Poster # 1075
Board # 520
SOT 2013
San Antonio, TX

STUDYING DRUG-INDUCED CHOLESTASIS WITH A NOVEL CELLULAR MODEL CO-EXPRESSING THE MAJOR BILE SALT TRANSPORTERS IN THE LIVER

Mark S. Warren, John X. Zhang, Jane Huang, Pascal Druzgala and Yong Huang
Optivia Biotechnology Inc., 115 Constitution Dr. Suite 7, Menlo Park, CA, USA

OPTIVIA Biotechnology Inc.
www.optiviabio.com

ABSTRACT

Drug induced cholestasis and hepatocellular injury are two major manifestations of drug-induced liver injury (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 identified as the major transporters modulating hepatic clearance of bile salts and their conjugates.

We recently demonstrated that canalicular excretion and intracellular retention of bile salts can be studied with a polarized MDCK cell model concomitantly expressing three major bile salt transporters in the liver, i.e., OATP1B1, NTCP and BSEP. To evaluate the utility of this model in studying drug-induced cholestasis, we tested 20 drugs and their major metabolites that have been reported to cause cholestasis in humans. More than half of the drugs or their metabolites, including traglitozone, benzbromarone and bosentan, significantly inhibited B>A transcellular transport (canalicular excretion) of [^{3}H]Taurocholate at clinically relevant concentrations, suggesting that blocking transporter-mediated canalicular excretion of bile salts is a major mechanism of drug induced-cholestasis in vivo. Furthermore, a few drugs, such as Rifampicin, markedly elevated intracellular concentrations of Taurocholate (which has been suggested to lead to hepatocellular damage), suggesting that at high concentrations, these drugs are more likely to cause cholestatic hepatitis than the others. Other agents, such as estradiol-17-beta-glucuronide, tamoxifen and pyrazinamide, did not exhibit significant inhibitory effect on Taurocholate transport under the test conditions, which suggests there may be other mechanism(s) involved.

In summary, our study demonstrates that the novel OATP1B1/NTCP/BSEP triple transporter expression model can be a useful, economical tool for early-stage screening and for mechanistic study of compounds with cholestasis liability.

INTRODUCTION

Drug-induced liver injury (DILI) is a serious and significant problem. More than 40% of hepatitis cases in adults over 50 are due to DILI [1]. Drug toxicity is not only a primary reason for the failure of pharmaceuticals during drug development, but drug-induced liver injury is also the single most frequent reason for removing approved medications from the market [2]. With more than 1000 drugs and supplements reported to cause liver injury and more than 50% of fulminant hepatic failure cases due to drug toxicity [3,4], identifying drugs with the potential of causing DILI as early in the development pathway as possible is of paramount importance.

One major mechanism of DILI is due to changes in bile composition within the hepatocytes. The primary bile salts, cholate and chenodeoxycholate, are synthesized in the hepatocytes from cholesterol, and are then secreted into the canaliculi, flowing into the gallbladder and then into the duodenum. In the small intestine, bile salts are essential for the digestion and absorption of lipids and fat-soluble vitamins. Along the small intestine, bile salts are nearly quantitatively reabsorbed and transported back to the liver via the portal circulation, where they are taken up by the hepatocytes, and begin this enterohepatic circulation cycle again [5].

Transporters are responsible for the movement of bile salts in hepatocytes. Uptake across the basolateral (sinusoidal) membrane occurs primarily in a sodium-dependent manner and is mediated by the sodium-taurocholate cotransporting polypeptide, NTCP (SLC10A1), as well as by the sodium-independent organic anion-transporting polypeptides, or OATPs (SLCO family members), which are also mediators of drug and xenobiotic uptake. Export across the apical (canalicular) membrane occurs against a steep gradient and is mediated by the Bile Salt Export Pump, BSEP (ABCB11), which is the rate limiting step in the overall transport from the portal blood into the bile. BSEP is essential for keeping the intracellular level of bile salts low in hepatocytes, as these compounds can be cytotoxic due to their detergent properties, which can lead to mitochondrial stress and eventually to cell death. Since bile formation is an iso-osmotic process, bile salts are a major driving force for the generation of canalicular bile flow. When bile flow is reduced, a pathophysiological condition results called cholestasis. Under these conditions, bile salts are metabolized to sulphated and glucuronidated forms, which can be excreted into the bile by multidrug resistance proteins MRP2 (ABCC2), or back into the blood by MRP3 (ABCC3) and MRP4 (ABCC4). These salvage systems can help to reduce potentially cytotoxic levels of bile salts inside the hepatocytes.

