Explaining complex phenomena by discovering, validating, and falsifying in silico mechanistic hypotheses: a demonstration focused on multiple features of acetaminophen hepatotoxicity

Graduate Program in Bioengineering UC Berkeley / UCSF

Andrew K. Smith, Glen E.P. Ropella, and **C. Anthony Hunt** Bioengineering and Therapeutic Sciences, UCSF

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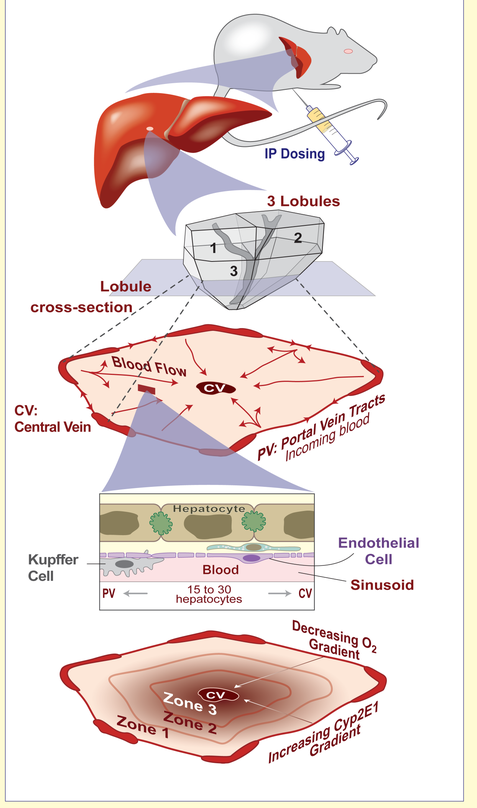
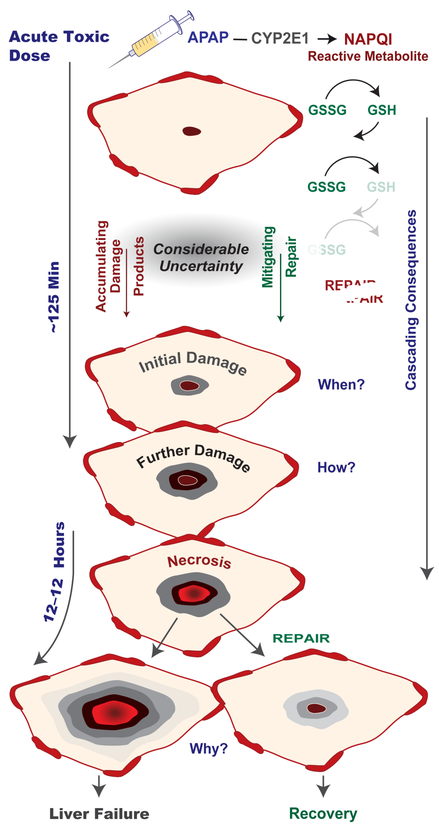
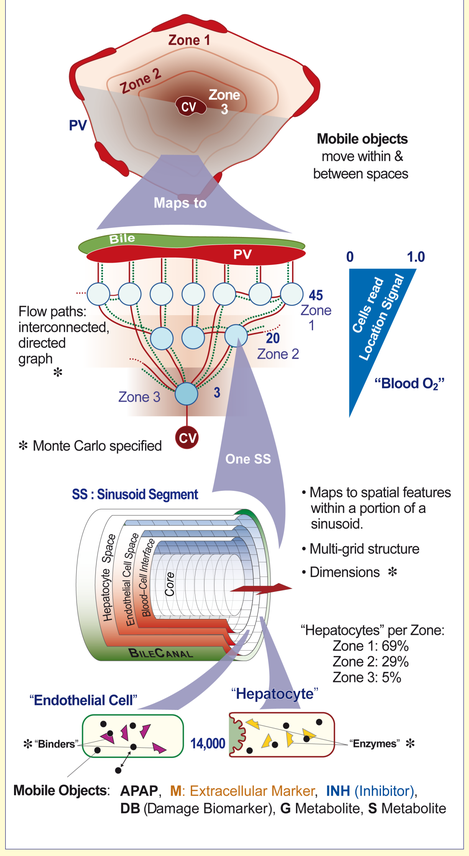
a Multiscale Biology – Key Features



b APAP Hepatotoxicity – Key Features



c In Silico Liver – Key Features



Objectives

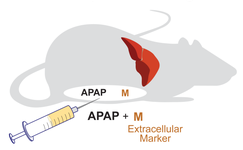
Achieve two new validation targets: They are uniquely characteristic of APAP toxicity. Do so without compromising validation targets already achieved.

