Abstract #412

Quantitative Modeling Uncovers a Potential Limitation in the Putative Mechanism of CCl_4 Hepatotoxicity

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Abstract

Drug-induced liver injury (DILI) is one of the leading causes of drug development failures and drug withdrawals. DILIsym® is being developed to identify and mitigate DILI risk through in silico analysis of compounds. The DILIsym® representation of the innate immune response was initially based on acetaminophen (APAP) data. Carbon tetrachloride (CCl_4), a compound with hepatotoxic similarities to APAP, was simulated for further evaluation of the innate immune response to liver injury. DILIsym® simulation results for CCl_4 pharmacokinetics were consistent with published PK data. CCl_4 is generally thought to induce hepatotoxicity via a free radical metabolite which drives lipid peroxidation. However, when CCl_4 was simulated via DILIsym®, free radical generation and lipid peroxidation to levels consistent with the public literature were insufficient to drive hepatotoxicity. Analysis demonstrated that saturation of the metabolic pathway generating the free radical limited the extent of lipid peroxidation and thus the extent of cell death. In comparison, the APAP metabolic pathway has a greater dynamic range, resulting in higher levels of lipid peroxidation and cell death. Papers were identified in which lipid peroxidation independent mechanisms of cell death were suggested, including lipid peroxidation independent mitochondrial toxicity. The CCl_4 representation was modified to induce mitochondrial electron transport chain (ETC) inhibition. Concurrent induction of lipid peroxidation and ETC inhibition mechanisms permitted reconciliation of simulated hepatotoxicity with published data. For example, >3x ALT elevations were simulated at doses <50 mg/kg, with the highest ALT elevations (>1000 U/L) at doses >100 mg/kg in mice. Simulated CCl_4 was then used to evaluate and further refine the innate immune response. In summary, quantitative analysis of CCl_4 in DILIsym® not only permitted evaluation of the innate immune response, but also suggested a limitation in the putative primary mechanism of hepatotoxicity.

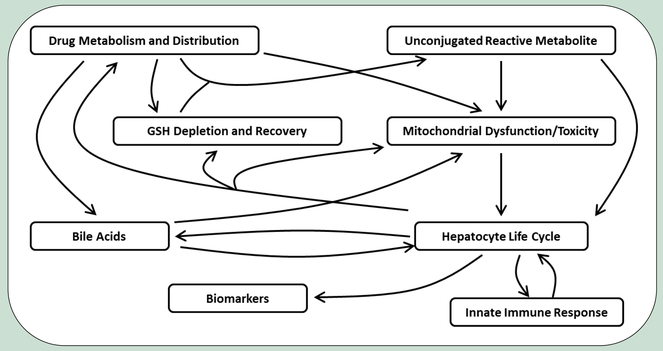
Introduction

DILIsym® is a multi-scale mechanistic model of drug-induced liver injury (DILI). The model contains a physiologically-based pharmacokinetic model of drug distribution and metabolism in the liver and several mechanisms of toxicity. Acetaminophen (APAP) has been used as an exemplar compound for reactive metabolite mediated oxidative stress and hepatotoxicity based on the wealth of available data.

The immune response has been implicated in various forms of DILI. As a first step to incorporating immune responses in DILI, macrophage participation in APAP overdose has been added to DILIsym®. To increase confidence that the macrophage model was adaptable to other compounds, carbon tetrachloride (CCl_4) was tested. Similar to APAP, the liver metabolizes CCl_4 to a reactive metabolite (RM). RM-generated oxidative stress is the putative primary mechanism of hepatotoxicity. There are also published data illustrating the recruitment of the immune response in CCl_4 hepatotoxicity.

We re-parameterized the DILIsym® compound physiologically based pharmacokinetic (PBPK) model and compound metabolism model to represent CCl_4. We selected RM-induced oxidative stress as the mechanism of hepatotoxicity and then ran simulations to explore the relationship between CCl_4 hepatotoxicity and recruitment of the immune response.

