# MECHANISTIC MODELING PREDICTS DRUG-INDUCED HYPERBILIRUBINEMIA THAT INVOLVES INHIBITION OF ENZYMES AND TRANSPORTERS

Kyunghee Yang[1], Jeffrey L Woodhead[1], Paul B Watkins[2], Scott Q Siler[1], and Brett A Howell[1]

[1]DILIsym Services Inc. Research Triangle Park, NC 27709;

[2]UNC School of Medicine & UNC Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

## ABSTRACT

**BACKGROUND**: Elevated serum ALT and bilirubin indicates high risk of fatal drug-induced liver injury. However, drugs also can increase serum bilirubin in the absence of hepatic injury by inhibiting enzymes and/or transporters. The bilirubin sub-model within DILIsym® (the product of a public-private partnership involving scientists from industry, academia, and the FDA) was updated to predict drug-induced hyperbilirubinemia.

**METHODS:** The bilirubin sub-model was optimized to bilirubin levels in patients with inherited disorders of bilirubin disposition: Rotor syndrome (RS), Gilbert syndrome (GS), and Dubin-Johnson syndrome (DJS). Indinavir (INV)-mediated hyperbilirubinemia was simulated using an INV PBPK model and its inhibition constants for UGT1A1 (6.8 µM) and OATP1B1 (4.1 µM).

**RESULTS**: Simulations recapitulated conjugated hyperbilirubinemia in RS/DJS and unconjugated hyperbilirubinemia in GS [serum total bilirubin (TB): 2-7, 5-12, and 2-13 mg/dL, respectively]. After administration of 800 mg INV TID for 1 month, simulations predicted unconjugated hyperbilirubinemia (pre- and post-treatment serum TB: 0.55 and 0.69 mg/dL), which is consistent with reported clinical data (pre- and post-treatment serum TB: 0.5±0.28 and 0.84±0.36 mg/dL).

**CONCLUSION:** Mechanistic modeling of bilirubin can be used to predict drug-induced hyperbilirubinemia, which is not related to liver injury.

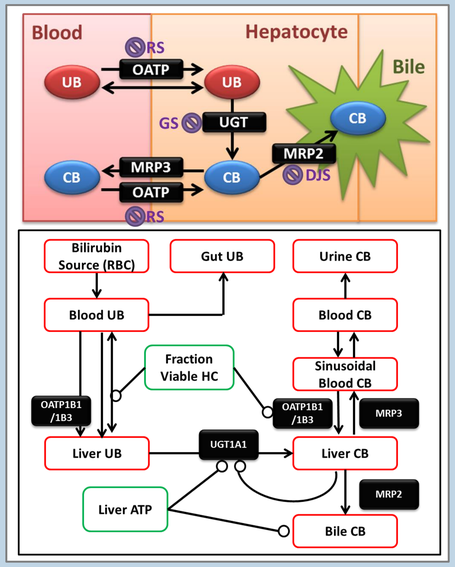
## INTRODUCTION

- Bilirubin, the product of heme breakdown from red blood cells, is exclusively eliminated by liver. Thus, circulating bilirubin is widely used as a diagnostic biomarker for liver function.
- Drug-induced hyperbilirubinemia may occur as a result of drug-induced liver injury (DILI). However, drugs also can increase serum bilirubin with no or minimal hepatic injury by inhibiting enzymes and/or transporters, as manifested in patients with genetic disorders of bilirubin metabolism and transport.
- DILIsym® is a mechanistic, multiscale model of DILI that integrates pharmacokinetic and *in vitro* toxicity data to predict *in vivo* hepatotoxicity in humans and preclinical animals [1].
- Indinavir is a protease inhibitor that is used to treat HIV. Asymptomatic, unconjugated hyper-bilirubinemia is observed in 6-25% of HIV patients receiving indinavir [2]. Indinavir is a potent inhibitor of UGT1A1 and OATP1B1 [3].
- Acetaminophen causes dose-dependent hepatotoxicity (ALT elevations). At overdose levels, acetaminophen increases both plasma ALT and bilirubin [4,5].

