# Predicting drug-induced liver injury in vitro

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## Abstract

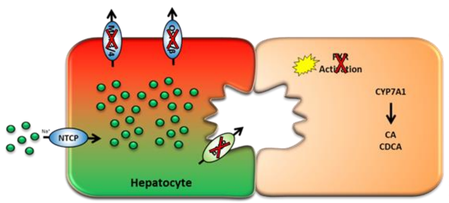
Drug-induced liver injury (DILI) is a common reason for late stage drug failure and remains difficult to predict. The C-DILI™ Assay uses Sandwich-Cultured Transporter Certified™ Human Hepatocytes (SCHH) cultured under physiological relevant concentrations of bile acids and lipids, allowing for determination of alterations in bile acid disposition and hepatotoxicity. In this study, the hepatotoxicity potential and mechanism (cholestatic versus general) was assessed using the C-DILI™ Assay for four preclinical candidates (BIO1 through BIO4) found to be hepatotoxic in repeat-dose rat studies as indicated by histopathology and/or clinical chemistry changes. All four compounds were inactive in a BSEP inhibition assay at concentrations up to 100 µM (unless limited by solubility). SCHH were exposed to test article under standard conditions or bile acid sensitization conditions to assess hepatotoxicity potential and mechanism using the LDH leakage profile across the media conditions. Concentration-dependent increases of LDH were observed in SCHH treated with BIO1, BIO2, and BIO3 under both media conditions, suggesting that these three test articles have the potential to cause hepatotoxicity through a general toxicity mechanism rather than impairing bile acid homeostasis. In contrast, LDH leakage was 13X greater under sensitization media versus standard conditions following the lowest BIO4 treatment examined, suggesting that BIO4 has the potential to disrupt bile acid homeostasis in hepatocytes leading to bile acid-dependent or cholestatic hepatotoxicity. The C-DILI™ Assay results for general hepatotoxicity were consistent with rodent findings, however, based on this assay, only BIO4 would have the potential to cause cholestatic hepatotoxicity in humans.

## C-DILI™ Assay Experimental Design/ Methods

- Cryopreserved hepatocytes were thawed and suspended in propriety hepatocyte seeding medium (QualGro™ Seeding Medium) at a density of 0.8 million viable cells/mL onto BioCoat® 96-well cell culture plates.
- Cells were allowed to attach for 2-4 hours, rinsed, and fed with warm seeding medium.
- 18-24 hours later, cells were fed and overlaid with propriety culture medium (QualGro™) supplemented with Matrigel® (0.25 mg/mL) and maintained in QualGro™ Hepatocyte Culture Medium for 4 days until used in studies.
- Hepatocytes were then treated with QualGro™ Sensitization or QualGro™ Standard Human Culture Medium containing solvent control DMSO (0.1%), positive and negative controls CsA, Troglitazone, and Ketoconazole, and test articles BIO1-BIO4.
- Following 24 hours of exposure, culture plates were harvested for ATP (controls only) and LDH analysis. Cellular ATP content was determined using the CellTiter-Glo® Assay Kit [Promega (Madison, WI)]. LDH content of media was determined using the CytoTox-ONE® Assay Kit (Promega).

## C-DILI™ Assay Experimental Design/ Methods

**Figure 1. Bile Acid-Induced (e.g. Cholestatic) Hepatotoxicity**




The C-DILI assay is a comprehensive assay that integrates effects on BSEP, OST, MRP3/4, and FXR using SCHH to assess cholestatic hepatotoxicity potential. SCHH repolarize and form networks of bile canaliculi-like structures and reestablish vectorial flow of bile acids while maintaining other mature hepatocyte functions, including nuclear receptor signaling and metabolism. These are necessary biological processes involved in bile acid homeostasis that an appropriate model system must support to prospectively evaluate a new chemical entity's (NCE) cholestatic DILI potential.

Inhibition of BSEP alone is not sufficient to result in cholestatic hepatotoxicity due to the activation of FXR increasing basolateral efflux (OST) of bile acids and preventing hepatotoxicity[1]. However, if those same compounds also antagonize FXR and prevent the hepatocyte from reducing intracellular concentrations of bile acids, then those compounds would increase the potential for cholestatic hepatotoxicity[1]. NCE that disrupt multiple pathways of the bile acid homeostasis mechanisms have higher cholestatic hepatoxicity potential.

## Results

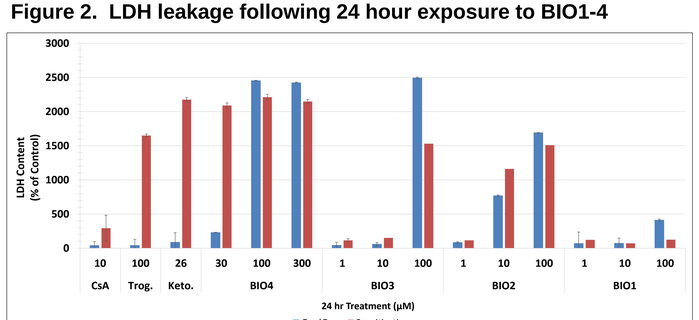
**Table 1. LDH leakage following 24 hour exposure to BIO1-4**

