Extrapolating *In Vitro* Metabolic Interactions to Isolated Perfused Liver: Predictions of Metabolic Interactions between *R*-Bufuralol, Bunitrolol, and Debrisoquine

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ABSTRACT: Drug–drug interactions (DDIs) are a great concern to the selection of new drug candidates. While *in vitro* screening assays for DDI are a routine procedure in preclinical research, their interpretation and relevance for the *in vivo* situation still represent a major challenge. The objective of the present study was to develop a novel mechanistic modeling approach to quantitatively predict DDI solely based upon *in vitro* data. The overall strategy consisted of developing a model of the liver with physiological details on three subcompartments: the sinusoidal space, the space of Disse, and the cellular matrix. The substrate and inhibitor concentrations available to the metabolizing enzyme were modeled with respect to time and were used to relate the *in vitro* inhibition constant (K_i) to the *in vivo* situation. The development of the liver model was supported by experimental studies in a stepwise fashion: (i) characterizing the interactions between the three selected drugs (*R*-bufuralol (BUF), bunitrolol (BUN), and debrisoquine (DBQ)) in microsomal incubations, (ii) modeling DDI based on binary mixtures model for all the possible pairs of interactions (BUF–BUN, BUF–DBQ, BUN–DBQ) describing a mutual competitive inhibition between the compounds, (iii) incorporating in the binary mixtures model the related constants determined *in vitro* for the inhibition, metabolism, transport, and partition coefficients of each compound, and (iv) validating the overall liver model for the prediction of the perfusate kinetics of each drug determined in isolated perfused rat liver (IPRL) for the single and paired compounds. Results from microsomal coincubations showed that competitive inhibition was the mechanism of interactions between all three compounds, as expected since those compounds are all substrates of rat CYP2D2. For each drug, the K_i values estimated were similar to their K_m values for CYP2D2 indicative of a competition for the same substrate-binding site. Comparison of the performance between the novel liver physiologically based pharmacokinetic (PBPK) model and published empirical models in simulating the perfusate concentration–time profile was based on the area under the curve (AUC) and the shape of the curve of the perfusate time course. The present liver PBPK model was able to quantitatively predict the metabolic interactions determined during the perfusions of mixtures of BUF–DBQ and BUN–DBQ. However, a lower degree of accuracy was obtained for the mixtures of BUF–BUN, potentially due to some interindividual variability in the relative proportion of CYP2D1 and CYP2D2 isoenzymes, both involved in BUF metabolism. Overall, in this metabolic interaction prediction exercise, the PBPK model clearly showed to be the best

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Abbreviations: AUC, area under the curve; BUF, bufuralol; BUN, bunitrolol; C_C, drug concentration in the cellular matrix; C_{Cu}, free drug concentration in hepatocytes; C_D, drug concentration in the Disse space; C_{Du}, free concentration in the space of Disse; C_i, intracellular drug concentration; C_{PS}, drug sinusoid concentration in previous segment; C_S, drug concentrations in sinusoids; C_{Su}, free drug concentrations in sinusoids; C_u, free drug concentration; DBQ, debrisoquine; DDI, drug–drug interaction; EF, rapid exchange factor; Fu_C, unbound fraction in hepatocytes; Fu_D, fraction unbound in space of Disse; Fu_{mic}, fraction unbound in microsomal incubate; Fu_p, fraction unbound in plasma; IPRL, isolated perfused rat liver; K_i, inhibition constant; K_m, affinity constant; K_U, affinity constant for uptake; PA, permeability × area product; PA_z, permeability × area product of a given segment; PBPK, physiologically based pharmacokinetic; $P_{C_{Du}}$, cell-to-unbound drug in Disse ratio; $P_{C:Du}$, hepatocyte-to-buffer ratio; $P_{C:W}$, cellular matrix to water (i.e., buffer) ratio; Q, blood flow rate; Q_{HA}, blood flow in hepatic artery; Q_L, liver blood flow; Q_{PV}, portal venous blood flow; VF, volume fraction of the segment; V_{max}, maximal velocity; U_{max}, maximal rate for uptake; 1-OHBUF, 1-hydroxybufuralol; 4-OHBUN, 4-hydroxybunitrolol; 4-OHDBQ, 4-hydroxydebrisoquine.

