Establishing Model Mechanism-Based Causal Linkages Between APAP-Induced Hepatic Necrosis and Serum ALT

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ABSTRACT

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Bottom line: Two variants of a Virtual Model Mechanism provide individualized matches to 18 targeted plasma ALT values in mice. The Mechanisms provide a plausible multilevel causal explanation for zonation of APAP-induced liver injury features in mice and ALT release from individual hepatocytes at 3, 4.5, and 6 h following an i.p. dose of 300 mg/kg of APAP.

We demonstrate the scientific utility of conducting virtual experiments to posit plausible causal links between APAP within individual hepatocytes in vivo, ALT release, and plasma ALT. Model Mechanism entities are concrete and organized so that the activities occurring during execution produce a strong behavioral analogy to the referent biology. I.e., they are reasonably biomimetic.

We extend the Virtual Model Mechanism from Smith et al. [2]. Stress resulting from accumulation of damage products (MitoD and nonMD) above a threshold triggers ALT release. Released ALT accumulates in Mouse Body. We hypothesize that versions E & E' of the Model Mechanisms in Figure 4 have mouse counterparts.

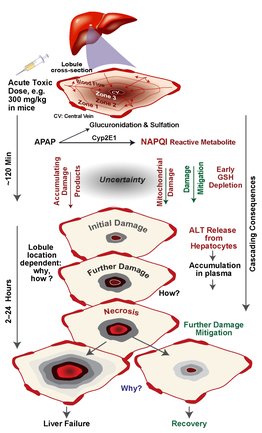
We directly map measurement of ALT objects in Mouse Body to wet-lab counterparts using a simple scaling: 1000 ALT objects in Mouse Body corresponds to 580 IU of ALT/ml plasma. We also provide a plausible explanation for inter-individual differences in how those mice respond to APAP-induced liver injury.

OBJECTIVE

The objective for this work is to obtain quantitative mappings from measurements Virtual Model Mechanism simulations to the individual plasma ALT values measured at 3, 4.5, and 6 h (following i.p. APAP doses 300 mg/kg) that fall within 10% of the reported measurement (the validation targets).

We start with the final Mechanism for APAP metabolism, disposition, and hepatotoxicity described by Smith et al. [2] diagrammed in Figure 3, and extended it to include mechanism features intended to plausibly explain ALT release from HPCs and accumulation in plasma as diagrammed in Figure 4.

Features of APAP-induced Hepatic Necrosis



METHODS

Technical Details

We use the MASON toolkit coupled with agent-oriented modeling methods. A vMouse is treated as a form of data. We manage vMice configuration files using the Subversion version control tools in Assembla plus those at https://github.com/ and at https://simtk.org/m. The entire toolchain is open-source.

Experiments are run using local hardware and virtual machines on Google Compute Engine, running 64-bit Debian 7. Quality assurance details, along with practices for validation, verification, sensitivity analysis, and uncertainty quantification are described in Smith et al. [2]. Data and code are available at (https://simtk.org/projects/aili and https://simtk.org/home/isl/).

The **targeted plasma ALT measurements** are from [5]. Mice (6 per time point) were dosed i.p. with 300 mg/kg APAP (a toxic but not lethal dose).

Achieving an Explanatory Virtual Model Mechanism

Methods for developing Virtual (computational) Model Mechanisms and for conducting Virtual Experiments are provided in [2-4]. For the results presented, all methods are identical to those detailed by Smith et al. [2].

Three Essential definitions

Biological mechanism [1] : biological entities and activities organized temporally and spatially so that they are responsible for generating the phenomenon to be explained

A **Model Mechanism** [1] : a well-defined mechanism-oriented explanation for the target phenomena (e.g., attributes that characterize APAP hepatotoxicity in mice) having three features.

1. Relevant information about the phenomenon is organized in text supported by drawings, sketches and mathematics.
2. The descriptive explanation (sufficient to meet the definition of biological mechanism) is sufficiently detailed to conceptualize how mappings are established between features of the explanation and particular measurements.
3. A working hypothesis is provided about how the mappings in (2) might extend to the actual causal explanation.

A **Virtual Model Mechanism** [1] : all features are instantiated incrementally in software and verified. Model Mechanism entities during execution are concretized objects utilizing defined spaces. Four requirements are specified early in the workflow to guide software engineering, mechanism instantiation and simulation refinements.

1. Arguments are presented that Model Mechanism entities and activities are biomimetic. i.e., they are sufficiently analogous to their mouse counterparts according to prespecified, criteria. **However, it is not necessary that they precisely model the biology.**
2. During execution, features are measured analogous to how their wet-lab counterparts are (or might be) measured, and they match or mimic measurements of the target phenomenon within some tolerance.
3. A Virtual Model Mechanism represents variables, parameters, and I/O in units defined by other system components so that they are internally consistent.
4. As needed, a Virtual Model Mechanism can express quantitative predictions.
   More important, it can be falsified quantitatively.

