Prediction of Clinical Hepatotoxicity of Clinical Drug Candidates using the Novel C-DILI™ Assay: A Retrospective Case Study

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ABSTRACT

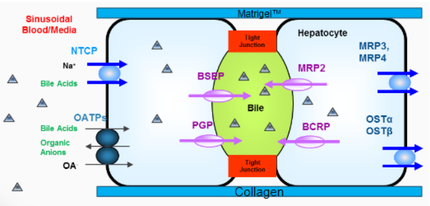
Drug-induced liver injury (DILI) is difficult to predict and arises through multiple mechanisms, one of the key pathways involves inhibition of uptake and efflux transporters that modulate bile salt disposition. Assessing the potential for compounds to inhibit transporters usually entails measuring in vitro inhibition with transfected cells or inside-out vesicles. A major limitation is that these assays do not represent a physiologically-relevant liver model. In contrast, the C-DILI model uses Transporter-Certified™ hepatocytes, that are exposed to physiological relevant concentrations of bile acids and lipids which allows for rapid determination of alterations in bile acid disposition and hepatotoxicity. Such a model was used to determine the hepatotoxicity potential of 3 clinical candidates. Sandwich-cultured human hepatocytes were treated with BMS compounds A, B, and C at concentrations representing multiples of clinically observed or predicted human plasma levels in the absence (standard) and presence (sensitization) of bile salts. After a 24 hour exposure, cells were analyzed for toxicity by measuring intracellular ATP depletion and LDH leakage into media. BMS-A at 100 µM (57x highest observed human Cmax) in sensitization media demonstrated a 210% increase in LDH leakage while ATP was reduced to 79%, however LDH and ATP were essentially unchanged in standard media. BMS-B exhibited no major changes to LDH or ATP in either media at any concentration. At 25 µM (20x highest predicted human Cmax), BMS-C exerted a 173% increase in LDH leakage while ATP content decreased to 87% in the sensitization media. The results suggest that BMS-A has a high risk of cholestatic hepatotoxicity at 100 µM while BMS-C has medium potential at 25 µM to induce cholestatic hepatotoxicity. During clinical trials in healthy subjects, BMS-A and BMS-B increased ALT and AST, however these changes were generally <2x ULN and occurred in less than 10% of subjects. BMS-C was terminated due to hepatic lipidosis in pre-clinical species that manifested at 1.5x the projected human Cmax after an efficacious dose. Taken into context, the C-DILI assay demonstrated an overall low in vitro risk of cholestatic hepatotoxicity of BMS candidates as toxicity was only observed at levels >20x observed/projected human Cmax.

INTRODUCTION

During the course of drug development, assessing the potential for clinical candidates to cause toxicity is critical to establish safety margins and dosing schemes in human clinical trials. However, despite exhaustive tox studies in multiple species, there remains the possibility that preclinical toxicity may not reflect toxicity in humans. It becomes imperative then to establish preclinical models that can accurately determine compound safety in a more physiologically relevant system.

The liver is one such system that requires careful consideration. Figure 1 shows an in vitro model of the liver, in the form of sandwich-cultured hepatocytes (SCH) for which hepatocytes have polarized to form basolateral (blood facing) and apical (bile facing) membranes.

FIGURE 1. SCH System



Each of these membranes possess transporters that are responsible for the uptake and efflux of endogenous compounds, metabolites, and xenobiotics.

A major advantage the SCH system provides is that in addition to uptake and efflux transporters, metabolic enzymes are also active. Furthermore, the system possesses intact regulatory pathways that can be modulated in response to external stimuli. This permits the SCH system to adapt to high exposures of test compounds through regulation of metabolism and transporters, and improve the prediction of hepatotoxicity (e.g. under stress condition when bile acids are elevated).

