein (upper panel) or gram of liver (lower panel). Horizontal line: median; +: mean; boxes: 25th-75th percentiles; whiskers: non-outlier range. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. MP: Membrane protein.

Figure 4. The Simcyp liver cirrhosis population PBPK module, which incorporates changes in physiology including CYP expression/activity due to cirrhosis but not changes in transporter expression, was used to predict repaglinide disposition in liver cirrhosis patients (A). Incorporation in the model of changes in OATP1B1 expression due to cirrhosis improved the prediction of repaglinide disposition in liver cirrhosis patients (B). The observed data are from Hatrop et al. (Hatorp et al., 2000).

Table 1. MRM parameters of peptides (calibrator and internal standard) selected for targeted analysis of human hepatobiliary transporters. The labeled amino acid residue of the internal standard is shown in bold and italic.

Transporter	Signature peptides	Parent Ion (z=2)	Product Ions (z=1)		Cone (V)	CE[1] (V)	RT[2] (min)	On-column calibration range (femtomole)[3]
			1	2				
BCRP	SSLLDVLAAR	523.0	757.4	644.3	40	18	21.0	2.76-88
	SSLLDVLAA***R***	527.8	654.5	767.5				
BSEP	STALQLIQR	515.2	529.1	657.1	32	18	15.4	2.92-93
	STALQLIQ***R***	520.3	539.4	667.4				
MATE1	GGPEATLEVR	514.7	816.9	688.0	30	14	12.2	3.46-111
	GGPEATLEV***R***	519.8	627.4	698.4				
MRP2	LTIIPQDPILFSGSLR	885.4	989.1	1329.7	30	26	22.2	2.29-73
	LTIIPQDPILFSGSL***R***	890.5	999.6	1339.7				
MRP3	ADGALTQEEK	531.1	634.3	747.3	30	16	8.2	1.96-125
	ADGALTQEE***K***	535.3	642.3	755.4				
MRP4	AEAAALTETAK	538.2	733.0	875.4	30	17	9.8	6.41-103
	AEAAALTETA***K***	542.3	741.4	883. 5				
NTCP	GIYDGDLK	440.7	547.1	710.1	30	13	12.5	3.42-219
	GIYDGDL***K***	444.7	555.3	718.3				
OATP1B1	NVTGFFQSFK	587.9	860.5	961.4	35	17	20.1	1.33-85
	NVTGFFQSF***K***	591.9	868.5	969.5				
OATP1B3	IYNSVFFGR	551.8	826.5	526.6	30	15	18.0	1.31-84
	IYNSVFFG***R***	556.8	635.2	836.6				
OATP2B1	VLAVTDSPAR	514.8	646.3	816.4	30	16	11.2	1.56-100
	VLAVTDSPA***R***	519.9	656.3	826.4				
OCT1	LSPSFADLFR	576.7	476.6	768.0	25	23	21.9	1.38-88
	LSPSFADLF***R***	581.8	778.4	865.4				
P-gp	NTTGALTTR	467.6	618.3	719.4	30	16	8.4	1.50-96
	NTTGALTT***R***	472.6	628.3	729.5				

1 CE: Collision energy; [2] RT: Retention time; **3** The low end of this range reflects the lower limit of quantification of the assay

Table 2. Demographics of liver tissue donors.

	Alcoholic cirrhosis	Hepatitis C cirrhosis	Control
Sample size	27	30	36
Age (year)	55±8	53±8	47±14
Sex	Male: 25; Female: 2	Male: 18; Female: 12	Male: 18; Female: 18
Race	C[1]: 25; AI[2]: 1; NR[3]: 1	C: 25; AA[4]: 4; H[5]: 1	C: 33; AA: 2; A[6]: 1
Albumin (g/dL)	2.9±0.6	2.6±0.6	NR
Alkaline phosphatase (units per liter)	171±96	112±54	NR
Alanine transaminase (units per liter)	NR	146±243	NR
Aspartate aminotransferase (units per liter)	219±550	223±388	NR
Creatine (mg/dL)	1.6±0.7	NR	NR
INR	2.6±1.1	1.7±0.5	NR
Total bilirubin (mg/dL)	9.5±8.9	3.6±2.9	NR

[1]C: Caucasian; [2]AI: American Indian; [3]NR: Not reported; [4]AA: African American; [5]H: Hispanic; [6]A: Asian.

