Is the Mechanism of APAP Toxicity In Vivo & In Vitro Really the Same?
A Model Mechanism Based Explanation of the In Vitro–in Vivo Disconnect

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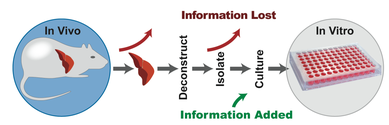
NEEDED

Credible, Knowledge-Based In Vitro–to–In Vivo Translations and Predictions

PROBLEM

There is often an in vitro–to–in vivo (IVIV) disconnect. Knowledge-based translation is often problematic.



APPROACH

Current approaches use correlation and extrapolation. Herein we offer an alternative approach.

CONTEXT

- Pharmacological and Toxicological Phenomena => acetaminophen (APAP) pharmacology/toxicology: In Vivo = Mice; In Vitro = Hepatocyte 2D Cultures.
- It is infeasible to study an actual biological system to learn where, how, and why in vivo–to–in vitro changes occur.
- It is well understood that IVIV disconnect causes include: 1) loss of 3D contextual features, and 2) hepatocytes often behave differently in vitro.
- By better understanding the contributions of those two sources, we can close this IVIV disconnect.

SPECULATION

Speculation: We can use M&S methods (e.g. Virtual Experiments) to pursue plausible mechanism-based models of explanation for specific IVIV disconnect phenomena. By insisting that methods are generalizable, we will be on a path to close IVIV disconnects.

OBJECTIVE

Demonstrate feasibility of using virtual experiment methods to explain quantitatively contributions to the IVIV disconnect caused by loss of 3D hepatic contextual features

HYPOTHESIS

Test this hypothesis: temporal values necrosis trigger events (toxicity) will be essentially the same because analog hepatocytes (aHPCs) function the same in both Mouse and Culture Analogs, identical APAP exposures.

DEFINITION

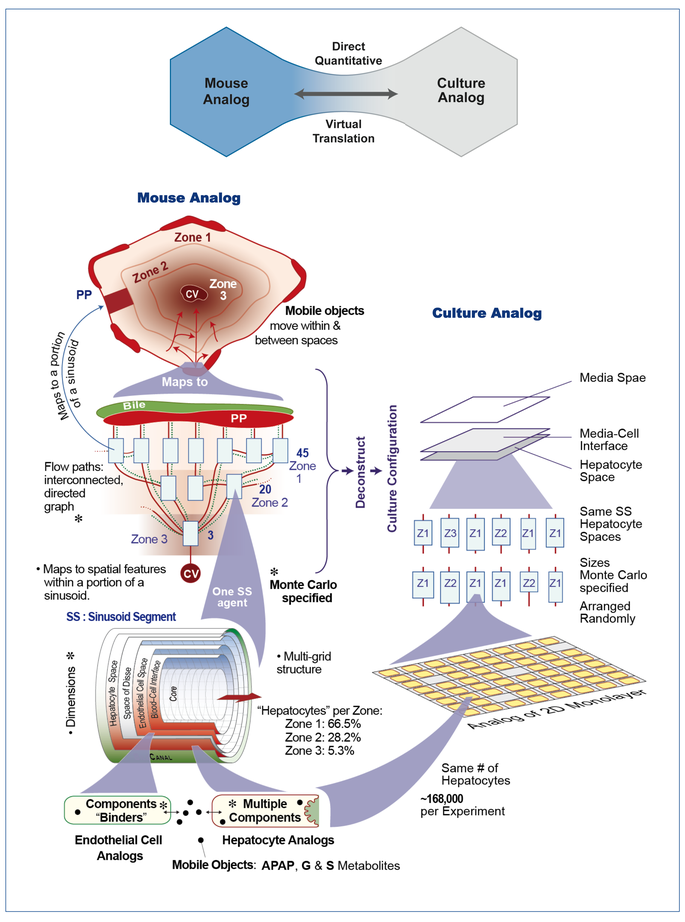
Mechanism – We adopt this definition of mechanism [1]: a mechanism involves entities and activities organized in such a way that they are responsible for the phenomenon to be explained. In addition to a phenomenon, an explanatory mechanism exhibits four essential features [2]: 1) Components (e.g., entities and activities, modules); 2) Spatial arrangement of components; 3) Temporal aspects of components; and 4) Contextual locations (e.g., location within a hierarchy).

