Effect of albumin on human Cytochromes P450 kinetics: extrapolation of *in vivo* clearance from *in vitro* data

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Abstract

It has been reported that addition of bovine serum albumin (BSA) to *in vitro* incubations improves estimation of kinetic parameters, intrinsic clearance ($CL_{int} = V_{max}/K_m$), by decreasing the K_m for drugs metabolised by cytochrome P450 2C9 (CYP2C9). However, the effect of BSA on other CYP isozymes was unclear. The aims of this study were to characterize effect of BSA on kinetics of specific pathways for CYP2C8, 2C19, and 3A4 whether the addition of BSA could improve the prediction of *in vivo* clearance by using human hepatic microsomal 6α-hydroxypaclitaxel, 5-hydroxyomeprazole, and omeprazole sulfone formations as markers for CYP2C8, 2C19, and 3A4 pathways, respectively. The metabolite formations were determined by HPLC. In the presence of 2% BSA, rate of CYP2C8-mediated 6α-hydroxypaclitaxel formation was well described by single enzyme Michaelis-Menten kinetic. Mean CL_{int} was significantly increased about 4 fold. Thus the extrapolation of *in vitro* CL_{int} to *in vivo* clearance for paclitaxel 6α-hydroxylation was more accurate in the presence of BSA in the incubation. 5-Hydroxyomeprazole formation in the presence of 2% BSA followed two enzyme Michaelis-Menten kinetics. In the presence of 2% BSA, the mean K_{m1} and V_{max1} for omeprazole 5-hydroxylation were decreased 4 and 2 fold, respectively which resulted in 2-fold increases in the mean CL_{int1}. Moreover, the mean K_m and V_{max} for CYP3A4-mediated omeprazole sulfoxidation were increased (15-19 fold) in the presence of 2% BSA therefore the mean CL_{int} was not significantly changed. BSA converted omeprazole sulfoxidation from two enzyme to single enzyme Michaelis-Menten kinetic. In conclusion, the addition of 2% BSA is likely to improve *in vitro* clearance prediction for CYP2C8-mediated paclitaxel 6α-hydroxylation. Moreover, BSA showed minor effect on the CYP2C19-mediated omeprazole 5-hydroxylation whereas had no effect on CYP3A4-mediated omeprazole sulfoxidation. The effect of albumin on individual CYP isoforms was variable; the use of BSA to improve prediction of *in vivo* intrinsic clearance seems to be possible with only CYP2C8.

Keywords: bovine serum albumin, cytochromes P450, *in vitro-in vivo* extrapolation

Introduction

In vitro approaches have been used to predict drug clearance in humans during drug development process. An *in vitro* intrinsic clearance (CL_{int}), generally determined from kinetic data of human liver microsomes (HLM) may be extrapolated to hepatic clearance using a mathematical equation. However, the microsomal CL_{int} values underpredict the *in vivo* CL_{int} and hepatic clearance (CL_H) of drug metabolized by cytochrome P450 (CYP).

Polyunsaturated fatty acids (PUFAs) released from the membranes of enzymes during the course of an incubation act as the inhibitors of several drug metabolizing enzymes (i.e. CYP2C9) (Rowland et al., 2008) resulting in overestimation of the K_m value. Consequently,

CL_{int} based on this value may underpredict *in vivo* CL_H. This effect was reversed by adding bovine serum albumin (BSA) to *in vitro* incubations. BSA improves estimation of kinetic parameters, CL_{int}, by decreasing the K_m for drugs metabolized by CYP2C9 (Rowland et al., 2008). In this regard, the universality of the albumin effect on other CYP isozymes is still arguable.

Therefore, the aims of present study were (i) to investigate effect of BSA on the kinetics of specific pathways for CYP2C8-mediated paclitaxel (PAC) 6α- hydroxylation, CYP2C19-mediated omeprazole (OMP) 5-hydroxylation, and CYP3A4-mediated OMP sulfoxidation using HLM as enzyme source and (ii) to investigate the effect of BSA on the clearance prediction.

