Virtual Experiments Falsify a Prevailing Mechanistic Explanation for Acetaminophen Induced Liver Injury and Enable Discovery of Plausible Alternative Mechanisms That May Help Explain Interindividual Variability

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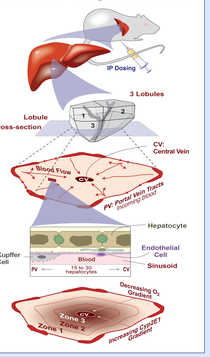
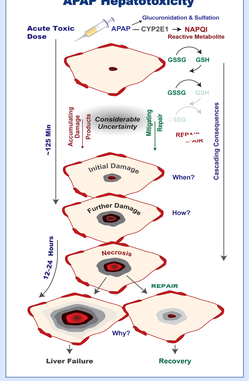
BACKGROUND

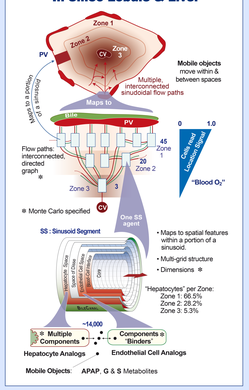
Multiscale Biological Features



APAP Hepatotoxicity



In Silico Lobule & 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 APAP dose in mice.

The Prevailing Explanatory Hypothesis

The weight of the evidence supports this hypothesis (Mechanism 1):

Location dependent differences in NAPQI formation (increasing PV to CV) within hepatic lobules (zonation) are necessary and sufficient to account for necrosis occurring first adjacent to the lobule's central vein (CV), and thereafter progressing in the PV (portal vein) direction.

However, challenging that hypothesis directly in mice is currently infeasible because doing so would require sequential intracellular measurements at different locations within hepatic lobules of the same mouse.

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**

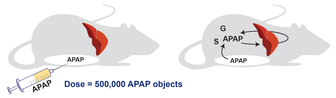
The Mechanism Is Composite

APAP hepatotoxicity is a consequence of multiple events at multiple scales that are composed over time

Mouse Level Events

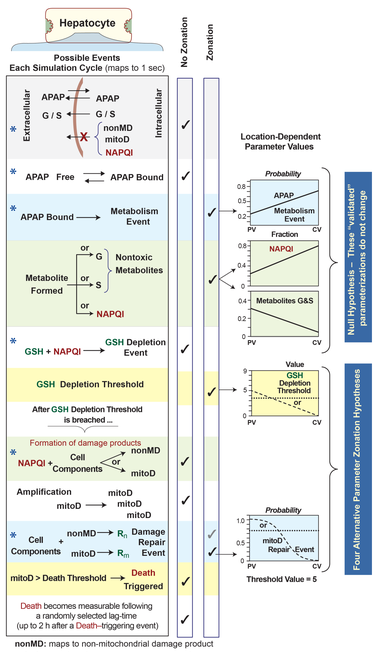
Simulated intraperitoneal dosing at t = 0

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

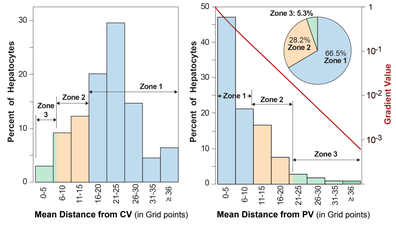


Dose = 500,000 APAP objects

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

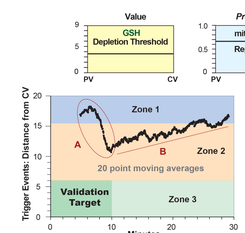


Number of Hepatocytes is Location Dependent Within Lobules



Mechanism 1: Null Hypothesis

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



Falsified

Problems

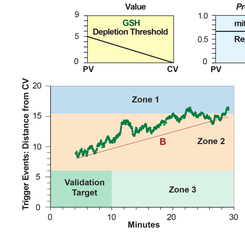
A: Such a pattern has not been reported

B: Acceptable trend, but should start in Zone 3

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

Alternate Mechanism 2

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



Falsified

Problems

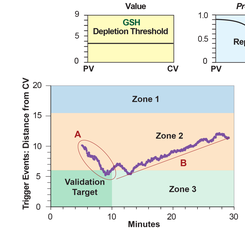
A: Solved

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

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

Alternate Mechanism 3

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



Falsified

Problems

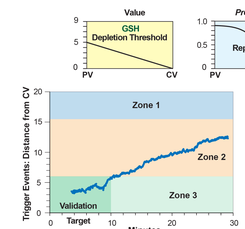
A: Pattern not reported

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 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

