RESEARCH ARTICLE

The Effects of Bovine Serum Albumin on Kinetic Characterization of Human Liver Microsomal CYP2C19 and CYP2E1 Activities

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Abstract

The addition of bovine serum albumin (BSA) to incubations of HLM or recombinant enzymes enhances the activities of several human cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes, including CYP1A2, CYP2C8, CYP2C9, UGT1A8, UGT1A9, UGT1A10, UGT2A1, UGT2B4, UGT2B7, and UGT2B15. Available evidence suggests that BSA increases the activities of these drug metabolizing enzymes by sequestering polyunsaturated fatty acids that are released from membranes, especially when using human liver microsomes (HLM) as the enzyme source. Despite the importance of the 'albumin effect' for the accurate determination of kinetic parameters, the effects of BSA on CYP enzymes other than CYP1A2, CYP2C8, and CYP2C9 are yet to be investigated. This study aimed to investigate the effects of BSA on kinetic characterization of human liver microsomal omeprazole (OMP) 5′-hydroxylation and chlorzoxazone (CZX) 6-hydroxlation pathways which are catalyzed by CYP2C19 and CYP2E1, respectively. When BSA (2% w/v) was added to incubations, the K_m and V_{max} values for CYP2C19-catalyzed OMP 5′-hydroxylation were decreased by approximately 75% and 50%, resulting in a 2-fold increase in CL_{int}. By contrast, BSA had a minimal effect on the K_m, V_{max}, and CL_{int} values for human liver microsomal CYP2E1-catalyzed CZX 6-hydroxylation. These data confirm that BSA selectively affects human liver microsomal CYP activities.

Keywords: Bovine serum albumin, chlorzoxazone, omeprazole, 5′-hydroxy-omeprazole, 6-hydroxychlorzoxazone

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ผลของซีรั่มอัลบูมินจากวัวที่มีต่อลักษณะทางไคเนติกของการทำงานของเอนไซม์ไซโตโครม พี450 2C9 และ 2E1 โดยใช้ไมโครโซมจากตับมนุษย์

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บทคัดย่อ

การเติมซีรั่มอัลบูมินจากวัว (bovine serum albumin; BSA) ลงในหลอดทดลองที่ทำ incubation โดยใช้ไมโครโซมจากตับมนุษย์หรือรีคอมบิแนนท์เอนไซม์มีผลเพิ่มการทำงานของเอนไซม์หลายชนิด เช่น เอนไซม์ไซโตโครม พี450 (cytochrome P450; CYP) CYP1A2, CYP2C8, CYP2C9, เอนไซม์ยูดีพี-กลูคิวโรโนซิวทรานสเฟอเรส (UDP-glucuronosyl-transferase; UGT) UGT1A8, UGT1A9, UGT1A10, UGT2A1, UGT2B4, UGT2B7 และ UGT2B15 มีหลักฐานการวิจัยรายงานว่าการที่ BSA เพิ่มการทำงานของเอนไซม์เหล่านั้นโดย BSA สามารถจับกับกรดไขมันไม่อิ่มตัวซึ่งถูกปลดปล่อยจากเซลล์เมมเบรนเมื่อใช้ไมโครโซมจากตับมนุษย์เป็นแหล่งเอนไซม์ ถึงแม้ว่าผลของอัลบูมินจะทำให้ค่าพารามิเตอร์ทางจลนศาสตร์ของเอนไซม์ CYP1A2, CYP2C8, และ CYP2C9 มีค่าถูกต้องยิ่งขึ้น อย่างไรก็ตามยังไม่มีการศึกษาผลของอัลบูมินต่อเอนไซม์ CYP อื่น ๆ การศึกษานี้มีวัตถุประสงค์เพื่อสำรวจผลของ BSA ต่อลักษณะทางจลนศาสตร์ของโอเมพราโซล 5′-ไฮดรอกซีเรชั่น และ คลอซอกซาโซน 6-ไฮดรอกซีเรชั่น ซึ่งถูกเปลี่ยนแปลงด้วยเอนไซม์ CYP2C19 และ CYP2E1 ตามลำดับ เมื่อเติม BSA (2% w/v) ลงในหลอดทดลองที่ทำ incubation พบว่า ค่า K_m และ V_{max} สำหรับโอเมพราโซล 5′-ไฮดรอกซีเรชั่นที่ถูกเปลี่ยนแปลงด้วยเอนไซม์ CYP2C19 โดยใช้ไมโครโซมจากตับมนุษย์เป็นแหล่งเอนไซม์ มีค่าลดลงประมาณ 75% และ 50% ตามลำดับ จึงมีผลให้ค่า intrinsic clearance (CL_{int}) มีค่าเพิ่มขึ้นประมาณ 2 เท่า ในทางตรงกันข้าม BSA มีผลเล็กน้อยต่อค่า K_m, V_{max}, และ CL_{int} สำหรับคลอซอกซาโซน 6-ไฮดรอกซีเรชั่น ซึ่งถูกเปลี่ยนแปลงด้วยเอนไซม์ CYP2E1 โดยใช้ไมโครโซมจากตับมนุษย์เป็นแหล่งเอนไซม์ จากข้อมูลเหล่านี้ยืนยันได้ว่า BSA มีผลอย่างจำเพาะเจาะจงต่อการการทำงานของเอนไซม์ CYP ซึ่งใช้ไมโครโซมจากตับมนุษย์เป็นแหล่งเอนไซม์