Overview of DILIsym®



Initial CCl_4 Simulation Results

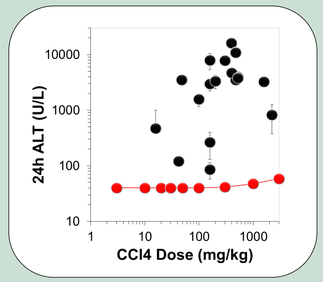


Fig 1a. Initial representation of CCl_4, red, fails to recapitulate observed mouse hepatotoxicity, black [8, 10-21]

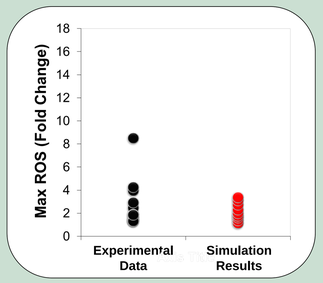


Fig 1b. Simulated CCl_4 (3-3000 mg/kg) generates oxidative stress but less than simulated APAP (Fig 2a) [15, 22-32]

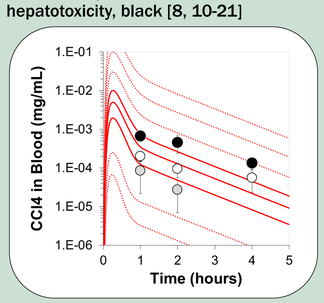


Fig 1c. PBPK simulations (1-3000 mg/kg). Solid red lines are the result of dosing as reported [1] and are consistent with data.

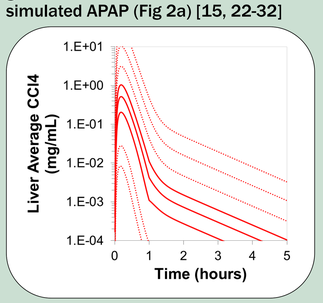


Fig 1d. Liver exposure increases with higher doses of CCl_4 (simulations as in Fig 1c)

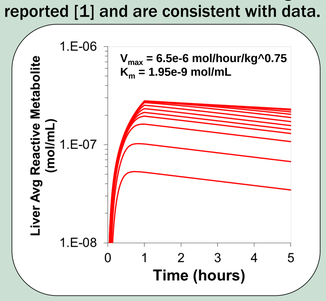


Fig 1e. RM generation becomes saturated at higher doses of CCl_4 (1-3000 mg/kg)

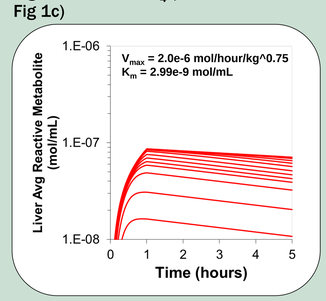


Fig 1f. Saturation persists with alternate published values for CCl_4 metabolism

Dual Mechanism CCl_4 Simulation Results

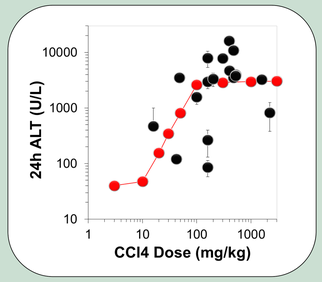


Fig 3a. Adding ETC inhibition permits CCl_4 simulations, red, consistent with the observed hepatotoxicity, black [8,10-21]

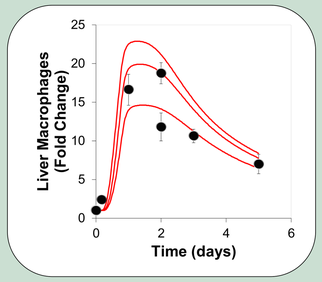
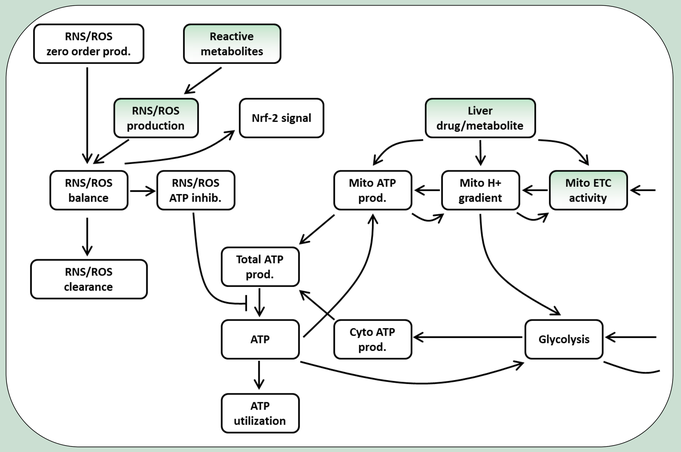