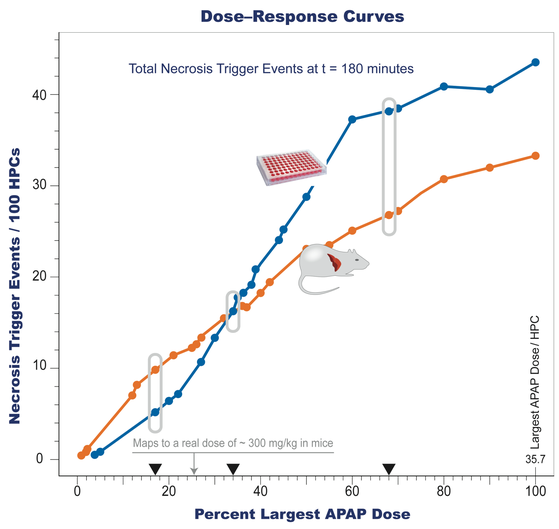
METHODS

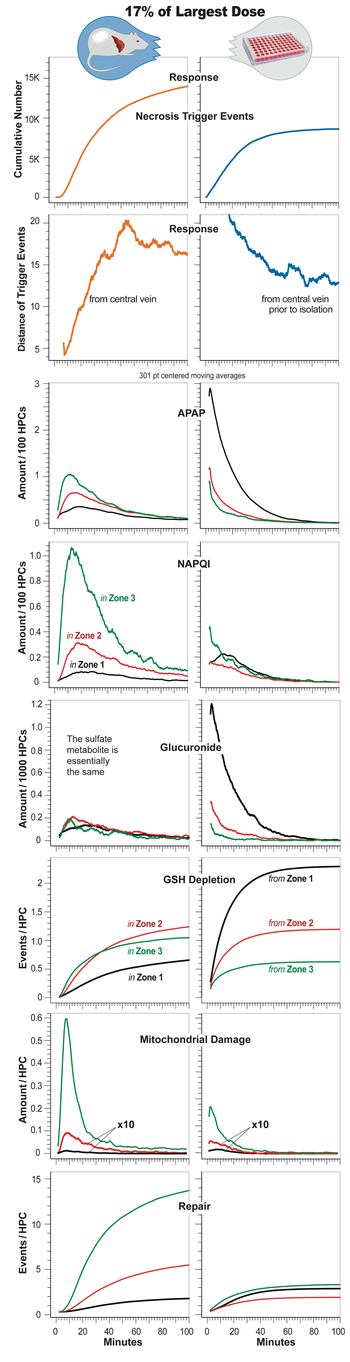
- Start with an established [3] multi-attribute, multiscale model that adequately explains multiple features of APAP hepatotoxicity in mice.
- Mimic the wet-lab procedure: isolate, and deconstruct the liver, isolate and culture hepatocytes.
- Verify that all analog hepatocytes (aHPCs) internal mechanisms are the same in both simulated culture and liver contexts.
- Configure all aHPCs into a Culture Analog that mimics commonly used 2D culture systems.
- Enable parallel virtual experiments in which APAP doses and number of exposed aHPCs are the same for Mouse and Culture contexts.
- Each aHPC "remembers" its location within the Liver Lobule. In that way we were able to compare how the same aHPC behaved during exposure to APAP in Mouse and Culture contexts.
- Response is occurrence of Necrosis Trigger Events.
- Record time-course measurements of other key aHPCs events.
- Conduct Dose-Response (D-R) experiments

RESULTS + EXPLANATIONS

- Theis falsified because the Mouse & Culture Analog Dose-Response curves are different. Thus, the virtual causal mechanisms within each system are different.
- Cell level spatial and temporal mechanisms shared by both Analogs behave the same.
- So, why are the mechanisms different? Hepatocytes in Mouse & Culture Analogs are heterogeneous, because parameterizations within Mouse Analogs are location dependent. Although the aHPCs are the same, exposure to APAP is different.
- In the Culture Analog, all aHPCs are exposed essentially simultaneously to the level of APAP in media adjacent to the Cells. However, within the Liver Analog, aHPCs are exposed to APAP sequentially. Upstream aHPCs "see" greater amounts than do downstream aHPCs.
- In the Liver Analog, within the same time interval, aHPCs that are most sensitive to APAP (those close to the central vein) have much higher intracellular levels of unbound APAP than do aHPCs further upstream.
- In the Culture Analog, during a given time interval, all aHPCs have essentially the same intracellular levels of unbound APAP.

