In Vitro–In Vivo Extrapolation of Clearance: Modeling Hepatic Metabolic Clearance of Highly Bound Drugs and Comparative Assessment with Existing Calculation Methods

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ABSTRACT: *In vitro–in vivo* extrapolation (IVIVE) is an important method for estimating the hepatic metabolic clearance (CL) of drugs. This study highlights a problematic area observed when using microsomal data to predict *in vivo* CL of drugs that are highly bound to plasma proteins, and further explores mechanisms for human CL predictions by associating additional processes to IVIVE disconnect. Therefore, this study attempts to develop a novel IVIVE calculation method, which consists of adjusting the binding terms in a well-stirred liver model. A comparative assessment between the IVIVE method proposed here and previously published methods of Obach (1999. Drug Metab Dispos 27:1350–1359) and Berezhkovskiy (2010. J Pharm Sci 100:1167–1783) was also performed. The assessment was confined by the availability of measured *in vitro* and *in vivo* data in humans for 25 drugs highly bound to plasma proteins, for which it can be assumed that metabolism is the major route of elimination. Here, we argue that a difference in drug ionization and binding proteins such as albumin (AL) and alpha-1-acid glycoprotein (AAG) in plasma and liver also needs to be considered in IVIVE based on mechanistic studies. Therefore, converting unbound fraction in plasma to liver essentially increased the predicted CL values, which resulted in much more accurate estimates of *in vivo* CL as compared with the other IVIVE methods tested. The impact on CL estimate was more apparent for drugs binding to AL than to AAG. This is a mechanistic rational for explaining a considerable proportion of the divergence between previously estimated and observed CL values. Human CL was predicted within 1.5-fold, twofold, and threefold of the observed CL for 84%, 96%, and 100% of the compounds, respectively. Overall, this study demonstrates a significant improvement in the mechanism-based prediction of metabolic CL for these 25 highly bound drugs from *in vitro* data determined with microsomes, which should facilitate the application of physiologically based pharmacokinetic (PBPK) models in drug discovery and development. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:838–851, 2012

Keywords: disposition; microsomes; hepatic clearance; metabolic clearance; unbound fraction; computational ADME; *in vitro–in vivo* extrapolation; IVIVE; pharmacokinetics; PBPK modeling

Abbreviations used: AL, albumin; AAG, alpha-1-acid glycoprotein; AFE, average fold error; AAFE, absolute average fold error; CL, clearance (referring to plasma kinetics); CL_{int}, intrinsic clearance; CCC, concordance correlation coefficient; fu_p, unbound fraction in plasma; $fu_{p\text{-}app}$, unbound fraction in plasma apparent; fu_{liver}, unbound fraction in liver; fu_{inc}, unbound fraction in incubations; $f_{unionized}$, fraction of drug unionized; F_I, ionization factor; IVIVE, *in vitro–in vivo* extrapolation; K_m, Michaelis–Menten affinity constant; pI, isoelectric point; PLR, plasma-to-liver concentration ratio; Q_{liver}, blood flow rate to liver, R_{BP}, blood-to-plasma concentration ratio; RMSE, root mean squared error; r, coefficient of correlation.

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INTRODUCTION

Prediction of hepatic metabolic clearance (CL) is important because it provides insight into the rate of elimination of drugs from the body and allows for a physiological interpretation of the potential route(s) of elimination and the magnitude of oral first-pass elimination for a candidate drug. Therefore, CL is an important parameter in selecting the size of the dose, and along with volume of distribution, it determines the half-life and therefore the frequency of dosing.[1] Although it is convenient to use *in vitro* data in drug discovery, *in vitro–in vivo* extrapolation (IVIVE) methods are often used to scale-up the *in vitro* intrinsic CL (CL_{int}) data from human liver preparations for predicting *in vivo* CL of drugs that are mainly eliminated by metabolism. Commonly used *in vitro* systems for the determination of CL_{int} include microsomal incubations, hepatocyte suspensions, and plated hepatocytes.[2–8]

