# Mathematically modeling CD8+ T cell-mediated drug induced liver injury (DILI): from ovalbumin to amodiaquine in mice

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## ABSTRACT

**OBJECTIVES**: Idiosyncratic drug-induced liver injury (iDILI) is poorly understood, but there is evidence to support that some iDILI events may be immune-mediated. Amodiaquine (AQ), a malaria medication, is thought to cause immune-mediated iDILI due its delay between treatment initiation and DILI onset and its ability to induce a more rapid injury upon drug re-challenge.[1] Previous work has been devoted to representing T cell-induced cytotoxicity in response to ovalbumin (OVA)-expressing hepatocytes in mice.[2] The current work aims to translate the OVA representation to recapitulate AQ-mediated hepatotoxicity in mice.

**METHODS:** An existing quantitative systems toxicology (QST) model (DILIsym®) was previously expanded to characterize CD8+ T cell responses to hepatocyte-expressed OVA.[2] This model has further been expanded to incorporate exposure of AQ via a physiologically-based pharmacokinetic (PBPK) representation which includes AQ metabolism to reactive metabolites (RMs). AQ RMs induce hepatocellular ER stress[3] as well as oxidative stress[4]. These mechanisms for intrinsic toxicity were evaluated for their potential to alter the hepatic microenvironment, making it more permissive for a CD8+ T cell response. The model representation of T cell interaction with hepatocyte-presented antigen was also updated to incorporate the putative lower T cell receptor affinity of AQ compared with OVA and to represent a function of cytokine/co-stimulation levels which modulate T cell activity. These modifications, along with the pathway for T cell exhaustion already built into the OVA model, allow for representing interference in pathways implicated by CTLA4 and PD-1 compared with a wild-type mouse. Simulations aimed to reproduce CD8+ T cell-mediated DILI in a PD-1$^{-/-}$ mouse model (bred on a C57BL/6 background) exposed to AQ with anti-CTLA4 treatment.[5,6]

**RESULTS** Simulations successfully captured differences in CD8+ T cell responses under different experimental conditions. No ALT elevations were predicted in simulated wild-type mice treated with AQ. Simulations designed to mimic inhibition of regulatory pathways, *i.e.*, PD-1$^{-/-}$ mice treated with anti-CTLA4, predicted CD8+ T cell-mediated ALT elevations consistent with levels seen in experimental data.[5,6]. Variations in cytokine/co-stimulation levels were simulated to assess T cell response sensitivity and range of response. These abstract results have been previously presented in part at Immunology2020, Honolulu, HI, 5/2020 and published in the conference proceedings as abstract 2395.

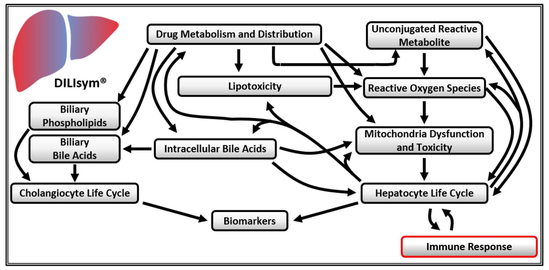
**CONCLUSION:** The results provided confidence in the QST model translation of OVA hepatotoxicity to AQ-induced DILI in mice. Results provided a basis for exploring AQ-induced DILI in humans using QST modeling with the goal of investigating immune-mediated iDILI risk for future compounds.

## INTRODUCTION

- Much work has been devoted to identifying the mechanisms that contribute to dose-dependent drug-induced liver injury (DILI)[9]. 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 suggest that iDILI events may be immune-mediated. Immune effects are implicated largely due to the delayed onset and for the rapid re-injury observed after resuming treatment. Immune involvement is 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.
- This work aims to expand DILIsym to reconcile clinical data implicating the immune response with mechanistic data characterizing liver-specific CD8+ T cell responses to hepatocyte-expressed OVA. After capturing the responses to hepatocyte-expressed OVA appropriately, it is expanded to reproduce mouse CD8+ T cell responses to hepatocyte-expressed amodiaquine (AQ) antigen.