1. **Necrosis** occurs first in hepatocytes close to CV (Zone 3) and then progresses outward toward PV.
2. **30 min** after APAP dose, an IP dose of SP600125 (INH; blocks Jun N-terminal kinase) significantly attenuates necrosis.

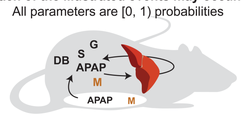
Challenge: Discover and improve, plausible analog mechanisms that stand as hypotheses that explain key features of hepatotoxicity and its inhibition in mice.

d Key Mouse-level Events

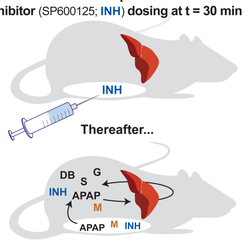
Simulated intraperitoneal dosing at t = 0



Thereafter, each simulation cycle, each of the illustrated events may occur.
All parameters are [0, 1) probabilities



Some Experiments
Inhibitor (SP600125; **INH**) **dosing at t = 30 min**



e Within each Hepatocyte, each simulation cycle, each of the following mechanisms may occur

All parameters are [0, 1) probabilities

Extracellular APAP ⇄ APAP Intracellular
G &/or S ⇄ G &/or S

APAP + "Enzymes" ⇄ APAP–"Enzymes"

APAP–"Enzymes" → G, S: nontoxic metabolites; N: reactive metabolite NAPQI (+"Enzymes")

GSH + N → GSSG, GSH depletion events

After GSH depletion threshold breached ...

N + unspecified cell components → **D1**: damage product; **D2**: damage product that can trigger Necrosis; **BM**: damage biomarker accumulates in "Mouse" (Intracellular)

D2 → D2 D2 D2 amplification

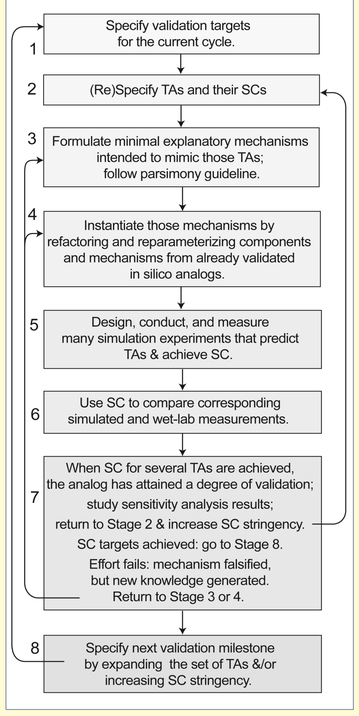
D1, D2 + unspecified cell components → **R**: repaired damage

If ∑ **D2** > *Cell's Necrosis Threshold* : **Necrosis** is triggered

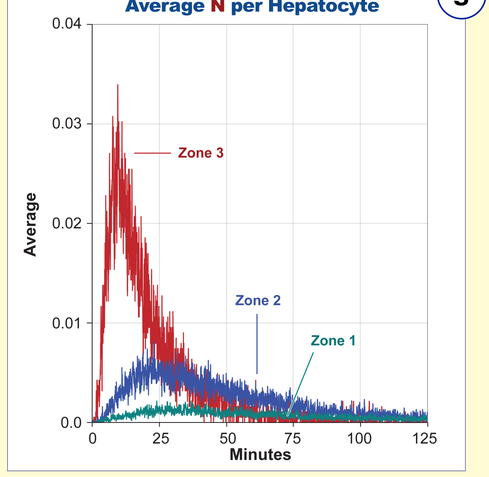
Necrosis triggered : Death scheduled randomly [3.8, 10.6 h]

