Abstract #141

Modeling of Furosemide in DILIsym™ Model Reveals Testable Hypotheses about Hepatotoxicity Mechanisms

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Abstract

A predictive, quantitative, mathematical model (DILIsym™) is under development as a public-private initiative based on the physiological processes involved in drug-induced liver injury. The model includes multiple scales, ranging from molecular/cellular interactions to organ-tissue level considerations. Simulated mice, rats, and humans accurately reproduce the hepatotoxic responses to acetaminophen and methapyrilene, providing validation of the DILIsym™ model for reactive metabolite mediated toxicity. Furosemide (FS) hepatotoxicity is also thought to be reactive metabolite mediated, but with some mechanistic differences. FS was also recently included in the DILIsym™ model, simulating the quantitative aspects of drug metabolism, covalent binding, glutathione, and ATP levels that are described in the literature and from unpublished experiments in mice dosed with 400 mg/kg. ALT levels were accurately predicted based on these inputs, and multiple hypotheses for the ATP reductions were tested with additional modeling and simulations. Dose-dependent necrosis and associated increases in ALT levels do not appear to be dependent upon mitochondrial toxicity. Rather, simulations that included substantial (4x) increases in cellular energy expenditure or decreases (75%) in mitochondrial ATP production due to substrate limitations were more consistent with the experimental hepatotoxic responses. Specific laboratory experiments that will test these hypotheses were identified and are currently underway, including providing glycolytic substrate to sustain ATP production and levels in mice treated with FS. Incorporating FS into the DILIsym™ model provided an increased understanding of the mechanisms associated with FS hepatotoxicity, identified gaps in knowledge, and suggested multiple testable hypotheses to close these gaps.

Introduction

Objectives of the DILI-sim Initiative

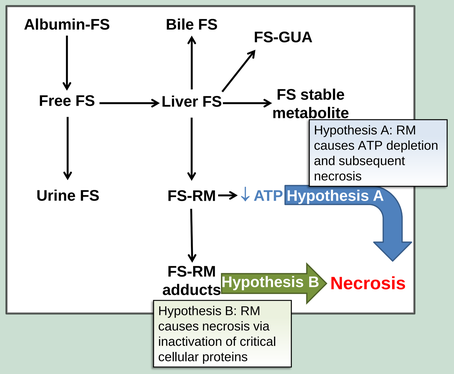
- To develop and validate a mechanistically based, mathematical model (DILIsym™) of drug induced liver injury (DILI) in order to inform and improve decision making at key points in the drug development life cycle
- To apply the DILIsym™ model to aid in understanding species differences with respect to DILI, including how pre-clinical safety testing results translate to human DILI response
- To use the DILIsym™ model to identify and evaluate non-standard mechanistically relevant DILI safety biomarkers, which can be used to monitor early functional events that ultimately may result in DILI during clinical trials and so enable improved personalized healthcare

Furosemide Hepatotoxicity

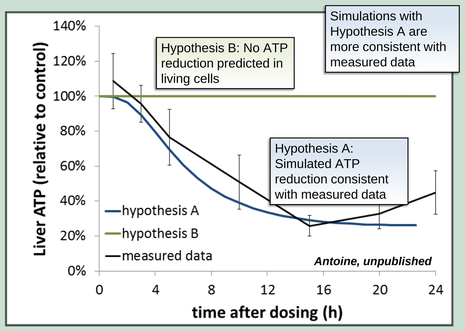
- Furosemide (Lasix) is a widely-used diuretic, with no reported instances of human hepatotoxicity
- Mitchell 1975 reported hepatotoxicity in mice treated with very high doses of furosemide; very high doses of furosemide saturate albumin binding sites, leading to high free circulating and liver furosemide, and ultimately increased generation of reactive metabolite
- In a collaboration between the Institute for Health Sciences and the MRC Center for Safety Science, wet lab experiments and simulations were used to examine the mechanistic underpinnings of furosemide hepatotoxicity in mice

Simulation Results, Measured Data, and DILIsym™ Model Overview

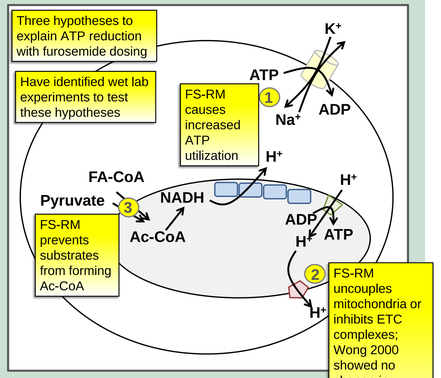
Furosemide Metabolism and Hepatotoxicity Mechanisms



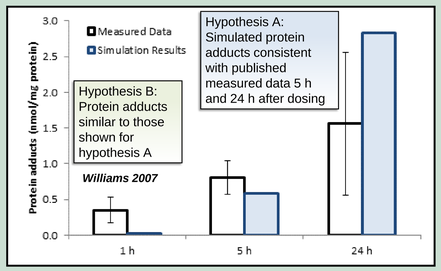
Liver ATP Simulation Results and Measured Data



