Use of B-CLEAR® Human Sandwich-Cultured Hepatocytes to Screen Compounds for Cholestatic Potential

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INTRODUCTION

Inhibition of the bile salt export pump (BSEP), which is localized to the canalicular membrane of hepatocytes and is the major efflux route for bile acids, has been implicated as a potential mechanism for drug-induced cholestasis and hepatotoxicity. Current screening methods using transfected models have not shown a strong correlation between BSEP inhibition potency and clinical cholestasis or hepatotoxicity. Bile acid homeostasis is tightly controlled through many mechanisms including multiple transport proteins that take up bile acids from the blood and efflux them into bile. The relative extent of inhibition of *both* uptake and efflux determines the intracellular accumulation of bile acids. A potential inhibitor's intracellular concentration is also important since it determines the extent of transport inhibition and drives toxicity. Thus, an *in vitro* model that has all transport proteins expressed, localized, and functioning as *in vivo* is critical to predict accumulation and the net effect of uptake and efflux inhibition, as it occurs *in vivo*.

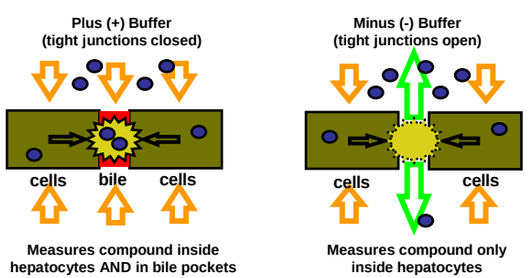

Figure 1. Schematic of B-CLEAR® technology

The goal of this study was to measure changes in uptake, efflux, and intracellular concentration of taurocholic acid (TCA) in B-CLEAR® human sandwich-cultured hepatocytes following exposure to the BSEP inhibitors pioglitazone (PIO), bosentan (BOS), or clozapine (CLOZ), and to relate the extent of inhibition to the intracellular concentrations of the inhibitors.

METHODS

Human Hepatocyte Isolation and Culture. Freshly isolated hepatocytes were cultured in 24-well BioCoat™ plates and overlaid with Geltrex™ 24 hours post-seeding. Culture medium was changed daily; uptake studies were performed on Day 7.

Experimental. Using B-CLEAR® technology, the effects of inhibitors on the hepatobiliary disposition of TCA were determined. Culture medium was removed from cells, and cells were washed twice and pre-incubated with 1 µM [^{3}H]TCA or 2.5 µM d_8-TCA for 10 minutes. The TCA was then aspirated and cells were washed and incubated in the presence or absence of inhibitor (1 µM PIO, 40 µM BOS, or 130 µM CLOZ) for 10 min. Cells were then washed to remove residual substrate, and lysates of cells+bile pockets and cells and were analyzed by scintillation counting or LC-MS/MS for accumulation of TCA. Triplicate samples were evaluated in n=3 human livers.

Sample Preparation and Analysis. For [^{3}H]TCA accumulation studies, radioactivity was measured in cell lysates by liquid scintillation counting. For measurement of accumulation of d_8-TCA and PIO, BOS, and CLOZ, cell lysates were prepared by extraction with 70% methanol in water containing internal standard. Lysates were filtered and evaporated to dryness under nitrogen. Dried samples were reconstituted, filtered and analyzed by LC-MS/MS, which employed reversed-phase HPLC and electrospray ionization.

Data Analysis. Total accumulation, cellular accumulation, intracellular concentration, and biliary excretion (BEI) were determined using B-CLEAR® technology. Data are expressed as percent of control (mean ± standard deviation).

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RESULTS AND DISCUSSION

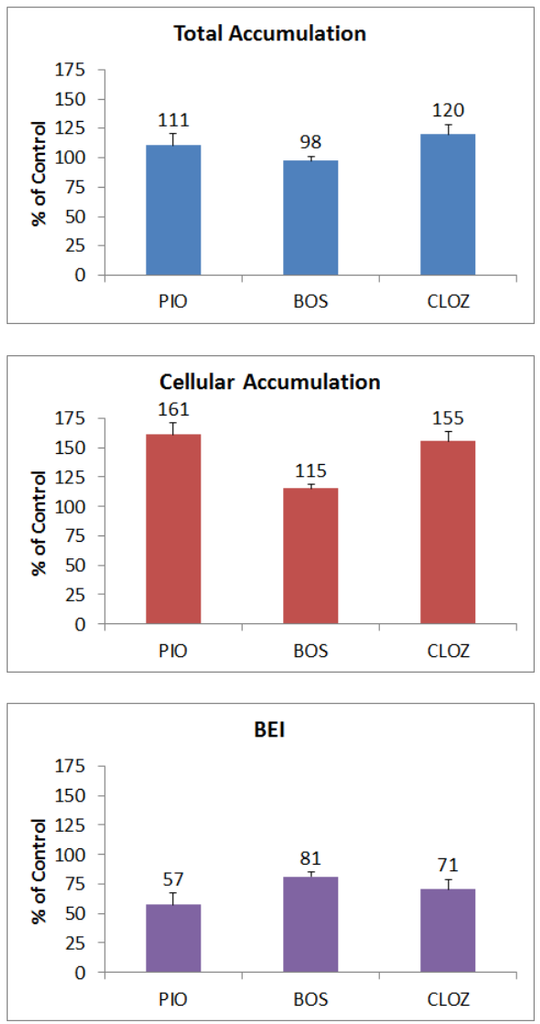

Figure 2. Total Accumulation (top), Cellular Accumulation (middle) and BEI (bottom) of TCA presented as % of control. Data represent mean ± SD of n=3 human donors.

	PIO	BOS	CLOZ
[Exposure] (µM)	1	40	130
[Intracellular] (µM)	57 ± 1	348 ± 7	4076 ± 331
Fold Above Exposure	57	9	31

Table 1. Intracellular concentration of PIO, BOS, and CLOZ following a 10 min incubation. Data represent mean ± SD of n=1 human donor.

CONCLUSIONS

In BSEP membrane vesicles, the reported IC_{50} for TCA transport is 3 µM for PIO, 126 µM for CLOZ, and 38 µM BOS.[1] However, our studies indicate that the magnitude of bile acid transport inhibition also depends on the intracellular accumulation of inhibitor within the hepatocyte.

- All three compounds had little effect on Total Accumulation of TCA (TCA accumulated in cells plus bile pockets)
- All three compounds inhibited canalicular efflux of TCA (BEI) and caused its intracellular accumulation to increase (Cellular Accumulation).
- The extent of compound accumulation corresponded to efflux inhibition and TCA accumulation, with PIO>CLOZ>BOS

These results indicate that *both* uptake and efflux processes, which together govern the intracellular concentration of both substrates and inhibitors, are important parameters in evaluating compounds' cholestatic potential. The B-CLEAR® model, which incorporates all of these parameters, as well as metabolism, is a useful model to evaluate the cholestatic potential of compounds.

1. Dawson et al., Drug Metab Dispos. 2012 Jan;40(1):130-8