# Comparison of *In Vitro–In Vivo* Extrapolation of Biliary Clearance Using an Empirical Scaling Factor Versus Transport-Based Scaling Factors in Sandwich-Cultured Rat Hepatocytes

PENG ZOU,[1] XINGRONG LIU,[2] SUSAN WONG,[2] MEIHUA ROSE FENG,[1] BIANCA M. LIEDERER[2]

[1]Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, Michigan 48109

[2]Genentech, Inc., Drug Metabolism and Pharmacokinetics, South San Francisco, California 94080

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**ABSTRACT:** Biliary clearance ($CL_b$) is often underestimated by *in vitro–in vivo* extrapolation from sandwich-cultured hepatocytes (SCHs). The objective of this study was to compare the performance of a universal correction factor with transporter-based correction factors in correcting underestimation of $CL_b$. The apparent *in vitro* $CL_b$ of a training set of 21 compounds was determined using the SCH model and extrapolated to apparent *in vivo* $CL_b$ ($CL_{b,\,app}$). A universal correction factor (10.2) was obtained by a linear regression of the predicted $CL_{b,\,app}$ and observed *in vivo* $CL_b$ of training set compounds and applied to an independent test set ($n = 20$); the corrected $CL_b$ predictions of 13 compounds were within twofold error of observed values. Furthermore, two transporter-based correction factors (Organic anion transporting polypeptides/multidrug-resistance-related protein 2 and diffusion/P-glycoprotein) were estimated by linear regression analysis of training set compounds. The applications of the two correction factors to the test set resulted in improved prediction precision. In conclusion, both the universal correction factor and transporter-based correction factors provided reasonable corrections of $CL_b$ values, which are often underestimated by the SCH model. The use of transporter-based correction factors resulted in an even greater improvement of predictions for compounds with intermediate-to-high $CL_b$ values. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2837–2850, 2013

**Keywords:** biliary excretion; hepatocytes; clearance; drug transport; hepatobiliary disposition; *in vitro/in vivo* correlations (IVIVC); organic aniontransporting polypeptide transporters; P-glycoprotein

## INTRODUCTION

The accurate prediction of hepatic clearance ($CL_H$) is an essential step in the identification of new chemical entities as drug candidates and in the estimation of human pharmacokinetics. $CL_H$ is determined by hepatic metabolism and biliary excretion. Reasonable prediction accuracy of $CL_H$ for compounds with high hepatic metabolism has been achieved by interspecies scaling and *in vitro–in vivo* extrapolation (IVIVE) from liver microsome or hepatocyte incubations.[1,2] By contrast, the quantitative prediction of $CL_H$ is still very challenging for compounds with low hepatic metabolism and high biliary excretion.[2]

*In vitro–in vivo* extrapolation from sandwich-cultured hepatocytes (SCHs) is an approach to estimating biliary clearance ($CL_b$); however, low bile or blood flow and the variable expression levels of influx and/or efflux transporters can result in a 10–100-fold underestimation of $CL_b$ in IVIVE from SCH.[3,4] Several reports reveal that the expression levels and activities of influx transporters such as Oatps, Ntcp, and Oct1 in rat hepatocytes were consistently and considerably reduced in the SCH model,[5–7] thus leading to underestimation of $CL_b$. Additionally, the expression levels of canalicular efflux transporters are inconsistent between different laboratories. Li et al.[8] reported a 40% decrease in the bile salt export pump (Bsep) protein level, a 50% decrease in the multidrug-resistance-related protein 2 (Mrp2) protein level, and

Additional Supporting Information may be found in the online version of this article. Supporting Information

*Correspondence to*: Bianca M. Liederer (Telephone +650-467-7343; Fax: 650-225-6452; E-mail: liederer.bianca@gene.com)

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a fivefold increase in the breast cancer resistance protein (Bcrp) level in sandwich-cultured rat hepatocytes (SCRHs) over 5 days in culture. Tchaparian et al.[7] observed dramatically increased protein levels of P-glycoprotein (P-gp), Bcrp, and Mrp 1, 2, 3, and 4 over 4 days in culture. In Borlak's report,[5] the expression levels of canalicular transporters P-gp and Mrp2 were similar to those determined *in vivo*.

