P-83 Evaluation of the CYP metabolic activities in the presence of albumin in human liver microsome

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Introduction

Cytochrom P450 (CYP) isoforms are responsible for the metabolism of the majority of drugs in human. An intrinsic clearance (CL_{int}) determined from *in vitro* intrinsic clearance can be used to predict the *in vivo* hepatic metabolic clearance (CL_H) of drugs in humans. However, *in vitro-in vivo* extrapolation (IV-IVE) commonly underestimates the *in vivo* CL_{int} and CL_H of drugs metabolized by CYP isoforms. Recently, it was reported that the addition of albumin to the *in vitro* incubation system increased the metabolic activities of some CYP isoforms due to inhibition of the effect of polyunsaturated long-chain fatty acids (PUFAs). Therefore, we evaluated the effect of bovine serum albumin (BSA) on the metabolic activities of CYP isoforms in human liver microsomes (HLM). We found that some CYP isoforms were observed to show enhancement of $CL_{u,met}$ in the presence of albumin.
In this presentation, We have summerised the effect of albumin on the metabolic activity of each CYP isoform in HLM.

Method

<Measurement of CYP metabolic activities using HLM and BSA>

Table 1 Reaction conditions for each CYP isoform

CYP isoform	Metabolic activity	* Model substrate concentration (μM)	BSA concentration
CYP1A2	Phenacetin *O*-deethylation	6	0, 1, 2, 4 %
CYP2B6	Bupropion hydroxylation	20	
CYP2C8	Amodiaquine *N*-deethylation	0.5	
CYP2C9	Diclofenac 4'-hydroxylation	0.8	
CYP2C19	*S*-(+)- Mephenytoin 4'-hydroxylation	6	
CYP2D6	(±)- Bufuralol 1'-hydroxylation	2	
CYP3A	Testosterone 6β-hydroxylation	12	
CYP3A	Midazolam 1'-hydroxylation	0.2	
CYP3A	Nifedipine oxidization	6	

*The model substrate concentrations were set to around 1/5 K_m values based on internal data of Sekisui Medical Co., Ltd.

Incubation samples contained phosphate buffer (50 mM, pH 7.4) including fatty acid-free BSA (0,1,2 and 4 %), HLM (0.1 mg protein/mL, H0610, mixed gender, pool of 50, Sekisui Xeno Tech, LLC), NADPH generating system (Corning) and each model substrate (the concentrations were set to around 1/5 K_m). Reactions were performed at 37° C (The incubation time was 5 min : CYP2C8, 2C9 and 3A (Midazolam), 20 min : CYP1A2, 2B6, 2C19, 2D6, CYP3A (Testosterone) and CYP3A (Nifedipine)) and terminated by adding acetonitrile solution containing internal standard. The metabolite concentrations were determined by LC-MS/MS (HPLC system : LC-20ADvp, Shimadzu, MS/MS system :API4000 and API5500 QTRAP, SCIEX).

<Protein binding of each CYP model substrates in HLM and BSA>

Protein binding was determined by equilibrium dialysis using Rapid Equilbrium Dialysis Device (RED Device, Thermoischer Scientific). Protein solution samples (Donor side) contained phosphate buffer (50 mM, pH 7.4) including fatty acid-free BSA (0,1,2 and 4 %), HLM (0.1 mg protein/mL) and each model substrate (the concentrations were set to around 1/5 K_m). Phosphate buffer (50 mM, pH 7.4) was added to the acceptor side. The RED device was sealed and transferred to a shaker, placed in a refrigerator at 4° C for 24 hour. After reaching equilibrium, equilibrium samples were collected and mixed with methanol solution containing internal standard. The samples were analyzed by LC-MS/MS.

<Caluclation of metabolic activity>

Metabolic activity of each CYP isoform was calculated according to the following equation.

$$\text{Metabolic activity (pmol/mg protein/min)} = \frac{\text{Marker metabolite concentration (nM)}}{\text{HLM concentration (mg protein/mL)} \times \text{Incubation time (min)}}$$

<Calculation of fraction unbound (f_u)>

The fraction unbound in the microsomal binding of each CYP model substrate was calculated to the following equation .

$$f_u = \frac{\text{Peak area ratio in acceptor side}}{\text{Peak area ratio in donor side}}$$

<Calculation of $CL_{u,met}$>

$CL_{u,met}$ of each CYP isoform was calculated to the following equation.

$$CL_{u,met}\ (\mu L/min/mg\ protein) = \frac{\text{Metabolic activity (pmol/mg protein/min)}}{\text{Model substrate concentration (μM)} \times f_u}$$

Results

Table2 $CL_{u,met}$ and f_u each CYP isoform by HLMs in the presence and absence of BSA