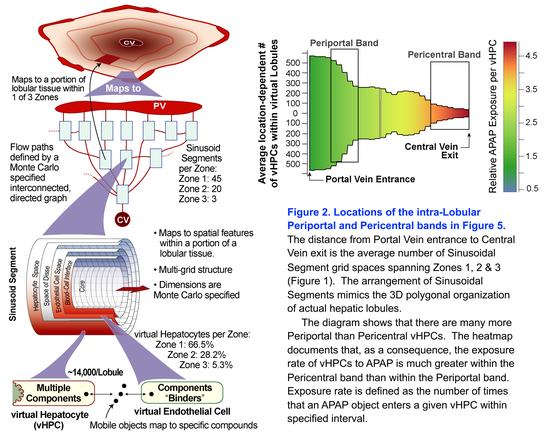


Figure 1. The multilevel biomimetic organization of virtual entities within a vLobule. One Monte Carlo vLobule maps to a small random sample of PV-to-CV portions of several mouse lobules.

Figure 2. Locations of the intra-Lobular Periportal and Pericentral bands in Figure 5. The distance from Portal Vein entrance to Central Vein exit is the average number of Sinusoidal Segment grid spaces spanning Zones 1, 2 & 3 (Figure 1). The arrangement of Sinusoidal Segments mimics the 3D polygonal organization of actual hepatic lobules.

The diagram shows that there are many more Periportal than Pericentral vHPCs. The heatmap documents that, as a consequence, the exposure rate of vHPCs to APAP is much greater within the Pericentral band than within the Periportal band. Exposure rate is defined as the number of times that an APAP object enters a given vHPC within specified interval.

Figure 3. The Final Mechanism from Smith et al. [2] for APAP metabolism, disposition, and hepatotoxicity

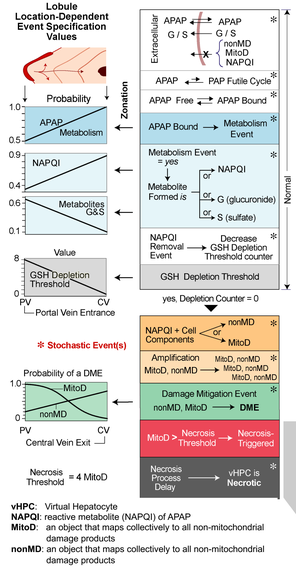
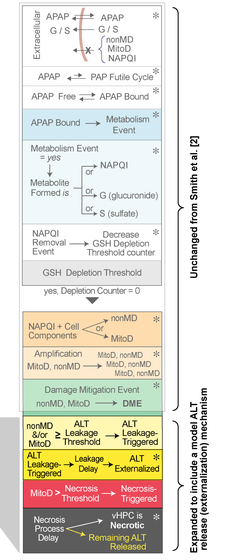


Figure 4. Expanded Mechanisms explored in this work



vHPC: Virtual Hepatocyte
NAPQI: reactive metabolite (NAPQI) of APAP
MitoD: an object that maps collectively to all non-mitochondrial damage products
nonMD: an object that maps collectively to all non-mitochondrial damage products

RESULTS

The simulation profiles in Figures 5 and 6 provide a plausible explanation for the target plasma ALT values. It was evident from the start of this work that explaining the 3 h data would be most challenging. The process of identifying plausible Model Mechanism explanations involved exploring variants to first achieve or exceed the validation target for the 6 & 4.5 h values, and then seeking variants that could also match all six 3 h values.

Bottom Line: Using a fixed ALT-Release Threshold, Model Mechanisms E and E' (described below) provide an individualized match to all plasma ALT values at 3, 4.5, and 6 h. The virtual & wet-lab means, SDs, and SEMs values for the virtual and wet-lab data sets are identical. Model Mechanisms A-D (also described below) were judged to be marginally less parsimonious.

Model Mechanism E: ALT-Release (0-6 h) = *f*(ALT-Release Threshold, MitoD (but not nonMD), Leakage Delay, transition to Necrotic).
ALT release is the combined consequence of release triggered by MitoD exceeding the ALT Release Threshold and subsequent release occurring when a vHPC transitions from Necrosis-triggered to Necrotic. The Min/Max for the stochastically determined ALT Leakage Delay are 2700 (0.75 h)/18000 (5h), and for and Necrosis Delay they are 7200 (2 h)/21600 (6 h).

For **E**, we specify that inter-individual variation in simulated ALT release is caused by individual differences in the cumulative influence of "environmental factors" on the operation of the entire Model Mechanism, causing measurements of phenomena for each individual to be amplified or diminished relative to the population average. Thus, individual values deviate randomly from the population average. That theory seems to hold for 14 of the 18 mice, **but not for the four smaller 3 h values.**

Model Mechanism E': The Min/Max for the stochastically determined Leakage Delay and Necrosis Delay used by **E** are both increased to 8800 (2.44 h)/23200 (6.44 h).
We modified **E** to **E'** at 3 h to account for the four apparent "outliers" by specifying the following. The cumulative influence of environmental factors on **E** for (only) those four mice is different from that for the other 14 mice. We specified that a consequence is prolongation of both Leakage Delay and Necrosis Delay times, but without significant impact on all other **E** features.