One strategy for correcting the underestimation of $CL_b$ is to incorporate a universal empirical correction factor determined by correlating $CL_b$ predicted from SCRHs with the observed *in vivo* $CL_b$.[9] A reasonable correlation between $CL_b$ predicted by SCRHs and *in vivo* $CL_b$ was observed among drugs that undergo similar uptake (Organic anion transporting polypeptides, Oatps) and efflux mechanisms (Mrp2 or Bcrp) such as angiotensin II receptor blockers, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)reductase inhibitors, and β-lactam antibiotics.[9–11] On the contrary, for compounds in which different combinations of influx and efflux transporters are involved in the biliary excretion, or when passive diffusion is involved, the universal correction factor might not work. To address this issue, Li et al.[4] proposed using the ratio of the protein amount of canalicular efflux transporters (Mrp2, Bsep, and Bcrp) in rat liver to that in SCRH as a correction factor. Improved prediction accuracy was achieved by this method; however, this strategy ignored the decreased expression levels of sinusoidal influx transporters in SCRHs, which might seriously affect the predictability of the SCRH model.

In the current study, a universal correction factor and a series of correction factors based on the activities of both influx and efflux transporters were examined for IVIVE of $CL_b$. A universal correction factor was estimated by linear regression analysis of 21 compounds in a training set. To determine whether transporter-specific correction factors can improve IVIVE, the compounds in the training set were divided into transporter-specific subgroups based on uptake and excretion mechanisms. Although both the universal correction factor and the transporter-specific correction factors provided reasonable $CL_b$ predictions when applied to the independent test set; the use of transporter-specific correction factors resulted in a greater improvement of prediction precision.

## MATERIALS AND METHODS

### Chemicals

Topotecan, benazeprilat, rosuvastatin, rosuvastatin-d6, candesartan, atorvastatin, olmesartan, deferasirox, temocaprilat, irinotecan, and octreotide acetate were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Trovafloxacin and fluvastatin were obtained from Torcris Bioscience (Ellisville, Missouri) and Cayman Chemical Company (Ann Arbor, Michigan), respectively. Probenecid was supplied by Santa Cruz Biotechnology (Santa Cruz, California). [$^3$H]Taurocholate (5 Ci/mmol; purity >97%) was purchased from PerkinElmer Life and Analytical Sciences, Inc. (Waltham, Massachusetts). All other chemicals were purchased from Sigma–Aldrich (St. Louis, Missouri).

### Metabolic Stability Assay

Cryopreserved rat hepatocytes (Celsis IVT, Baltimore, Maryland) were used for the hepatocyte stability assay. The cryopreserved hepatocytes were thawed in InVitroGRO$^{TM}$ HT (Celsis IVT, Baltimore, Maryland) medium and centrifuged at 100*g* for 10 min. Cells were resuspended in Dulbecco's Modified Eagle Medium (DMEM) (Gibco®, Grand Island, New York), and viability was assessed by the trypan blue exclusion method. Hepatocytes with viability greater than 80% were used in the study. The compounds (1 μM in DMEM) were incubated with hepatocytes ($0.5 \times 10^6$ cells/mL) in a $CO_2$ incubator with 95% air/5% $CO_2$ at 37°C and 95% humidity. At 0, 10, 30, and 60 min, aliquots of the incubation mixture were taken out and the reaction was quenched by adding two volumes of acetonitrile containing 100 nM of rosuvastatin-d6. The mixtures were then centrifuged at 2095*g* for 10 min to precipitate the protein. The supernatants were diluted with two volumes of water and transferred into a 96-well assay plate to measure the disappearance of parent compounds by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The predicted hepatic metabolic clearance ($CL_{met}$) was calculated using the following previously reported equation[2]:

$$CL_{met} = (Q_P \times f_u \times CL_{int})/(Q_P + f_u \times CL_{int}) \quad (1)$$

where $Q_p$ represents the rat hepatic plasma flow rate (40 mL*min$^{-1}$*kg$^{-1}$), $CL_{int}$ represents the intrinsic clearance, and $f_u$ represents the unbound fraction in rat plasma, which was collected from the literature (Table 1).

### Hepatocyte Culture

Sandwich-cultured rat hepatocytes (B-CLEAR®), which were isolated from male Wistar rats and cultured in 24-well plates, were purchased from Qualyst, Inc. (Durham, North Carolina). Hepatocytes were cultured in the medium provided by Qualyst, Inc. at 37°C in a humidified incubator with 95% air/5% $CO_2$. Medium was changed daily. On day 4 after hepatocyte seeding, the SCRHs were subjected to accumulation studies.

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