Prediction of Multidrug Resistance Protein 3 (MDR3) Inhibition-mediated Cholestatic Drug-induced Liver Injury (DILI) Using Quantitative Systems Toxicology (QST) Modeling

J. J. Beaudoin[1], J. Adiwidjaja[2], K. Yang[1], J. L. Woodhead[1,*]

[1]QSP Solutions, Simulations Plus, Inc., RTP, NC; [2]PBPK Solutions, Simulations Plus, Inc., Lancaster, CA

***Contact information**: jeff.woodhead@simulations-plus.com

SimulationsPlus

BACKGROUND & PURPOSE

- DILI is a primary cause of acute liver failure and reason for the termination of drug development programs[1,2]
- To successfully predict and prevent DILI events, it is critical to understand the various types of underlying DILI mechanisms
- Inhibition of hepatic efflux transporters is a well-recognized mechanism that can lead to DILI (*e.g.*, bile salt export pump (BSEP) inhibition-mediated accumulation of toxic bile acids (BAs) in hepatocytes)
- MDR3 inhibition is a key mechanism that can manifest into cholestatic DILI, clinically defined by alkaline phosphatase (ALP) >2x upper limit of normal (ULN) in combination with a major elevation of γ-glutamyl-transferase (GGT) and alanine aminotransferase (ALT)/ALP (fold ULN) <2, and characterized by cholangiocellular injury[3,4]
- MDR3 is a phospholipid (PL) floppase that translocates PLs to the apical side of the canalicular membrane where PLs can form mixed micelles with biliary BAs, thereby reducing BA monomer-induced injury to cholangiocytes[5]
- This important hepatic function can be compromised by compounds that inhibit MDR3 activity, and could result in the development of clinically defined cholestatic liver injury[2,6]
- A computational QST model for this phenomenon in humans has recently been developed[7]
- In the current work, MDR3 inhibitors with and without cholestatic DILI liability were used to validate this novel QST model of cholestatic DILI

METHODS

- DILIsym® (version 8A), a commercially available QST model of DILI, was extended to mechanistically represent MDR3 inhibition-mediated cholestatic DILI
- This model consists of previously developed representations of BA homeostasis, mitochondrial function, oxidative stress, innate immunity, among other submodels important to liver health and injury, that are solved computationally in the DILIsym software[8-14]
- To predict MDR3 inhibition-mediated cholestatic DILI, new relevant features were mathematically represented in DILIsym (**Fig. 1**)[7]
- A variety of publicly available clinical data with and without drug effects was used to calibrate and validate the updated model and to construct a new virtual population (SimPops®) of healthy volunteers (n=285) representing variability in both BA toxicity and cholestasis mechanisms
- Physiologically based pharmacokinetic (PBPK) models of four selected MDR3 inhibitors were developed in GastroPlus® (version 9.8.2) to inform the hepatocellular exposure of these drugs (**Fig. 2**)
- Dosing protocol-specific exposure predictions along with *in vitro* MDR3 and BSEP inhibition potential data (*e.g.*, half-maximal inhibitory concentration, IC_{50}) were implemented in the extended DILIsym model to evaluate cholestatic DILI predictions for each of the MDR3 inhibitors (**Fig. 3**)

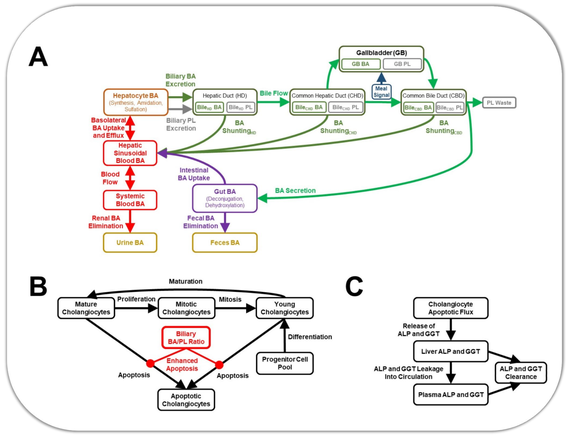


Fig. 1: The novel cholestatic liver injury model[7] represents features related to (A) bile acid and phospholipid homeostasis, (B) the cholangiocyte life cycle for each of the three bile duct segments, and (C) cholestatic liver injury biomarkers.