Table 3. Frequency of OATP1B1SNPs detected in the alcoholic and hepatitis C cirrhosis human liver tissue

Marker ID	Variant	Change for variant	Frequency (n)		
			Wild-type	Heterozygous variant	Homozygous variant
Alcoholic cirrhosis					
rs4149015	-11187G>A	Promoter	23	4	0
rs2306283	388A>G	N130D	12	12	3
rs4149056	521T>C	V174A	19	7	1
rs4149057	571T>C	L191L	6	10	10
rs2291075	597C>T	F199F	10	13	3
rs11045819	463C>A	P155T	23	4	0
Hepatitis C cirrhosis					
rs4149015	-11187G>A	Promoter	24	6	0
rs2306283	388A>G	N130D	7	14	9
rs4149056	521T>C	V174A	25	5	0
rs4149057	571T>C	L191L	10	12	8
rs2291075	597C>T	F199F	8	14	8
rs11045819	463C>A	P155T	22	7	1

Figure 1

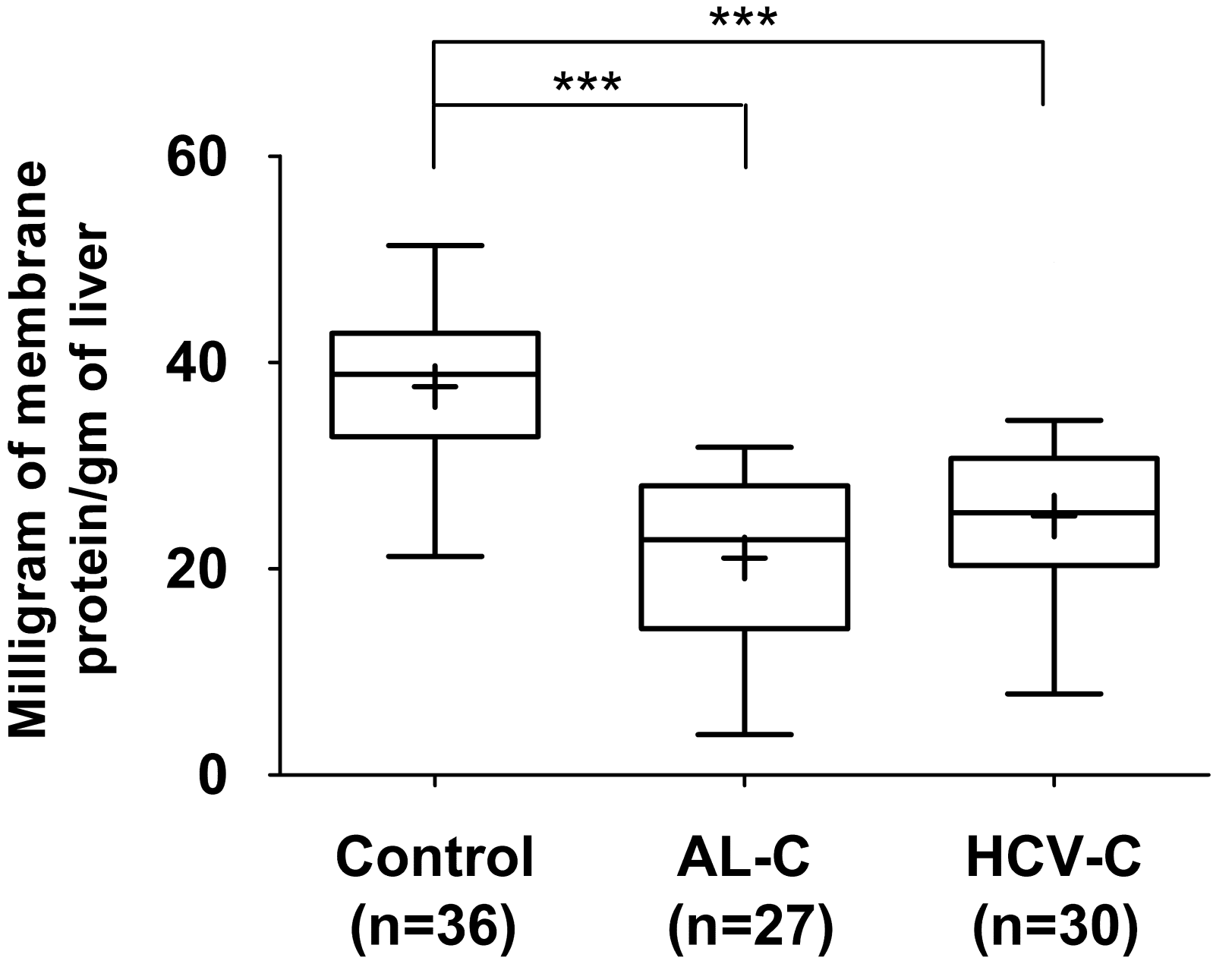

***
***
60
40
20
0
Milligram of membrane protein/gm of liver
Control (n=36)
AL-C (n=27)
HCV-C (n=30)

Figure 2

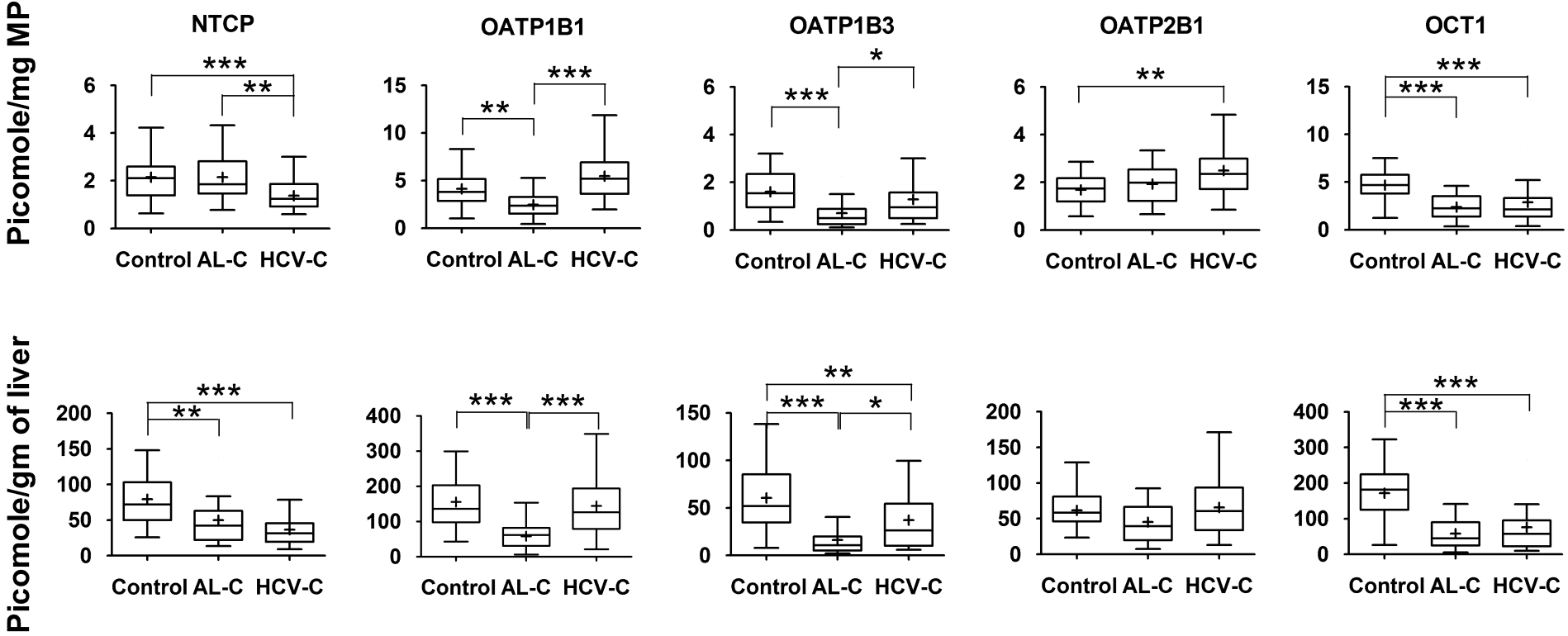

Picomole/mg MP
NTCP
OATP1B1
OATP1B3
OATP2B1
OCT1
Control AL-C HCV-C
Picomole/gm of liver