Therapeutics that interfere with these transporters, especially BSEP, are often associated with cholestasis and eventually liver damage. A recent study of more than 200 compounds demonstrated a strong correlation between the degree of BSEP interference and the severity of liver injury [6]. However, inhibition of BSEP alone is not always a perfect predictor of potential liver injury, as some compounds with low IC_{50} values are not known to have effects on liver, while others with higher IC_{50} values are associated with liver injury – perhaps because of the involvement of multiple other transporters *in vivo*.

RESULTS

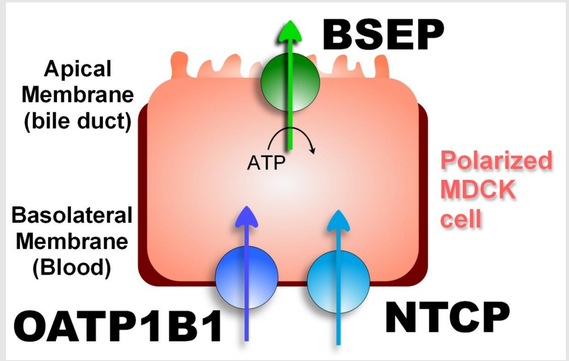

Figure 1. MDCK model co-expressing major bile salt transporters in the liver. OATP and NTCP transport bile salts into polarized MDCK monolayer cells, while BSEP effluxes the bile salts across the apical membrane.

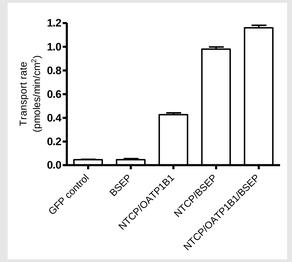

Figure 2. Transcellular transport of taurocholate in various transfected monolayers. BSEP alone does not increase transport, but a >10-fold increase results when NTCP is expressed in addition to BSEP. NTCP/OATP1B1 inhibitors have a pronounced effect on the NTCP/OATP1B1/BSEP expressing cells.

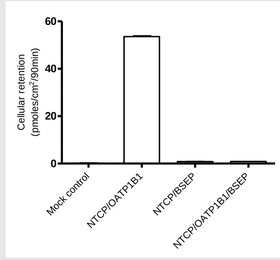

Figure 3. Intracellular accumulation of taurocholate in various transfected monolayers. Removal (or inhibition) of BSEP from the NTCP/OATP1B1/BSEP-expressing cells results in a dramatic increase in cellular retention of taurocholate.

Compound	Therapeutic area	Liver effects
Benzbromarone	antigout	Withdrawn due to liver injury
Bosentan	antiphypertension	Cholestasis
Chlorpromazine	antipsychotic	Cholestasis
Clavulanic acid	antibacterial	mixed hepatic injury
Erythromycin	antibacterial	cholestasis (black box warning)
Fenofibrate	dyslipidemia	Elevated liver enzymes
Fenofibric acid	dyslipidemia	Elevated liver enzymes
Glimepiride	antidiabetic	Cholestasis
Glyburide	antidiabetic	Cholestasis
Pyrazinamide	antibacterial	drug-induced hepatitis
Rifampicin	antibacterial	Hepatotoxic
Rosiglitazone	antidiabetic	Association
Sulindac	NSAID	Cholestatic hepatitis
Sulindac sulfide	NSAID	Sulindac metabolite
Sulindac sulfone	NSAID	Sulindac metabolite
Tamoxifen	antineoplastic	Elevated liver enzymes
Troglitazone	antidiabetic	Withdrawn due to fatal liver injury

Table 1. Drugs with known liver effects that were tested in the model co-expressing BSEP, OATP1B1, and NTCP. Both transcellular transport and intracellular accumulation were measured.

