In Vitro Evaluation of *Boswellia serrata* Extract (BSE) as an Inhibitor of CYP450 Using Cryopreserved and Fresh Human Hepatocytes and Human Liver Microsomes

Amy Roe[1], Chris Black[2], Ken Brouwer[2], Jonathan Jackson[2]

[1]Procter & Gamble, Cincinnati, OH 45040, [2]Qualyst Transporter Solutions, Durham, NC 27713

#462

INTRODUCTION

As the use of dietary supplements increases, the potential for drug-dietary supplement interaction should be evaluated. *Boswellia serrata* (gum resin) has traditionally been used to treat various inflammatory diseases. Potent *in vitro* inhibition (≥65%) across major drug metabolizing enzymes (CYP1A2/2C8/2C9/2C19/2D6/3A4) using pooled human liver microsomes (pHLM) has been reported in the literature for levels of *Boswellia serrata* extract (BSE) as low as 1 µg/mL.[1] This apparent potent and promiscuous level of inhibition by an herbal ingredient led us to question the relevance of the results in the *in vitro* test system used (pHLM), when compared to a more physiologically-relevant model such as hepatocytes.

This study evaluated the ability of BSE to inhibit CYP2C9 and CYP3A4/5 in pHLM, cryopreserved suspended human hepatocytes, and a fully integrated sandwich-cultured human hepatocyte system (SCHH). The aim of these studies was to understand potential *in vitro* system differences in studying herb-drug interactions. This work focused on CYP2C9 and CYP3A4/5 since these isoforms were previously shown to be the most potently inhibited by BSE. Also, CYP2C9 is a major isoform involved in NSAID metabolism, which could be used concomitantly with BSE in patients suffering from inflammatory diseases such as osteoarthritis. Thus, we utilized the CYP2C9 substrate, ibuprofen, to study a potentially clinically-relevant HDI using SCHHs. Additionally, CYP3A4 metabolizes >50% of all prescription medicines, making it a critical isoform to include in HDI assessments.

METHODS

Test Article:

BSE (gum resin), purchased from Verdure Sciences (Noblesville, IN), was used in the pHLM and cryopreserved human hepatocyte studies. The Verdure Sciences BSE contained a total boswellic acids content of 32.4% by weight (based on Certificate of Analysis). For the initial pHLM and cryopreserved suspended hepatocyte studies, a stock solution of 5 mg/mL was prepared for IC50 evaluations. From this stock solution, working solutions of 0.001, 0.01, 0.05, 0.1, 0.5 and 1 mg/mL were prepared for testing. BSE (gum resin), purchased from Chromadex, Inc (Irvine, CA), was used in the SCHH studies. The Chromadex BSE contained a total boswellic acids content of 42.9% by weight (based on Certificate of Analysis). For the SCHH studies, stock solutions of 7.5, 23.7, 75, and 237 mg/mL were prepared, and working solutions of 0.75, 2.37, 7.5, 23.7, and 75 µg/mL were tested. In general, incubation conditions were based on standard procedures.[2]

Test Systems:

Human microsomes and cryopreserved hepatocytes in suspension - The initial *in vitro* metabolic systems used in these studies were pHLM (N=16 donors) and Cryostax™, single-freeze cryopreserved human hepatocytes (N=10 donor pool). This work was conducted at Xenotech, LLC (Lenexa, KS).

Sandwich-cultured human hepatocytes(SCHH) - Qualyst B-CLEAR® technology utilizes a fully integrated hepatic cell system that supports physiologically relevant drug metabolism and drug transport (uptake and efflux) with proven *in vitro* to *in vivo* correlation to provide clinically relevant data. Sandwich-cultured hepatocytes exhibit both phase I and Phase II drug metabolizing enzymes and maintain key regulatory pathways necessary to model hepatic induction while preserving *in vivo*-like localization of key uptake and efflux transporters.

Enzyme Activity Assays:

IC50 values were determined for direct-, time- and metabolism-dependent inhibition of CYP2C9 and CYP3A4/5 by BSE using pHLM and cryopreserved hepatocytes.

Enzyme	Reagent Use	Name
CYP2C9	Substrate	Diclofenac
	Substrate metabolite	4′-Hydroxydiclofenac
	Internal Standard	4′-Hydroxydiclofenac-d_4
	Direct Inhibition positive control	Sulfaphenazole
	Metabolism-dependent inhibition positive control	Tienilic acid
CYP3A4	Substrate	Midazolam
	Substrate metabolite	1′-Hydroxymidazolam
	Internal standard	1′;-Hydroxymidazolan-d_4
	Direct inhibition positive control	Ketoconazole
	Metabolism-dependent inhibition positive control	Troleandomycin

IC50 values were determined for direct inhibition of CYP2C9 and CYP3A4 by BSE us SCHH.