Inhibition criterion : P_{inh} = (**INH** count) / (D2 + 1)
PRNG draw from uniform [0.0, 1.0)
if PRNG draw ≤ P_{inh}, then delete scheduled death event

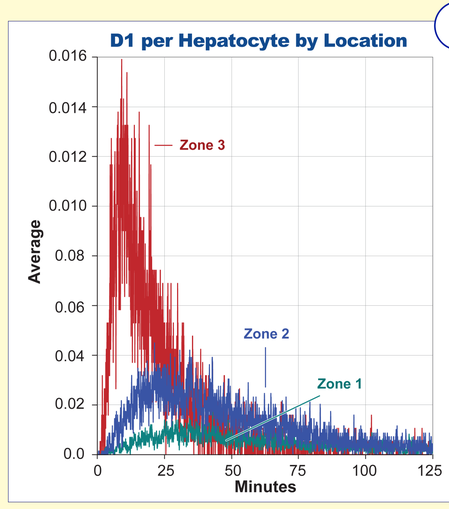
f Iterative Refinement (IR) Protocol



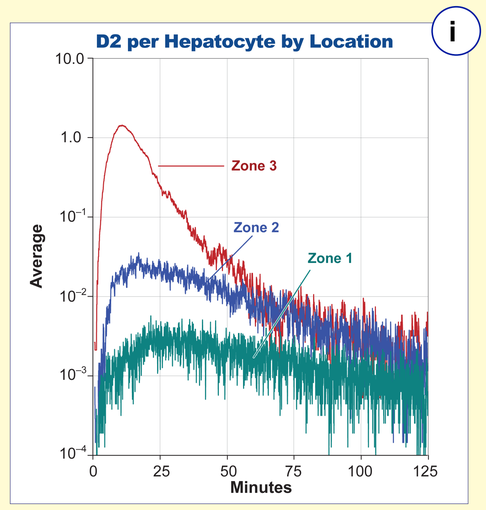
g Average N per Hepatocyte



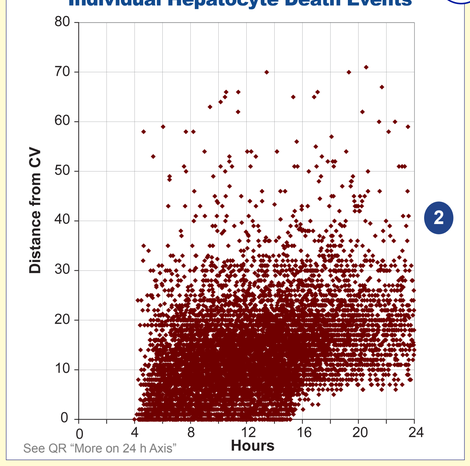
