Virtual Experiments Falsify a Prevailing Mechanistic Explanation for Acetaminophen Induced Liver Injury and Enable Discovery of Plausible Alternative Mechanisms

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Key words: *virtual experiments, mechanistic explanation, liver injury, acetaminophen toxicity, concrete mechanisms, agent-based, multi-attribute*

Acetaminophen induced liver injury is a multifaceted phenomenon potentially causing liver failure. A precondition to developing strategies that avoid, disrupt, and/or reverse liver damage is a reliable causal cascade description. Yet even after three decades of intense investigation, a cause-effect relationship remains elusive. The weight of the evidence supports this hypothesis: zonation (spatial heterogeneity) within hepatic lobules of NAPQI formation (the reactive metabolite) can account for necrosis occurring first adjacent to the lobule's central vein (Mechanism Composite 1). However, the hypothesis cannot be tested directly in mice because doing so requires sequential intracellular measurements at different locations within hepatic lobules, which is currently infeasible.

We challenged and refuted—falsified—Mechanism Composite 1 using virtual experiments with concrete mechanisms. To date, objects of acetaminophen toxicity experiments have included animal models, organotypic hepatocyte cultures, and organ-on-chip systems. But controlling key variables during such experiments has proven problematic. Consequently, results have fallen short of expectations. We experimented on virtual mice containing a concretized biomimetic liver lobule model of Mechanism Composite 1, which had already achieved multiple multiscale qualitative and quantitative validation targets. Virtual hepatocyte objects can exhibit lobule-location dependent behaviors. We first achieved quantitative validation targets for acetaminophen clearance and metabolism. We then implemented and verified a concrete, parsimonious version of Mechanism Composite 1 and imposed several evidence-based constraints. A parameter space search failed to produce a parameterization that could achieve the key validation target: necrosis first occurs adjacent to the lobule's central vein. We posited that at least one additional feature must also exhibit zonation to achieve the validation target. We developed and challenged two alternative hypotheses: either amount of GSH (Mechanism Composite 2) or amount of mitochondrial damage (Mechanism Composite 3) must exhibit zonation in addition to NAPQI formation. Alternative mechanisms were evaluated based on their composed behavior (phenotype). Mechanism composites can be selected (for or against) based on whole or decomposed pattern/phenotype. Both hypotheses were falsified.

We then challenged a fourth hypothesis: zonation in amount of available GSH *and* mitochondrial damage repair (in addition to NAPQI formation) are required to achieve the validation target. That mechanism achieved the validation target.

In silico outcomes are multiscale consequences of two location-dependent counteracting mechanisms. We hypothesize that corresponding mechanisms occur within mouse lobules upon exposure to a toxic APAP dose: two types of intracellular injury initiated by NAPQI: mitochondrial (mitoDamage) & non-mitochondrial (nonMD) damage. The pace of repair of (recovery from) mitochondrial injury determines if Necrosis is triggered or not.

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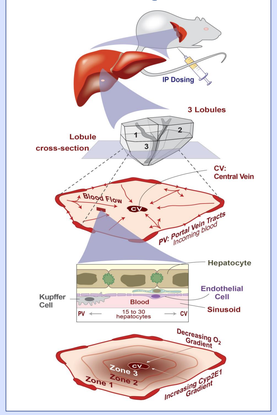
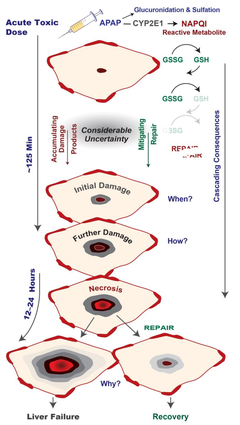
BACKGROUND

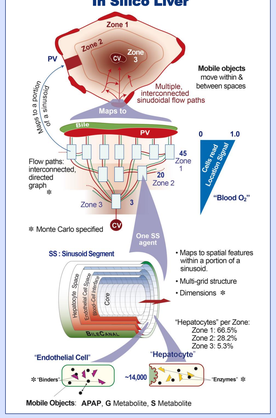
Multiscale Biological Features



APAP Hepatotoxicity



In Silico Liver



Important Features of Acetaminophen Hepatotoxicity

- Mechanisms of acetaminophen (APAP) hepatotoxicity have been under intensive investigation for several decades.
- APAP overdose causes multiple interrelated molecular level events
- But their relative importance in causing hepatocellular death is ***still*** not well understood. That is because their relative importance is location dependent within hepatic lobules
- NAPQI depletes intracellular GSH and then covalently binds to proteins
- GSH depletion causes accumulation of ROS and RNS, increasing oxidative stress & intracellular damage
- Increasing damage and stress leads to mitochondrial dysfunction and DNA fragmentation
- Absent adequate compensatory repair of damage and amelioration of oxidative stress, necrosis is triggered.