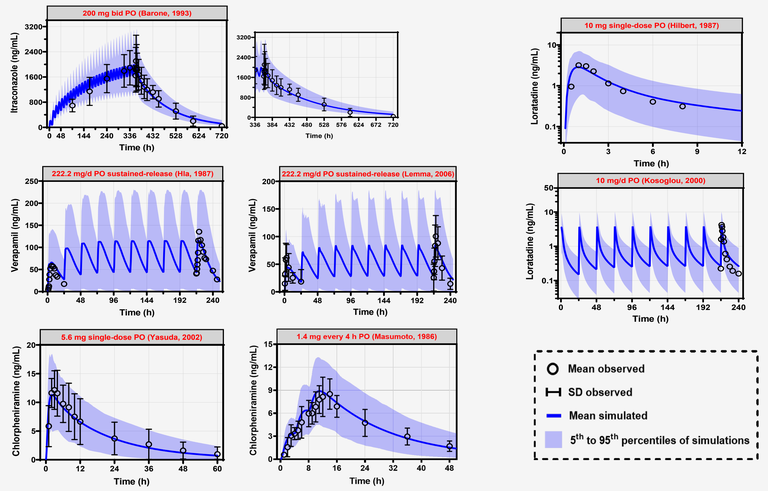
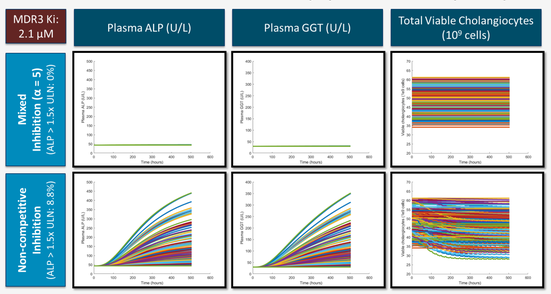


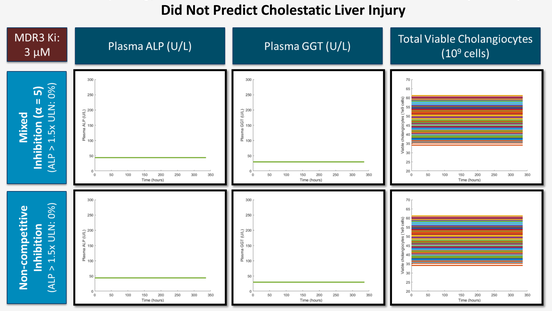
Fig. 2: Upon literature review, the anti-fungal itraconazole and anti-hypertensive verapamil were deemed suitable MDR3 inhibitors with cholestatic DILI liability (*e.g.*, elevations of ALP and GGT were observed clinically), while the two antihistamines and MDR3 inhibitors chlorpheniramine and loratadine were deemed appropriate negative controls. PBPK model simulations for each of the four compounds showed consistency with clinically observed PK data for multiple datasets and dosing routes[15-21]. Predicted PK parameters (*e.g.*, C_{max}, C_{min}, AUC) following various single- and repeat-dose regimens generally fell within 1.25-fold (0.80-1.25) of the clinically reported metrics. bid, two times a day; PO, by mouth

RESULTS

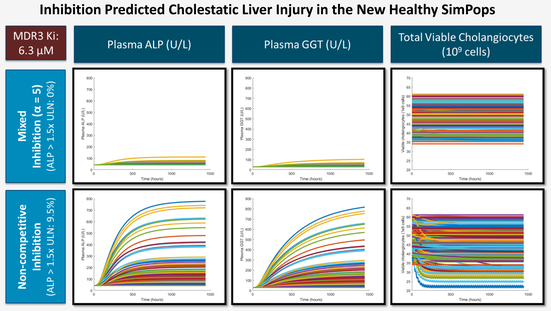
Itraconazole (200 mg bid PO for 3 Weeks) Simulations with Non-Competitive MDR3 Inhibition Predicted Cholestatic Liver Injury in the New Healthy SimPops



Loratadine (10 mg/d PO for 2 Weeks) Simulations in the New Healthy SimPops Did Not Predict Cholestatic Liver Injury



Verapamil (222 mg/d PO for 60 d) Simulations with Non-Competitive MDR3 Inhibition Predicted Cholestatic Liver Injury in the New Healthy SimPops



Chlorpheniramine (4 mg qid PO for 2 Weeks and 60 d) Simulations in the New Healthy SimPops Did Not Predict Clinically Relevant Cholestatic Liver Injury

