Computational Exploration of the Role of a Prototypical Damage-Associated Molecular Pattern (DAMP) Molecule in Acetaminophen Hepatotoxicity

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Abstract

Background. Drug-induced liver injury (DILI) is a major source of acute liver failure and is one of the leading causes of drug development failures. As such, there remains an important unmet need for earlier identification and mitigation of DILI risk. The DILIsym™ model is a mechanistic representation of DILI in preclinical species and humans designed to address this need. The first generation model focused on hepatocyte drug interactions, with limited representation of inflammation. To facilitate the quantitative investigation of innate immune responses in DILI, we have expanded the representation to include liver macrophages, liver sinusoidal endothelial cells, and various mediators. The model represents the current understanding of mechanistic links between hepatocyte death, immune cell activation, mediator production, and mediator effects on hepatocyte death and regeneration. Selection of model parameters was informed by the literature. Mediator profiles and local accumulation of macrophages in the liver were aligned with reported data from acetaminophen (APAP) hepatotoxicity. HMGB1 is a classic alarmin or damage-associated molecular pattern (DAMP) molecule that can induce immune cell activation and is represented in the model. HMGB1 can be released from dying cells, including acetaminophen-exposed hepatocytes [1,2] and can also be produced by activated immune cells [3]. Several reports demonstrate that in mice, neutralization of HMGB1 reduces APAP-mediated ALT elevation [4–6].

Results. In the model, HMGB1 neutralization reduces APAP-mediated ALT elevation (max 30% reduction) in the baseline simulated mouse. The simulated improvement fell within the range reported in the experimental literature (10-70% reduction). The model parameters were varied to create earlier or higher HMGB1 profiles. An earlier HMGB1 profile did not improve the effect of HMGB1 neutralization on APAP hepatotoxicity. In contrast, HMGB1 neutralization was more effective in alleviating APAP hepatotoxicity when HMGB1 levels were higher, largely due to an increase in HMGB1-mediated immune activation (55% reduction). Lastly, model parameters were varied such that HMGB1-mediated immune cell activation led to a greater proportion of immune (e.g., TNF-α) mediated hepatocyte death relative to reactive-metabolite mediated hepatocyte death. It was initially surprising to observe that while the degree of liver injury was similar, this change led to slower progressing liver injury. Closer examination revealed that less reactive-metabolite mediated cell death reduced the level of DAMP release, which slowed immune cell activation and the subsequent immune-mediated injury. HMGB1 neutralization was also more effective in alleviating APAP hepatotoxicity in this scenario (55% reduction).

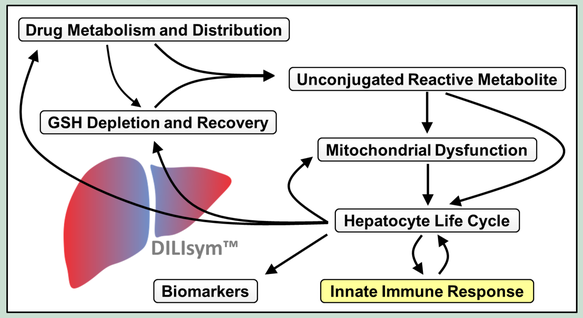
Conclusion. This research reports on how different HMGB1 profiles are predicted to translate to HMGB1 neutralization response and illustrates the application of the DILIsym™ model to test hypotheses regarding the role of the innate immune response in DILI.

Acknowledgements

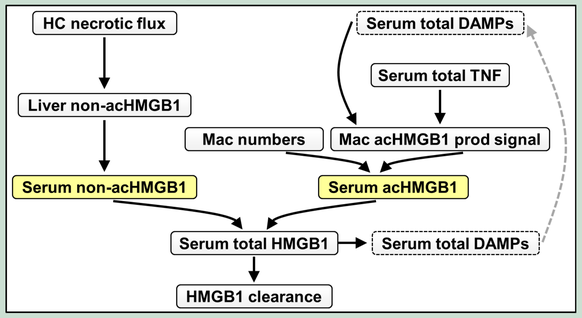
Funding provided by members of the DILI-sim Initiative. See *www.DILIsym.com* for more information.

DILIsym™ Model Design, APAP ± anti-HMGB1 Data, and Simulation Results

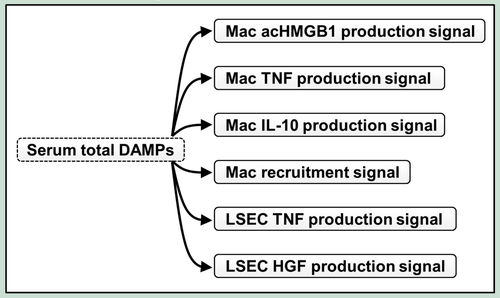
DILIsym™ - Innate Immune Response



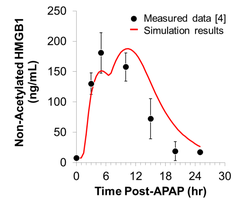
DILIsym™ - HMGB1 Production



DILIsym™ - HMGB1 Effects

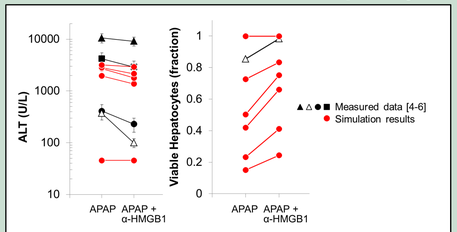


APAP-Induced HMGB1



- Simulated results consistent with measured data [4]
- Strain and dose differences between measured data and simulations
- Strain differences present a potential source of variability in response to blocking HMGB1