Focus

- This work focuses on the first 24 hours following a toxic dose

The Prevailing Explanatory Hypothesis

The weight of the evidence supports this hypothesis:

It is the zonation of NAPQI formation that accounts for necrosis occurring first close to the lobule's central vein (CV), and thereafter progressing in the PV (portal vein) direction.

Science Demands...

…that we challenge explanatory hypotheses

However, the above hypothesis cannot be tested directly in mice because doing so requires sequential intracellular measurements, which are currently infeasible.

•Mathematical descriptions can challenge hypotheses about relationships among changes in parameters and output

•They cannot challenge (falsify) competing explanations of how those phenomena were generated

•Challenging an explanatory hypothesis requires contrasting (experimenting on) competing explanations for how the same phenomenon may be generated

Our Solution

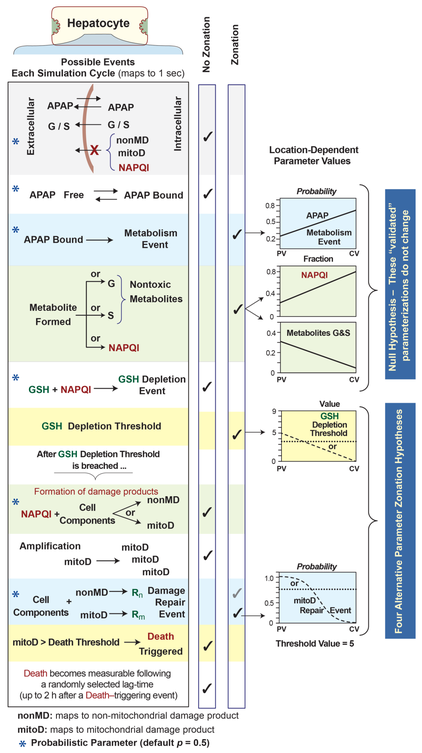
- Concretize and experiment on competing plausible **in silico** mechanistic explanations for how the above pattern of necrosis may be generated

Mouse Level Events

Simulated intraperitoneal dosing at t = 0

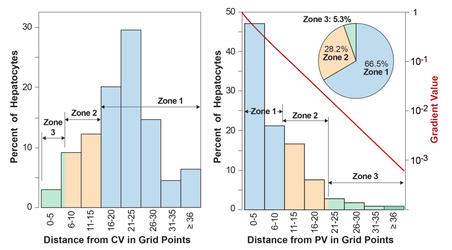
Thereafter, each simulation cycle, each of the illustrated events may occur

Within each Hepatocyte, each simulation cycle, □ each of the following mechanistic events may occur



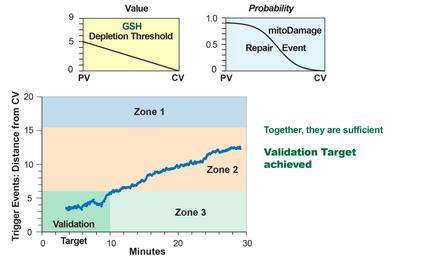
nonMD: maps to non-mitochondrial damage product
mitoD: maps to mitochondrial damage product
Probabilistic Parameter (default p = 0.5)

Number of Hepatocytes is Location Dependent Within Lobules



Alternate Mechanism Composite 4

Hypothesis: Enabling decreasing PV-to-CV Zonation of both GSH Depletion and mitoDamage Repair is sufficient to achieve the Validation Target

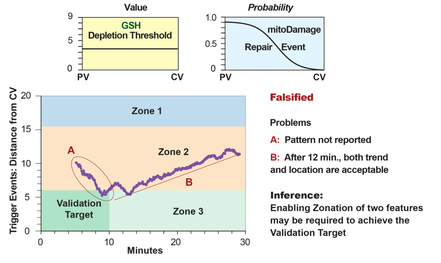


Together, they are sufficient

Validation Target achieved

Alternate Mechanism Composite 3

Hypothesis: Enabling mitoDamage Repair to decrease dramatically PV-to-CV rather than being zone independent (Mechanism Composite 1) is sufficient to achieve the Validation Target



Falsified

Problems

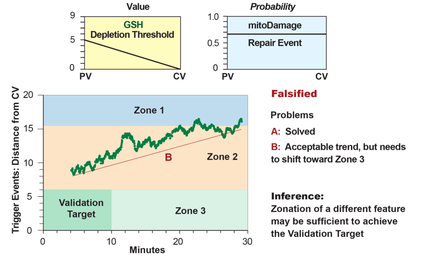
A: Pattern not reported

B: After 12 min., both trend and location are acceptable

Inference:
Enabling Zonation of two features may be required to achieve the Validation Target

Alternate Mechanism Composite 2

Hypothesis: Enabling GSH Depletion to decrease PV-to-CV rather than being zone independent (Mechanism Composite 1) is sufficient to achieve the Validation Target



