A NOVEL OATP/NTCP/BSEP TRIPLE EXPRESSION MODEL FOR STUDYING CANALICULAR BILE SALT TRANSPORT AND DRUG-INDUCED CHOLESTASIS

Mark S. Warren, Alan Kosaka, Mirza Jahic, Jane Huang, and Yong Huang
Optivia Biotechnology Inc., 115 Constitution Dr. Suite 7, Menlo Park, CA, USA

P234

OPTIVIA Biotechnology Inc.
www.optiviabio.com

ABSTRACT

Drug-induced cholestasis and hepatocellular injury are two major manifestations of drug-induced liver disease (DILI). Research has shown such injuries are often attributed to inhibition of bile salt transporters in the liver. NTCP, OATP1B1, BSEP and MRP2 have been shown to play major roles in hepatic clearance of bile salts and their conjugates. Because of the challenges in obtaining cell lines stably expressing these transporters, it is difficult to conduct in vitro studies on vectorial transport of bile salts and drug-induced cholestasis. Here we present a novel cell model that concomitantly expresses **OATP1B1, NTCP and BSEP transporters in polarized MDCK cells**, along with detailed functional characterization of this model.

Using a novel transient transfection technology called Opti-Expression, high levels of transporters have been consistently expressed – individually and in various combinations – in polarized MDCK cell monolayers. To demonstrate the effect of each transporter on bile salt transport, B>A flux and intracellular retention of [^{3}H]Taurocholate (TC) were measured in polarized MDCK cells expressing either GFP as a control, BSEP only, OATP1B1/NTCP, NTCP/BSEP or OATP1B1/NTCP/BSEP, by transfecting cells with a similar number of plasmid copies for each transporter. Our results demonstrated: 1) cells expressing BSEP alone did not lead to increased TC transport compared to the control cells, indicating that vectorial transport of low-permeability TC requires both basal and apical transporters working in concert; 2) B>A flux of TC in cells expressing both NTCP and BSEP (the NTCP/BSEP and OATP1B1/NTCP/BSEP models) was significantly higher than the rest; 3) compared to the NTCP/BSEP model, the triple expression system showed slight increases in B>A flux, suggesting OATP1B1 transports TC less efficiently than NTCP does. Furthermore, vectorial TC transport in the triple-expression system was drastically reduced by inhibitors of BSEP and/or NTCP, such as Rifampicin and Bromosulfophthalein (BSP). It was also demonstrated that the B>A flux of TC mediated by NTCP/OATP1B1/BSEP was time- and dose-dependent. On the other hand, OATP1B1/NTCP-expressing cells had over 100 times higher intracellular TC compared to the control cells, while co-expressing BSEP resulted in more than 90% reduction of intracellular TC. These results clearly demonstrate that NTCP is the dominant basal uptake transporter and BSEP is the major apical efflux transporter of taurocholate, and that, collectively, NTCP and BSEP are responsible for excretion of taurocholate in the liver.

A number of cholestasis-inducing agents, such as Rifamipin, Troglitazone, and Bosentan, were tested using our triple expression system. Interestingly, depending on their relative potency of inhibiting NTCP and BSEP, these agents showed distinctive effects on intracellular TC retention, suggesting some agents are more likely to cause mixed cholestatic and hepatocellular damage in the liver than others. Our data also suggested that OATP1B1 can be an important modulator of intracellular BSEP inhibition, resulting in intrahepatic cholestasis through enhanced cellular entry of low permeability drugs that are substrates of OATP1B1. Our studies demonstrate that this OATP1B1/NTCP/BSEP triple expression model can be a useful tool to study bile salt transport, drug-induced cholestasis and hepatocelluar toxicity.

BACKGROUND

Bile salts are essential for the digestion and absorption of lipids and fat-soluble vitamins, yet are amphipathic molecules displaying detergent properties, causing toxicity in cells such as hepatocytes. Thus, anything disrupting the normal enterohepatic circulation of bile salts can result in problems ranging from mild cholestasis to severe and potentially fatal liver injury. The primary basolateral transporter responsible for uptake of bile salts into hepatocytes from the blood and/or sinusoid is NTCP, with lesser roles from members of the OATP family. The transporter nearly exclusively responsible for apical efflux of unconjugated bile salts into the canaliculus is BSEP. In addition to bile salts, BSEP has been shown to transport and be inhibited by a variety of xenobiotics. Therapeutics that interfere with BSEP function, such as bosentan, erythromycin, and nafazodone, are often associated with liver liabilities. Hepatotoxicity remains a primary cause of failure during drug development, drug approval, and the post-approval withdrawal of licensed drugs. Due to these issues, there is a strong need for an in vitro system amenable to screening of research-stage molecules that could indicate potential negative effects on bile function.

