Abstract #132

Mechanistic Modeling Reveals the Most Important Unknowns in Bile Acid-Mediated DILI

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Abstract

BSEP inhibition and consequent bile acid (BA) buildup has been proposed to be an important mechanism in drug-induced liver injury (DILI). There are many gaps in the knowledge of BA homeostasis and its disruption by transporter inhibitors. Modeling can help us understand which of these knowledge gaps should be filled first in order to provide maximum understanding. We have constructed a model of BA homeostasis and toxicity within DILIsym™, a mechanistic model of DILI that includes bile acid flux into and out of hepatocytes, the role of the farnesoid X receptor (FXR) in the regulation of BA transport and synthesis, and gallbladder BA release and recirculation. We first analyzed our system's behavior in the presence of a simulated BSEP inhibitor. We predict a 134% increase in total periportal hepatocyte content of amide conjugated chenodeoxycholic acid (CDCA) and a 311% increase in sulfate-conjugated lithocholic acid (LCA). However, the simulated BSEP inhibition resulted in a decrease in unconjugated CDCA, amide-conjugated LCA, and unconjugated LCA. Significant zonal effects were also witnessed; accumulation was 6x greater as a percentage of baseline in centrilobular hepatocytes, though centrilobular concentrations remained lower than periportal concentrations. We next performed a sensitivity analysis on our system's response to BSEP inhibition in order to determine which parameter has the greatest effect on hepatocyte content of BAs and hence BSEP inhibitor-induced hepatotoxicity. We found that the most important unknowns in the system are the level of BSEP expression in the cell and the magnitude of the FXR-mediated regulatory signal, while other parameters such as the affinity constants for basolateral CDCA and LCA transport were less important. We conclude that the highest priorities for wet lab research to inform our model and enhance our understanding of BSEP inhibitor mediated DILI are: 1) quantifying the relationship among intrahepatocyte concentrations of individual bile acids, FXR activation, and transporter activity; and 2) quantifying the toxicity caused by CDCA amide conjugates and LCA sulfate conjugates.

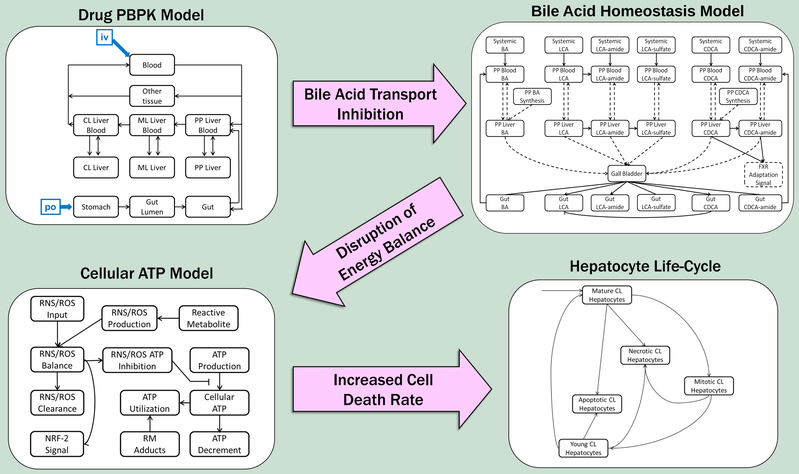
Introduction

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 as well as several mechanisms of toxicity. We use the DILIsym model to explore the relationship between BSEP inhibitors and bile acid buildup within hepatocytes.

DILI caused by BSEP inhibitors is thought to be the result of a buildup of toxic bile acids within liver cells. This buildup will lead to oxidative stress, ATP decline, and potentially apoptosis or necrosis. We have constructed a model of basic bile acid circulation and its disruption by transporter inhibitors. We have also included representations of the metabolism and transport of two bile acids known to cause toxicity *in vitro*: chenodeoxycholic acid (CDCA) and lithocholic acid (LCA).

There are several gaps in the understanding of bile acid homeostasis and disruption, as well as several potential risk factors for bile acid buildup. Modeling can help to determine which knowledge gaps should be addressed first, as well as to identify the individual characteristics that are most likely to place someone at risk for toxicity.

Bile Acid Model Overview



Simulation Results in Baseline Model

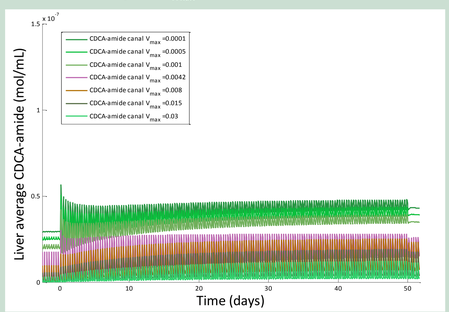
Baseline Human Relative Increase in Bile Acid AUC After Model Inhibitor Dosing Periportal Region

Bile acid species	AUC without drug (mol-h/mL)	AUC with drug (mol-h/mL)	Percent change
Bulk BA	4.99E-05	1.52E-04	205%
LCA	3.95E-10	1.12E-10	-72%
LCA-amide	7.01E-08	6.10E-08	-13%
LCA-sulfate	1.60E-06	6.59E-06	311%
CDCA	5.52E-10	4.42E-10	-20%
CDCA-amide	2.14E-05	5.01E-05	134%