DILI-sim Initiative

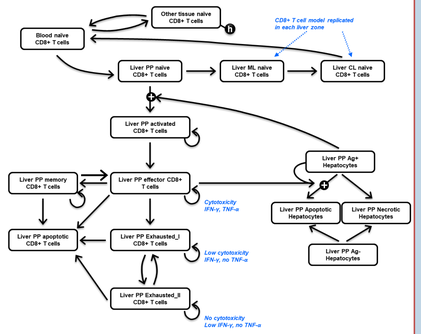
## RESULTS



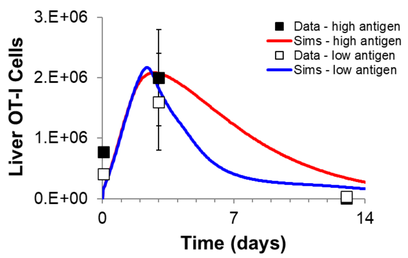

The mechanistic model for CD8+ T cell-mediated injury was added to DILIsym, an already existing QST/QSP model that uses in *vitro data*, predictions of compound exposure, and known liver biochemistry to predict toxicity liability for novel compounds. The CD8+ T cell submodel is added to the current representation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

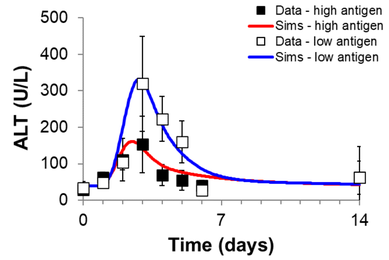
The mechanistic model for CD8+ T cell-mediated injury contains three major segments, each interacting in order to produce desired responses.

- In the upper part of the diagram, the naïve T cell portion of the model represents a simplified circulation of cells among the liver zones, blood, and other body tissues. This facilitates steady state naïve cell modeling as well as injection experiments.
- In the lower left part of the diagram, a representation of T cells becoming activated, gaining an effector phenotype, and moving into other states is shown. Antigen regulates all these steps, though only activation is shown in order to simplify the diagram. Phenotypic differences between effectors and exhausted cells are indicated in the diagram
- On the right portion of the diagram, the existing DILIsym hepatocyte life cycle has been modified in order to support antigen presentation and CD8+ killing of Ag presenting hepatocytes.




- Hepatocyte-expressed OVA comparator data[7] was utilized in order to calibrate the CD8+ T cell life cycle and responses to high and low percentages of hepatocytes expressing OVA.
- Critical aspects of response included overall level and timing of response, as measured by total liver OT-I cells; and antigen clearance via hepatocyte killing, as measured by plasma ALT levels.
- OVA responses are calibrated to a full effector phenotype response at low levels of antigen as evidenced by more antigen clearance (greater peak plasma ALT) and a dysregulated phenotype response at high levels of antigen evidenced by less antigen clearance (less peak plasma ALT).

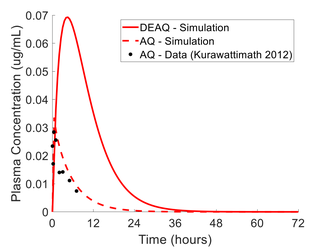



| | Low antigen | | | | | High antigen | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OT-I T cells | AQ T cells | AQ T cells Low exhaustion | AQ T cells Low coinhibition | AQ T cells Low exhaustion Low coinhibition | OT-I T cells | AQ T cells | AQ T cells Low exhaustion | AQ T cells Low coinhibition | AQ T cells Low exhaustion Low coinhibition |
| Naïve T cells | 5e6 OT-I cells[a] | 120 AQ liver T cells[b] | 120 AQ liver T cells[b] | 120 AQ liver T cells[b] | 120 AQ liver T cells[b] | 5e6 OT-I cells[a] | 120 AQ liver T cells[b] | 120 AQ liver T cells[b] | 120 AQ liver T cells[b] | 120 AQ liver T cells[b] |
| TCR affinity | 1 | 0.01 | 0.01 | 0.01 | 0.01 | 1 | 0.01 | 0.01 | 0.01 | 0.01 |
| Net co-stimulation | 1 | 0.1 | 0.1 | **1** | **1** | 1 | 0.1 | 0.1 | **1** | **1** |
| Effector-to-Exhaustion | 1 | 1 | **0.01** | 1 | **0.01** | 1 | 1 | **0.01** | 1 | **0.01** |
| ALT (U/L)[c] Cmax (OT-1) or 6 weeks (Drug) | 330 | 20 | 20 | 105 | 210 | 160 | 20 | 22 | 20 | 640 |