คำสำคัญ: ซีรั่มอัลบูมินจากวัว, คลอซอกซาโซน, โอเมพราโซล, 5′-ไฮดรอกซีโอเมพราโซล, 6-ไฮดรอกซีคลอซอกซาโซน

Introduction

An intrinsic clearance ($CL_{int} = V_{max}/K_m$) determined from in vitro kinetic data 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 under-predicts the in vivo CL_{int} and CL_H of drugs metabolized by cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes.[1,2] It has been proposed that the under-prediction of in vitro CL_{int}, and hence CL_H, arises largely from the over-estimation of K_m due to the presence of inhibitory polyunsaturated long-chain fatty acids (PUFAs) released from membranes during the course of an in vitro incubation.[3,4] In particular, arachidonic acid, linoleic acid, and oleic acid are potent inhibitors of several human liver microsomal drug metabolizing enzymes, including CYP1A2, CYP2C8, CYP2C9, CYP2C19, UGT1A9, UGT2B4, and UGT2B7.[5-9]

Experimental approaches to improve the prediction accuracy of in vivo CL_H using IV-IVE have been explored in recent years. Previous studies from this and other laboratories have demonstrated that the addition of either bovine serum albumin (BSA; 0.1% to 2% w/v), fatty acid-free human serum albumin (FAF-HSA; 2% w/v), or human intestinal fatty acid binding protein (IFABP; 1% w/v) to incubations increases the prediction accuracy of the hepatic clearances of drugs metabolized by CYP1A2[7], CYP2C8[6], CYP2C9[3,10,11], UGT1A9[12], UGT2B4[13], and UGT2B7.[4,10,14] Increased activities of recombinant UGT1A7, UGT1A8, UGT1A10, UGT2A1, and UGT2B15 have also been reported in the presence of BSA (0.1% w/v), although the effects of BSA were substrate dependent.[15] In most instances, the increased activities of these CYP and UGT enzymes observed in the presence of BSA, FAF-HSA, or IFABP arose mainly from a reduction in K_m values, although V_{max} was also variably altered with some substrate/enzyme combinations.

In humans, CYP and UGT enzymes are responsible for the metabolism of the majority of drugs and xenobiotics. However, compared to UGT, the effects of albumin on CYP enzymes are incompletely understood. Early studies of the so-called 'albumin effect' focused on CYP2C9. Supplementation of incubations of human liver microsomes (HLM) with BSA (2% w/v) increased the activity of phenytoin hydroxylation and tolbutamide hydroxylation, mainly via a reduction in the K_m values for these two metabolic pathways.[3,11] More recent studies have also shown that BSA increased the enzyme activities of human liver microsomal CYP1A2-catalyzed phenacetin *O*-deethylation and lidocaine *N*-deethylation, and CYP2C8-catalyzed paclitaxel 6α-hydroxylation, primarily via a decrease in K_m.[6,7] It has been demonstrated that these effects of BSA arise from sequestration of released PUFAs which inhibit CYP1A2[7], CYP2C8[6], CYP2C9[3], as well as UGT1A9[12], UGT2B4[13], and UGT2B7[4]. By contrast, addition of albumin to microsomal incubations does not enhance the activities of UGT1A1[12], UGT1A4[16], UGT1A6[12], and CYP3A4[7], presumably because these enzymes are only weakly inhibited by PUFAs. Arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid, linoleic acid, and oleic acid potently and competitively inhibit CYP2C9- and CYP2C19-mediated reactions, with K_i values ranging from 1.7 to 4.7 μM and 2.3 to 4.7 μM, respectively. These PUFAs have also been reported to competitively inhibit metabolic reactions catalyzed by CYP2E1 and CYP3A4, but with K_i values > 10 μM.[9] To date, however, the effects of BSA on the kinetics of reactions catalyzed by CYP2C19 and CYP2E1 have not been characterized.

