**ABSTRACT**

Drug-induced interference with normal bile flow can result in relatively mild and transient cholestasis, or it can result in much more severe hepatic injury, leading to drug-induced liver injury (DILI). Drug hepatotoxicity is a common reason for the failure of pharmaceuticals during drug development and DILI is the most common reason for removing approved medications from the market. Identifying drug candidates with the potential to cause DILI as early as possible in the development pathway is of paramount importance, and having tools available to conduct SAR studies to retain beneficial drug properties while minimizing the DILI risk of those candidates could prove invaluable.

We have developed an integrated approach for assessing the DILI potential of drugs by combining two assay systems: a recombinant cell based system expressing key transporters involved in drug uptake and bile flow, and a holistic primary hepatocyte culture model which has been shown to more accurately predict intrinsic clearance, metabolite generation, and hepatic drug potential of drugs. Using the first system, a panel of drugs known to have hepatic liabilities was tested against MDCK-II cells co-transfected with OATP1B1, NTCP, and BSEP transporters. Several of these drugs associated with cholestasis, but not necessarily hepatotoxic, were shown to inhibit bile salt uptake transporters (NTCP, OATP1B1), which would result in decreased bile flow. Drugs like rifampicin, which are known to be hepatotoxic, inhibited the bile salt efflux transporter (BSEP) but not the uptake transporter (NTCP), resulting in high cellular retention of bile acids, which could lead to cell death due to their detergent-like properties. Drugs that inhibit both uptake and efflux transporters may lead to cholestasis, but are less likely to lead to hepatic injury as they will not cause high intracellular concentrations of bile salts.

Using the second system, a selection of drugs were tested in the Hurel hepatocyte model to assess toxicity in the absence and in the presence of a mixture of bile acids. Drugs such as ritonavir and cyclosporine were found to increase hepatotoxicity in the presence of bile acids. Drugs which were found to not increase cellular retention of bile acids in the first system, like erythromycin, showed no increase in hepatotoxicity in the presence of bile acids in the second system. Unlike transfected cell lines, which do not have significant metabolic capability, the primary hepatocytes are capable of activating potential toxins due to drug metabolites and their longer term effects on bile acid accumulation. If bile acid enhanced toxicity arises due to a metabolite, the mechanism of that toxicity can then be further defined by using the first system.

Combining the two assay systems results in a novel and powerful approach to assess the DILI potential of drugs and their metabolites.

**RESULTS**

**Mechanistic transporter models:** MDCK-II cells were maintained in DMEM with low glucose and 10% FBS. Cells were seeded in 24-well plates (PCG-0.4 μm) and then transfected using a novel in situ transfection technology, Opti-Expression®[^4^], which allows consistent and effective transfection of polarized cell monolayers. Cells were either transfected with one or more plasma membrane encoding the appropriate transporter gene(s) or GFP as a mock control. Assays were conducted by placing test compounds in basal chamber, measuring transmonolayer bile salt transport into apical chamber and its cellular accumulation.

**Effect on TCA secretion**

**Effect on Cellular TCA retention**

**Effect on Apical TCA efflux**

**Mechanical study using clearly defined MDCK models expressing various transporters**

**RESULTS (CONT'D)**

**Mechanistic study using clearly defined MDCK models expressing various transporters**

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**REFERENCES**


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**Figures:**

- Fig. 1: A) Simple schema of hepatic clearance pathways of bile salts (BS) and drugs, and sites of drug induced inhibition of major BS transporters. B) Phase image of human co-culture hepatocytes and CDF staining of the bile canalculus.
- Fig. 2: The use of mechanistic recombinant models to determine contribution of individual transporters to bile salt accumulation. (A) Basal apical transmembrane transporter, and (B) Intracellular accumulation of taurocholate in MDCK cells expressing various combinations of transporters.
- Fig. 3: Drugs with reported DILI were tested with OATP1B1/NTCP/BSEP MDCK model for their net effects on bile metabolite transport and transporter inhibition. (A) Transporter inhibition (B) Intracellular accumulation (both are dependent on NTCP and BSEP). (C) Apical efflux (primarily mediated by BSEP).
- Fig. 4: Mechanistic study using clearly defined MDCK models expressing various transporters revealed that Rifampicin is a substrate of P-gp, but is not transported by NTCP nor BSEP, and its intracellular concentration heavily depends on P-gp.
- Fig. 5: P-gp Dramatically reduces the intracellular concentrations of Rif and Des-Rif in cells expressing P-gp. Thus, P-gp may be a key determinant of the hepatic concentration and liver toxicity of Rif and Des-Rif.
- Fig. 6: By decreasing their intracellular concentrations, P-gp significantly reduces the relative importance of Rifampicin as a substrate of P-gp, but is not transported by NTCP nor BSEP, and its intracellular concentration heavily depends on P-gp.
- Fig. 7: Protease inhibitors Ritonavir and Bosentan dramatically reduce the intracellular concentrations of Rif and Des-Rif in MDCK models through P-gp inhibition. Which suggests that when co-administered with Rif, they may aggravate Rif hepatotoxicity.