Falsified

Problems

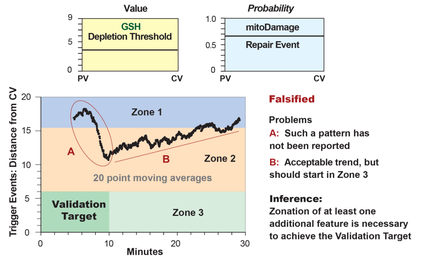
A: Solved

B: Acceptable trend, but needs to shift toward Zone 3

Inference:
Zonation of a different feature may be sufficient to achieve the Validation Target

Mechanism Composite 1: Null Hypothesis

Hypothesis: Enabling reactive metabolite (NAPQI) formation alone to ***increase*** PV-to-CV is sufficient to achieve the Validation Target



Falsified

Problems

A: Such a pattern has not been reported

B: Acceptable trend, but should start in Zone 3

Inference:
Zonation of at least one additional feature is necessary to achieve the Validation Target

All results averaged over 12 Monte Carlo Trials

Prerequisite Requirements

A concretized Multiscale Mouse Model that satisfactorily:

- Mimics micro- and mesoscale hepatic anatomical features
- Mechanisms during execution are biomimetic within and across multiple scales
- Achieves the following quantitative validation targets

Prerequisite Validation Targets Already Achieved

•Within ± 1 SD for single pass outflow profiles of APAP, atenolol, antipyrine, labetalol, diltiazem, propranolol, prazosin, and sucrose

•Within ± 1 SD for whole mouse APAP blood levels following IP dosing

•Within ± 1 SD for APAP hepatic extraction ratio

•Within ± 1 SD for NAPQI, glucuronide, & sulfate metabolites

•Intrinsic clearance of APAP (per Hepatocyte) increases at least 50% PV-to-CV

•Relative production of NAPQI (as percent of total metabolites) increases at least 50% PV-CV

New Validation Targets Achieved

- Amelioration of some types of oxidative damage (nonMD) increases PV-to-CV
- APAP in plasma peaks prior to 20 min after IP dose
- Little NAPQI formation in Zone 1
- Rapid GSH depletion: > 50% depleted within 30 min. after IP dose
- Measurements of hepatotoxicity occur in a temporally progressive, central (CV) to peripheral (PV) pattern
- Some necrosis evident at 2 hours
- Peak Necrosis occurs at ~ 8 hours
- Periportal hepatocytes are spared
- At 30 minutes, NAPQI adducts are approximately twice that at 15 minutes

Key Validation Target for This Work

Necrosis trigger events occur first in Zone 3, close to CV

Thereafter, they increase in the PV direction

New Insight, New Hypothesis

When necrosis threshold exhibits no zonation, the in silico outcomes of simulated APAP injury within the first 24 hours are multiscale consequences of two location-dependent counteracting mechanisms. We hypothesize that corresponding mechanisms occur within mouse lobules upon exposure to a toxic APAP dose:

Two types of intracellular injury initiated by NAPQI: mitochondrial (mitoDamage) & non-mitochondrial (nonMD) damage

The pace of repair of (recovery from) mitochondrial injury determines if Necrosis is triggered or not.

Methodological Take-Home Messages

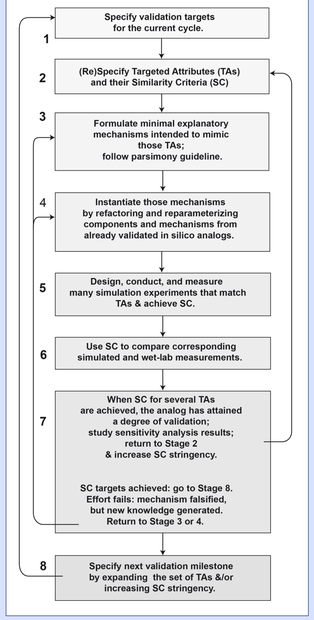
- Virtual experiments falsify a prevailing composite mechanistic explanation for acetaminophen induced liver injury and enable discovery of plausible alternative mechanisms, evaluated based on their composed behavior (phenotype)
- Composite mechanisms can be selected (for or against) based on whole or decomposed pattern/phenotype

METHODS

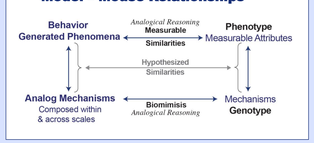
The current work uses the MASON toolkit

Only composite Mechanisms can be evaluated (falsified) Mechanism components cannot be evaluated

Core Method Iterative Refinement Protocol



Model – Mouse Relationships



About Monte Carlo Lobules

About Qual/Quant Validation

About Agents

About In Silico Liver

About Analogs

Glossary

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