Omeprazole (OMP) has been used as a selective probe substrate for measuring CYP2C19 activity in vitro and in vivo.[17,18] In particular, the high affinity component of OMP 5′-hydroxylation, to form 5′-hydroxyomeprazole (5′-OH OMP), reflects CYP2C19 activity in vitro.[17,19,20] Similarly, available evidence indicates that the conversion of chlorzoxazone (CZX) to 6-hydroxychlorzoxazone (6-OH CZX) by HLM is catalysed predominantly by CYP2E1.[21] Thus, the present study aimed to characterize the effect of BSA supplementation on the kinetics of human liver microsomal CYP2C19-catalyzed OMP 5′-hydroxylation and CYP2E1-catalyzed CZX 6-hydroxylation in order to provide a more complete understanding of the effect of BSA on the activities of CYP enzymes.

Materials and Methods

Chemicals and reagents

Omeprazole (OMP), chlorzoxazone (CZX), 6-hydroxychlorzoxazone (6-OH CZX), and bovine serum albumin (BSA; product number A7906) were purchased from Sigma Aldrich (Sydney, Australia). 5′-Hydroxyomeprazole (5′-OH OMP) was provided by AstraZeneca (Molndal, Sweden). β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Sigma Aldrich (Sydney, Australia). All other reagents and solvents were of analytical grade.

Human liver microsomes

Human livers (H7, H10, H12, H13, and H40) for the OMP 5′-hydroxylation study were obtained from the human liver bank of the Department of Clinical Pharmacology, Flinders University, Australia while human livers (H0, H1, H4, H5, and H11) used for the CXZ 6-hydroxylation experiments were provided by the human liver bank of the Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Thailand. Approval for the use of human liver tissues in xenobiotic metabolism studies was obtained from Flinders Clinical Research Ethics Committee and Khon Kaen University Ethics Committee for Human Research. All livers were obtained within 60 min of death, and were immediately sliced and frozen in liquid nitrogen. The frozen livers were stored at -80°C until used. Human liver microsomes were prepared by differential centrifugation.[22]

Omeprazole 5′-hydroxylation assay

Incubation samples (300 µL) contained phosphate buffer (0.1 M, pH 7.4), HLM (0.6 mg/mL), and OMP (1-500 µM). After a 5 min pre-incubation, reactions were initiated by the addition of NADPH regenerating system (1 mM NADP, 10 mM glucose 6-phosphate, 2 IU/mL glucose 6-phosphate dehydrogenase, and 5 mM $MgCl_2$). Incubations were then continued for 20 min at 37°C in a shaking water bath. Reactions were terminated by the addition of ice-cold acetonitrile (900 µL) and then samples were centrifuged at 10,000 g. The supernatant layer (800 µL) was evaporated to dryness using a Mivac modular concentrator (Genevac Limited, Suffolk, United Kingdom). The residue was reconstituted in 120 µL of 25% acetonitrile: 75% 50 mM phosphate buffer pH 8.5 (v/v) and an aliquot (20 µL) was injected into the HPLC system. Analytes were separated on a reversed–phase, Synergi Hydro RP C18 column (150×3.0 mm id, 4 µm particle size; Phenomenex,

Torrance, CA) at 25°C. The mobile phase was a linear gradient comprising 0.01% formic acid in 5% acetonitrile (mobile phase A) and 100% acetonitrile (mobile phase B): initial conditions were 90% A /10% B, increasing to 70% A /30% B using a linear gradient over 8.5 min and then to 40% A/60% B over 0.1 min, which was held for 0.9 min before returning to the starting conditions. The mobile phase flow rate was 1.0 mL/min and analytes were monitored by UV detection at 302 nm. Under the HPLC conditions described above, respective retention times for 5′-OH OMP and OMP were 4.4 and 6.6 min.

