Modeling Insights on Species Differences in Response to CD8+ T cell-mediated Liver Injury

Zackary R. Kenz[a], Christina Battista[a], Lisl K.M. Shoda[a]

[a]DILIsym Services, Inc., a Simulations Plus company, Research Triangle Park, NC

ABSTRACT

OBJECTIVES: Idiosyncratic drug-induced liver injury (iDILI), which is typically rare and often severe, has led to black box warnings or drug withdrawals from the market, with adverse effects on patients and drug development [1]. T cell-mediated immune responses have been implicated for many iDILI drugs[1]. Previous work has expanded an existing quantitative systems toxicology (QST) model (DILIsym®) to include mouse CD8+ T cell responses to hepatocyte-expressed OVA[2] and mouse CD8+ T cell responses to hepatocyte-expressed amodiaquine (AQ) antigen[3]. This work aims to reproduce human CD8+ T cell responses to hepatocyte-expressed AQ antigen and explore putative mechanisms underlying cross-species differences.

METHODS: Simulating human responses involved human AQ dosing protocols and species differences in the represented biology, including numbers of human naïve antigen-specific CD8+ T cells[4,5]. The simulated mouse was treated with 250mg/kg/day AQ[6]. The simulated human was treated with 600mg AQ per week[7].

RESULTS AQ exposure in a simulated mouse resulted in delayed, mild hepatotoxicity, illustrated by alanine aminotransferase (ALT) elevations >100U/L after roughly two weeks of treatment. There was no progression to severe liver injury on continued drug treatment through 10 weeks, consistent with published data[6]. In a simulated human, AQ exposure resulted in delayed hepatotoxicity, illustrated by ALT elevations 120U/L after roughly 4.5 weeks of simulated AQ treatment. With continuing AQ treatment, the simulated human progressed to severe liver injury, evidenced by ALT >1000U/L and TB >2mg/dL, consistent with AQ DILI[7]. Relative expansion of CD8+ T cells was similar between species, as was their cytotoxic potential. A surprising variable that differentiated the severity of injury was the relative recovery potential of mouse *vs*. human hepatocytes. Simulations include hepatocyte proliferation with subsequent liver regeneration. Hepatocyte proliferation rates were previously optimized to available acetaminophen recovery data[8,9] and were unaltered for these simulations. The parameterization is permissive for balanced injury and recovery in the simulated mouse but generates progressive injury with insufficient recovery in the simulated human. These abstract results have been previously presented in part at Immunology2020, Honolulu, HI, 5/2020 and published in the conference proceedings as abstract 2352.

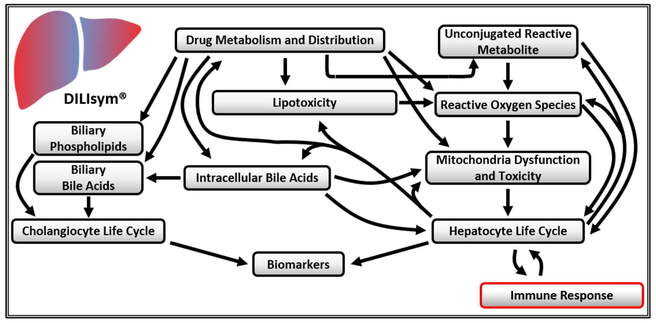
CONCLUSION: The results provide proof-of-concept that leveraging data from mouse studies of CD8+ T cell responses in the liver can translate to a human representation consistent with published data. The results illuminate a potential mechanism for cross-species differences in susceptibility to T cell-mediated injury. Further simulations and experimental follow-up will be conducted to confirm or refute the model findings.

INTRODUCTION

- Much work has been devoted to identifying the mechanisms that contribute to dose-dependent drug-induced liver injury (DILI). However, idiosyncratic DILI (iDILI), or rare and often severe adverse reactions that are not necessarily dose-dependent, remain difficult to predict, and can be extremely costly, both for patient health and drug development programs.
- Recent publications have suggested that iDILI events may be immune-mediated. Immune effects have been implicated largely due to the delayed onset and for the rapid re-injury observed after resuming treatment. Immune involvement has been further supported by the identification of HLA risk alleles for some drugs.
- DILIsym® software applies a quantitative systems toxicology (QST) approach to the understanding of dose-dependent DILI by integrating *in vitro* mechanistic toxicity data, *in vivo* dynamic drug exposure, known biochemistry, and known patient characteristics to predict the hepatotoxic risk for novel therapeutics.
- Previous work has expanded DILIsym to reconcile clinical data implicating the immune response with mechanistic data characterizing liver-specific CD8+ T cell responses to hepatocyte-expressed OVA. The model was further expanded to reproduce mouse CD8+ T cell responses to hepatocyte-expressed amodiaquine (AQ) antigen.
- We now aim to reproduce human CD8+ T cell responses to hepatocyte-expressed AQ antigen and explore the mechanisms underlying species differences following AQ administration.