Four BMS clinical candidate compounds were selected for susceptibility to induce hepatocyte injury following acute compound exposure using human SCH. The incubation concentrations were determined according to human clinical data listed in the table below:

TABLE 1. CLINICAL PARAMETERS

Compound	Clinical Reference Dose	Total Cmax (µM)	Max Observed ALT (U/L)	Max Observed AST (U/L)	Notes
BMS-A	200 mg QD	1.75	64 (ULN=47)	52 (ULN=44)	SAD & MAD studies "clean"
BMS-B	200 mg QD	3.31	94 (ULN=47)	48 (ULN=44)	SAD & MAD studies "clean"
BMS-C	700 mg QD (estimated)	1.27 (estimated)	-	-	Lipidosis in preclinical tox studies
BMS-D	200 mg QD	1.59	307 (ULN=55)	150 (ULN=50)	Clinical trials halted

METHODS

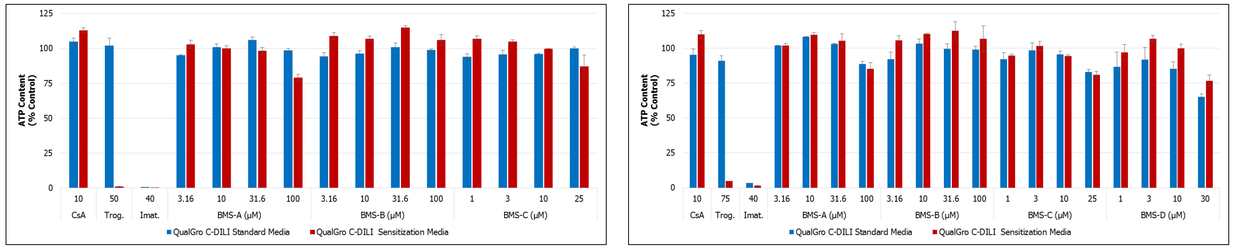
- Sandwich-cultured human hepatocytes (SCHH) were established by thawing Transporter Certified™ cryopreserved cells according the manufacturer's instructions. Once thawed the cells were suspended in QualGro™ Seeding Medium at a density of 0.8 million viable cells/mL and seeded onto BioCoat® 96-well cell culture plates purchased from Corning (Corning, NY).
- After a culture time of 18-24 hours, the seeding medium was removed, and the cells were fed and overlaid with QualGro™ Culture Medium supplemented with 0.35 mg/mL Matrigel®.
- Cells were maintained in QualGro™ Culture Medium until day four of culture. On day four of culture, SCHH were utilized in the C-DILI™ assay, a QTS proprietary assay (patent pending). Briefly, SCHH hepatocytes were exposed to Cyclosporin A (CsA, 10 µM) or Troglitazone (50-75 µM), Imatinib (40 µM), and BMS clinical candidate compounds (multiple concentrations) for 24 hours. Compounds were diluted directly into C-DILI culture or C-DILI sensitization medium, a QTS proprietary medium (contains a physiological mixture of bile acids and lipids).
- Following the exposure period cellular ATP was determined using CellTiter-Glo™ luminescent cell viability assay from Promega (Madison, WI). LDH leakage was determined using CytoTox-ONE™ homogeneous membrane integrity fluorescence assay from Promega. Both assays were performed according to the manufacturer's instructions. Luminescence and fluorescence were measured with a BioTek (Winooski, VT) Synergy 4 Plate Reader.
- The extent of cellular injury, as determined by LDH leakage, was applied to predict a relative risk of hepatotoxicity using the relationship:

DILI Risk Level	
LDH Content Range	Risk Level
0-150%	Low
151-199%	Medium
≥200%	High

- Each treatment group was conducted in triplicate wells across two Transporter Certified™ cryopreserved hepatocyte lots. The hepatocytes in donor #1 (lot DJJ) were from an adult Caucasian male while the hepatocytes in donor #2 (Lot FEA) were from an adult Caucasian female.
- Data are shown as mean ± standard deviations of n=3 replicates.

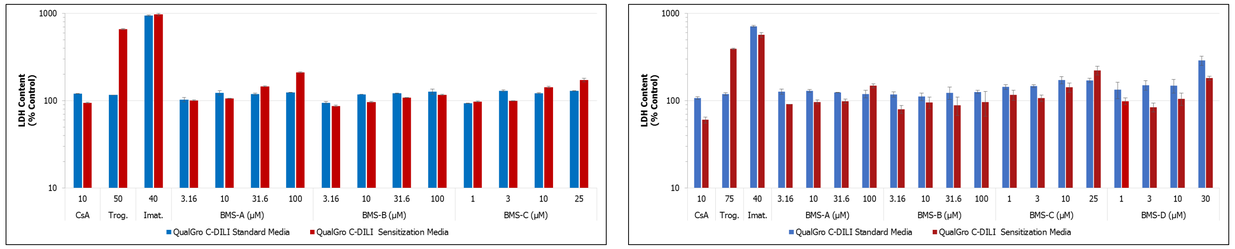
RESULTS

FIGURE 2. INTRACELLULAR ATP DEPLETION AFTER 24 HOUR TREATMENT IN TWO HEPATOCYTE DONORS



Intracellular ATP depletion was measured in donor DJJ (left panel) or donor FEA (right panel) after a 24 hour incubation in the presence of negative and positive controls or increasing concentrations of BMS compounds.