MATERIALS AND METHODS

Combinations of these transporters were expressed in MDCK cells using Optivia's proprietary *in situ* transfection technology, Opti-Expression. Transfections used similar numbers of plasmid copies for each transporter. Intracellular retention and B→A flux of ^{3}H-Taurocholate (TC) were measured in cells expressing either GFP as a control, BSEP only, OATP1B1/NTCP, NTCP/BSEP or OATP1B1/NTCP/BSEP.

RESULTS

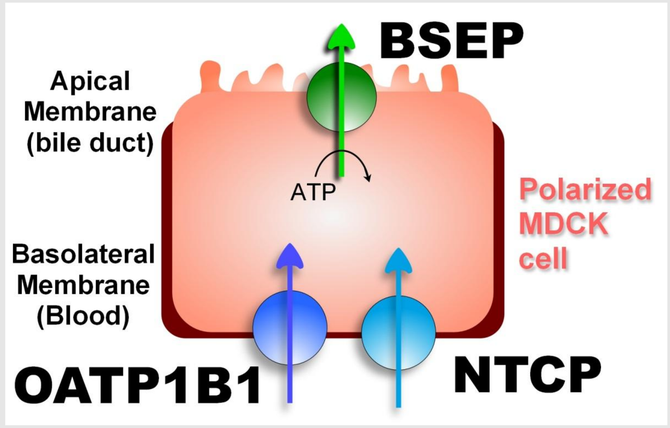

Figure 1. MDCK model co-expressing major bile salt transporters in the liver. OATP and NTCP will transport bile salts (BS) into the polarized MDCK monolayer cells, while BSEP will efflux those bile salts across the apical membrane.

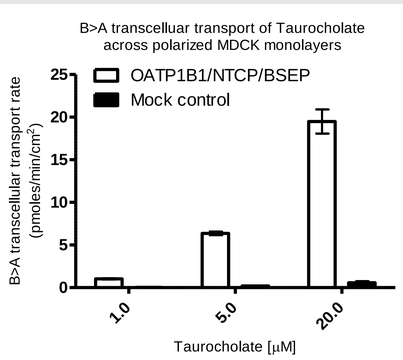

Figure 2. Dose response of the B→A flux of Taurocholate.

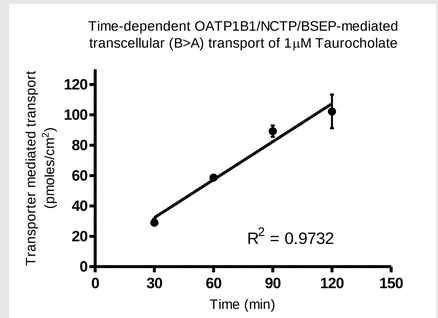

Figure 3. The OATP1B1/NTCP/BSEP triple expression model shows that taurocholate transcellular transport is linear for up to two hours in these cells.

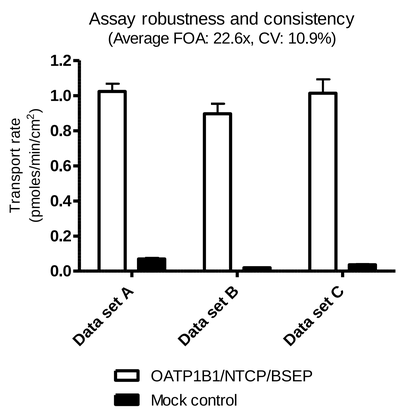

Figure 4. The OATP1B1/NTCP/BSEP triple expression model shows interday/intraday consistency and an average 22.6-fold increase in B → A taurocholate transport over control cells.

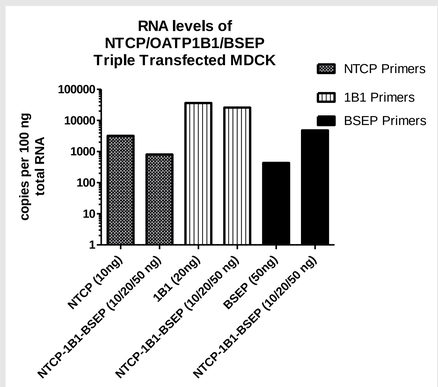

Figure 5. RNA levels demonstrate that transporter expression is not substantially altered between single and triple transfections

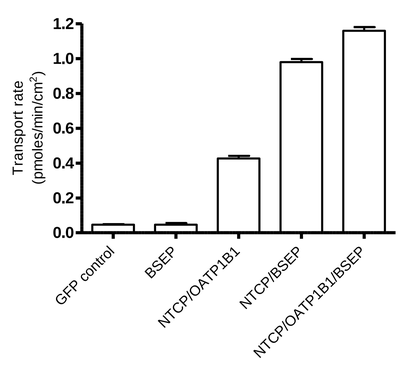

Figure 6. Transcellular transport of taurocholate in various transfected monolayers