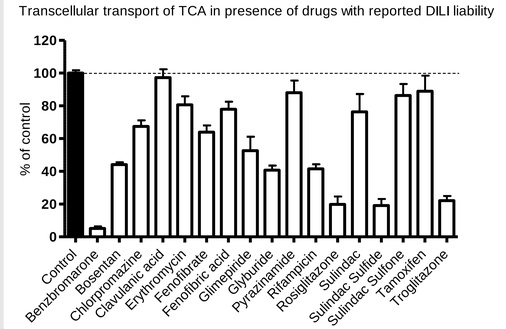

Figure 4. OATP1B1/NTCP/BSEP-mediated flux of taurocholate in the presence of various cholestasis-inducing agents. Fenofibrate and tamoxifen were tested at 20 µM; troglitazone and rosiglitazone were tested at 30 µM; all others were tested at 50 µM. Disrupting the flow of bile salts may be a major mechanism of drug-induced cholestasis in vivo as several of these agents show significant decreases in bile salt transport in this model.

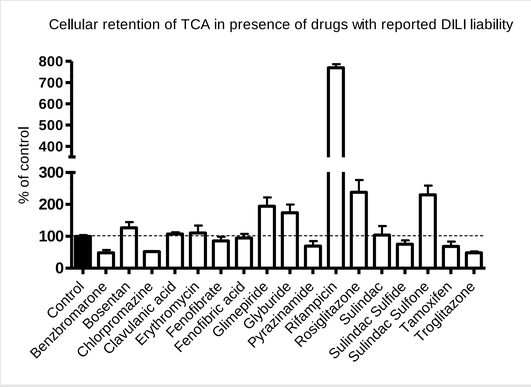

Figure 5. Intracellular retention of taurocholate in the presence of various cholestasis-inducing agents in OATP1B1/NTCP/BSEP-expressing cells. Concentrations used were the same as in Figure 4. Several of these drugs, notably rifampicin, significantly elevate the intracellular concentration of taurocholate. In vivo, such an elevation could lead to hepatocellular damage, as the detergent-like properties of bile salts are toxic to cells.

DISCUSSION

- A unique cell-based model has been developed to examine the effects that drugs will have on the human transporters involved in bile salt transport.
- This human transporter model may be useful in identifying cholestasis-inducing drugs that interfere with bile salt transport, as well as those that block bile salt excretion and cause hepatocelluar toxicity via elevated intracellular bile salt levels.
- Comparing results from cells expressing OATP1B1, NTCP, and BSEP to results from cells expressing any one or two of these transporters can give detailed mechanistic information that is not currently available in any other cell-based or animal model.
- Similar models can be developed to look at the equivalent transporters in pre-clinical species.

REFERENCES

1. Pauli-Magnus C, Meier PJ. Hepatobiliary transporters and drug-induced cholestasis. Hepatology. 2006 Oct;44(4):778-87.
2. Temple RJ, Himmel MH. Safety of newly approved drugs: implications for prescribing. JAMA. 2002 May 1;287(17):2273-5.
3. Lee, WM. Drug-induced hepatotoxicity. N Engl J Med. 2003 Jul 31;349(5):474-85.
4. Padda MS, Sanchez M, Akhtar AJ, Boyer JL. Drug-induced cholestasis. Hepatology. 2011 Apr;53(4):1377-87.
5. Steiger B. Role of the bile salt export pump, BSEP, in acquired forms of cholestasis. Drug Metab Rev. 2010 Aug;42(3):437-45.
6. Morgan RE, Trauner M, van Staden CJ, Lee PH, Ramachandran B, Eschenberg M, Afshari CA, Qualls CW Jr, Lightfoot-Dunn R, Hamadeh HK. Interference with bile salt export pump function is a susceptibility factor for human liver injury in drug development. Toxicol Sci. 2010 Dec;118(2):485-500.