Drug induced liver injury (DILI) has led to the withdrawal of drugs from the market. One postulated mechanism of DILI is cholestasis caused by inhibition of the bile salt efflux pump (BSEP). Inhibition of BSEP can be measured in vitro with BSEP expressing vesicles. However, not all drugs identified as BSEP inhibitors cause DILI as there are many pathways involved in cholestatic toxicity. An in vitro system which models in vivo transporter expression and functionality more completely may better predict the potential of drugs to cause cholestasis or DILI. We evaluated the hepatobiliary disposition of taurocholic acid (TCA) in the presence of cholestatic agents using B-CLEAR® human sandwich-cultured hepatocytes.

**Background & Purpose**

Drug-induced liver injury (DILI) is characterized as liver injury due to intake of medications or xenobiotics, which leads to liver abnormalities or dysfunction.1 There are a large number of drugs that have been withdrawn from the market due to acute DILI, including, troglitazone (antidiabetic and anti-inflammatory), benzotriazone (glut), and allopurinol (purine aldehyde dehydrogenase).2 The mechanism behind DILI is multifaceted and can be exacerbated by unpredictable metabolism and bodily response, as well as the complex relationship between an individual genetic makeup and environmental risk factors.3 Furthermore, several factors may contribute to hepatobiliary injury and cholestasis, including, hepatocellular necrosis, the production of reactive metabolites (and oxidative stress) during cytochrome P450 metabolism of the parent drug, activation of stress signaling, and mitochondrial dysfunction.4

Bile acids are water soluble and products of cholesterol metabolism which are highly regulated by metabolism, excretion, absorption, and feedback mechanisms, in order to limit their intracellular accumulation. It is hypothesized that dysfunction to the bile salt efflux pump (BSEP) may contribute to the mechanism of acute cholestasis, and over-expression of BSEP in vitro models of BSEP inhibition may not accurately predict the potential for DILI. Therefore, an in vitro model which better reflects in vivo transporter expression and activity may better predict the in vivo level sensitivities to cause DILI in the clinic. The purpose of this work was to evaluate the use of B-CLEAR® human sandwich-cultured hepatocytes to investigate the hepatobiliary disposition of taurocholic acid (TCA) in the presence of cholestatic agents.

**Materials & Methods**

**Materials:** Human hepatocytes were isolated and purified at Xenotech, LLC and characterized for use at Qualyst Transporter Solutions, LLC. Seeding, overlay, and culturing media, and Plus (+) and Minus (-) Buffer Assay Buffers were obtained from Qualyst Transporter Solutions. (μM) taurocholic acid (TCA) was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA, USA). Bovine serum albumin, carboxylmethyl, cisapride monohydrate, cyclosporine A, etoposide, glibenclamide, nimesulide, propofol, sulpiride, and troglitazone were obtained from Sigma (St. Louis, MO, USA). Eastmead and oximes were obtained from Cayman Chemicals (Ann Arbor, MI, USA).

**Cell Culture:** Human hepatocytes were seeded at a density of 1.5x10^5 cells/ml in 24 well culture plates. Morphology: 48 hours after seeding, morphology was determined. After overnight (0.25-2mg/ml) was added to the wells and allowed to polymerize overnight. Cells were fed every 5 days and kept at 37°C, 95% relative humidity, and 5% CO2.

B-CLEAR® Assay: On day 0, cells were removed from the incubator and rinsed 3 times with PBS (+) Buffer. Cells were then pre-incubated in 300 μl PBS (+) or Buffer for 10 min at 37°C with one of the following inhibitors: cyclosporine, cyclosporine A (Ca), cisapride monohydrate, cyclosporine, fluvalastin, glibenclamide, nimesulide, propofol, sulpiride, troglitazone. Pre-incubation buffer was replaced and replaced with 300 μl PBS (+) or buffer containing 25 μM taurocholic acid (TCA) and the inhibitors previously listed and incubated in the presence of 4% DMSO for a total of 30 min at 37°C. At 5 minutes post-inhibitor addition times in PBS (+) or buffer, the cells were washed three times in PBS (±) buffer. The cells were then added to the assay buffer (1XenoTech, LLC, 16825 W. 116th St., Lenexa, KS, USA 2Qualyst Transporter Solutions, LLC, 2810 Meridian Parkway, Durham, NC, USA)

**Conclusions**

The B-CLEAR® human hepatocyte sandwich cultured assay demonstrates the ability to utilize the accumulation of a probe substrate to assess both inhibition of both uptake as well as efflux. Many xenobiotics which inhibit efflux out of hepatocytes can lead to a risk of hepatotoxicity. It is important to note that a compound capable of inhibiting uptake into hepatocytes may lead to a risk of systemic toxicity. Therefore, the use of an in vitro system which more accurately models in vivo conditions can better predict the overall effect of xenobiotics on uptake, clearance and potential toxicity. These data presented here demonstrate that hepatobiliary uptake and clearance are complicated processes which are influenced by a number of factors. The Quantitative Transporter Solutions B-CLEAR technology panel with Xenotech-produced sandwich-cultured hepatocytes are able to better assess inhibition of both uptake and efflux in hepatocytes. Future directions include a correlation of toxicity utilizing human sandwich-cultured hepatocytes in vitro to better understand the effect of acute and chronic exposure to these clinically relevant cholestatic agents.

References


**Table 1. Summary of screening results for clinically relevant cholestatic agents**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Cholestasis Agent</th>
<th>Concentration (µM)</th>
<th>IC50 of TCA (µM)</th>
<th>BEI (%)</th>
<th>Kp value</th>
<th>% Change of Control Kp</th>
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<tr>
<td>Solvent Control</td>
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<td>0.1% DMSO</td>
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<td>Carbozyzine</td>
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<td>Cisapride Monohydrate</td>
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</table>

**Table 2. Mean total accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 1. Nicardipine total accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 2. Eastmeid total accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 3. Fluvoxamine total accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 4. Cyclopamine A total accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 5. Olmesartan medoxomil total accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 6. Chlopamide total accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 7. TCA accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 8. BBP accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 9. Eastmeid accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 10. Fluvoxamine accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 11. TCA accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 12. Eastmeid accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 13. Fluvoxamine accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 14. TCA accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 15. Eastmeid accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 16. Fluvoxamine accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 17. TCA accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 18. Eastmeid accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 19. Fluvoxamine accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 20. TCA accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 21. Eastmeid accumulation (pmol/mg) and average BEI (%) (+/−)

**Figure 22. Fluvoxamine accumulation (pmol/mg) and average BEI (%) (+/−)