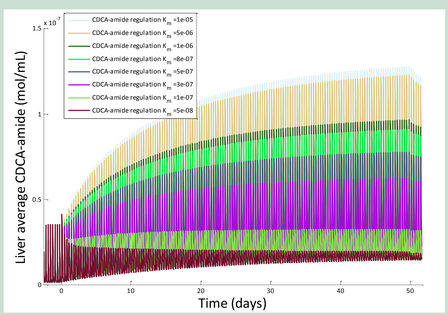
Baseline Human Relative Increase in Bile Acid AUC After Model Inhibitor Dosing Centrilobular Region

Bile acid species	AUC without drug (mol-h/mL)	AUC with drug (mol-h/mL)	Percent change
Bulk BA	1.02E-05	1.10E-04	980%
LCA	2.91E-11	1.52E-11	-48%
LCA-amide	1.20E-08	5.80E-08	382%
LCA-sulfate	1.56E-07	3.19E-06	1947%
CDCA	4.54E-11	6.28E-11	38%
CDCA-amide	2.69E-06	2.94E-05	994%

Parameter Sweep for CDCA-amide Canalicular Transport V_{max} in the baseline model



Parameter Sweep for CDCA-amide regulatory response Km in the baseline model



References

1. Howell et al. (2012) *J Pharmacokinet Pharmacodyn* 2012 39(5):527-41.
2. Woodhead et al. (2012) *J Pharmacol Exp Ther* 342(2):529-40.

Population Results

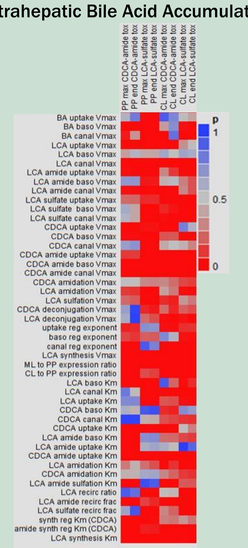
Table of Most Important Parameters for CDCA-amide Accumulation in Hepatocytes

Variable	Correlation coeff.
CDCA-amide canalicular transport Vmax	-0.2945
Canalicular regulation scaling factor	0.1874
CDCA-amide liver uptake Km	-0.1811
CDCA-amide basolateral transport Vmax	-0.1579
LCA synthesis Km	0.1478
CDCA liver uptake Km	-0.1379
CDCA-amide regulatory response Km	0.1287
LCA uptake Vmax	-0.1237
CDCA regulatory response Km	-0.1231
LCA synthesis Vmax	-0.1056

Table of Most Important Parameters for LCA-sulfate Accumulation in Hepatocytes

Variable	Correlation coeff.
LCA synthesis Vmax	0.3040
Fraction of LCA recirculated	0.2463
CDCA-amide basolateral transport Vmax	0.2457
LCA canalicular transport Km	0.2100
LCA synthesis Km	-0.2055
LCA-amide sulfation Vmax	0.1463
LCA-sulfate canalicular transport Vmax	-0.1409
Fraction of LCA-sulfate recirculated	0.1307
CDCA basolateral transport Vmax	0.1299
Bulk bile acid liver uptake Vmax	0.1278

Statistical Significance of Parameter Correlations to Intrahepatic Bile Acid Accumulation



Methods

- **We simulated the three-times-daily dosing of a model BSEP inhibitor over the course of 50 days.**
  - **Accumulation of bulk bile acids, CDCA and its amide conjugates, and LCA and its amide and sulfate conjugates were measured for the periportal and centrilobular regions of the liver.**
- **We simulated the same BSEP inhibitor protocol on a population of simulated individuals (SimPops™)**
  - **SimPops™ [1,2] contained variability in 47 model parameters, including transporter activity, affinity constants for each bile acid for transporters, amidation and sulfation activity and affinity, gut metabolism such as LCA synthesis from CDCA and LCA and CDCA deconjugation, and regulation of bile acid synthesis and transport.**
  - **A multivariate sensitivity analysis was performed on the accumulation of LCA-sulfate and CDCA-amide.**

Conclusions

- **CDCA-amide and LCA-sulfate accumulated the most in liver cells.**
  - **LCA-amide and unconjugated CDCA and LCA decreased in the periportal region; all bile acid species except for unconjugated LCA increased in the centrilobular region.**
  - **Increase in centrilobular region was 6x greater than increase in periportal region, though absolute concentration of bile acids remains higher in the periportal region.**
- **CDCA-amide accumulation is most affected by the efflux transporter expression levels, the magnitude of FXR regulation, and the affinity of CDCA species for uptake transporters.**
- **LCA-sulfate accumulation is most affected by the extent of LCA synthesis in the gut and the efficiency of the recirculation of LCA species into the blood from the ileum.**

Future Directions

- **We have used *in vitro* experiments on unconjugated bile acids in sandwich-cultured hepatocytes to construct a relationship between bile acid accumulation in cells and ATP decline.**
  - **Abstract #504**
  - **We will use this relationship to model potential drug-induced toxicity in exemplar compounds**
- **We propose directions for future experimentation:**
  - **Determining zonal differences in bile acid toxicity**
  - **Determining the relationship between CDCA-amide and LCA-sulfate and toxicity**
  - **Quantifying the effect of CDCA and CDCA-amide on FXR activation, and the subsequent effect on transporter activity at the cell membrane**

Acknowledgements

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