FIGURE 3. LDH LEAKAGE INTO MEDIA AFTER 24 HOUR TREATMENT IN TWO HEPATOCYTE DONORS



LDH leakage from hepatocytes into extracellular media was measured in donor DJJ (left panel) or donor FEA (right panel) after a 24 hour incubation in the presence of negative and positive controls or increasing concentrations of BMS compounds.

TABLE 2. SUMMARY OF ATP AND LDH ENDPOINTS

Compound	Conc. (µM)	QualGro C-DILI Standard Media				QualGro C-DILI Sensitization Media				Hepatotoxicity Potential (Mechanism)
		ATP Content		LDH Content		ATP Content		LDH Content		
		% Control	Std. Dev.	% Control	Std. Dev.	% Control	Std. Dev.	% Control	Std. Dev.	
DMSO 0.1%	NA	100	4	100	1	100	2	100	1	NA
CsA	10	105	2	120	1	113	2	94.7	1.0	Low Hepatotoxicity
Troglitazone	50	102	5	116	1	1.02	0.12	659	9	High Hepatoxicity (Cholestatic)
Imatinib	40	0.597	0.051	946	12	0.270	0.042	973	34	High Hepatotoxicity (General)
BMS-A	3.16	95.2	0.4	103	6	103	3	101	1	Low Hepatotoxicity
	10	101	2	123	7	100	2	106	0	Low Hepatotoxicity
	31.6	106	2	119	3	98.2	2.5	146	2	Low Hepatotoxicity
	100	98.6	1.4	124	1	79.0	2.4	210	4	High Hepatoxicity (Cholestatic)
BMS-B	3.16	94.3	2.6	94.7	3.1	109	2	87.0	1.8	Low Hepatotoxicity
	10	96.4	1.9	118	0	107	2	96.3	1.6	Low Hepatotoxicity
	31.6	101	3	121	1	115	1	108	1	Low Hepatotoxicity
	100	99.0	0.7	127	8	106	4	117	1	Low Hepatotoxicity
BMS-C	1	94.0	2.1	93.7	0.5	107	2	97.9	0.6	Low Hepatotoxicity
	3	95.7	3.0	130	3	105	1	99.3	0.5	Low Hepatotoxicity
	10	96.0	0.3	122	1	100	0	143	4	Low Hepatotoxicity
	25	100	1	130	1	87.1	8.3	173	8	Medium Hepatotoxicity (Cholestatic)

Tabulated summaries for ATP depletion and LDH leakage after a 24 hour incubation in SCHH donor DJJ (left table) or donor FEA (right table). Color coding in tables corresponds to DILI Risk Level as detailed in the Methods section.