Calibration curves were constructed using 5′-OH OMP concentrations ranging from 1-4 μM and 1-6 μM for experiments performed in the absence and presence of BSA, respectively. 5′-OH OMP formation was quantified by comparison of the peak areas obtained from incubation samples with those of the calibration curves. The overall assay reproducibility for the conversion of OMP to 5′-OH OMP was determined in the same batch of HLM (n = 9) at OMP concentrations of 5 and 200 μM. Within-day coefficients of variation for OMP 5′-hydroxylation were 3.7 and 6.8% at OMP concentrations of 5 and 200 μM, respectively.

Chlorzoxazone 6-hydroxylation assay

Incubations (500 μL) contained phosphate buffer (0.1 M, pH 7.4), HLM (0.3 mg/mL), and CZX (15-500 μM). After a 5 min pre-incubation, reactions were initiated by the addition of NADPH (final concentration 1 mM). Incubations were performed for 20 min at 37°C in a shaking water bath. Reactions were subsequently terminated by the addition of 85% orthophosphoric acid (50 μL) and cooling on ice for 10 min. After addition of phenacetin (20 μL, 0.2 mM), the assay internal standard, the incubation mixture was saturated with ammonium sulfate (0.5 g) and was then extracted with 5 mL of dichloromethane/isopropanol (85:15 %v/v). The organic layer was separated by centrifugation (5,000 g for 10 min), transferred to a clean glass tube, and evaporated to dryness under a stream of N_2. The residue was reconstituted in 120 μL of 25% acetonitrile in 0.5 % glacial acetic acid and a 20 μL aliquot was injected into the HPLC. For reactions containing BSA (2% w/v), a higher CZX concentration range (50-1,200 μM) was used.

The analytes were separated on a Nucleosil C18 column (4.6 mm×150 mm id, 5 μM particle size, Phenomenex, Torrance, CA) with an isocratic mobile phase comprising 25% acetonitrile in 0.5% glacial acetic acid. The mobile phase flow rate was 1.5 mL/min and analytes were monitored by UV detection at 287 nm. Under these conditions, the respective retention times for 6-OH CZX, phenacetin, and CZX were 2.9, 5.8, and 8.5 min. 6-OH CZX formation was quantified by comparison of the peak height ratios obtained from incubation samples with a calibration curve in the range 0.5-4 μM. The overall assay reproducibility for the conversion of CZX to 6-OH CZX was determined in the same batch of HLM (n = 9) at CZX concentrations of 30 and 300 μM. Within-day coefficients of variation for CZX 6-hydroxylation were 5.4 and 5.6% at CZX concentrations of 30 and 300 μM.

Binding of omeprazole and chlorzoxazone to HLM and BSA

The binding of OMP and CZX to HLM, and mixtures of 2% BSA with HLM was measured by equilibrium dialysis using a Dianorm equilibrium dialysis apparatus according to the general method of McLure et al.[23] The concentrations of

OMP and CZX used in dialysis experiments spanned the concentration ranges used in kinetic experiments. The dialysis cell assembly was immersed in a water bath maintained at 37ºC and rotated at 12 rpm for 3.5 and 4 h for OMP and CZX, respectively. The fraction unbound of OMP or CZX in incubations ($fu_{,inc}$) was calculated by dividing the concentration of OMP or CZX in the buffer compartment by the concentration in the protein compartment. [23]

Kinetics and statistics analysis for omeprazole 5′-hydroxylation and chlorzoxazone 6-hydroxylation

Data points represent the mean of the duplicate measurements (<10% variance). Kinetic parameters for OMP 5′-hydroxylation and CZX 6-hydroxylation were derived by fitting expressions for the Michaelis-Menten equation, two enzyme Michaelis-Menten equation, substrate inhibition equation, or the Hill equation (see below) to experimental data. Fitting was performed with EnzFitter (version 2.0.18.0; Biosoft, Cambridge, UK) based on the unbound OMP or CZX concentration present in incubations. Goodness of fit for each equation was assessed from comparison of the parameter standard error of fit, 95% confidence intervals, coefficient of determination (r^2), and F-statistic. Statistical comparisons of kinetic parameters (paired *t*-test or Wilcoxon Signed Rank test) were performed using SPSS version 20. Values of $p < 0.05$ were considered significant.