h D1 per Hepatocyte by Location



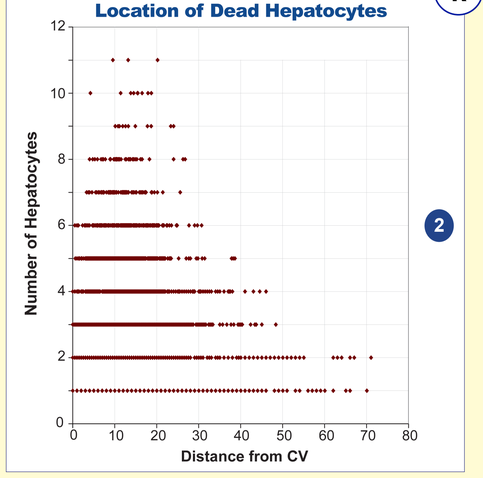
i D2 per Hepatocyte by Location



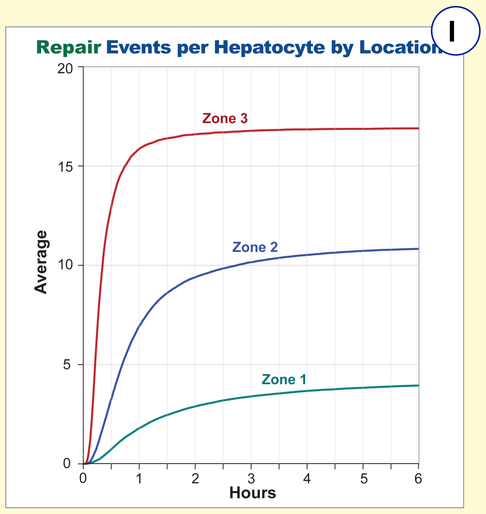
j Individual Hepatocyte Death Events



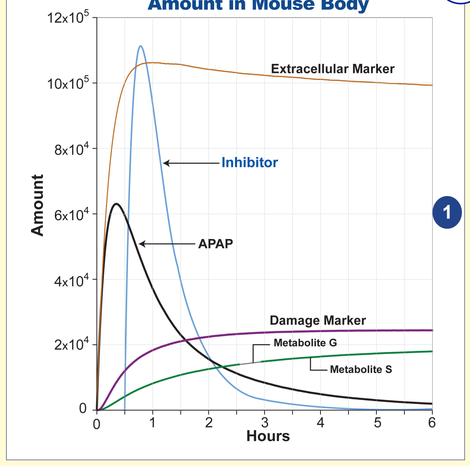
k Location of Dead Hepatocytes



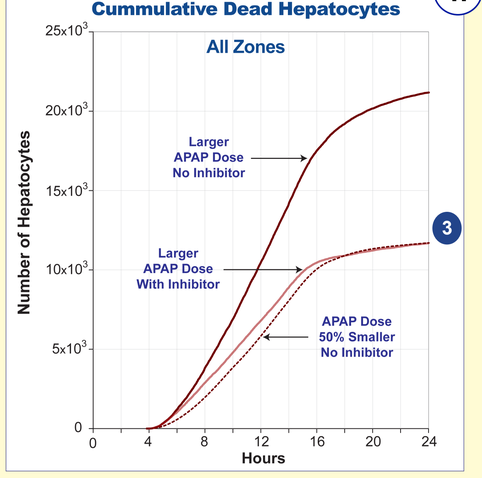
l Repair Events per Hepatocyte by Location