Compound	Conc. (µM)	QualGro C-DILI Standard Media				QualGro C-DILI Sensitization Media				Hepatotoxicity Potential (Mechanism)
		ATP Content		LDH Content		ATP Content		LDH Content		
		% Control	Std. Dev.	% Control	Std. Dev.	% Control	Std. Dev.	% Control	Std. Dev.	
DMSO 0.1%	NA	100	0	100	17	100	7	100	3	NA
CsA	10	95.3	4.1	107	4	110	3	60.1	4.6	Low Hepatotoxicity
Troglitazone	75	91.0	3.5	119	5	4.77	0.04	393	7	High Hepatoxicity (Cholestatic)
Imatinib	40	3.32	0.03	710	15	1.41	0.25	567	35	High Hepatotoxicity (General)
BMS-A	3.16	102	0	127	8	102	2	91.2	0.0	Low Hepatotoxicity
	10	108	0	129	4	110	2	97	5	Low Hepatotoxicity
	31.6	103	0	124	1	105	5	99	6	Low Hepatotoxicity
	100	88.6	1.9	119	12	85.3	4.3	148	7	Low Hepatotoxicity
BMS-B	3.16	92.1	5.1	118	8	106	3	79.2	8.3	Low Hepatotoxicity
	10	103	3	111	11	110	1	95.1	14.5	Low Hepatotoxicity
	31.6	99.7	3.4	123	20	112	7	88.6	20.8	Low Hepatotoxicity
	100	99.1	2.4	126	5	107	10	96.7	30.8	Low Hepatotoxicity
BMS-C	1	92.2	4.7	143	9	94.8	1.0	116	15	Low Hepatotoxicity
	3	98.6	5.3	147	6	102	3	107	9	Low Hepatotoxicity
	10	95.6	2.3	172	17	94.6	0.8	142	17	Low Hepatotoxicity
	25	83.1	1.6	171	10	80.8	2.7	223	26	High Hepatoxicity (Cholestatic)
BMS-D	1	86.8	10.5	134	28	97.0	5.6	98.4	8.6	Low Hepatotoxicity
	3	91.7	8.8	151	19	107	2	84.0	9.6	Low Hepatotoxicity
	10	85.4	5.0	148	27	99.9	3.2	105	17	Low Hepatotoxicity
	30	65.2	2.2	288	35	76.7	4.0	181	10	High Hepatotoxicity (General)

DISCUSSION

The hepatotoxicity category was determined based upon the LDH leakage profile across media conditions. Briefly, marked LDH leakage in only bile acid sensitization media suggests a cholestatic hepatotoxicity mechanism while marked LDH leakage in both media conditions suggests a general hepatotoxicity mechanism.

CONTROLS: Treatment with CsA, a negative control, was not observed to increase LDH leakage or decrease ATP content in SCHH under either QualGro (standard culture conditions) or Sensitization (bile acid fortified) media culture conditions as expected. In contrast, troglitazone and imatinib treatment increased LDH secretion markedly in SCHH. Troglitazone markedly increased LDH leakage and decreased ATP content in SCHH cultured under sensitization conditions only. In contrast, imatinib treatment significantly increased LDH leakage and reduced ATP content in SCHH under both culture conditions. These results demonstrated that the positive controls induced toxicity through their respective mechanisms as expected and demonstrated that culture conditions were adequate to detect and distinguish general from cholestatic hepatotoxicity.

BMS-A: Dose-related LDH leakage reaching > 200% of control was observed in one (DJJ) of the two SCHH preparations under sensitization media conditions only following 24 hours of exposure to 100 µM BMS-A. A concomitant decrease in ATP (>20%) was also observed in DJJ under sensitization media conditions only following treatment with 100 µM BMS-A. These results suggested that BMS-A has **high** hepatotoxicity (cholestatic) potential at concentrations ≥ 100 µM.

BMS-B: No marked LDH leakage or loss of ATP content was observed across either media condition examined in two separate SCHH cultures treated with 3.16 to 100 µM BMS-B. These results suggested that BMS-B has **low** hepatotoxicity potential across the concentrations examined.

BMS-C: Dose-related LDH leakage and loss of ATP was observed in both SCHH preparations under sensitization media conditions only following 24 hours of exposure to 25 µM BMS-C. LDH leakage > 200% of control was observed in one (FEA) of the two SCHH preparations under sensitization media conditions treated with 25 µM BMS-C. A concomitant decrease in ATP (≈20%) was also observed in FAE under sensitization media conditions only following treatment with 25 µM BMS-C. Taken together, these results suggested that BMS-C has **high** hepatotoxicity (cholestatic) potential at concentrations ≥ 25 µM.

BMS-D: Dose-related LDH leakage and loss of ATP was observed in SCHH preparation FAE under both media conditions following 24 hours of exposure to 30 µM BMS-D. LDH leakage >200% of control and a concomitant loss of ATP >30% of control was observed in SCHH under standard media conditions treated with 30 µM BMS-932481. These results suggested that BMS-D has **high** hepatotoxicity (general) potential at concentrations ≥ 30 µM.

SUMMARY

Overall these results suggest that the hepatotoxicity potential of BMS-C ≥ BMS-D > BMS-A >> BMS-B.