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

| Compound | Conc. (µM) | QualGro Standard | | | | QualGro Sensitization | | | | Hepatotoxicity Potential (Mechanism) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ATP Content | | LDH Leakage | | ATP Content | | LDH Leakage | | |
| | | % of Control | Std. Dev. | % of Control | Std. Dev. | % of Control | Std. Dev. | % of Control | Std. Dev. | |
| CsA | 10* | 117 | 6.8 | 73.4 | 4.5 | 121 | 4.3 | 99.4 | 7.0 | Low Hepatotoxicity |
| Troglitazone | 100* | 97.9 | 4.5 | 110 | 3.2 | 0.197 | 0.033 | 2030 | 58 | High Hepatoxicity (Cholestatic) |
| Ketoconazole | 12 | 105 | 3.2 | 166 | 48.0 | 22.7 | 3.9 | 1100 | 20 | High Hepatotoxicity (Cholestatic) |
| BIO4 | 30 | | | 147 | 3.0 | | | 1910 | 220 | High Hepatoxicity (Cholestatic) |
| | 100 | | | 5100 | 500 | | | 3480 | 160 | High Hepatotoxicity (General) |
| | 300 | | | 6260 | 140 | | | 3410 | 120 | High Hepatotoxicity (General) |
| BIO3 | 1* | | | 58.4 | 0.75 | | | 62.1 | 0.30 | Low Hepatotoxicity |
| | 10* | | | 4.12 | 5.8 | | | 82.1 | 25 | Low Hepatotoxicity |
| | 100* | | | 1690 | 460 | | | 1630 | 270 | High Hepatotoxicity (General) |
| BIO2 | 1* | | | 81.6 | 2.8 | | | 77.7 | 12 | Low Hepatotoxicity |
| | 10 | | | 122 | 32 | | | 120 | 32 | Low Hepatotoxicity |
| | 100 | | | 3280 | 130 | | | 1740 | 14 | High Hepatotoxicity (General) |
| BIO1 | 1 | | | 84.2 | 14 | | | 61.0 | 13 | Low Hepatotoxicity |
| | 10* | | | 71.4 | 5.2 | | | 46.5 | 6.5 | Low Hepatotoxicity |
| | 100 | | | 180.0 | 30.0 | | | 48.7 | 1.9 | Medium Hepatotoxicity (General) |

*Average N=2 replicates (Grubbs outlier test), all others N=3

**Figure 2. LDH leakage following 24 hour exposure to BIO1-4**




## Discussion

- The hepatotoxicity mechanism was determined based upon the LDH leakage profile of cells cultured in standard QualGro™ Human Medium versus bile acid sensitization conditions, QualGro™ Sensitization Medium (Figure 2 and Table 1).
- LDH leakage across both media conditions suggested the following rank order (highest to lowest) of hepatotoxicity potential: BIO4 >BIO3 ≥ BIO2 >> BIO1.
- LDH leakage was 13X greater under sensitization media vs. standard conditions following 30 µM BIO4 suggesting that BIO4 has the potential to disrupt the bile acid homeostasis mechanism in hepatocytes at this concentration.
- Increases of LDH leakage > 1630% ± 270% of control were observed in SCHH treated with ≥ 100 µM BIO4, BIO3, or BIO2 under both media conditions, suggesting that all 3 test articles have the potential to cause general hepatotoxicity at concentrations ≥ 100 µM.
- LDH leakage in SCHH following BIO1 treatment was < 200% of solvent control under both media conditions evaluated suggesting that BIO1 had the least hepatotoxicity potential of the test articles evaluated.
- The C-DILI™ Assay results were consistent with rodent findings (Table 2), Based on this assay, only BIO4 would have the potential to cause cholestatic hepatotoxicity in humans.

**Table 2. Correlation between rat study liver injury findings and cDILI™ results**

| BIO # | study length | summary of liver findings | Plasma Cmax Range (uM) | cDILI assay conc (uM) | cDILI assay results | Hepatoxicity Mode | BSEP Inhibition |
|---|---|---|---|---|---|---|---|
| BIO1 | 5 d | vacuolation (glycogen-like) & necrosis (mild – moderate), elevated liver enzymes | 4.8 - 36.2 | 1 | low | N/A | >100µM |
| | | | | 10 | low | N/A | |
| | | | | 100 | medium | General | |
| BIO2 | 14 d | necrosis (hep and bile duct)& bile duct hyperplasia (mild), elevated liver enzymes | 8.2 - 15.0 | 1 | low | N/A | >10µM (limited by solubility) |
| | | | | 10 | low | N/A | |
| | | | | 100 | high | General | |
| BIO3 | 28 d | No liver histopathology findings; elevated liver enzymes | 41.5 - 154.2 | 1 | low | N/A | >100µM |
| | | | | 10 | low | N/A | |
| | | | | 100 | high | Direct | |
| BIO4 | 14 d | hypertrophy, reactive sinusoidal linings, vacuolation, necrosis (mild-moderate), elevated liver enzymes | 100.9 -161.7 | 30 | high | Cholestatic | >14µM (limited by solubility) |
| | | | | 100 | high | General | |
| | | | | 300 | high | General | |

### References:

1. Jackson JP et al. Cholestatic DILI: A Function of BSEP Inhibition and FXR Antagonism. Appl In Vitro Tox 2018 4(3): advanced online publication DOI: 10.1089/aivt.2018.0011

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