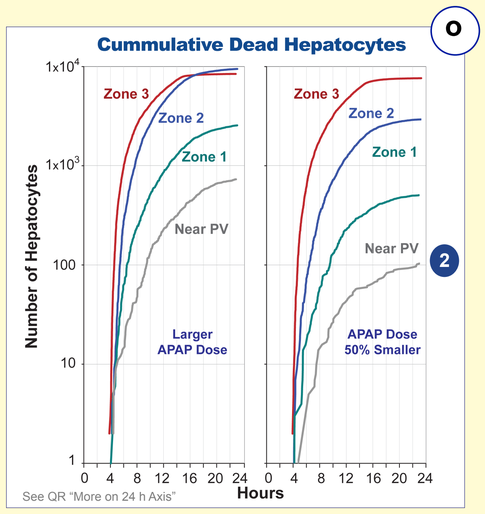
m Amount in Mouse Body



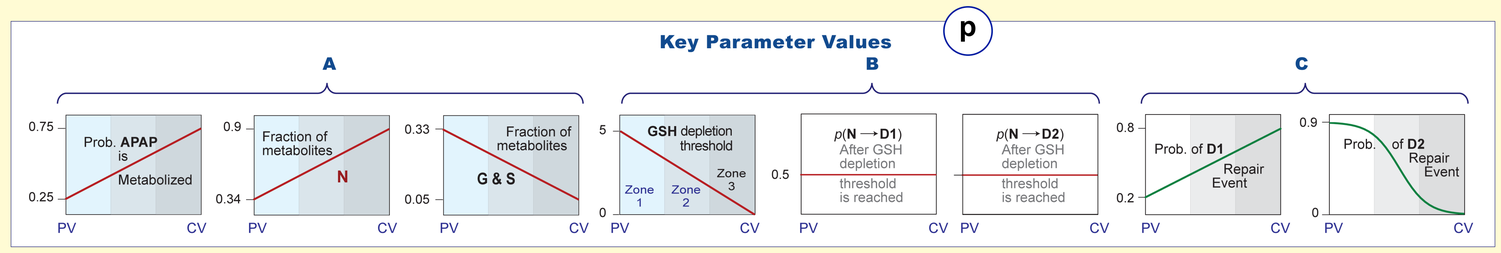
n Cummulative Dead Hepatocytes



o Cummulative Dead Hepatocytes



p Key Parameter Values



Validation Targets Already Achieved

- **APAP pharmacokinetics, hepatic clearance and metabolite ratios** See QR "More on In Silico Liver"
- **Single pass hepatic disposition profiles for eight compounds**
- **APAP PK measurements in mice following IP dosing**
- **Lobule zonation of metabolism (responsible enzymes exhibit different PV-to-CV patterns)**
- **NAPQI production increases PV-to-CV**

Approach

Challenge plausible explanatory mechanistic hypotheses by experimenting on agent oriented In Silico Liver (ISL) analogs. See QR "More on Agents" and also QR "More about Analogs"

ISL mechanisms are concrete and biomimetic.

Mechanisms: comprised of nested modular spaces, modules, and components.

***Prespecify* and achieve key validation targets**

A targeted attribute (TA) is phenomenon to be explained.

Validation targets are achieved when measurements of simulated and wet-lab phenomenon are quantitatively similar according to a *prespecified* similarity criteria (SC). For more, see QR "More on Qual/Quant Validation".

Achieve each validation target by cycling through the Iterative Refinement Protocol (IR Protocol).

Hypotheses:

Within each hepatocyte, there is a critical level in the accumulation of some macromolecular damage products: **a tipping point.** When reached, necrosis is triggered. The simplest tipping point scenario requires rapid accumulation of considerably more Damage Product in Zone 3.

Inhibitor reduces total cell deaths.

More on Monte Carlo Lobules

More on Qual/Quant Validation

More on Agents

More on In Silico Liver

More about Analogs

More on 24 h Axis

Glossary

Methods

Agent-based & Agent Oriented Analogs

We started with an ISL that had already achieved several validation targets.

Core Method: the Iterative Refinement Protocol

When making mechanistic granularity decisions, we adhered tightly to a strong parsimony guideline.

Simulating inhibition required:

1. A separate set of INH objects that would percolate simultaneously with APAP objects through and interact with analog components
2. Giving analog components the ability to distinguish between APAP and INH yet avoid interaction; and
3. Achieving estimates of INH PK properties following IP dosing
4. If INH criterion is met, cancel Death event

All experiments: 1 "APAP" = 8.33 x 10^{-4}% dose
Infusion dose: 120,000 "APAP" object
One experiment: 24 Monte Carlo lobules. See QR Monte Carlo

Results

Parameter Values without INH

A: These three location-dependent parameter values enabled achieving multiple quantitative validation targets.

B: Because GSH decreases PV-CV, we specified that the GSH depletion threshold should do the same. *p*(N → D1 & D2) was set arbitrarily at 0.5 and was not changed thereafter.

C: These parameterizations were arrived at following several IR Protocol mechanism falsifications. See QR "More on ISL" for details.

Map of APAP Mouse profile to APAP blood levels following an IP APAP dose achieved quantitative SC. (1)

Key qualitative validation target achieved: more dose dependent Hepatocyte Death occurs first adjacent to CV. Thereafter, more Death occurs further from CV. (2)

Following INH dosing, Hepatocyte Death was reduced. The reduction was comparable to that observed when APAP dose was reduced by 50%. (3)

MSM Take Home Points

Using in silico experimentation *alone*, we:

- **demonstrated improving mechanistic Insight.**
- **discovered a plausible analog mechanism that explains how key multiscale features of hepatotoxicity may be generated and inhibited in mice. The mechanism is a concrete, coarse grain hypothesis. It is plausible because multiple validation targets were achieved.**
- **generated new knowledge by *falsifying* several dozen analog mechanisms that seemed possible initially because they achieved early validation targets.**

The methods used make it straightforward to iteratively reuse and refine components to establish new, more explanatory mechanisms that achieve new validation targets.

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