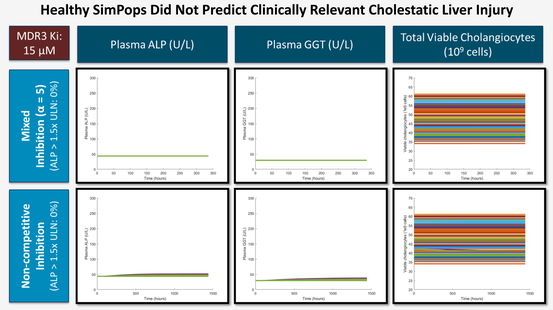


Fig. 3: In DILIsym simulations, the hepatic exposure predictions from the validated PBPK models were used along with 1) literature-reported IC_{50} values for MDR3 and BSEP, 2) mixed/non-competitive modes of inhibition for MDR3 (*i.e.*, assuming different binding sites for inhibitor and PL substrate), and 3) the DILIsym default assumption of mixed inhibition (α=5) for BSEP. Itraconazole (MDR3 IC_{50}: 2.1 μM; BSEP IC_{50}: 3 μM) at 200 mg bid PO and verapamil (MDR3 IC_{50}: 6.3 μM; BSEP IC_{50}: 178.9 μM) at 222 mg/d PO predicted ALP >1.5x ULN, GGT elevations and a reduction in viable cholangiocytes in ≥8.8% of the SimPops. On the other hand, chlorpheniramine (MDR3 IC_{50}: 15 μM) at 4 mg qid PO and loratadine (MDR3 IC_{50}: 3 μM; BSEP IC_{50}: 29 μM) at 10 mg/d PO did not predict clinically relevant cholestatic DILI signals in the SimPops, consistent with the no-DILI-concern classification of both compounds. bid, two times a day; PO, by mouth; qid, four times a day

CONCLUSION

- The novel cholestatic DILI representation in DILIsym predicted hepatotoxicity for the two DILI-associated MDR3 inhibitors itraconazole and verapamil, while no hepatotoxicity signals were predicted for the two clean MDR3 inhibitors loratadine and chlorpheniramine
- Hepatic exposure, MDR3 inhibition potential and MDR3 mode of inhibition were important drivers of the predicted cholestatic liver injury
- This work shows that QST modeling is a promising approach to reasonably predict clinically defined cholestatic DILI liability in humans

REFERENCES

1:**Padda et al**. Hepatology 53, 1377–1387 (2011); 2:**Mosedale and Watkins**. CPT 101, 469–480 (2017); 3:**Yang et al**. J. Pharm. Sci. 102, 3037–3057 (2013); 4:**Mosedale and Watkins**. J. Med. Chem. 63, 6436–6461 (2020); 5:**Boyer**. Compr. Physiol. 3, 1035–1078 (2013); 6:**Morita and Terada**. Biomed. Res. Int. 954781 (2014); 7:**Beaudoin et al**. Front. Pharmacol. 13:1085621 (2023); 8:**Woodhead et al**. CPT Pharmacometrics Syst. Pharmacol. 3, e123 (2014a); 9:**Woodhead et al**. Front. Pharmacol. 5:240 (2014b); 10:**Yang et al**. Pharm. Res. 32, 1975–1992 (2014); 11:**Howell et al**. J. Pharmacokinet. Pharmacodyn. 39, 527–541 (2012); 12:**Howell et al**. Toxicol. Lett. 226, 163–172 (2014); 13:**Woodhead et al**. J. Pharmacol. Exp. Ther. 342, 529–540 (2012); 14:**Shoda et al**. Gene Regul. Syst. Bio 11:1177625017696074 (2017); 15:**Barone et al**. Antimicrob Agents Chemother. 37, 778-784 (1993); 16:**Hla et al**. Br J Clin Pharmacol. 24, 661-664 (1987); 17:**Lemma et al**. CPT 79, 218-230 (2006); 18:**Yasuda et al**. Br J Clin Pharmacol. 53, 519-525 (2002); 19:**Masumoto et al**. J Chromatogr. 381, 323-329 (1986); 20:**Hilbert et al**. J Clin Pharmacol. 27, 694-698 (1987); 21:**Kosoglou et al**. Br J Clin Pharmacol. 50, 581-589 (2000)

FUNDING

This work was funded in part by the DILI-sim Initiative, a partnership between QSP Solutions, Simulations Plus, Inc., and multiple pharmaceutical companies, and by a Simulations Plus Postdoctoral Fellowship awarded to J.A.

CONFLICTS OF INTEREST

J.J.B, J.A., K.Y. and J.L.W are employees of Simulations Plus Inc.

www.simulations-plus.com