Michaelis Menten equation:

$$v = \frac{V_{max.}[S]}{K_m + [S]} \qquad \text{(Equation 1)}$$

where ν is the rate of metabolite formation, V_{max} is the maximal velocity, K_m is the Michaelis constant (substrate concentration at 0.5 V_{max}), and [S] is the substrate concentration.

Two-enzyme Michaelis Menten equation:

$$v = \frac{V_{max1}.[S]}{K_{m1} + [S]} + \frac{V_{max2}.[S]}{K_{m2} + [S]} \qquad \text{(Equation 2)}$$

where V_{max1} and V_{max2} are the maximal velocities of the high and low affinity component, and K_{m1} and K_{m2} are the Michaelis constants of the high and low affinity component.

Substrate inhibition equation:

$$v = \frac{V_{max}}{1 + (K_m/[S]) + ([S]/K_{si})} \qquad \text{(Equation 3)}$$

where K_{si} is the constant describing the substrate inhibition interaction.

The Hill equation:

$$v = \frac{V_{max}.[S]^n}{S_{50}{}^n + [S]^n} \qquad \text{(Equation 4)}$$

where S_{50} is the substrate concentration resulting in 50% of V_{max}, and n is the Hill coefficient.

Results

Binding of omeprazole and chlorzoxazone to HLM and BSA

The binding of OMP and CZX to HLM in the presence and absence of 2% BSA (w/v) is expressed as the unbound fraction present in the incubation mixture (fu_{inc}). Binding of OMP and CZX to HLM was negligible in the absence of BSA (mean fu_{inc} range 0.97 to 0.98). However, OMP and CZX bound significantly to mixtures of HLM plus BSA (2% w/v). The binding of OMP to HLM plus BSA was concentration-independent over the OMP concentration ranges investigated. Mean fu_{inc} values for OMP binding to HLM plus BSA were 0.26±0.03. The binding of CZX to 2% BSA plus HLM was concentration dependent, with fu_{inc} values ranging from 0.16 to 0.38 across the CZX concentration range (50-1,200 µM). For incubations containing BSA, the concentrations of OMP and CZX present in reaction mixtures were corrected for binding in the calculation of kinetic parameters.

Kinetics of omeprazole 5′-hydroxylation and chlorzoxazone 6-hydroxylation

In the absence and presence of BSA, 5′-OH OMP formation by HLM exhibited biphasic kinetics (Figure 1). Derived kinetic parameters are shown in Table 1. In all five livers studied, BSA (%w/v) significantly decreased the K_m for the high affinity component (K_{m1}) of OMP 5′-hydroxylation. Mean K_{m1} values in the absence and presence of BSA were 8.6±6.8 µM and 2.4±1.8 µM, respectively. The mean V_{max} value for the high affinity component (V_{max1}) of OMP 5′-hydroxylation from incubations performed in the presence of BSA (49.7 pmol/min.mg) was also significantly lower than the V_{max} (97.3 pmol/min.mg) observed in the absence of BSA. Consequently, addition of BSA to incubations of HLM resulted in an approximate 2-fold increase in CL_{int} (13.8±8.4 vs. 27.1±12.4 µL/min.mg protein).

By contrast, there was no consistent effect of BSA on the K_m values for the low affinity component (K_{m2}) of 5′-OH OMP formation (catalyzed by CYP3A4) in the 5 livers and the difference in this parameter was not significantly different. Addition of BSA to incubations resulted in non-significant increases in the V_{max} value for the low affinity component (V_{max2}) of OMP 5′-hydroxlation (239±77.4 vs. 269±92 pmol/min.mg; $p < 0.314$) and in the CL_{int} value for the low affinity component (CL_{int2}) of 5′-OH OMP formation (2.34±1.47 vs. 2.75±0.33 µL/min.mg protein).

In the absence of BSA, data for 6-OH CZX formation by HLM were variably described by the Michaelis-Menten, substrate inhibition or Hill equations (Table 2, Figures 2A, 2B, and 2C). However, for the two livers exhibiting positive co-operative kinetics, deviation from hyperbolic (i.e. Michaelis Menten) kinetics was minor, with Hill coefficients of 1.16 and 1.21 (Table 2). Thus, V_{max}/S_{50} values for these livers approximate CL_{int}. Similarly, substrate inhibition was weak ($K_m << K_{si}$)

for the single liver exhibiting substrate inhibition kinetics in the absence of BSA. By contrast, 6-OH CZX formation by HLM in the presence of BSA exhibited Michaelis-Menten kinetics (Figures 2A, 2B, and 2C). The derived kinetic parameters are given in Table 2. Assuming that S_{50} approximates K_m (see above), BSA (2% w/v) had no significant effect on the K_m/S_{50} and V_{max} values for CZX 6-hydroxylation (Table 2).

