QUANTITATIVE SYSTEMS TOXICOLOGY (QST) TO INVESTIGATE MECHANISMS CONTRIBUTING TO CLINICAL BILIRUBIN ELEVATIONS

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Some patients given Drug X experienced concomitant ALT and bilirubin elevations. Key questions addressed in this project include: Are the bilirubin elevations observed following Drug X administration due to severe hepatotoxicity? Can QST modeling (i.e., DILIsym software) address this question? Can QST modeling provide a mechanistic explanation that would otherwise explain the observed bilirubin elevations?

ABSTRACT

BACKGROUND: Some patients treated with Drug X experienced elevations in serum bilirubin with concomitant ALT elevations, potentially indicative of severe liver injury. However, Drug X directly alters bilirubin transporters and enzymes, potentially leading to bilirubin elevations absent liver injury. Distinguishing between these two possibilities is critical to inform drug development decisions. DILIsym®, a QST platform of drug induced liver injury (DILI), was used to investigate the interpretation of putative Drug X-related elevations in liver biomarkers.

METHODS: The initial investigation estimated hepatocyte loss by approximating the clinical ALT profiles through imposed hepatocyte death[1], then checked for concomitant bilirubin elevations[2]. Then, the potential for Drug X mediated altered bilirubin disposition to account for observed bilirubin elevations was investigated[3]. Simulations combined Drug X exposure predictions from a PBPK model with mechanistic bilirubin inhibition parameters derived from the *in vitro* assays in a simulated population (SimPops®).

RESULTS: Simulated hepatocyte loss that resulted in ALT profiles mimicking clinical data were not sufficient to yield clinically significant bilirubin elevations, suggesting ALT and bilirubin elevations were decoupled and thus did not reflect severe liver injury.

Simulation results combining Drug X exposure and the mechanistic interaction of Drug X with bilirubin transporters and enzymes were consistent with timing, but underestimated magnitude, of clinical bilirubin elevations, suggesting that altered bilirubin disposition had the potential to cause clinically observed bilirubin elevations but a mechanism might be missing. Inclusion of newer data on MRP2 expression allowed simulations to account for observed serum bilirubin elevations.

CONCLUSIONS: DILIsym investigations suggested that observed bilirubin elevations did not reflect serious liver injury and might be a result of altered bilirubin disposition..

INTRODUCTION

- DILIsym software applies a quantitative systems toxicology (QST) approach to investigate dose-dependent DILI by integrating *in vitro* mechanistic toxicity data, *in vivo* predictions of dynamic drug exposure, known biochemistry, and intra-patient variability to predict hepatotoxic risk for novel therapeutics.
- Transaminase and bilirubin elevations were observed in multiple patients treated with Drug X. DILIsym was used to (a) investigate whether simultaneous elevations in ALT >3x ULN and bilirubin >2x ULN were consistent with severe liver injury as defined in Hy's Law cases, and (b) provide *in vivo* context by which mechanisms for altered bilirubin disposition might account for clinical observations.

REFERENCES

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2. Longo, Diane M., et al. Refining liver safety risk assessment: application of mechanistic modeling and serum biomarkers to cimaglermin alfa (GGF2) clinical trials. Clin. Pharmacol. Ther. 102.6, 961-969 (2017).
3. Yang, K. *et al.* Systems pharmacology modeling of drug-induced hyperbilirubinemia: Differentiating hepatotoxicity and inhibition of enzymes/ transporters. *Clin. Pharmacol. Ther.* 101, 501–509 (2017).

ACKNOWLEDGEMENTS

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DISCLOSURES

CB, BH, and LS are employees of DILIsym Services, Inc.

METHODS

Estimating hepatocyte loss based on clinical ALT profiles

- Apply hepatocyte death via direct apoptosis (no specific mechanism) to replicate observed ALT profiles from two clinical patients as previously described[1] (Figure 1a)
- Compare resulting simulated bilirubin profiles to clinical bilirubin elevations

Figure 1. Simulations increase hepatocyte apoptotic flux leading to ALT release from dying hepatocytes , and simulated ALT elevation.