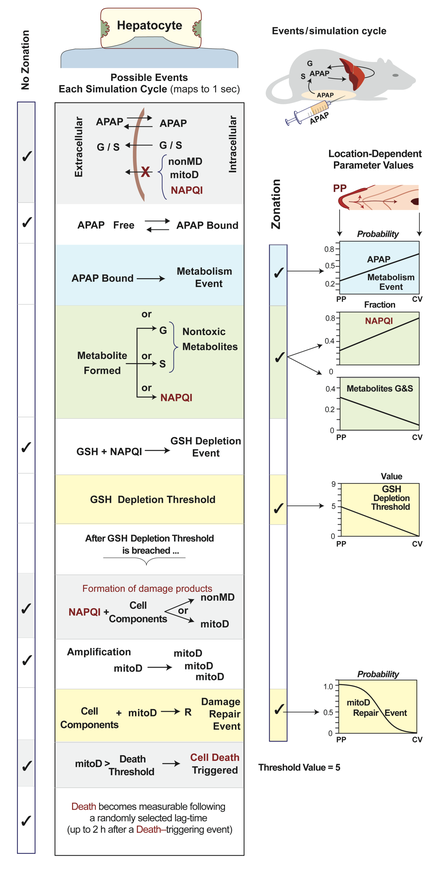






HEPATOCYTE LEVEL EVENTS

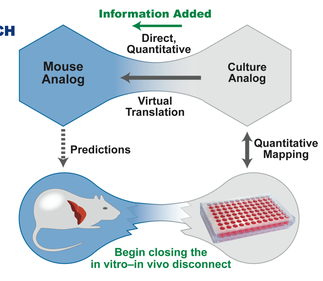
Within each Hepatocyte, each simulation cycle, each of the following events may occur



CONCLUSIONS

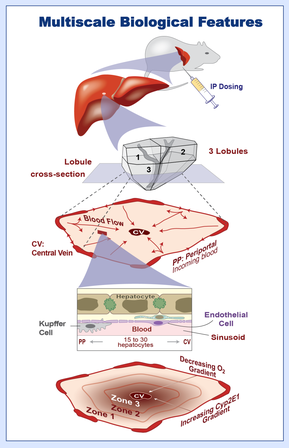
- We hypothesize that the different virtual mechanisms have real in vivo and in vitro counterparts.
- There is a loss of spatial organization of aHPCs (from Mouse to Culture); therefore, identifiable structural differences help explain the IVIV disconnect in APAP hepatotoxicity.
- A virtual Culture-to-Mouse translation can be used as a credible (knowledge & mechanism-based) method to begin closing the the IVIV disconnect.

NEW APPROACH

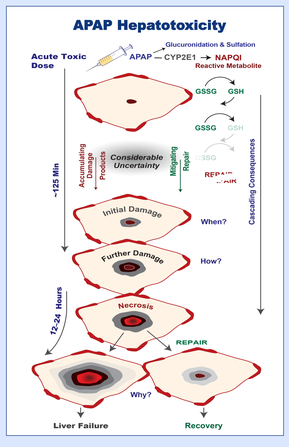


BACKGROUND

Multiscale Biological Features



APAP Hepatotoxicity



References

1. Bechtel W, Abrahamsen A. Explanation: A mechanist alternative. Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences. 2005; 36:421-41. doi:10.1016/j.shpsc.2005.03.010
2. Darden L. Thinking again about biological mechanisms. Philosophy of Science. 2008; 75(5):958-69. doi:10.1086/594538
3. Smith AK, Petersen BK, Ropella GE, Kennedy RC, Kaplowitz N, Ookhtens M, Hunt CA. Competing Mechanistic Hypotheses of Acetaminophen-Induced Hepatotoxicity Challenged by Virtual Experiments. PLOS Computational Biology. 2016 Dec 16;12(12):e1005253. doi10.1371/journal.pcbi.1005253