Figure 3

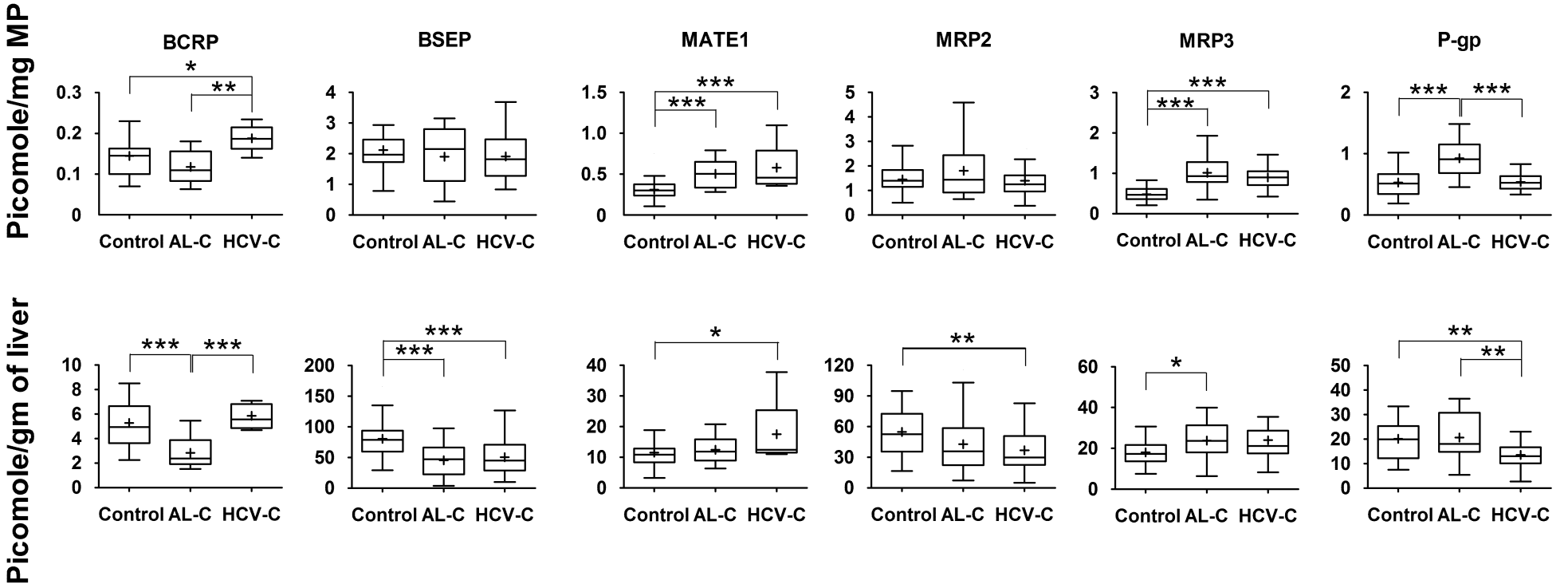
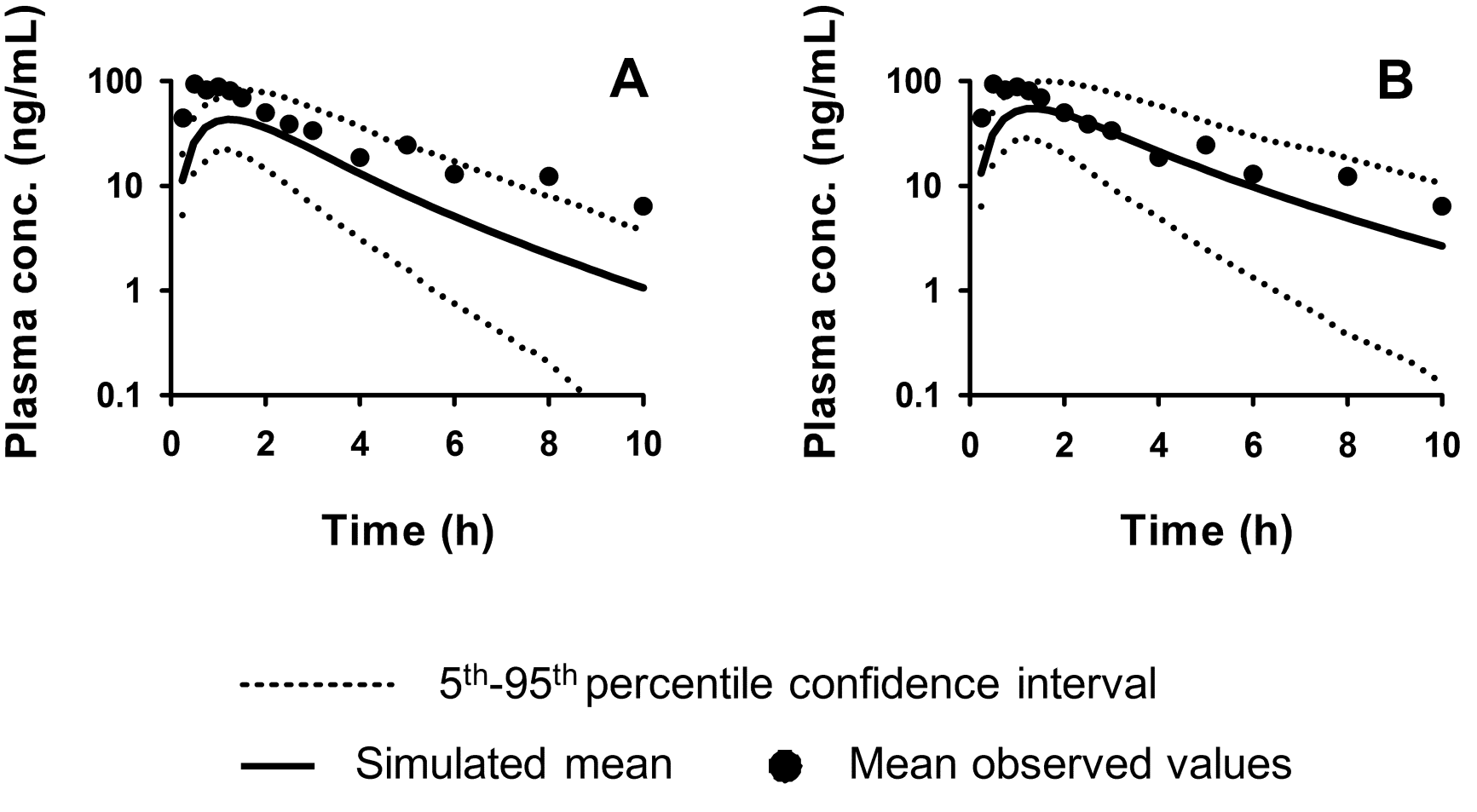

Picomole/mg MP
Picomole/gm of liver
BCRP
BSEP
MATE1
MRP2
MRP3
P-gp
Control AL-C HCV-C

Figure 4


A
B
Plasma conc. (ng/mL)
100
10
1
0.1
0
2
4
6
8
10
Time (h)
5th-95th percentile confidence interval
Simulated mean
Mean observed values