DILI-sim Initiative

## RESULTS

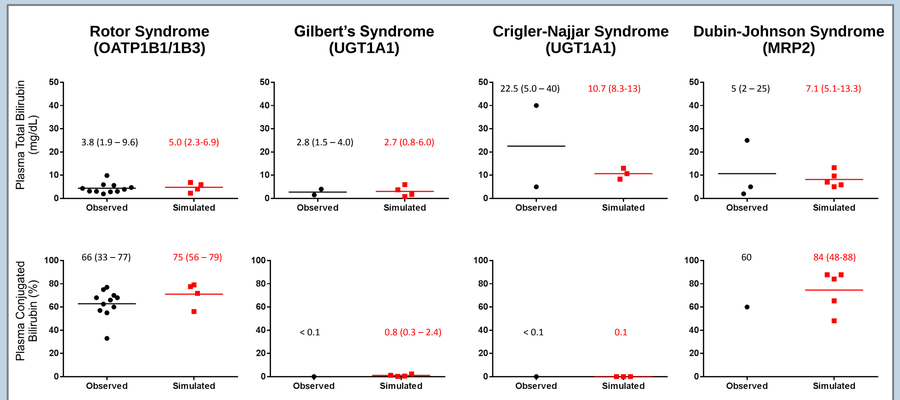
### Bilirubin Disposition




**Diagrams of hepatobiliary disposition of bilirubin and the bilirubin sub-model structure within DILIsym®.** CB, conjugated bilirubin; DJS, Dubin-Johnson syndrome; GS, Gilbert's syndrome; HC, hepatocytes; MRP, multidrug resistance-associated protein; OATP, organic anion transporting polypeptide; RBC, red blood cell; RS, Rotor syndrome; UB, unconjugated bilirubin; UGT, UDP glucuronosyltransferase.

- UB is taken up into hepatocytes by OATP1B1/1B3 or passive diffusion, and metabolized to CB by UGT1A1. CB is excreted into bile via MRP2, or undergoes blood-hepatocyte recirculation by MRP3 and OATP1B1/1B3.

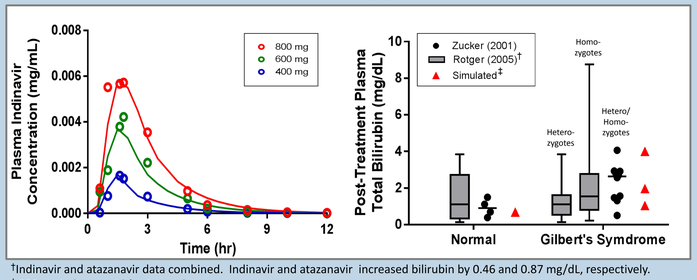
### Bilirubin Levels in Patients with Inherited Disorders of Bilirubin Metabolism and Transport




**Simulations reasonably recapitulated conjugated hyperbilirubinemia in Rotor Syndrome (RS) and Dubin-Johnson Syndrome (DJS) patients, and unconjugated hyperbilirubinemia in Gilbert's Syndrome (GS) and Crigler-Najjar Syndrome (CNS) patients.** Top panel represent observed (black circles) and simulated (red squares) plasma total bilirubin levels. Bottom panel represent percent conjugated bilirubin in plasma compared to plasma total bilirubin. Horizontal bars represent mean values of each group. Numbers represent median (lower range−upper range) for each group. Simulated patients have 70-95% impaired OATP1B1/1B3 function (RS), 30-60% impaired UGT1A1 function (GS), 70-90% impaired UGT1A1 function (CNS), or 50-90% impaired MRP2 function (RS), respectively.

- Normal Ranges of plasma TB and % plasma CB are 0.1-1.2 mg/dL and ~3.2%, respectively [6]. Respective baseline values in the DILIsym® bilirubin sub-model are 0.55 mg/dL and 3.6%.
- RS is a genetic disorder characterized by near-complete loss of OATP1B1/1B3 expression. RS patients present conjugated hyperbilirubinemia, suggesting that 1) there exist non-OATP-mediated hepatic uptake pathway(s) for UB, and 2) OATP1B1/1B3 is involved in blood-hepatocyte shuttling of CB [7].
- GS patients have 30~50% of normal UGT1A1 function, whereas CN is a rare genetic disorder with near-complete loss of UGT1A1 function. GS and CN patients present unconjugated hyperbilirubinemia [8].
- DJS patients, characterized by conjugated hyperbilirubinemia, have mutations in genes encoding MRP2. DJS patients have decreased MRP2 function and present adaptive increase in MRP3 expression [8].

### Indinavir-Mediated Hyperbilirubinemia




†Indinavir and atazanavir data combined. Indinavir and atazanavir increased bilirubin by 0.46 and 0.87 mg/dL, respectively.
‡ 800 mg indinavir tid for 1 month

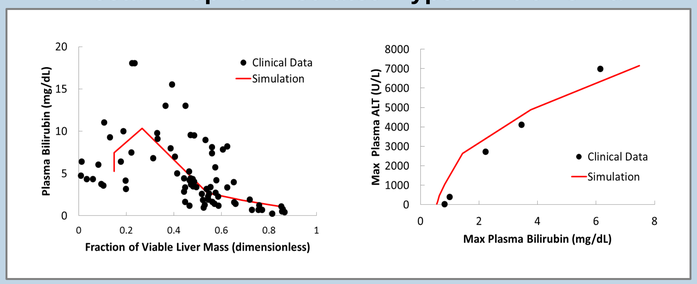
**The DILIsym® bilirubin sub-model predicts indinavir-mediated unconjugated hyperbilirubinemia that involves inhibition of OATP1B1 and UGT1A1.**