Enzyme	Reagent Use	Name
CYP2C9	Substrate	Ibuprofen
	Substrate metabolite	3-hydroxy-ibuprofen
	Direct inhibition positive control	Fluconazole
CYP3A4	Substrate	Midazolam
	Substrate metabolite	1′-hydroxy-midazolam
	Direct inhibition positive control	Ketoconazole

METHODS

The effect of adding 4% bovine serum albumin (BSA) to incubations was also tested in the SCHH enzyme activity assays. In general, enzyme activity assay conditions were based on standard procedures.[2]

Hepatotoxicity Assessment of BSE in SCHH:

LDH and ATP depletion assays were used to evaluate potential hepatotoxic effects related to exposure to BSE prior to choosing final dose concentrations. Tamoxifen and Aflatoxin were used as positive controls (i.e., hepatotoxic compounds). BSE and positive controls were compared to untreated controls. Hepatocyte cultures were visually inspected after 24 and 72 hours of various treatment conditions.

RESULTS

Table 1. *In vitro* evaluation of *Boswellia serrata* extract as an inhibitor of human CYP2C9 and CYP3A4/5 using pHLM and cryopreserved hepatocytes

Enzyme	Enzyme reaction	Test system	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition (requires addition of NADPH)		
			IC_{50} (µg/mL)[a]	Inhibition observed at 50 µg/mL (%)[b]	IC_{50} (µg/mL)[a]	Inhibition observed at 50 µg/mL (%)[b]	IC_{50} (µg/mL)[a]	Inhibition observed at 50 µg/mL (%)[b]	Potential for time- or metabolism-dependent inhibition[c]
CYP2C9	Diclofenac 4′-hydroxylation	Microsomes	11	84	9.8	87	12	87	No
		Hepatocytes	> 50	25	> 50	30	NA	NA	No
CYP3A4/5	Midazolam 1′-hydroxylation	Microsomes	1.4	98	1.6	99	1.8	100	No
		Hepatocytes	> 50	32	47	52	NA	NA	No

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.
b Inhibition observed (%) is calculated with the following formula (results are rounded to two significant figures):
Inhibition observed (%) = 100% - percent solvent control.
c Potential for time- or metabolism-dependent inhibition was determined by comparison of IC_{50} values with and without pre-incubation and with and without NADPH-generating system present in the pre-incubation, by comparison of the observed inhibition (%) for all pre-incubation conditions and by visual inspection of the IC_{50} plots.
NA Not applicable. NADPH is inherently present within the hepatocyte test system.
These studies were conducted at Xenotech, LLC (Lenexa, KS)

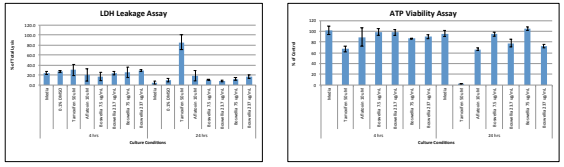


Figure 1. Cytotoxicity assessment at 4 and 24 hours- (above) and cell morphology(below) at 24 and 72 hours post-exposure to BSE.

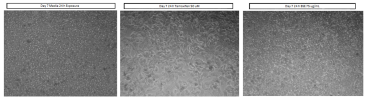


Table 2. *In vitro* evaluation of *Boswellia serrata* extract as an inhibitor of CYP2C9 using sandwich-cultured human hepatocytes. Comparison of IC50 values across *in vitro* systems.

Sandwich-Cultured Human Hepatocytes								
	No BSA	4% BSA		4% BSA				
BSE Concentration (µg/mL)	β - Boswellic Acid (µM)	CYP2C9 Mean ± SD % Remaining Activity	CYP2C9 Mean ± SD % Remaining Activity	Estimated IC_{50} (µg/mL)	β-Boswellic Acid Estimated IC_{50} (µM)	Estimated IC_{50} β - Boswellic Ext. IC_{50} (µM)	PHUM Estimated IC_{50} (µg/mL)	Suspended Cryopreserved Hepatocytes Estimated IC_{50} (µg/mL)
0.00	0.00	100 ± 9.08	100 ± 11.2					
0.750	0.317	100 ± 16.9	105 ± 4.02					
2.37	1.00	95.4 ± 5.85	93.1 ± 7.37					
7.50	3.17	68 ± 8.46	85 ± 10.4	>75	>31.7	>31.7	11	>50
23.7	10.0	84.4 ± 8.54	70.9 ± 0.997					
75.0	31.7	144 ± 6.36	98.1 ± 15.0					

RESULTS

Table 3. *In vitro* evaluation of *Boswellia serrata* extract as an inhibitor of CYP3A4/5 using sandwich-cultured human hepatocytes. Comparison of IC50 values across *in vitro* systems.