Methods

Kinetics of PAC 6α-hydroxylation, OMP 5-hydroxylation, and OMP sulfoxidation.
Incubation samples contained phosphate buffer (0.1 M, pH 7.4), HLM, BSA (0 or 2%), NADPH generating system, and selective substrates (PAC or OMP). Reactions were performed at 37°C and terminated. The metabolite concentrations were determined by validated HPLC techniques. Incubation conditions were optimized to ensure linearity with respect to protein concentration and incubation time for each reaction.

Data Analysis
Kinetic parameters for all metabolic pathways in the absence and presence of 2% BSA were generated by fitting experimental data to the single or two Michaelis-Menten or substrate inhibition kinetics. Fitting was performed with EnzFitter (version 2.0.18.0; Biosoft, Cambridge, UK) based on the unbound concentration present in incubations. Statistical analysis (*t*-test or Mann-Whitney Rank Sum test) was performed using SigmaStat version 3.11. Values of *p* less than 0.05 were significant.

Intrinsic clearances (CL_{int}) were determined as V_{max}/K_m and subsequently scaled to the whole liver CL_{int} assuming microsomal yield of 40 mg microsomal protein/g of liver, and a liver weight of 1500 g. *In vivo* CL_H was then predicted using expression for the well-stirred model.

Results

Kinetics of PAC 6α-hydroxylation, OMP 5-hydroxylation, and OMP sulfoxidation by HLM in the absence and presence of 2% BSA.
In the absence of 2% BSA, kinetic results for 6α-hydroxypaclitaxel formation by HLM were well described by Michaelis-Menten or substrate inhibition models while in the presence of 2% BSA exhibited single enzyme Michaelis-Menten kinetic (Fig. 1A). 5-Hydroxyomeprazole formation in the absence and presence of 2% BSA followed two enzyme Michaelis-Menten kinetics (Fig. 1B). In the absence of BSA, OMP sulfoxidation exhibited biphasic kinetics (Fig. 1C). Addition of 2% BSA to incubations resulted in Michaelis-Menten kinetic (Fig. 1C).
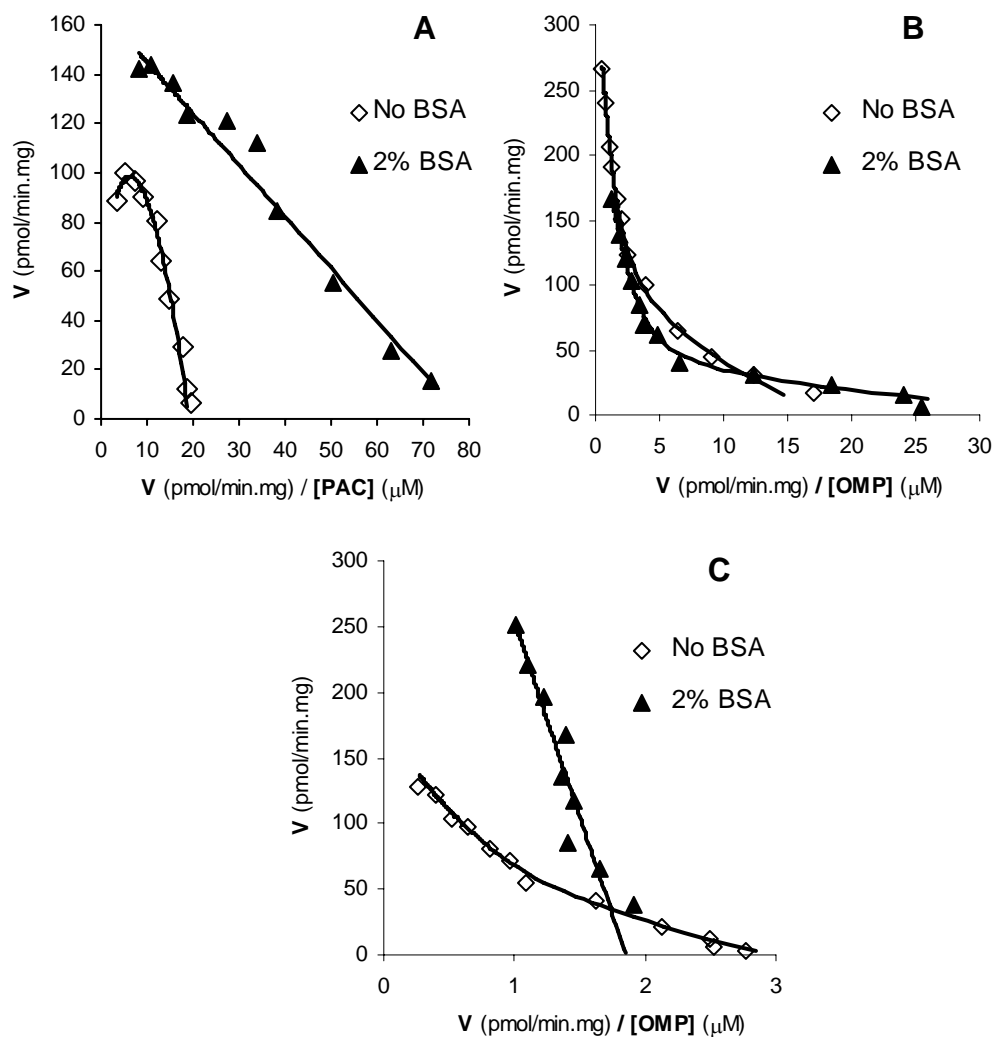
Figure 1. Representative Eadie Hofstee plots for PAC 6α-hydroxylation (A), OMP 5-hydroxylation (B), and OMP sulfoxidation (C) by HLM in the absence and presence of 2% BSA.