The central issue is whether the traditional assumption for drug access to the hepatocytes holds true for IVIVE. This assumption is that equilibrium between the free and protein-bound drug is instantaneous, such that the metabolism process is driven by a constant supply of unbound drug concentration in plasma.[1–9] The equilibrium model generally refers to a well-stirred or parallel tube. There is evidence that drug extraction from the liver is sensitive to changes in the plasma protein binding, in particular at very low free fraction values.[9] However, the role and definition of unbound fraction in plasma (fu_p) has long been a subject without a clear consensus because the kinetics of plasma protein binding under *in vivo* conditions is not fully understood, especially for highly bound drugs. As a result, it has been questioned whether CL can be predicted from *in vitro* parameters in the absence of *in vivo* measurements. Indeed, commonly reported discrepancies in IVIVE calculations include systematic underestimation and overestimation of *in vivo* CL when binding corrections measured *in vitro* (e.g., fu_p) and no binding corrections (i.e., direct scaling), respectively, are used, particularly for highly bound drugs.[2,4,5,8,10] Among the most probable reasons are the omissions of extrahepatic metabolism, significant errors in experimental assessment of fu_p, as well as CL_{int} and/or inappropriate estimations of unbound drug available for metabolism in liver. The latter is the most commonly referenced reason because many studies have reported greater drug uptake into liver than that predicted based upon the existing models using free fraction of drug in arterial serum (i.e., fu_p).[3–12]

Of the many studies that investigated this aspect, the ones investigating the impact of experimental settings on CL estimates seem to be of most importance. It has been observed that less accurate CL predictions were obtained with plasma-free and microsomal incubations as compared with those from incubations using plasma.[2,5,8] Blanchard et al.[3–5] and Chao et al.[6,7] assumed that adding plasma to the *in vitro* incubations would better mimic the protein binding/uptake/metabolism interplay that happens under dynamic *in vivo* conditions. Indeed, Blanchard et al.[5] determined CL_{int} using hepatocyte incubations containing undiluted plasma and consequently incorporated the resulting apparent CL_{int} into the well-stirred model. These authors observed that the *in vivo* CL values predicted with these experimental settings may increase up to 18-fold as compared with plasma-free incubations. Furthermore, such settings significantly reduced the systematic bias in the estimation of CL and provided a better correlation between the predicted and observed CL values for several highly bound drugs.[5] Berezhkovskiy et al.[12] suggested that the improvements could be due to the direct account of plasma protein binding in the incubation medium. These authors presented a mathematical exercise that assumed that the values of fu_p and hence the predicted CL values would increase only when the plasma in the incubations is diluted (i.e., fu_p would increase du to the impact of dilution). However, the calculations of Berezhkovskiy et al.[12] (diluted plasma) and observations of Blanchard et al.[5] (undiluted plasma) are not in total accordance.

The available evidence on the role of extracellular binding proteins on liver metabolism supports the notion that ionic interactions between the protein—drug complex and hepatocyte cell surface would supply more unbound drug to the cell membrane, and hence this should result in a greater uptake (or intracellular concentration) than that predicted based on the free concentration in plasma.[13] In other words, we should observe an enhanced uptake of the drug by passive diffusion (or transport processes), and hence a greater than expected CL_{int} and metabolism *in vivo*. In this context, Burczynski et al.[13] observed that the CL of palmitate, a compound highly bound to albumin (AL), was significantly larger in the presence of AL in incubations as compared with when no AL was added, which are in accordance with Blanchard et al.[5] Related to this, other studies seem to indicate that adding AL in the incubation medium may result in greater nonspecific binding of the AL–drug complex as compared with controls because smaller Michaelis–Menten affinity constant (K_m) values were observed.[14,15] Thus, these observations might result in whole-liver fu (fu_{liver}) significantly larger than fu_p under *in vivo* conditions, and hence explain why the consideration of protein binding inside the whole-liver compartment could be important for more accurate IVIVE calculations of drugs eliminated principally by metabolism.

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