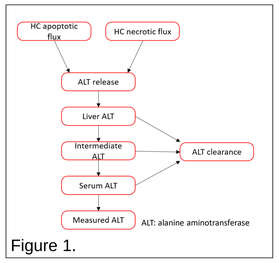

Figure 1.

Mechanistic simulations of Drug X

- Physiologically-based pharmacokinetic (PBPK) model for Drug X: Drug X liver exposure predicted in GastroPlus® using available *in vivo* and *in vitro* pharmacokinetic data
- Mechanisms of altered bilirubin disposition: Simulations apply IC_{50} values from *in vitro* assays to describe interaction of Drug X with bilirubin transporters and enzymes (Figure 2)
- Bilirubin SimPops®: Simulations conducted in N=285 individuals with variability in bilirubin biochemistry
- Software customization: DILIsym equations modified to include Drug X mediated inhibition of MRP2 expression based on newer data; creation of new SimCohorts of N=16 individuals most sensitive to altered bilirubin disposition based on the SimPops results

Figure 2. Simulated Drug X modulates bilirubin transporters and enzymes (*in grey italics*) according to the IC_{50} values determined from *in vitro* data. Simulations provide *in vivo* context for altered bilirubin disposition based on direct drug effects.

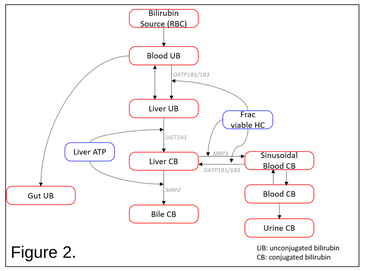

Figure 2.

RESULTS

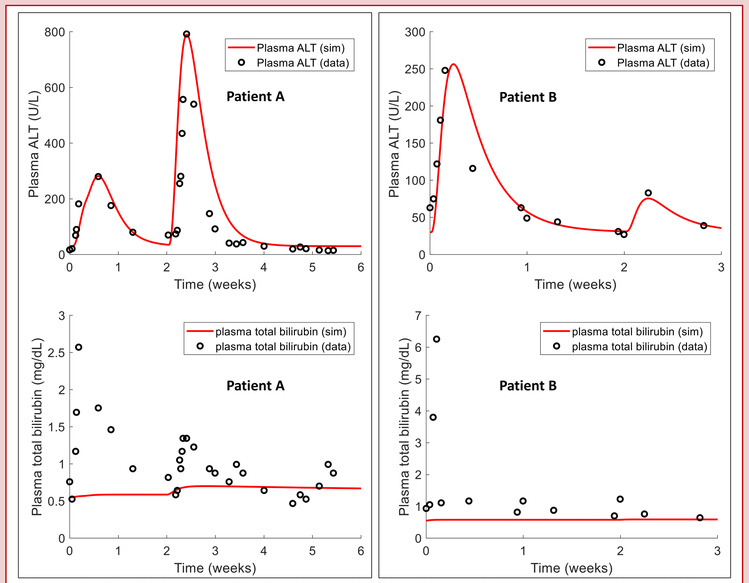

Figure 3. Simulations predict liver injury driving observed ALT elevations is insufficient to account for observed bilirubin elevations. Hepatocyte apoptosis was optimized to result in simulated ALT profiles that align with data from Patient A (top left) and Patient B (top right). The same simulation results were then used to compare how hepatocyte death (indicated by ALT elevations) impacted bilirubin elevations. Simulations that reproduce ALT elevations failed to yield clinically significant bilirubin elevations, suggesting that clinically-observed bilirubin elevations are not a result of severe liver injury.

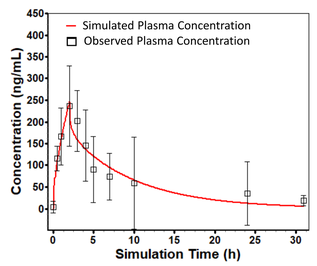

Figure 4. Simulated plasma profile following single dose of Drug X in the baseline human. Simulated plasma profile (red) was optimized to observed plasma profiles (black boxes). Training and validation data sets were evaluated.