Table 1. Kinetic parameters for omeprazole 5′-hydroxylation by HLM generated in the absence and presence of BSA

Liver	Without BSA					
	K_{m1}	V_{max1}	CL_{int1}	K_{m2}	V_{max2}	CL_{int2}
HLM7	5.4	148	27	115	291	2.53
HLM10	21	134	6.5	353	179	0.51
HLM12	8.3	95	12	75	325	4.31
HLM13	4.0	30	7.7	47	140	3.00
HLM40	5.0	79	16	191	261	1.37
Mean ± S.D.	8.6±6.8	97±47	13.8±8.4	156±123	239±77.4	2.34±1.47

Liver	With 2% BSA					
	K_{m1}	V_{max1}	CL_{int1}	K_{m2}	V_{max2}	CL_{int2}
HLM7	2.0	79	41	109	298	2.73
HLM10	5.1	52	10	126	275	2.18
HLM12	3.2	62	20	136	401	2.95
HLM13	0.9	25	29	53	157	2.96
HLM40	0.9	31	37	73	213	2.94
Mean ± S.D.	2.4±1.8	50±22*	27±12*	99±35	269±92	2.75±0.33

Units: K_{m1} and K_{m2}, μM; V_{max1} and V_{max2}, pmol/min.mg protein; CL_{int1} and CL_{int2}, μL/min.mg protein
*Significantly different ($p < 0.05$) from parameters derived from incubations without BSA

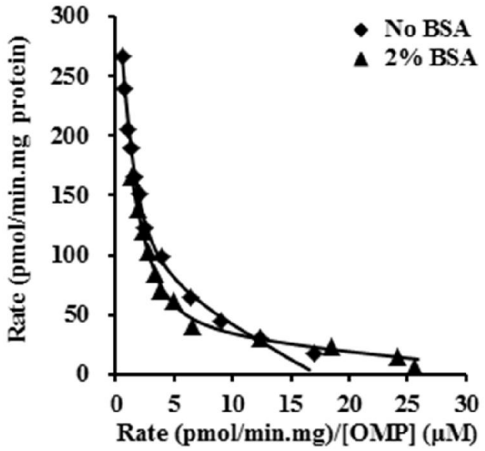

Figure 1.
Representative Eadie-Hofstee plots for omeprazole 5′-hydroxylation by HLM in the absence and presence of BSA. Each point represents the mean of duplicate measurements (< 10% variance) while curves are from model fitting.

Table 2. Kinetic parameters for chlorzoxazone 6-hydroxylation by HLM generated in the absence and presence of BSA

Liver	Without BSA						
	K_m	K_{si}	S_{50}	n	V_{max}	V_{max}/ K_m	V_{max}/ S_{50}
HLM0	133	-	-	-	1587	12	-
HLM1	129	1003	-	-	1100	8.5	-
HLM4	96	-	-	-	1805	19	-
HLM5	-	-	63	1.16	691	-	11
HLM11	-	-	58	1.21	511	-	8.9
Mean ± S.D.	119±20	-	61	1.19	1139±557	13±5.2	10.0

Liver	With 2% BSA		
	K_m	V_{max}	V_{max}/ K_m
HLM0	127	1364	11
HLM1	89	1098	12
HLM4	85	1663	20
HLM5	86	1501	17
HLM11	67	679	10
Mean ± S.D.	91±22	1261±386	14±4.2

Units: K_m, K_{si}, S_{50}, µM; V_{max}, pmol/min.mg protein; n = Hill coefficient