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predictor of perfusate kinetics compared to more empirical models. The present study demonstrated the potential of the mechanistic liver model to enable predictions of metabolic DDI under *in vivo* condition solely from *in vitro* information. J Pharm Sci 99:4406–4426, 2010

Keywords: metabolism *in vitro*; *in vitro–in vivo* extrapolation; IVIVE; drug interaction; DDI; microsomes; liver model; isolated perfused liver; metabolic clearance; PBPK modeling

INTRODUCTION

DDI are of great concern for the pharmaceutical industry because they can increase or decrease systemic and organ exposure which can result in possible adverse effects or loss of pharmacological action. Many of the reported DDI have been shown to occur at the level of metabolism by cytochrome P450 isoenzymes. Metabolic inhibition is the most prominently reported DDI. The *in vitro* characterization of these types of interactions is relatively straightforward but the success rate of existing approaches to extrapolate the *in vitro* data to the *in vivo* situation is relatively modest. In this context, several authors used *in vitro* assays to characterize and estimate DDI of several drugs under *in vivo* condition.[1–8] Although generally accepted to improve the accuracy of *in vitro–in vivo* predictions of DDI, the assessment of DDI by using a generic and mechanistic tool based only upon *in vitro* data represents a challenge. The main issues that might be addressed to develop an ideal extrapolation tool of DDI are (i) determination of the relationship between apparent *in vitro* and *in vivo* K_i's (or K_m's) and (ii) estimation/prediction of the substrate and inhibitor concentration available at the active site of the enzyme under *in vivo* condition, and hence the temporal kinetics of both substrate and inhibitor in liver tissue. Seldom is the *in vitro* determined apparent K_i value corrected for possible nonspecific binding in the *in vitro* assays, which might be done similarly as for the K_m estimation for substrate.[9–12] Therefore, these mentioned issues were not yet fully investigated.

Apart from the *in vitro* assays, most of the other attempts used to quantify the *in vitro–in vivo* extrapolations of metabolic interactions rely also on *in vivo* input data. In this case, characterization of DDI is often restricted to the information provided by the AUC *in vivo*, usually estimated before and after drug interaction using the alteration in the intrinsic clearance.[13–15] Since this static approach has recently been improved to account for important processes involved in mechanistic models of drug interaction, such as the gut wall metabolism,[16] the temporal aspect of the inhibitor kinetics remains neglected. Therefore, the inhibitor kinetics in liver tissue has not been considered for the prediction of the substrate clearance. Hence, a fixed inhibitor concentration was used, often referred to as a maximal therapeutic inhibitor concentration or the maximal inlet concentration of the inhibitor. This concentration may not reflect the actual concentration available to the metabolizing enzymes and certainly does not account for the inhibitors concentration–time profile and concentration gradient in the hepatic acinus.

Several efforts adopted the PB approach for the investigation of drug PK and DDI.[17–19] Moreover, some authors have recently considered the dynamic changes of the hepatic concentration of the inhibitor for the simulation of midazolam PK under intravenous condition using a PBPK modeling approach.[20] However, even if the kinetics of the inhibitor has been taken into account, it was not predicted from *in vitro* data but rather described from previous *in vivo* experiments. This was possible because several authors had recourse to clinical data to optimize/fit the input parameters involved in this specific PBPK model of DDI. Several PBPK models rely on those fitting procedures of human concentration–time profiles to correct for poor *in vitro–in vivo* correlations when performing predictions in human.[21–26] Recently, a promising *in vitro*-based PBPK model of DDI has also been proposed to predict drug PK by accounting for dynamical changes in inhibitor concentration in both gut and liver tissues after oral absorption.[27] Most of the pharmacokinetic parameters predicted by this recent PBPK method of DDI were predicted within a twofold error range either in the absence or presence of metabolism-based inhibition, but it only described the liver as being a simple well-stirred model. The same is true for the PBPK frameworks of chemical–chemical interactions developed in environmental toxicology,[28–30] but with an advantage of simulating the PK of volatile chemicals in binary, ternary, and quaternary mixtures in contrast to the pharmaceutical sciences where only binary mixtures kinetic were simulated.[17–26] Furthermore, some PBPK methods are developed to a degree that they are commercially available as software tools.[31,32] The commercially available models have not been published in details because of proprietary reasons. These models are therefore of limited use when more complex situations need the consideration of additional PK processes. This aspect does not make full use of valuable information on DDI.

An alternative approach would be to develop a mechanistic model, specifically for the liver, based only upon *in vitro* input data. In other words, the free drug concentrations in the cells can be estimated