Drug X *In vitro* IC_{50} values		
Bilirubin Transporter/Enzyme	**Units**	**DILIsym parameter value**
OATP1B1 IC_{50}	µM	1.4*
OATP1B3 IC_{50}	µM	18.9
MRP2 IC_{50}	µM	314
MRP3 IC_{50}	µM	39.85
UGT1A1 IC_{50}	µM	15.3

*Used for OATP inhibition constant in DILIsym (a conservative approach)

Table 1. *In vitro* assessment of Drug X on bilirubin transporters and enzymes. Experimental data characterizing Drug X inhibition of bilirubin transporters and enzymes were directly translated as IC_{50} values within DILIsym.

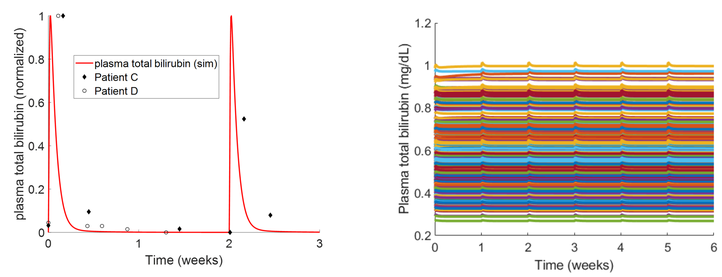

Figure 5. Simulations predict Drug X *in vivo* exposure and effects on bilirubin transporters and enzymes recapitulate timing but not magnitude of observed bilirubin elevations. Using predicted exposure and measured IC_{50} values for the effect of Drug X on bilirubin transporters and enzymes, DILIsym predicted minimal changes in bilirubin. When normalized to the maximum value (left), the simulated timing for bilirubin elevations (red line, one simulated individual) was consistent with the timing observed in clinic (shown in 2 patients: black diamond, black open circle). SimPops results (right) supported the argument that altered bilirubin disposition might plausibly account for bilirubin elevations, but additional considerations not yet included would be required to reproduce the magnitude of observed bilirubin elevations.

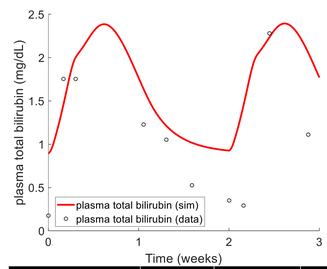

Figure 6. Simulations predict changes in Drug X mediated MRP2 expression combined with inhibition of transporters and enzymes can account for clinically observed bilirubin elevations. With inclusion of Drug X mediated inhibition of MRP2 expression, DILIsym predicted bilirubin elevations that could exceed 2 mg/dL. Illustrative results from one simulated individual shown. Both timing and magnitude of simulated bilirubin elevations were consistent with clinical data.

Reduction in MRP2 expression	TBIL > 2x ULN	TBIL > 1.5x ULN	TBIL > 2x baseline	TBIL > 1.5x baseline
10%	0/16	0/16	0/16	0/16
20%	0/16	0/16	0/16	0/16
30%	0/16	0/16	0/16	0/16
40%	0/16	0/16	0/16	0/16
50%	0/16	0/16	0/16	6/16
60%	0/16	3/16	2/16	15/16
70%	2/16	11/16	11/16	16/16
80%	9/16	13/16	13/16	16/16
90%	12/16	15/16	15/16	16/16

Table 2. Use of simulations to evaluate MRP2 required for clinical bilirubin elevations. Uncertainty in the relationship between *in vitro* and *in vivo* inhibition of MRP2 expression was addressed by evaluating 10-90% inhibition. Results illustrate that with >50% MRP2 reduction, bilirubin canalicular efflux was compromised, resulting in clinically relevant bilirubin elevations.

CONCLUSIONS

- Using hepatocyte loss to reproduce ALT elevations under-predicted clinically observed bilirubin elevations, suggesting clinical observations following administration of Drug X do not reflect severe liver injury
- Simulation results combining Drug X exposure and mechanistic interaction with bilirubin transporters and/or metabolism are consistent with the timing, but under-predict magnitude, of bilirubin elevations
- Simulations predict that Drug X-mediated reduction in MRP2 expression in conjunction with inhibition of bilirubin transporters and enzymes by Drug X can account for observed bilirubin elevations

DILI-sim Initiative

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