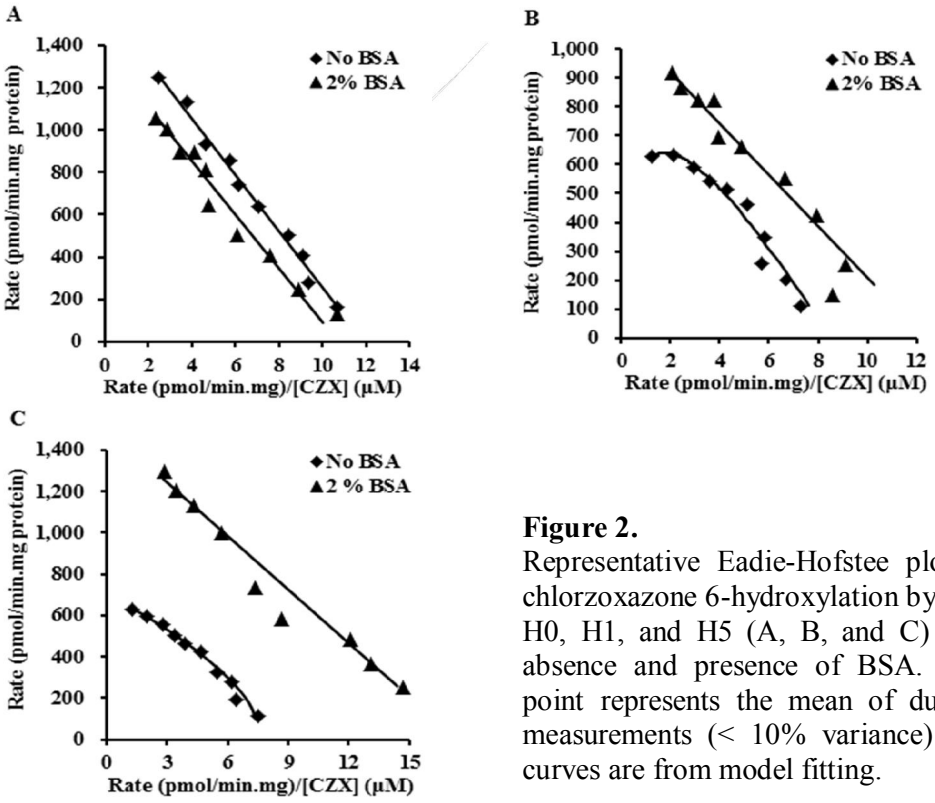

Figure 2.
Representative Eadie-Hofstee plots for chlorzoxazone 6-hydroxylation by HLM H0, H1, and H5 (A, B, and C) in the absence and presence of BSA. Each point represents the mean of duplicate measurements (< 10% variance) while curves are from model fitting.

Discussion and Conclusion

This study characterized the effects of albumin (BSA; 2% w/v) supplementation on human liver microsomal CYP2C19 and CYP2E1 activities. OMP 5′-hydroxylation and CZX 6-hydroxylation were used as the in vitro 'probes' for CYP2C19 and CYP2E1, respectively. BSA (2% w/v) decreased the K_m (by ~75%) and V_{max} (by ~50%) values of the high affinity component for OMP 5′-hydroxylation, with a consequent 2-fold increase in CL_{int}. By contrast, BSA did not significantly alter the kinetic parameters for human liver microsomal CZX 6-hydroxylation.

The K_m for the high affinity component (viz. K_{m1}) for OMP 5′-hydroxyl-ation by HLM in the absence of BSA (5.0-21 μM, mean 8.6 μM; Table 1) is in accordance with previously published values (1.0-16 μM, mean ~6 μM).[17,19,24,25] The approximate 75% decrease in the mean value of K_{m1} for human liver microsomal OMP 5′-hydroxylation in the presence of BSA (2% w/v) is consistent with sequestration of inhibitory PUFAs by albumin. However, the mean V_{max} value for the high affinity component (V_{max1}) of OMP 5′-hydroxylation was also significantly decreased in the presence of BSA (by approximately 50%). As noted in the Introduction, it has been shown previously that addition of BSA to incubations of HLM and recombinant enzymes significantly decreases the K_m values for substrates of CYP1A2, CYP2C8, CYP2C9, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7 and UGT2B15, although for some of these enzymes the effects of albumin were substrate dependent. Effects of albumin on V_{max} are more variable. For example, addition of BSA to incubations of HLM appears not to affect the V_{max} values for substrates of CYP1A2[7], CYP2C9[3], and UGT2B7[4] but may alter the V_{max} values of substrates of CYP2C8[6], CYP2C9[3], UGT1A7[15], UGT1A8[15], UGT1A10[15], UGT2A1[15], UGT2B4[13], UGT2B15[15], and UGT2B17[15]. Effects of BSA on the V_{max}'s for UGT1A9 substrates remains controversial.[12,26] Generally, however, the effects of BSA on V_{max} values tend to be of lesser magnitude than effects on K_m. Furthermore, effects of BSA may depend on the enzyme source, that is HLM and the various systems used to express recombinant human CYP and UGT enzymes.

