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Rapid Communication

Supplemental Analysis of the Prediction of Hepatic Clearance of Binary Mixtures of Bisphenol A and Naproxen Determined in an Isolated Perfused Rat Liver Model to Promote the Understanding of Potential Albumin-Facilitated Hepatic Uptake Mechanism

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ABSTRACT

The hepatic clearance (CL) of bisphenol A (BPA) in the isolated perfused rat liver (IPRL) model has been studied for the impact of albumin (ALB) binding and coadministration with naproxen (NAP) in a companion manuscript (Bounakta et al. *Xenobiotica*. 2017;3:1-13.). We reported that the extrapolations of hepatic CL of BPA, NAP, and the binary mixtures between 2 ALB concentrations did not follow the free drug hypothesis; however, potential ALB-facilitated hepatic uptake mechanism(s) were highly suspected. Therefore, the objective of the present study was to reanalyze the IPRL data to provide a deeper quantitative extrapolation of CL; however, the focus was made on the impact of ALB binding on the intrinsic clearance (CL_{int}) versus unbound CL_{int} instead of only the global hepatic CL to verify whether the concept of ALB-facilitated hepatic uptake still holds for these 2 additional parameters for binary mixtures. Firstly, the variations in CL_{int} that were observed between the IPRL model using no ALB and ALB in the perfusates were compared to the corresponding variations in the unbound fraction measured in the perfusates (fu_p) according to the free drug hypothesis, or to the variations in the fu_p values adjusted for potential ALB-facilitated uptake mechanism (i.e., $fu_{p\text{-adjusted}}$). The parameter $fu_{p\text{-adjusted}}$ showed a greater predictability compared to fu_p (average fold error ~ 1 vs. 5.2), suggesting that both the free and bound drug moieties should be available for hepatic uptake. Secondly, the supplemental data analysis showed a greater decrease in unbound K_m than in V_{max} resulting in increased uptake CL_{int} of the unbound drug (V_{max}/unbound K_m) with increased ALB concentration at a given substrate concentration, which is compatible with an ALB-facilitated hepatic uptake mechanism. Interestingly, the unbound CL_{int} increased by a factor that corresponds to the bound drug moiety also assumed available for hepatic uptake. These additional findings corroborate the recent literature. Overall, this study showed the importance of quantifying any differential of ALB concentration (*in vitro* vs. *in vivo* or hypoalbuminemia *in vivo* vs. hyperalbuminemia *in vivo*) in the IPRL-based, *in vitro*-to-*in vivo* or *in vivo*-to-*in vivo* extrapolation—based or physiologically based pharmacokinetics—based CL prediction of chemical-drug interactions between xenobiotics significantly bound to ALB.

Introduction

The impact of albumin (ALB) binding on the hepatic clearance (CL) *in vivo* of enzyme and transporter substrates has been extensively analyzed for single-drug formulations as reviewed by Poulin et al.[1,2] and Poulin and Haddad.[3] Recently, Fukuchi et al.[4] published another analysis on the ALB binding effect on CL for a single hepatic transporter substrate. Furthermore, a companion article extended this evaluation to chemical-drug interactions,[5] whereas Mao et al.[6] studied drug-drug interactions instead. Therefore, several drugs significantly bound to ALB administered either in single-drug formulations or in mixtures as well as different experimental settings (i.e., isolated perfused rat liver [IPRL], isolated or suspended hepatocytes or cultured cells overexpressing transporters) were already challenged, and, hence, the results of these experiments were not limited to few examples, which should satisfy the criterion of robust validation exercises. At present, these published studies were all compatible with a so-called potential

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ALB-mediated hepatic uptake of the bound drug moiety, which deviates to the free drug hypothesis.

It was therefore confirmed that conventional *in vitro*-to-*in vivo* or *in vivo*-to-*in vivo* extrapolation (IVIVE) procedures based on the free drug concentration hypothesis (i.e., correcting for the unbound fraction in plasma only; fu_p) significantly underestimated CL *in vivo* for drugs significantly bound to ALB when the input data were obtained in an experimental setting containing no ALB; this was reported for several single-drug formulations and binary mixtures.[1,3,5] The second observation was a greater decrease in the observed unbound Michaelis-Menten constant (unbound K_m) than in the observed maximal elimination rate (V_{max}) resulting in increased uptake intrinsic clearance (CL_{int}) of the unbound drug moiety (V_{max}/unbound K_m) with increased ALB concentration in the system, which is incompatible with the free drug concentration hypothesis.[4,6] The third observation was that the CL_{int} for the total drug moiety varied with the ALB concentrations at a given substrate concentration, but the variation in CL_{int} was estimated more accurately from the corresponding variations in the expected bound and free drug level in the incubation medium or the perfusate compared to the variations in the free drug level only.[3] Overall, these validation exercises showed several examples where the free drug concentration hypothesis seems to be violated for those drugs significantly bound to ALB.