BSA increased mean CL_{int} for PAC 6α-hydroxylation about 4 fold due to significantly decreasing mean K_m with a minor effect on mean V_{max} (Table 1). In addition, BSA increased mean CL_{int1} (2 fold) for OMP 5-hydroxylation. Although BSA reduced the K_m (4 fold) for OMP 5-hydroxylation, V_{max} was also decreased (2 fold) (Table 1). For OMP sulfoxidation, BSA increased both of K_{m1} and V_{max1} for this pathway (15-19 fold) thus the mean CL_{int1} was not significantly changed (Table 1).

Comparison of predicted CL_H to reported *in vivo* CL_H for PAC 6α-hydroxylation.

In the absence of 2% BSA, mean predicted CL_H of PAC 6α-hydroxylation by HLM was under-predicted from reported *in vivo* CL_H around 17-fold. Whereas the mean predicted CL_H of PAC 6α-hydroxylation in the presence of 2% BSA resulted in 5-fold underprediction (12.3 vs 65.2 L/h). The *in vivo* CL_H was determined to be 65.2 L/hr (data from Smorenburg et al. 2003; the approximately administrated dosage was 175 mg/m^2 3 hour infusion).

Table 1. Effect of 2% BSA on the kinetic parameters of PAC 6α-hydroxylation, OMP 5-hydroxylation, and OMP sulfoxidation.

Metabolic pathways	K_m[a] (μM)	K_{si}[b] (μM)	V_{max}[a] (pmol/min/mg)	CL_{int}[a] (μL/min/mg)
CYP2C8-mediated paclitaxel 6α-hydroxylation				
without BSA	8.66±4.15	41.2±26.6	167±78.1	19.6±3.17
with 2% BSA	2.23±0.34[*]		154±22.8	70.6±16.6[*]
CYP2C19-mediated omeprazole 5-hydroxylation				
without BSA	8.63±6.83		97.3±46.7	13.8±8.39
with 2% BSA	2.39±1.80		49.7±22.4	27.1±12.4
CYP3A4-mediated omeprazole sulfoxidation				
without BSA	16.5±11.5		63.0 ± 60.2	3.16±1.50
with 2% BSA	307±133[*]		965±272[*]	3.62±1.92

Data are mean ± S.D. ([a] n = 5, [b] n = 3), [*] Significant difference ($p \leq 0.05$) to control.

Conclusions

The present study reveals that BSA shows variable effects on each CYP isoform. The prediction of *in vivo* clearance from *in vitro* kinetic data for CYP2C8-catalysed PAC 6α-hydroxylation is likely to be improved in the presence of 2% BSA. In addition, BSA had minor effect on the CYP2C19-mediated OMP 5-hydroxylation while no effect on CYP3A4-mediated OMP sulfoxidation for improving *in vitro* CL_{int} prediction.

References

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