DILI-sim Initiative

RESULTS



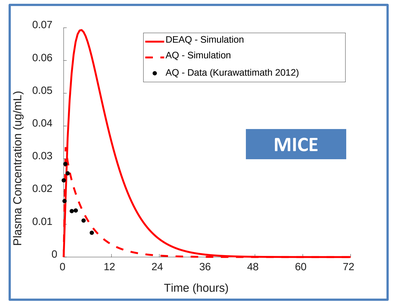
The mechanistic model for CD8+ T cell-mediated injury was added to DILIsym, an already existing QST/QSP model that uses in *vitro data*, predictions of compound exposure, and known liver biochemistry to predict toxicity liability for novel compounds. The CD8+ T cell submodel is added to the current representation of immune responses in DILI that include effects from macrophages, neutrophils, and immune mediators.

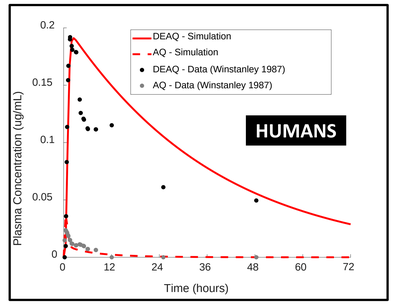
Patient	AQ dosing (mg/week)	Time to 1st sign / symptoms (days)	Max measured ALT elevation (xULN)	Max measured TB elevation (mg/dL)	Reference
1*	800	56	6.5	-	Larrey 1986
2*	500	21	4	0.9	Larrey 1986
3	600	42	6	-	Larrey 1986
4	200	37	3	1	Larrey 1986
5	600	77	38	18	Larrey 1986
6	400	82	11	21	Larrey 1986
7	600	104	77	40	Larrey 1986
1	700	110	54	31	Bernuau 1988
2	200 / 400	334	47	32	Bernuau 1988
3	600	103	30	47	Bernuau 1988
1*	600	36	28	22	Raymond 1989
1	200	75	37	24	Markham 2007

** Re-start of AQ after patient recovery resulted in ALT elevation within days*

Observed ALT elevations associated with AQ treatment are not dose-dependent and occurred anywhere from 21-334 days after start of treatment. When patients were re-challenged with AQ, ALT elevations were observed within days of continued AQ treatment.

Compared to mouse data, human injury can be more severe in both ALT levels and in bilirubin elevations.





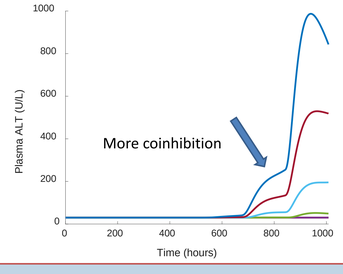
Mouse (top) and human (bottom) PBPK representations for AQ and its metabolites were developed and optimized to fit published mouse [10] and human [11] PK profiles. Notably, in humans DEAQ, the major metabolite of AQ, is found in higher concentrations in the plasma and persists longer than its parent compound. AQ and DEAQ are further metabolized to reactive metabolites, which contribute to observed liver injury.

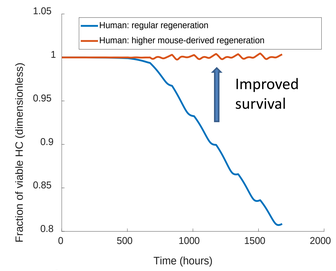
Simulation Results Exhaustion knockout, full avidity	Mouse		Human	
Dosing Protocol	250 mg/kg QD (in feed)		600 mg weekly	
Antigen level (Average %)	10%	30%	10%	30%
Peak ALT† (U/L)	280	680	450	2000
Peak total bilirubin† (mg/dL)	0.55	0.55	1	13
Time to injury (wks post first dose)	2	2	4	4

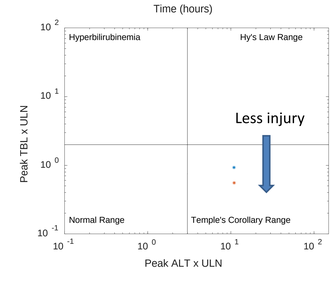
ALT baseline:
Mouse 20 U/L
Human 30 U/L
Bilirubin baseline:
Mouse 0.55 mg/dL
Human 1 mg/dL

Utilizing the mouse-calibrated CD8+ T cell model in humans yields qualitatively similar behavior in a susceptible scenario where the exhaustion pathway is knocked out and the cells receive full avidity (cytokine/costimulatory) signaling. When compared to mouse simulations, peak ALT in humans is higher with higher antigen, and onset of injury is delayed compared to the first AQ dose. One key difference, consistent with human data, is simulated bilirubin elevations in the human which were not seen in the mouse. This aspect is potentially related to hepatocyte regeneration differences in species, explored to the right. While these high and low Ag cases show potential variability in humans, subsequent examinations are planned to refine the model and include inter-individual variability in various model pathways which would lead to differential responses to drug-induced iDILI.

