Hepatocyte Composition-Based Model as a Mechanistic Tool for Predicting the Cell Suspension: Aqueous Phase Partition Coefficient of Drugs in *In Vitro* Metabolic Studies

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ABSTRACT: This study is an extension of a previously published microsome composition-based model by Poulin and Haddad (Poulin and Haddad. 2011. J Pharm Sci 100:4501–4517), which was converted to the hepatocyte composition-based model. The first objective was to investigate the ability of the composition-based model to predict nonspecific binding of drugs in hepatocytes suspended in the incubation medium in *in vitro* metabolic studies. The hepatocyte composition-based model describes the cell suspension–aqueous phase partition coefficients, which were used to estimate fraction unbound in the incubation medium (fu_{inc}) for each drug. The second objective was to make a comparative analysis between the proposed hepatocyte composition-based model and an empirical regression equation published in the literature by Austin et al. (Austin RP, Barton P, Mohmed S, Riley RJ. 2004. Drug Metab Dispos 33:419–425). The assessment was confined by the availability of experimentally determined *in vitro* fu_{inc} values at diverse hepatocyte concentrations for 92 drugs. The model that made use of hepatocyte composition data provides comparable or superior prediction performance compared with the regression equation that relied solely on physicochemical data; therefore, this demonstrates the ability of predicting fu_{inc} also based on mechanisms of drug tissue distribution. The accuracy of the predictions differed depending on the class of drugs (neutrals vs. ionized drugs) and species (rat vs. human) for each method. This study for hepatocytes corroborates a previous study for microsomes. Overall, this work represents a significant first step toward the development of a generic and mechanistic calculation method of fu_{inc} in incubations of hepatocytes, which should facilitate rational interindividual and interspecies extrapolations of fu_{inc} by considering differences in lipid composition of hepatocytes, for clearance prediction in the physiologically-based pharmacokinetics (PBPK) models. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2806–2818, 2013

Abbreviations used: AFE, average fold error; C_h, hepatocyte concentrations; CL, clearance; CL_{int}, intrinsic clearance; CCC, concordance correlation coefficient global; D_{ow}, *n*-octanol–buffer partition coefficient considering drug ionization; F_{wm}, fractional volume of water equivalent in the cell suspension of the incubation medium; F_{we}, fractional content of water equivalent in the erythrocytes; F_{nlm}, fractional volume of neutral lipids equivalent in the cell suspension of the incubation medium; F_{prm}, fractional volume content of protein equivalent in the cell suspension of incubation medium; F_{nle}, fractional content of neutral lipid equivalent in the erythrocyte; F_{aplm}, fractional volume content of acidic phospholipids equivalent in the cell suspension of the incubation medium; F_{aple}, fractional volume content of acidic phospholipids equivalent in the erythrocytes; fu_{inc}, unbound fraction in the incubation medium; fu_p, unbound fraction in plasma; HCM, hepatocyte composition-based model; I_m, ionization term for the incubation medium; I_e, ionization term for the erythrocyte; I_p, ionization term for the plasma; IVIVE, *in vitro–in vivo* extrapolation; P_{csa}, cell suspension–aqueous phase partition coefficient in the incubation medium; P_{nla}, neutral lipid–aqueous phase partition coefficient in the incubation medium; P_{apla}, acidic phospholipid–aqueous phase partition coefficient in the incubation medium; P_{ea}, erythocyte–aqueous phase partition coefficient; P_{pra}, protein–aqueous phase partition coefficient in the incubation medium; P_{ow}, *n*-octanol–buffer partition coefficient; pK_a, ionization constant; RBP, blood–plasma ratio *in vitro*; RMSE, root-mean-square error.

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INTRODUCTION

Although it is convenient to use *in vitro* data in drug discovery, *in vitro–in vivo* extrapolation (IVIVE) methods are used to scale up the *in vitro* intrinsic clearance (CL_{int}) obtained from incubated liver preparations for predicting *in vivo* hepatic clearance (CL) of drugs. For more accurate IVIVE, the unbound fraction in the incubation medium (fu_{inc}) should be taken into account in the upscaling process of the CL_{int} determined *in vitro*.[1–6] In the *in vitro* binding studies, the drug in the incubation medium (e.g., microsomal or hepatocyte preparations) distributes among diverse phases (i.e., lipid, macromolecules, and water), which should also be the case under *in vivo* conditions. Recently, a microsome composition-based model was developed to predict fu_{inc} in microsomal incubations based on the corresponding lipid composition and some readily available physicochemical properties.[7] The proposed microsome composition-based model can be viewed as a combination of two distinct processes, namely the nonspecific hydrophobic binding to neutral lipids and the ionic binding to acidic phospholipids. On the basis of the comparisons made using several rat and human datasets, the mechanistic model that made use of microsome composition data compares well with those empirical regression equations that relied solely on physicochemistry.[7] Because fu_{inc} obtained in hepatocytes in suspension is also an important parameter generated in drug discovery for CL prediction, an extension of the recently published composition-based model from microsomal data to hepatocyte data was necessary.

The purpose of this study was to investigate the ability of the composition-based model to predict fu_{inc} in incubations made from rat and human hepatocytes in *in vitro* metabolic studies. The second objective was to make a comparative assessment between the hepatocyte composition-based model proposed in this study and an existing regression equation published in the literature.[8]

METHODS

The overall strategy is divided in two steps. The first step consists of adjusting a recently published microsome composition-based model[7] to include lipid composition data for the hepatocytes incubations instead. The second step compares the prediction performance of the proposed mechanistic method with an empirical regression equation published in the literature by Austin et al.[8]

Datasets

Data for several structurally unrelated drugs were collected for which the input parameters for binding and physicochemistry are experimentally determined and available in the literature, bringing the total number of drugs studied to 92 (25 acids, 37 neutrals, 30 bases).[1–20] The hepatocyte binding data in rats (Sprague–Dawley) and humans were compiled from several sources and methods: viable suspended hepatocytes using oil centrifugation (using live cells), dialysis (using dead cells), and ultrafiltration (using dead cells).[8–13] The value of fu_{inc} represents the ratio of drug concentrations between the aqueous phase of the incubation medium (free drug) and hepatocyte binding in cell suspension (total drug). When more than one measured fu_{inc} value was available in the literature for a drug, all values were reported in this study; therefore, the mean observed fu_{inc} value was used for comparison with the calculated value from each prediction method.

Modeling Assumptions

Compounds were divided into acidic, basic, and neutral classes according to differences in mechanisms of tissue distribution among the classes of drugs.[7] It was assumed that distribution of the current drugs into the incubated hepatocytes was not impeded by limited diffusion and/or transport processes in the binding studies as the *in vitro* experiments were conducted at equilibrium conditions. Therefore, the nonspecific binding to lipids is the main mechanism governing *in vitro* fu_{inc} for each drug assuming concentration independency of fu_{inc} as explained below. The other modeling assumptions are the same as previously published by Poulin and Haddad.[7] The datasets are compiled in Table 1.

Development of the Hepatocyte Composition Based Model for Prediction of fu_{inc}

The only difference between a previously published microsome composition-based model[7] and the current model developed for hepatocyte is the lipid composition data used as input parameters. In other words, the model equations are the same but not the lipid composition data. The corresponding equations of the hepatocyte composition-based model are displayed in Table 2. For more details, please refer to the original source.[7] Briefly, the hepatocyte composition-based model describes the cell suspension–aqueous phase partition coefficient (P_{csa}) referring to the ratio of drug concentration between the hepatocytes in suspension