RAPID COMMUNICATION

Prediction of Total Hepatic Clearance by Combining Metabolism, Transport, and Permeability Data in the *In Vitro–In Vivo* Extrapolation Methods: Emphasis on an Apparent Fraction Unbound in Liver for Drugs

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ABSTRACT: Poulin and coworkers recently proposed in three manuscripts (2012. J Pharm Sci 101:838–851; 2012. J Pharm Sci 101:4308–4326; and 2013. J Pharm Sci 102:in press) a novel *in vitro*-to-*in vivo* extrapolation (IVIVE) method for clearance (CL) involving estimation of apparent fraction unbound in liver (fu_{liver}) based on albumin-facilitated hepatic uptake and correction of unbound drug according to the ionization fraction either side of the plasma membrane. This novel IVIVE method has improved the prediction accuracy, and, hence, reduced the bias in predicting hepatic metabolic CL referring to plasma kinetics of several acidic/neutral drugs in preclinical species and humans either based on microsomal or hepatocyte data. So far, the prediction performance of this novel IVIVE method has been assessed for metabolic CL only. Because CL might also be governed by transporters effect and/or permeation limitation in addition to metabolism, an extension of the proposed IVIVE method from metabolic data to transport and permeability data was necessary. Therefore, it was assumed that the concept should also work for multiple CL processes predictions because it is applicable as long as the drug gets to the hepatocyte cell surface. In this study, the proposed IVIVE method was assessed using a large database of predictions of total hepatic CL in rats and humans by combining *in vitro* hepatocyte data on metabolism, transport, and permeability. The proposed IVIVE method is similarly effective in minimizing average prediction bias and improves accuracy unlike other IVIVE methods. Overall, the present study confirms the utility of the novel IVIVE method for predicting total hepatic CL of drugs under *in vivo* conditions either by considering metabolism data only or combining metabolism with transporter and permeability data. This study will facilitate the predictions of total hepatic CL in physiologically based pharmacokinetics (PBPK) model. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: hepatocytes; intrinsic clearance; unbound fraction; metabolism; transport; permeability; *in vitro–in vivo* extrapolation; *in vitro–in vivo* correlation; IVIVE; pharmacokinetics; physiologically based pharmacokinetics; PBPK modeling

INTRODUCTION

Poulin et al.[1–3] have argued that a difference in drug ionization and binding proteins in plasma and whole liver need to be considered in the *in vitro*-to-*in vivo* extrapolation (IVIVE) procedures. These authors have extended IVIVE knowledge by converting the fraction unbound in plasma (fu_p) to apparent fraction unbound in whole liver (fu_{liver}) to improve the predictions

Abbreviations used: AAG, alpha1-acid glycoprotein; AFE, average fold error; AL, albumin; CCC, concordance correlation coefficient global; CL, clearance; CL_{int}, intrinsic clearance; $CL_{int,app}$, intrinsic clearance apparent; $CL_{int,met}$, intrinsic clearance for metabolism; $CL_{int,inf}$, intrinsic clearance for active influx; $CL_{int,eff}$, intrinsic clearance for active efflux; $CL_{int,pass}$, intrinsic clearance for passive diffusion; $CL_{int,sec}$, intrinsic clearance for bile secretion; fu_{cells}; fraction unbound in cells, fu_{inc}, unbound fraction in incubation medium; fu_{liver}, unbound fraction in liver apparent; fu_p, unbound fraction in plasma; IVIVE, *in vitro–in vivo* extrapolation; Q_{liver}, blood flow rate to liver; SF, scaling factor; R_{BP}, blood–plasma ratio; RMSE, root mean squared error.