- PBPK model including saturable hepatic metabolism by CYP3A4 reasonably captures non-linear PK of indinavir observed at therapeutic doses [9].
- Simulations reasonably predict increase in plasma bilirubin (mostly UB) in normal patients after administration of indinavir [2,10].
- Simulations also indicate that indinavir induced hyperbilirubinemia is more pronounced in individuals possessing GS alleles, consistent with the clinical data [2,10]. Rotger et al. reported similar increase in median bilirubin levels between normal subjects and GS patients treated with atazanavir or indinavir (0.75 vs. 0.7-1.0 mg/dL) [10], whereas Zucker et al. reported greater bilirubin increase in GS patients compared to normal subjects after indinavir treatment (1.36±1.05 vs. 0.39±0.3 mg/dL) [2]. Simulations predict slightly greater bilirubin increase in GS patients compared with normal subjects (0.21-0.30 vs. 0.14 mg/dL).

**The DILIsym® bilirubin sub-model recapitulates hepatotoxicity-induced hyperbilirubinemia in patients overdosed with acetaminophen.**

- The bilirubin sub-model was optimized to recapitulate quantitative relationship between fraction of viable liver mass (determined from liver biopsy in overdose patients), plasma ALT, and plasma bilirubin levels observed in acetaminophen-overdose patients [4,5].
- Mechanistic modeling of DILI using proper PK and toxicity input panel data adequately predicted hyperbilirubinemia due to severe hepatotoxicity (e.g, troglitazone) [11].

### Acetaminophen-Mediated Hyperbilirubinemia




## METHODS

**DILIsym® bilirubin sub-model update** The bilirubin sub-model was updated to represent hepatic enzyme (UGT1A1)- and transporter (OATP1A1/1B1, MRP2, and MRP3)-mediated bilirubin disposition. Relevant parameters were optimized to bilirubin levels in patients with inherited disorders of bilirubin disposition: Rotor Syndrome (RS), Gilbert Syndrome (GS), Crigler-Najjar Syndrome (CNS), and Dubin-Johnson Syndrome (DJS). Bilirubin levels and fraction of viable hepatocytes in patients overdosed with acetaminophen were also employed in optimization to recapitulate the quantitative relationship between hepatocyte loss and plasma bilirubin levels observed in clinics.

**PBPK model development** A PBPK model of indinavir was developed in human using in vitro and in vivo pharmacokinetic data available [9, 12].

**Simulation of hyperbilirubinemia** Indinavir-mediated hyperbilirubinemia was simulated in the baseline human SimSingle™ using PBPK model predictions of indinavir disposition and in vitro inhibition data for OATP1B1, UGT1A1, and MRP2 ($IC_{50}$ = 6.8, 4.1, and >100 µM, respectively) [3].

| | |
|---|---|
| ***Update bilirubin model structure*** | Incorporate transporter-mediated hepatic uptake and biliary clearance as well as enzyme-mediated glucuronidation of bilirubin. |
| ***Optimize model parameters*** | Bilirubin levels in patients with inherited disorders of bilirubin metabolism and transport (e.g., Gilbert's syndrome, Dubin-Johnson syndrome, Rotor Syndrome) and acetaminophen overdose patients. |
| ***Simulate in vivo drug-bilirubin interactions*** | Represent exemplar compounds/drugs with reported interactions with bilirubin dynamics. |

## CONCLUSION

- Using a PBPK model of indinavir and *in vitro* enzyme/transporter inhibition data, the DILIsym® bilirubin sub-model predicted indinavir-mediated unconjugated hyperbilirubinemia.
- Mechanistic modeling of bilirubin can be used to differentiate hyperbilirubinemia induced by hepatotoxicity or enzyme/transporter inhibition.

## REFERENCES

[1] Shoda et al., Biopharm Drug Dispos. 2014, 35(1):33-49.
[2] Zucker et al., Proc Natl Acad Sci USA. 2001, 23;98(22):12671-6.
[3] Chang et al., Mol Pharm. 2013, 5;10(8):3067-75.
[4] Portmann et al., J Pathol. 1975, 117(3):169-81.
[5] Prescott et al., Br Med J. 1979, 3;2(6198):1097-100.
[6] Levitt et al., Clin Exp Gastroenterol. 2014, 2;7:307-28.
[7] Steeg et al., J Clin Invest. 2012, 122(2):519-28.
[8] Erlinger et al., Gastroenterology. 2014, 146(7):1625-38.
[9] Hsu et al., Antimicrob Agents Chemother. 1998, 42(11):2784-91.
[10] Rotger et al., J Infect Dis. 2005, 15;192(8):1381-6.
[11] Yang et al., Clin Pharmacol Ther. 2014, 96(5):589-98.
[12] Lin et al., Drug Metab Dispos. 1996, 24(10):1111-20.

## ACKNOWLEDGEMENTS

- This work was supported by members of the DILI-sim Initiative (www.DILIsym.com).

DILIsym® Services
USING MECHANISTIC MODELING TO SOLVE LIVER TOXICITY PROBLEMS