Simulated example exploration of the human CD8+ T cell model avidity response behavior using the 30% antigen level indicates a range of responses when the strength of the avidity signal is modulated. This indicates the human model has the potential to represent the behavior of immune checkpoint inhibitors which would modulate some combination of these two pathways.







Human simulations with 10% Ag conducted with a stronger hepatocyte regenerative boost during injury, akin to that of a mouse, show minimal hepatocyte loss and consequently minimal elevation in bilirubin. Since CD8+ T cells are still killing hepatocytes, ALT continues to be elevated in these individuals. These simulations show an example of inter-individual variables like liver recovery capability that could contribute significantly to clinical presentation of iDILI.

METHODS

Developing a mechanistic model of CD8+ T cell responses

- The well-characterized CD8+ T cell response to hepatocyte-expressed ovalbumin (OVA) was used to construct the mechanistic model.
- The model links antigen presenting hepatocytes to T cell activation, proliferation, and potential exhaustion.
- Cytotoxic T cell antigen clearance through apoptosis of hepatocytes, as the antigen-presenting cell; dying hepatocytes release ALT (already included in DILIsym) which is used as a biomarker of liver injury.
- The number of naïve antigen-specific CD8+ T cells is set for each species based on a survey of available literature

Physiologically-based pharmacokinetic (PBPK) model for amodiaquine (AQ)

- Human and mouse PBPK models are developed in DILIsym using available *in vitro* and *in vivo* pharmacokinetic data.
- The human and mouse PBPK models predict exposure for AQ as well as its metabolites, DEAQ and AQQI, and a reactive metabolite of DEAQ.
- AQ and its metabolites have the potential to induce ER stress and increase ROS production in an exposure-dependent manner, shifting the liver towards a less tolerogenic state and thus making it more permissive for an adaptive immune response.

Simulated CD8+ T cell responses to AQ administration

- Simulated mice receive 250 mg/kg/day AQ over a 12-hour period, consistent with AQ administered orally in the feed.
- Simulated humans received 600 mg AQ each week[10].
- The avidity signal is modulated to mimic treatments such as immune checkpoint inhibitors that may impact the presentation of DILI.

Species-specific differences in responses to AQ

- Mouse-derived regeneration parameters were used in human AQ simulations to predict differences in liver injury and/or survival.

CONCLUSION

- Leveraging data and prior modeling of CD8+ T cell responses in mice provides the necessary groundwork to translate and construct a human representation for iDILI.
- Representation of amodiaquine PBPK, and particularly the long half life of the major metabolite DEAQ (and the resulting reactive metabolite) was successful and crucial in assessing human AQ iDILI as well as cross-species differences
- Simulated human responses qualitatively and, to some extent, quantitatively recapitulate the behavior seen in clinical data
- The mechanistic model allows for investigating potential cross-species differences in susceptibility to T cell-mediated injury
- The examination in humans is a preliminary exploration of the qualitative behavior of the model, with future refinement and examination of inter-individual variability planned

REFERENCES

1. Kullak-Ublick. Gut 66:1154-1164 (2017)
2. Kenz. ACoP9 Poster W-076 (2018)
3. Kenz. Immunology2020 2395 (2020)
4. Alanio. Blood 115:3718-3725 (2010)
5. Bechara. Front. Immunol. 10:1331 (2019)
6. Mak. Chem Res Toxicol. 28:1567-1573 (2015)
7. Larrey. Ann. Intern. Med. 104:801-803 (1986)
8. DeAngelis. Hepatol. 42:1148-1157 (2005)
9. Portmann. J Pathol. 117:169-181 (1975)
10. Kurawattimath, et al. Eur J Drug MetabolPharmacokinet(2012);37:23–30
11. Winstanley. Eur. J. Clin. Pharmacol. 33: 331-333 (1987)

ACKNOWLEDGEMENTS

- This current work was supported by members of the DILI-sim Initiative.

DILIsym® Services
USING MECHANISTIC MODELING TO SOLVE LIVER TOXICITY PROBLEMS