Model Mechanisms A & B: ALT-Release = *f*(ALT-Release Threshold, MitoD, nonMD, Leakage Delay, Necrotic).
A & **B** use different delay intervals for ALT release. All remaining ALT is released when a vHPC transitions from Necrosis-triggered to Necrotic. As with **E**, there are an individualized stochastically determined delay intervals before ALT Release begins and for Necrosis Delay.

Model Mechanism C: ALT-Release = *f*(Necrotic); i.e., all ALT release is a consequence of Necrosis. Release occurs when a vHPC transitions from Necrosis-triggered to Necrotic.

Model Mechanism D: This is the same as **E**, except that triggering ALT release is a function of nonMD rather than MitoD. ALT-Release = *f*(ALT-Release Threshold, nonMD (but not MitoD), Leakage Delay, Necrotic).

Figure 5. Key measurements of virtual metabolism, disposition, hepatotoxicity and ALT release for Model Mechanism E corresponding to the results in Figure 6. The locations of the intra-Lobular Periportal and Pericentral bands are provided in Figure 2.

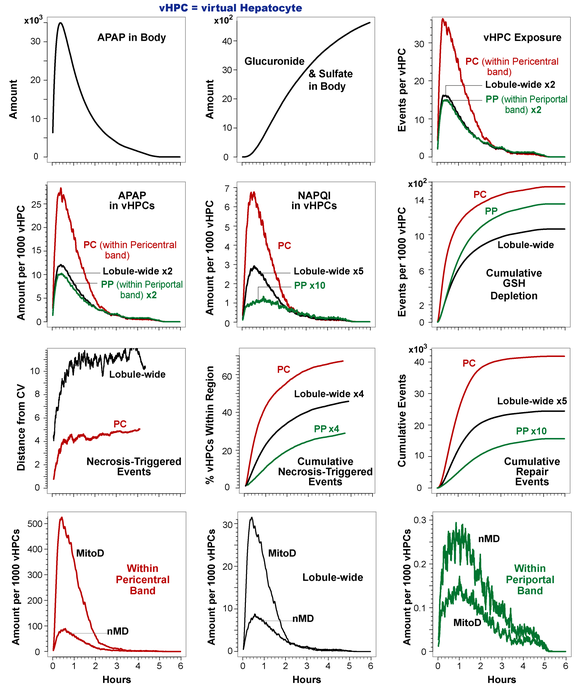
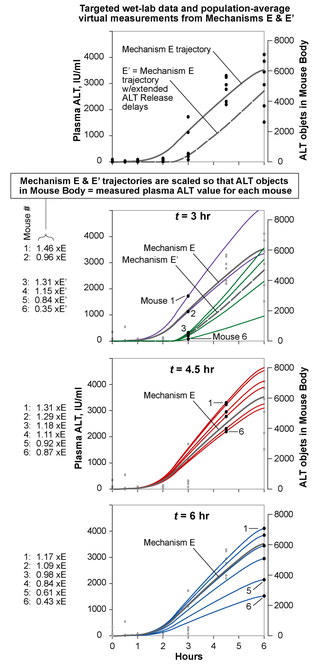


Figure 6. Virtual profiles providing the current best (marginally parsimonious) explanations for the three sets of plasma ALT values.



CONCLUDING OBSERVATIONS

Two variants of the Model Mechanisms in Figure 4 and the simulation profiles in Figures 5 and 6 provide the current best explanations for the target plasma ALT values. They provide a plausible multilevel causal explanations for both zonation of APAP-induced liver injury features and ALT release from individual hepatocytes at 3, 4.5, and 6 h following an i.p. dose of 300 mg/kg of APAP. We directly map measurement of ALT objects in Mouse Body to wet-lab counterparts using a simple scaling: 1000 ALT objects in Mouse Body corresponds to 580 IU of ALT/ml plasma.

We simulate inter-individual variation by multiplying the scaled output by r, a random draw from a normal distribution having mean = 1.0. The Mechanism E and E' profiles in Figure 6 for r = 1 are hypothesized to map the population average plasma ALT profiles for mice. After combining the individual deviations from the virtual population average trajectories for E and E', all 18 plasma ALT measurements were consistent with being distributed randomly about the population average. The statistical results for the 18 deviations are as follows:

μ (deviation) = 0.99, SD = 0.303, SEM = 0.0714, CV = 0.306, consistent with wet-lab measurements.

We hypothesize that differences in how matched mice experience the same environment contribute to inter-individual differences in how they respond to APAP. An analogous process in mice would mean that environmental factors, as experienced by an individual mouse, influences (amplifies or diminishes) the overall quantitative operation of the mechanism under study rather than particular features of the mechanism.

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