Title: Explanatory, Concrete Insight into Location-Dependent, Multiscale Aspects of Drug-Induced Liver Injury

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Acetaminophen (APAP) is the cardinal example of a growing class of xenobiotics that are converted in part into a reactive, hepatotoxic metabolite. Cell level pathogenesis within lobules (the liver's multicell functional unit) is location dependent, a phenomenon called zonation. Blood exits lobules through its central vein (CV). Pathology always begins adjacent to the CV, the location often called zone 3, and progresses toward the lobule's portal vein (PV), called zone 1. Damage can become irreversible. Cascading damage can cause death. Effective treatment formulation will benefit from insight into concrete features of how causal toxicity cascade may unfold. A long-standing hypothesis has been that the root cause is that expression levels of responsible CYP enzymes within zone 3 are much greater than in zones 1 and 2. Results from virtual experiments provided evidence against that hypothesis. We used a validated, multiscale, multi-attribute, In Silico Liver (ISL) model family (discrete event, OO, agent oriented and agent directed). An ISL is an evolving suite of hypotheses about how livers function. We implemented independent, medium-grained, parsimonious, mechanisms within each hepatocyte (H) and validated the ISL iteratively against coarse-grained measures of xenobiotic fraction in perfusate during single-pass perfusions. Enzymes (E) convert APAP objects within each H to three metabolites, A & B (not reactive), and N, which maps to reactive NAPQI. The ratio is held constant. N depletes GSH and can cause formation of a damage product, D, which maps collectively to the variety of molecular damage products actually formed. In early experiments we specified that the correlation between cumulative D formation and the probability of observable pathology is zone independent. In rats, the PV-CV gradient in CYPs responsible for N formation ranges from 1:4 to 1:10. Using the 1:4 ratio such that the probability of unbound APAP metabolism/sec was 0.5 in each zone, formation of D in zone 1 was > 2.5x that in zone 3. Clearly, to begin being explanatory, ISL mechanisms would need to be more complicated. With a focus on validation targets, the consequences of incremental changes were explored. In one experiment, the PV-CV enzyme gradient was increased to 1:10. Further, the following PV-CV gradient was implemented for the probability of unbound APAP metabolism/sec: A: 0.33-0.05; B: 0.33-0.05; and N: 0.33-0.9. So doing caused D in zone 3 to be 50% greater than in zone 1. However, given biological variability, that improvement cannot account for the observed, robust, pathology zonation pattern. Results of further iterative mechanism refinement experiments are providing concrete support for the hypothesis that zonation of additional multiscale mechanistic features is a precondition to consistent, dose dependent onset of H necrosis in zone 3.