Sandwich-Cultured Human Hepatocytes								
	No BSA	4% BSA	No BSA	No BSA	4% BSA	4% BSA		
BSE Concentration (µg/mL)	β - Boswellic Acid (µM)	CYP3A4/5 Mean ± SD % Remaining Activity	CYP3A4/5 Mean ± SD % Remaining Activity	Estimated IC_{50} (µg/mL)	β-Boswellic Acid Estimated IC_{50} (µM)	Estimated IC_{50} β - Boswellic Ext. IC_{50} (µg/mL)	PHUM Estimated IC_{50} (µg/mL)	Suspended Cryopreserved Hepatocytes Estimated IC_{50} (µg/mL)
0.00	0.00	100 ± 31.9	100 ± 25.7					
0.750	0.317	91.8 ± 0.991	69 ± 13.5					
2.37	1.00	88.3 ± 9.25	62.7 ± 6.85					
7.50	3.17	75.9 ± 5.62	55.1 ± NA	>75	>31.7	17.2	7.3	1.4	67.0
23.7	10.0	64.7 ± 3.63	65.4 ± 18.9					
75.0	31.7	63.7 ± 0.661	29.5 ± 5.35					

Figure 2

Boswellia Serrata Inhibition of OHMDZ Formation in SCHH

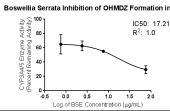
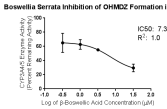


Figure 3

Boswellia Serrata Inhibition of OHMDZ Formation in SCHH



DISCUSSION & CONCLUSIONS

- BSE was a direct inhibitor of CYP2C9 and CYP3A4/5 in pHLM with IC50 values of 11 µg/mL and 1.4 µg/mL; respectively (Table 1). These values were similar to previous literature.[1]
- Direct inhibition of CYP2C9 and CYP3A4/5 in suspended cryopreserved hepatocytes occurred at IC50 values >50 µg/mL (Table 1) .
- No marked changes were observed in cell morphology, ATP, or LDH at the concentrations examined following 4 hr exposure in SCHH (Figure 1). A 28% decrease in ATP was observed following 24 hr of exposure to BSE at 237 ug/mL These data suggested that BSE was well tolerated in SCHH following 24 hr of exposure at concentrations ≤75 ug/mL.
- Direct inhibition of CYP2C9 and CYP3A4/5 using fully-integrated SCHH occurred at IC50 values > 75 µg/mL when BSA was not added to the incubations. When 4% BSA was added to the incubations, the IC50 value did not change for CYP2C9 (Table 2 and Figure 2); however, the IC50 value for CYP3A4 decreased to 17.2 µg/mL (Table 3 and Figure 3).
- The direct inhibition of CYP2C9 and CYP3A4/5 by BSE appears to be dependent upon the *in vitro* test system used to measure the IC50 value.
  - These data indicate that pHLM may be overly conservative in predicting inhibition potential of herbal extracts due to unrealistically high exposure levels.
  - A more integrated system such as SCHH which maintains transport capability (uptake and efflux) is likely more relevant.
- β-Boswellic acid (β-BA) is a major constituent (~20%) of the BSEs used in these *in vitro* studies. Therefore, we estimated IC50 values of β-BA towards CYP2C9 and CYP3A4 activity in the SCHH studies.
  - The IC50 value of β-BA was 31.7 µM for CYP2C9 with or without 4% BSA and CYP3A4 without BSA.
  - The presence of 4% BSA decreased the IC50 value for CYP3A4 to 7.3 µM.
  - This value is similar to what was observed for purified β-BA inhibition of CYP3A4 using baculovirus-infected insect cells; IC50 = 6.3 µM.[1]
  - The estimated IC50 for β-BA established in our SCHH studies is also within range of reported Cmax plasma levels for β-BA from human clinical studies with *Boswellia*; which further supports that the SCHH system is a suitable *in vitro* model for predicting clinically-relevant HDI interactions.[4]
- Additional work is underway to fully characterize the BSEs used across these *in vitro* HDI studies. Once completed, an overall prediction of clinically-relevant interactions may be further defined by combining data from analytical characterization, *in vitro* HDI studies, published clinical studies, and product formulation considerations.

REFERENCES

1. Frank A and Unger M, (2006) J Chromatogr A, 1112:255-262
2. FDA Guidance: Drug Interaction Studies (2012)
3. Kimoto E et al. (2012) Mol Pharmaceutics, 9:3535-3542
4. Sterk V et al. (2004) Planta Med, 70: 1155-1160.