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of hepatic clearance (CL) of drugs by using common liver models (i.e., well-stirred or parallel tube). The only intention was to adjust the value of fu_p commonly used in the liver prediction models. In other words, we have extended knowledge by factoring in events occurring at the hepatocyte cell surface that promote drug uptake. The rational is that under *in vivo* conditions more unbound drug to the hepatocyte cell membranes is expected due to potential protein-facilitated uptake mechanisms and drug ionization effect. First, potential ionic interactions between the protein-bound drug complex and hepatocyte cell surface may enhance dissociation and facilitate the release of the bound ligand effectively supplying more unbound drug to the cell membrane for uptake, and hence, hepatic uptake (or intracellular concentration) is greater than expected compared with when the fraction unbound in plasma is considered. In other words, the reservoir of drugs in liver would increase. This process was observed particularly for the plasma protein charged positively at the physiological pH, the albumin (AL), compared with alpha1-acid glycoprotein (AAG), probably because the hepatocyte cell membranes are rich in negatively charged phospholipids to which AL may interact.[4,5] Furthermore, this concept should be applicable when no AL or a little amount of AL is added in the incubation mediums. Second, in the *in vitro* experiments used for plasma protein binding estimates excess of buffer at pH 7.4 was used. In contrast, under *in vivo* conditions there is a pH gradient between the intracellular and extracellular aqueous phases. Therefore, the *in vitro* fu_p value was adjusted for drug ionization effect potentially occurring in liver under *in vivo* conditions compared with *in vitro* in plasma. Accordingly, an apparent fu_{liver} that corrects the *in vitro* fu_p value for both the protein-facilitated uptake and drug ionization effect was used in common liver models to scale the intrinsic metabolic clearance ($CL_{int,met}$), and, hence, a novel mechanistic IVIVE method was proposed.[1–3]

The concept of apparent fu_{liver} is in accordance with a recent study that had highlighted various examples from the literature where tissue unbound drug concentrations have demonstrated a superior correlation, at least with regard to efficacy, compared with the plasma unbound drug concentrations.[6] Therefore, it appears that referring only to the fraction unbound in plasma for predicting some biological processes in tissues might not be always valid. Thus, the novel IVIVE based on fu_{liver} showed a significant improvement in the predictions of metabolic CL of drugs over other IVIVE methods either derived empirically or mechanistically.[1–3,7] In addition, the novel IVIVE method is based on a mechanistic framework, and uses solely *in vitro* input data compared with previous empirical methods that need *in vivo* data first for their development;[7,8] this novel mechanistic framework based on *in vitro* data is exactly the kind of predictive approach that is needed in early drug discovery and development.

So far, the use of apparent fu_{liver} in IVIVE's was validated with drugs for which liver metabolism was the main route of elimination. However, the concept of fu_{liver} should also work for drugs being transported into the hepatocytes and/or the distribution is permeability limited, as long as the drug gets into the cells either by a protein-facilitated uptake process or not. Therefore, we hypothesize that the role of protein-facilitated uptake should also be factored for drugs for which hepatic CL is determined by multiple processes in liver. Indeed, there are several cases where the metabolic CL_{int} *in vitro* does not accurately predict the *in vivo* result.[8,9] The objective of this study was to verify if the novel concept of apparent fu_{liver} also works when *in vitro* hepatocyte data on metabolism, transport, and permeability are all taken into account for total hepatic CL predictions referring to plasma kinetics.

METHOD

The method consisted of comparing total hepatic CL values observed *in vivo* in rats and humans with predicted CL derived from *in vitro* hepatocyte data on metabolism, transport, and permeability for several drugs. The drugs studied were substrates for several transporters. Here, the novel IVIVE method of Poulin et al.[1–3] was compared with three other IVIVE methods commonly used in the literature,[8–11] namely, the conventional and empirical methods. Therefore, a total of four IVIVE methods were challenged in this study by using the same drug datasets. Finally, we present a sensitivity analysis to demonstrate the relationships between fu_p *in vitro* and apparent fu_{liver} estimated for the *in vivo* conditions for various classes of drugs to show how the different IVIVE methods vary with the fractions unbound used as input parameters.

Comparative Analysis of IVIVE Methods

The IVIVE methods used in this study for the prediction of hepatic metabolic CL referring to plasma kinetics are described by the following equation based on the well-stirred liver model[1–3]:

$$\mathrm{CL} = \frac{Q_{\mathrm{liver}} R_{\mathrm{BP}} \mathrm{CL}_{\mathrm{int,met}} \mathrm{CF}}{Q_{\mathrm{liver}} R_{\mathrm{BP}} + \mathrm{CL}_{\mathrm{int,met}} \mathrm{CF}} \quad (1)$$

where Q_{liver} is the liver blood flow rate [65 mL/(min kg) for rats and 20.7 mL/(min kg) for humans], R_{BP} is the blood–plasma ratio, and CF is the correction factor equaling to (1) the ratio fu_p/fu_{inc} (where fu_{inc} is the fraction unbound in the incubation medium) for the conventional method, (2) the ratio fu_{liver}/fu_{inc} for

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