[a] *adoptive transfer of OT-I T cells (Ochel 2015, Cebula 2013)*
[b] *quantification of Ag-specific naïve CD8+ T cells (Jenkins 2012)*
[c] *ALT baseline in OT-1 model is 30 U/L; ALT baseline in drug-specific model is 20 U/L*

- In an experimental setting, antigen level is not necessarily known based on AQ exposure; low and high Ag cases along with simulated modulations of model pathways were examined to explore potential combinations consistent with data.
- In mouse, maximal ALT levels reached ranged from 100-200 U/L[5,6].
- Low antigen simulations in the mouse show the most readily obtained ALT level consistency with experimental data.
- Modulation of the costimulatory/coinhibitory conditions indicate the high antigen simulations have the capacity to match the data range (high ALT elevations) but further exploration in the parameter space for costimulation/coinhibition can be done to better capture observed liver injury and understand how to accurately represent the conditions for future drug simulations.




Simulated plasma profiles in the baseline mouse treated with IV bolus injection of 1 mg/kg amodiaquine. Amodiaquine parent simulation (dashed red line) and data[8] (solid black circles) and major metabolite DEAQ (desethylamodiaquine) simulation (solid red line) are shown. Minimal data for DEAQ in mice are available in the literature; the model was calibrated in part based on unpublished collaborator data as well as utilizing published human data on AQ metabolism. Of note, DEAQ persists in the plasma longer than AQ, but both AQ and DEAQ are further metabolized into reactive metabolites that contribute to observed AQ-mediated hepatotoxicity.

## METHODS

**Developing a mechanistic model of CD8+ T cell responses**

- Data from well-characterized CD8+ T cell responses to hepatocyte-expressed ovalbumin (OVA) are used to construct the mechanistic model.
- The model links antigen presenting hepatocytes to T cell activation, proliferation, acquisition of cytotoxic effector function, and potential differentiation to memory and exhausted phenotypes.
- The model dynamically represents cytotoxic T cell-induced apoptosis of antigen-expressing hepatocytes; dying hepatocytes release ALT (already included in DILIsym) which is a biomarker of liver injury.
- The number of naïve antigen-specific CD8+ T cells is set for each species.

**Ovalbumin (OVA) simulations**

- The amount of antigen is a key regulator in CD8+ T cell responses, and thus low or high amounts of hepatocytes expressing OVA-antigen are added into the system.
- The model parameters are calibrated to reproduce CD8+ T cell expansion in both high and low antigen expression scenarios and antigen clearance in the low antigen expression case. In the high antigen expression case, antigen is not cleared, exhausted T cells are dominant, resulting in lower ALT levels.

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

- A PBPK model for AQ in mice is developed in DILIsym using available *in vitro* and *in vivo* pharmacokinetic data.
- The PBPK model predicts 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 reactive oxygen species (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 orally over a 12-hour period.
- The parameters used to define the OVA-antigen CD8+ T cell response are used in the AQ simulations. The reactive metabolites of AQ provide neoantigen within the simulations to stimulate a CD8+ T cell response.
- Parameters surrounding the presentation of a neoantigen were not necessary in the OVA-antigen scenario and were therefore calibrated during these AQ simulations.

## CONCLUSION

- The mechanistic model provides the groundwork for quantitative insight into the role of antigen on T cell-mediated liver injury and sets the stage for further investigation of immune-mediated iDILI.
- The successful model translation of OVA-induced hepatotoxicity to AQ-induced hepatotoxicity in mice provides confidence in the QST model to be further refined and expanded to also capture and describe hepatotoxicity in other species and/or with other drugs.

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