CYP isoform	0 % BSA		1% BSA		2% BSA		4% BSA		* Ratio
	f_u	$CL_{u,met}$	f_u	$CL_{u,met}$	f_u	$CL_{u,met}$	f_u	$CL_{u,met}$	
CYP1A2	1.00	10.0	0.738	63.0	0.527	80.0	0.388	97.5	9.75
CYP2B6	1.00	5.90	0.862	11.4	0.857	10.2	0.810	9.82	1.66
CYP2C8	0.769	858	0.679	2030	0.503	2120	0.409	1800	2.10
CYP2C9	0.972	382	0.000410	31400	0.000263	25500	0.000137	19900	52.1
CYP2C19	0.945	0.672	0.980	2.28	0.897	2.19	0.686	2.77	4.12
CYP2D6	0.933	7.28	0.673	13.3	0.532	14.1	0.291	21.7	2.98
CYP3A (Testosterone)	0.945	50.1	0.318	68.9	0.164	67.4	0.0936	55.4	1.11
CYP3A (Midazolam)	0.784	849	0.0297	3770	0.0208	3010	0.0131	3150	3.71
CYP3A (Nifedipine)	0.832	47.9	0.148	157	0.0826	154	0.0474	188	3.92

* : These values were calculated using the following equation "$CL_{u,met}$ (4% BSA)/$CL_{u,met}$ (0% BSA)".

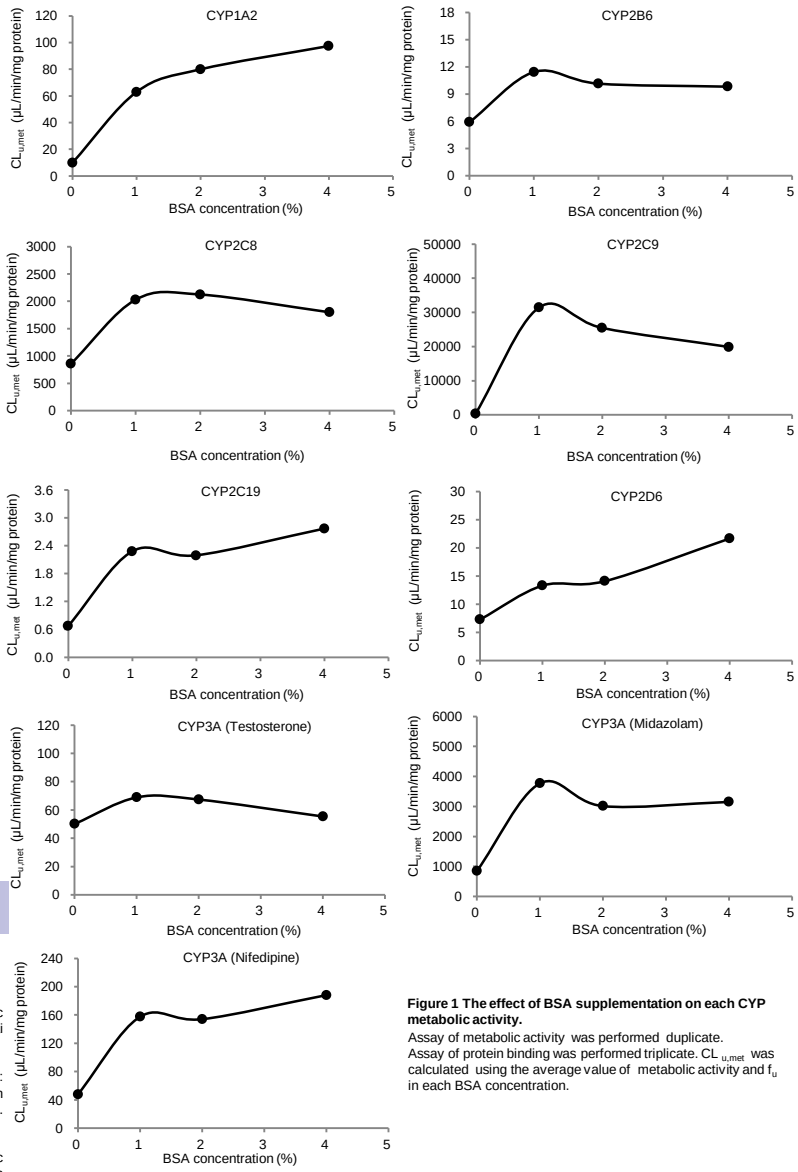

Figure 1 The effect of BSA supplementation on each CYP metabolic activity.
Assay of metabolic activity was performed duplicate.
Assay of protein binding was performed triplicate. $CL_{u,met}$ was calculated using the average value of metabolic activity and f_u in each BSA concentration.

References

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Discussion

This study characterized the effect of albumin (BSA) supplementation on human liver microsomal CYP activities. CYP1A2, 2C8, 2C9, 2C19, 2D6, 3A (Midazolam), and 3A (nifedipine) were observed to show enhancement of $CL_{u,met}$ in the presence of BSA (Table 2 and Figure 1). This enhancement in $CL_{u,met}$ may result from the trapping of PUFAs, a reported inhibitor for several metabolizing enzymes (Reference 2), present in HLM by the added BSA.
By contrast, CYP2B6 and CYP3A (Testosterone) did not show significant alteration in $CL_{u,met}$ by adding BSA (Table 2 and Figure 1). This study indicated that the effect of BSA on CYP activities in HLM is substrate dependent. We showed that the effect of BSA on the metabolic activity of each CYP isoform in HLM. These results may help us to evaluate the metabolic intrinsic clearance of CYP isoforms.

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