To overcome this problematic, the ALB-mediated hepatic uptake mechanism(s) was suggested to understand these published datasets because the free drug concentration hypothesis failed to explain the corresponding observations. Based on several evidences in the literature, a portion of the protein-bound drug moiety is suggested to become also available for hepatic uptake in addition to the free drug moiety for the compounds that are significantly bound to ALB, which supports the notion of potential ALB-facilitated uptake mechanism(s). The presence of ionic and hydrophobic interactions between the ALB-bound drug complex and the hepatocyte cell surface has been suggested to bring the bound drug moiety at the surface of hepatocytes for potentially more uptake and/or metabolism, but other hypotheses were proposed to support the presence of ALB-facilitated uptake mechanism(s). These hypotheses were all compiled in a recent review of the literature.[2] If this is true, the ALB-bound drug complex would have more affinity for the hepatocytes reducing the apparent K_m value. Interestingly, Mao et al.[6] and Fukuchi et al.[4] observed such a decrease in the K_m value in the presence of ALB in their incubation medium *in vitro*, and these observations were supported by a physiological modeling study for the *in vivo* condition, whereas Poulin et al.[2] compiled other studies were the K_m was also significantly reduced by the presence of ALB. The maximal velocity (V_{max}) also changed with the presence of ALB as discussed by several authors and compiled by Bounakta et al.,[5] but no rational explanation was provided for V_{max}. As a first step, an objective of the present study was to provide a deeper understanding of the variations in K_m and V_{max} with the ALB concentration particularly for the binary mixtures for which data are lacking in that domain. Therefore, the ALB-binding effect on V_{max} and K_m needs further investigation. Nonetheless, the supposition of interaction between the ALB-bound drug complex and the hepatocyte surface is acknowledged,[2,4] but to then say that it is possible to quantify this formalized mechanism required more analyses. However, whichever is the hepatic uptake mechanism of a bound drug in the presence of ALB compared to the absence of ALB in an experimental setting, the scaling across the ALB concentrations should be related to the differential of bound drug concentration between a system containing no ALB and a system containing the ALB, and similarly between the *in vitro* (incubation medium without ALB) and *in vivo* (liver with ALB) conditions; thus, the differential of ALB concentration between these matrices may provide a fairly good estimate of the differential of ALB-bound drug, and, hence, of the scaling factor quantifying the ALB binding effect.

In this context, Poulin et al.[1] were the first who tried to quantify this ALB-binding effect to derive an improved scaling factor to help the IVIVE-based prediction of CL. These authors assumed that each ALB-bound drug complex may interact with the hepatocyte surface; therefore, it was assumed that each drug molecule bound to an extracellular binding protein (i.e., ALB) may interact with the hepatocyte cell surface to deliver additional drug to the intracellular space than the actual unbound concentration. This supposes that the differential of ALB concentration between 2 experimental conditions may predict the differential of the protein-bound drug concentration that is also assumed available for uptake in the hepatocytes. Consequently, the differential of ALB concentration between plasma and liver *in vivo* was measured and used to estimate the corresponding differential of bound drug concentration. Hence, the value of fu_p measured *in vitro* in plasma (or in the incubation medium or a perfusate) was adjusted to mimic better the *in vivo* condition in the liver, which mainly consisted of converting the fu_p value with the plasma-to-liver concentration ratio (PLR) of ALB. The PLR is based on our earlier publications and involves real measurements of the ALB concentration differences in the liver and plasma where a drug can be bound to ALB. Therefore, this correction is not empirical because it is based on known physiological data. The adjustment of the *in vitro* value of fu_p provided a novel parameter named $fu_{p\text{-adjusted}}$ that estimates the potential contribution of the additional protein-bound drug complex in liver under *in vivo* condition. A pH gradient effect was also considered in the estimation of $fu_{p\text{-adjusted}}$ to reproduce the pH difference between the *in vitro* conditions (in plasma, in the perfusate or in the incubation medium) and *in vivo* conditions in the hepatocytes. Thus, $fu_{p\text{-adjusted}}$ of a significantly bound drug may become superior to fu_p to reflect the contribution of the additional ALB-bound drug moiety in liver.[1,2,5] Consequently, the use of $fu_{p\text{-adjusted}}$ significantly improved the IVIVE-based CL predictions compared to fu_p for several enzymatic and transporter substrates studied in different experimental settings. Furthermore, the variations in CL_{int} with the ALB concentration of the perfusate followed the variations in the corresponding values of $fu_{p\text{-adjusted}}$ but not of fu_p, which corroborates the IVIVE-based predictions.[3]

Because the novel scaling factor $fu_{p\text{-adjusted}}$ and the impact of ALB binding on CL (or CL_{int}) have been challenged mainly for single-drug formulations compared to binary mixtures (Poulin et al.[1,2]; Poulin and Haddad[3]; Fukuchi et al.[4] vs. Mao et al.[6]), an extension of the recent validation exercises from single-drug formulations to binary mixtures was also necessary to corroborate the observation that both the free and bound drug moieties could be implicated in the uptake process in liver for a drug bound to ALB and given in mixtures. Therefore, we have recently verified the impact of ALB binding and coadministration with naproxen (NAP) on the IVIVE-based prediction of hepatic CL of bisphenol A (BPA) using an IPRL model proxy to *in vivo*.[5] This published chemical-drug interaction study confirmed that the IVIVE method using $fu_{p\text{-adjusted}}$ of the perfusate extrapolated more accurately the CL between the 2 IPRL systems (i.e., from the system containing no ALB to the system containing ALB) compared to other IVIVE methods based on either fu_p or no binding correction. The same IPRL data can be examined more attentively to study the effect of $fu_{p\text{-adjusted}}$ versus fu_p on the CL_{int} versus the unbound CL_{int}. Hence, it is also of interest to quantify by which factor the CL_{int} of these binary mixtures would vary with the ALB concentrations of the perfusate at a given substrate concentration, and to verify whether the factors of variation would again follow the variations in $fu_{p\text{-adjusted}}$ or fu_p to corroborate