HMGB1 Blockade – ALT and Hepatocytes

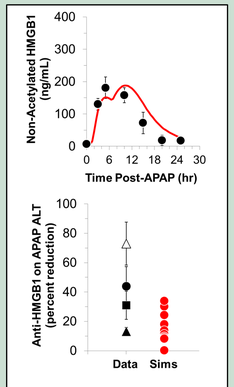


- Simulating different doses of APAP (150-400 mg/kg) yields different levels of injury
- Simulating HMGB1 blockade is hepato-protective across multiple doses, generally consistent with measured data [4-6]

Alternate Simulated Mice to Investigate Impact of Different HMGB1 Profiles, Different Role of Innate Immune Response

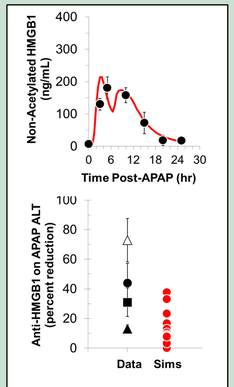
Alternate Mouse	Parameters Changed
Baseline	None
Earlier HMGB1 elevation	HC HMGB1 release rate HMGB1 clearance rate
Higher peak HMGB1	HC HMGB1 release rate
More immune-mediated HC cell death	ATP-dep necrosis Vmax TNF-dep necrosis Vmax

Baseline



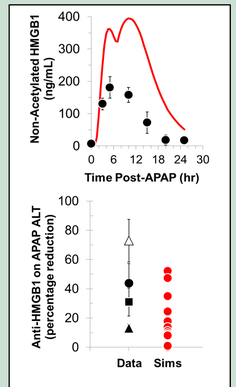
HMGB1 neutralization yields ~30% reduction in APAP ALT elevation

Earlier Peak HMGB1



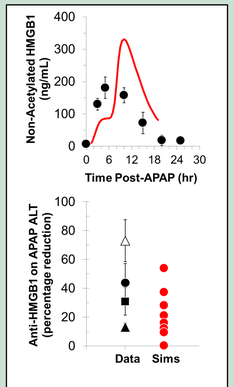
Earlier peak HMGB1 minimally impacts the relative efficacy of anti-HMGB1

Higher Peak HMGB1



Higher HMGB1 levels can increase the relative efficacy of anti-HMGB1

More Immune-Mediated HC Death



More immune-mediated death can increase the relative efficacy of anti-HMGB1

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Introduction

The DILIsym™ Model

- DILIsym™ is a mechanistic, multi-scale, mathematical model being developed through the DILI-sim Initiative to assist in the safety characterization of compounds in development
- The initial focus is on *in vitro* to *in vivo* preclinical and *in vivo* preclinical to first in human clinical translation [7-9]
- Simulated humans, dogs, rats, and mice are included
- Release of non-acetylated HMGB1 from dying hepatocytes is critical in the modeled initiation of the innate immune response; acetylated HMGB1 from macrophages can also contribute and is an indicator of immune cell activation
- HMGB1 neutralization is used to characterize the contribution of the innate immune response to overall hepatotoxicity

Methods

- Serum HMGB1 was optimized to provide reasonable agreement with measured data [4]
- Other aspects of the innate immune response (e.g., TNF-α levels) were similarly optimized
- The DILIsym™ model was also compared against a wide range of comparator data (e.g., GSH, ATP, dose-dependent ALT elevations, bile acids)
- HMGB1 neutralization was simulated to investigate the role of HMGB1 in hepatotoxicity in the baseline mouse
  - A range of APAP doses was used to account for variability in experimental protocols and relative responses
  - Maximum ALT levels were compared for APAP ± HMGB1 neutralization
- Alternate mice were created and tested to better understand the role of HMGB1 and the resultant innate immune response on hepatotoxicity

Key Findings

- HMGB1 neutralization was hepato-protective in the baseline simulated mouse, consistent with data [4-6]
- An earlier peak in HMGB1 (3 *vs.* 10 hours) minimally affected the level of protection achieved with HMGB1 neutralization
- Higher levels of HMGB1 (~2x) led to greater levels of protection when neutralizing HMGB1
  - Increased HMGB1 levels are inconsistent with available data, but might be observed between different mouse colonies or different mouse strains
- More immune-mediated hepatocyte death led to greater levels of protection when neutralizing HMGB1
  - As TNF-mediated (immune, extrinsic) death was increased, ATP-mediated (intrinsic) death was reduced to maintain comparable overall levels of HC loss
  - More TNF-mediated death shifted HMGB1 peak to later in the progression of liver injury, i.e., less early intrinsic death and therefore less early HMGB1 release
  - Maximum protection levels were constrained by the need for some intrinsic killing to provide initial HMGB1 release and trigger an innate immune response
- Neutralizing HMGB1 in alternate simulated mice illustrates the utility of the DILIsym™ model in testing alternate hypotheses on the role of the innate immune response in DILI

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