Fig 3b. With CCl_4 hepatotoxicity, simulations of macrophage accumulation are consistent with published data [8, 9]

Mitochondrial Dysfunction/Toxicity

To explore the possible contribution of mitochondrial dysfunction in CCl_4 hepatotoxicity, a second mechanism, CCl_4 RM-induced inhibition of the electron transport chain (ETC) activity was introduced.



Contrast to Acetaminophen

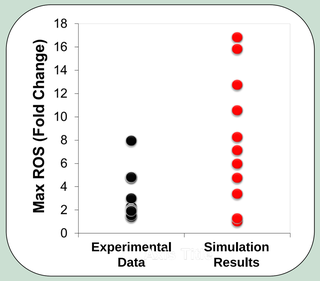


Fig 2a. In contrast to CCl_4, APAP RM generation is not limiting and can lead to extensive oxidative stress [33-42]
APAP, 50 – 800 mg/kg

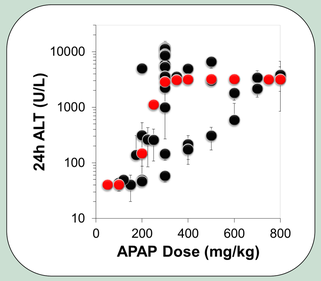


Fig 2b. In contrast to CCl_4, APAP RM-mediated oxidative stress is sufficient to simulate, in red, dose-dependent hepatotoxicity consistent with published data, in black [35, 43-58]

Methods

- We simulated mice given single doses of CCl_4 orally or intraperitoneally in aqueous or oil-based vehicle
- CCl_4 tissue distribution, fraction unbound, and clearance were estimated from the literature [e.g., 1-3] and tuned to match published time course profiles [1] (Fig 1c)
- CCl_4 metabolism was simplified to represent generation of the reactive metabolite (RM), trichloromethyl peroxy radical, implicated in CCl_4 hepatotoxicity [4]
  - RM generation was governed by V_{max} and K_m values, taken from the published literature [1]
  - Suicide inhibition of the CYP450 pathway was simplified to represent 95% inhibition after 1 hour exposure in mice [1]
- The same approach was taken for the exploration of rat CCl_4 responses. ***Rat simulations not shown.***

Results and Analysis

- In initial simulations, CCl_4-generated oxidative stress was unable to reproduce dose-dependent hepatotoxicity (Fig 1a, b)
- Analysis demonstrated that hepatotoxicity was limited by saturation of CCl_4 metabolism (Fig 1e)
  - Use of alternate V_{max} and K_m values for CCl_4 metabolism [5] led to saturation of RM generation at a lower level and did not change the hepatotoxicity outcomes (Fig 1f)
  - Further literature review suggested the possibility of direct mitochondrial effects [6, 7]
- CCl_4-mediated mitochondrial dysfunction, i.e., electron transport chain (ETC) inhibition, permitted the simulation of CCl_4 hepatotoxicity (Fig 3a)
- Simulated macrophage accumulation was consistent with experimental observations [8, 9] (Fig 3b)

Conclusions

- Quantitative modeling and simulation of CCl_4 suggests saturation of CCl_4 metabolism could limit RM-mediated oxidative stress and resultant hepatotoxicity
- Data on mitochondrial liability may indicate an underappreciated mechanism of toxicity
- Once CCl_4 dose-dependent hepatotoxicity was simulated, a reasonable accumulation of macrophages could be confirmed.

Acknowledgements

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References

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