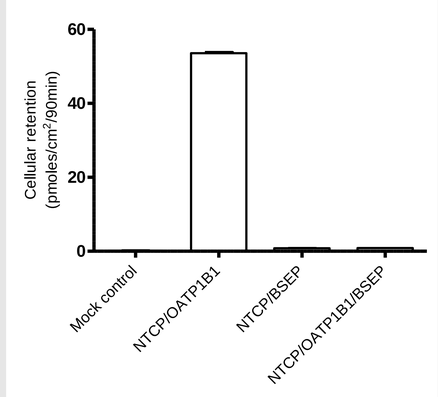

Figure 7. Intracellular accumulation of taurocholate in various transfected monolayers

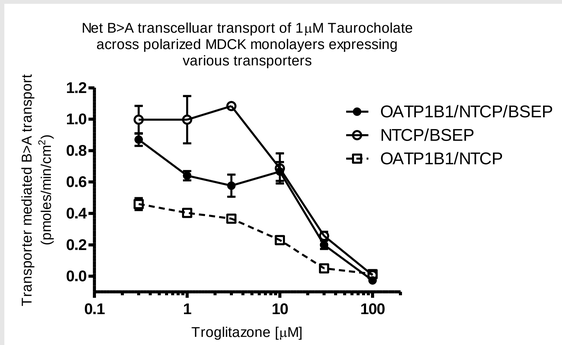

Figure 8. Transporter-mediated flux of taurocholate in the presence of troglitazone

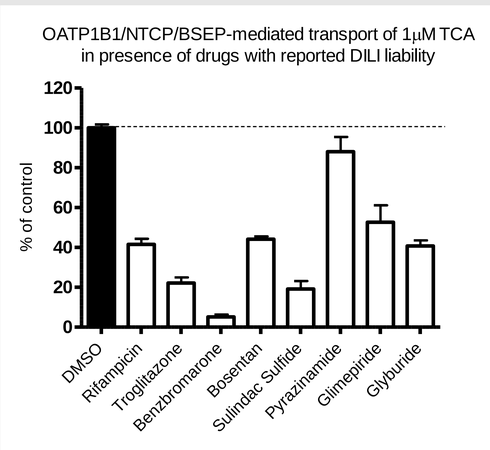

Figure 9. OATP1B1/NTCP/BSEP-mediated flux of taurocholate in the presence of various cholestasis-inducing agents. Troglitazone was tested at 30 μM, Pyrazinamide at 300 μM, all others were at 50 μM.

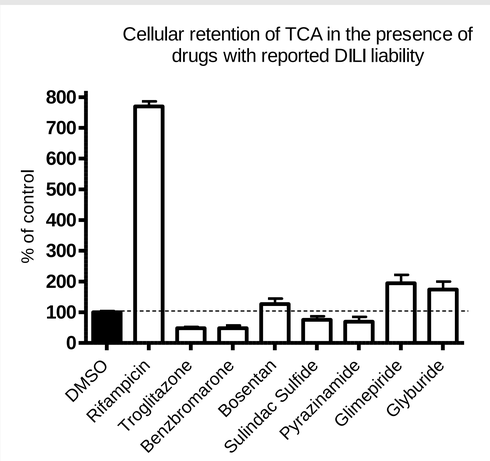

Figure 10. Intracellular retention of taurocholate in the presence of various cholestasis-inducing agents in OATP1B1/NTCP/BSEP-expressing cells. Concentrations used were the same as in Figure 9.

DISCUSSION

- This transiently transfected model offers a unique approach to potentially evaluate drug-induced cholestasis and liver injury.
- Different drugs show different effects on B → A transport and intracellular accumulation of taurocholate, depending on which transporter they inhibit.
- Troglitazone may inhibit basal uptake (OATP1B1/NTCP) more than apical efflux (BSEP), while Rifampicin primarily inhibits BSEP, increasing intracellular bile salt accumulation.
- This model may be useful in distinguishing between cholestasis-inducing drugs that interfere with bile salt transport, or those that block bile salt excretion and cause hepatocelluar toxicity via elevated intracellular bile salt levels.

CONCLUSION

- An in vitro assay model concomitantly expressing OATP1B1, NTCP and BSEP transporters in polarized MDCK cells has been developed and characterized. The effects of cholestasis-inducing agents on transcellular flux and intracellular retention of taurocholate was tested using this system, demonstrating the utility of this novel in vitro model in evaluating drug-induced liver damage.

REFERENCES

1. Steiger, B. (2010). Drug Metab. Reviews 42:437-445.
2. Morgan R.E. et al. (2010). Toxicol. Sci. 118:485-500.
3. Steiger B. et al (2007). Pflugers Arch. 453:611-620.