It has been reported previously that BSA (2% and 4% w/v) has no effect on human liver microsomal CYP2C19-mediated *S*-mephenytoin 4′-hydroxylation activity.[27] However, kinetic parameters (K_m and V_{max}) for S-mephenytoin 4′-hydroxylation were not determined in the previous study.[27] In the absence of full kinetic characterization it is premature to conclude that the latter observation indicates that the effect of BSA on human liver microsomal CYP2C19 activity is substrate dependent. The low affinity component of OMP 5′-hydroxylation is catalyzed by CYP3A4.[17] The lack of effect of BSA on the low affinity component of OMP 5′-hydroxylation observed here is consistent with a previous study from this laboratory that demonstrated BSA was without effect on the low affinity component of lidocaine N-demethylation, which is also catalyzed by CYP3A4.[7]

As indicated above, BSA was without effect on human liver microsomal CZX 6-hydroxylation. The kinetic parameters determined here for CZX 6-hydroxylation and those reported previously differ. In the absence of BSA, the K_m/S_{50} values for CZX 6-hydroxylation using HLM as the enzyme source (58-133 μM; Table 2) were greater than published values (30-39 μM).[21,28] The reason for these differences is unknown, although livers used for the CZX 6-hydroxylation study were from Thai donors.

Further, substantial inter-individual variability in CYP2E1 activity is known to occur.[29] Also of note, non- Michaelis-Menten kinetics (i.e. weak substrate inhibition (K_{si} >> K_m) and weak autoactivation (n ~ 1.2) were observed here for CZX 6-hydroxylation in the absence of BSA, whereas in the presence of BSA (2% w/v) CZX 6-hydroxylation followed Michaelis-Menten kinetics. A transition from Michaelis-Menten to weak substrate inhibition kinetics in the presence of BSA (2% w/v) has been observed for the glucuronidation of zidovudine by HLM and recombinant UGT2B7[30] and for codeine glucuronidation by HLM[13]. It is known that the binding of fatty acid analogs to CYP2E1 induces a conformational change, such that the size and topology of the enzyme active site are altered.[31] Thus, sequestration of fatty acids by BSA may interfere with their modulatory effects on the conformation of the CYP2E1 active, with consequent changes in enzyme kinetics. As with UGT, BSA appears to have a selective effect on CYP enzyme activities. It has been reported that the effects of BSA on glucuronidation activity may be UGT enzyme selective and substrate dependent.[15] Recent studies from this and other laboratories have demonstrated that BSA increases CYP1A2[7], CYP2C8[6], CYP2C9[3,10,11], UGT1A7[15], UGT1A8[15], UGT1A9[12], UGT1A10[15], UGT2A1[15], UGT2B4[13], and UGT2B7[4,10,15], and UGT2B15[15] activities. However, BSA is without effect on CYP2D6[27], CYP3A4[7,32], UGT1A1[12,15], UGT1A4[15,16], and UGT1A6[12,15] activities. The 'albumin effect' appears to be selective for enzymes inhibited by PUFAs. It has been reported that arachidonic acid is a moderately potent inhibitor of CYP2C19.[8,9] Consistent with this observation, BSA significantly increased CYP2C19-mediated OMP 5′-hydroxyl-ation. By contrast, arachidonic acid is a weak or non-inhibitor of CYP1B1, CYP2B6, CYP2D6, CYP2E1 and CYP3A4.[8,9] In agreement with weak inhibition of CYP2E1 by arachidonic acid, K_m values for human liver microsomal CZX 6-hydroxylation were unchanged by BSA in the present study.

In conclusion, this study showed that the addition of BSA (2% w/v) to incubations of HLM enhanced of CYP2C19-catalyzed OMP 5′-hydroxylation but not CYP2E1-catalyzed CZX 6-hydroxylation. Taken together with the results of previous studies, it is evident BSA selectively alters the activities of drug metabolizing enzymes. Thus, supplementation of incubations with BSA is necessary only for those enzymes and substrates whose activities are increased by BSA.

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