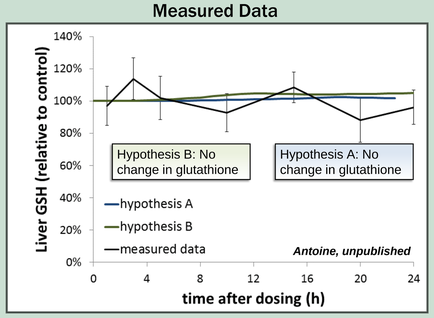
Testable Hypotheses to Explain Furosemide-Mediated ATP Reduction



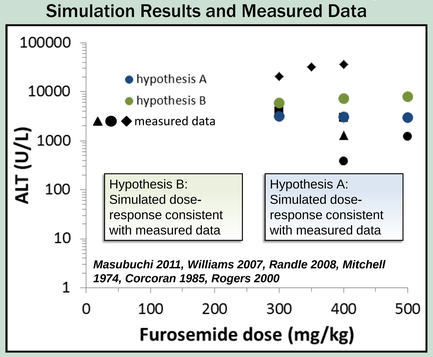
Furosemide Protein Adduct Simulation Results and Measured Data



Liver Glutathione Simulation Results and Measured Data



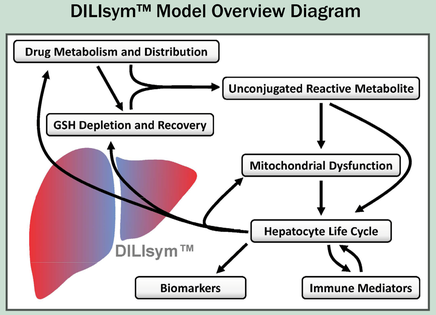
Furosemide Dose-Response Hepatotoxicity Simulation Results and Measured Data



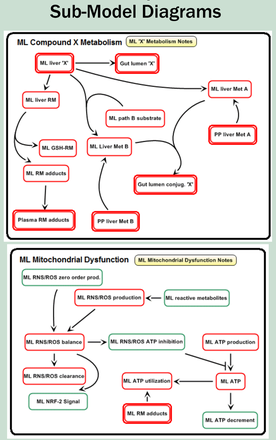
DILIsym™ v1A Model Overview

- **Multiple species: human, rat, mouse, and dog**
  - Population variability
- **The three primary acinar zones of liver represented**
- **Essential cellular processes represented to multiple scales in interacting sub-models**
  - Hepatocyte life-cycle
  - GSH depletion
  - Mitochondrial dysfunction
  - Cellular energy balance
  - Immune mediators
  - Dosing (IP, IV, Oral)
  - Drug metabolism
  - Pharmacokinetics
  - Transporter Inhibition
  - Biomarkers (ALT, AST, INR, bilirubin)
- **v1A focused on reactive metabolite-based hepatotoxicity**
  - Acetaminophen
  - Methapyrilene
  - Furosemide
  - Aflatoxin B1
  - AMAP
  - Single and multiple dose
  - Other compounds to be added
- **Enhancing DILIsym™ model to include BSEP inhibition/bile salt-mediated hepatotoxicity and mitochondria toxicity**
- **Compartment-based modeling**
  - >280 state variables
  - 'Form to function' connection
  - Ordinary differential equations
  - May expand to include alternative mathematical approaches

DILIsym™ Model Overview Diagram



Selected DILIsym™ Model Sub-Model Diagrams



Methods

- A mechanistic, mathematical model of DILI (DILIsym™), built using published and unpublished data in the MATLAB® platform was used to perform simulations
  - A middle-out approach was used to build the model. The model is multi-scale and currently comprised of ordinary differential equations.
  - Relevant mechanistic components of reactive metabolite-based hepatotoxicity are included
- CD-1 mice were dosed with 400 mg/kg furosemide or saline
  - Liver ATP (nmol/mg protein), GSH (nmol/mg protein), and ALT (U/L) were measured over the following 24 h
- Simulations of furosemide administration, metabolism, and effects in mice were performed to compare competing mechanistic hypotheses and determine plausibility when compared with measured data
- Additional, testable hypotheses were formulated to explain wet lab observations
  - Associated experiments also identified

Conclusions

- Wet lab experiments revealed novel finding of substantial liver ATP reductions following administration of 400 mg/kg furosemide to CD-1 mice
- Simulations support hypothesis A, that furosemide reactive metabolite-mediated ATP reduction leads to hepatocellular necrosis without reducing GSH; ATP levels are not predicted to be reduced on a per mg protein basis making hypothesis B simulation results incompatible with measured data
- Several hypotheses and associated experiments have been formulated to describe the furosemide-mediated reductions in liver ATP
- Simulations from the DILIsym™ model have provided results complimentary to those generated in the lab and have suggested avenues for further exploration

Future Directions

- Conduct wet lab experiments to test hypotheses and insights generated from modeling and wet lab work
- Continue to validate the current model (reactive metabolite, oxidative stress, and mitochondrial dysfunction based) with additional drugs
- Add additional DILI mechanisms such as direct mitochondrial toxicity, BSEP inhibition and other transporter effects, and pharmacology effects
- Expansion of the innate immune system, including explicit immune cell and immune mediator representation is under way
- Apply the DILIsym™ model to multiple test cases within the pharmaceutical industry and integrate it into the DILI-sim Initiative member companies' drug development process

Acknowledgements

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DILIsym™