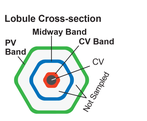
Validation Target achieved

All results averaged over 12 Monte Carlo Trials

Measurements of Cascading Causal Events within Lobules for Alternative Mechanism 4

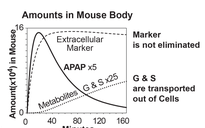
Single Toxic APAP dose given IP

Lobule Cross-section



To highlight zonation influences, contents of each Hepatocyte were measured within these three 5-grid-space-wide bands

Amounts in Mouse Body

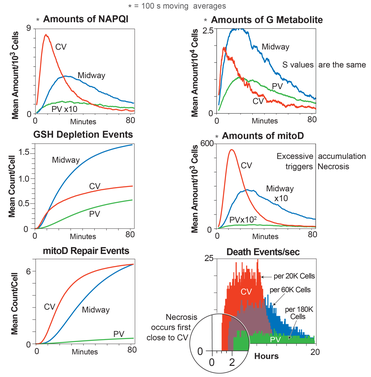


Marker is not eliminated

G & S are transported out of Cells

To facilitate verifications, G & S are allowed to accumulate in Mouse Body

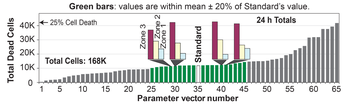
+ = 100 s moving averages



Observations on Sensitivity/Robustness Assessments for Alternative Mechanism 4

Sixty-four additional parameter vectors were judged to be plausibly biomimetic. All are available here: http://furm.org/lorentz2015/hepatox/65-Parameterizations.pdf (or use the QR code). From 1 – 7 parameter values were changed. Some changes involved adding or altering their PV-to-CV valuations.

Green bars: values are within mean ± 20% of Standard's value.

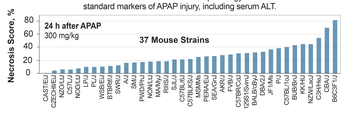


Each green case may map to different individual mice (same strain). Although total Dead Cells are similar, there is greater variation when Deaths are subdivided by zone (two inserts). Such differences are evidence of the concrete differences in individual mechanisms.

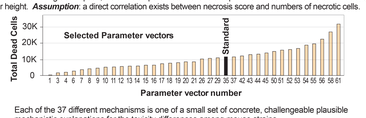
Mapping to Inter-strain Variability

Harrill et al. (doi: 10.1101/gr.090241.108) used inbred mouse strains as a model for human genetic diversity in response to APAP overdosing.

No correlation was found between pathology scores and standard markers of APAP injury, including serum ALT.



Given the following assumption, we selected parameter vectors that provided a reasonable match of relative bar height. ***Assumption***: a direct correlation exists between necrosis score and numbers of necrotic cells.



Each of the 37 different mechanisms is one of a small set of concrete, challengeable plausible mechanistic explanations for the toxicity differences among mouse strains.

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

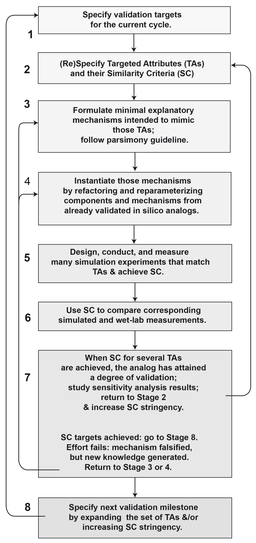
- **Virtual experiments falsify a prevailing composite mechanistic explanation for acetaminophen induced liver injury and enable discovery of plausible alternative mechanisms, evaluated based on their composed behavior (phenotype)**
- **Composite mechanisms can be selected (for or against) based on whole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

METHODS

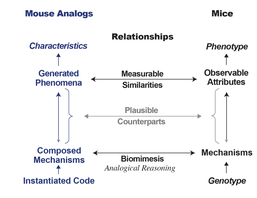
The current work uses the MASON toolkit

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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