MECHANISTIC MODELING WITH DILISYM® PREDICTS SPECIES DIFFERENCES IN CKA VIA MULTIPLE HEPATOTOXICITY MECHANISMS

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ABSTRACT

OBJECTIVE: To predict species differences in CKA-mediated hepatotoxicity using DILIsym®, a mechanistic model of drug-induced liver injury (DILI)

METHODS: Inhibitory effects of CKA on bile acid (BA) and bilirubin transporters were assessed using transporter-overexpressing vesicles and cells. CKA-mediated oxidative stress and mitochondrial dysfunction were determined in HepG2 cells. These in vitro data were used to define hepatotoxicity parameters and combined with PBPK sub-model simulations of hepatic compound exposure to predict DILI in DILIsym. Previously constructed human and rat simulated populations (SimPops™) that incorporate variability in the aforementioned toxicity mechanisms were utilized to determine the impact of inter-individual variability upon administration of single CKA doses of 900mg and 500mg/kg, respectively.

RESULTS: CKA induces oxidative stress (oxidative stress production rate constant=7278mL/mol/hr human, 9705 mL/mol/hr rat) and inhibits mitochondrial electron transport chain (ETC) flux (ETC inhibition coefficient=14.2 mM human, 1.42 mM rat). CKA inhibits human BSEP (IC_{50}=129.7 μM), rat Bsep (IC_{50}=94 μM), human MRP3 (IC_{50}=11.2 μM), human NTCP (IC_{50}=19.5 μM), rat Mrp2 (IC_{50}=68.5 μM), and human OATP (IC_{50}=0.84 μM)[1]. CKA was modeled as a noncompetitive inhibitor of BSEP/Bsep and MRP3/Mrp3 and a competitive inhibitor of NTCP/Ntcp. Due to lack of data, IC_{50} values for NTCP/Ntcp, MRP3/Mrp3, MRP2/Mrp2, and OATP/Oatp were assumed the same across species. Human SimPops predicted modest increases <1.5x upper limit of normal (ULN) in serum ALT, recapitulating the clinical data. Rat SimPops predicted ALT elevations >3xULN in 36.4% of the population, slightly underpredicting the 75% observed in preclinical trials. DILIsym recapitulated preclinical observations in bilirubin increase due to both DILI and bilirubin transporter inhibition. Rat SimPops predicted increases in total bilirubin >2xULN for 100% of the population, mirroring preclinical data. Conversely, no significant increases in bilirubin were observed in human SimPops, consistent with clinical observations.

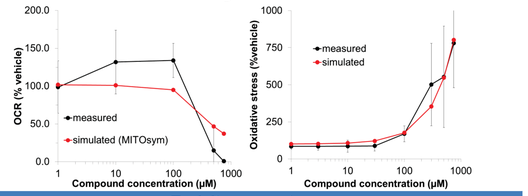
CONCLUSION: Using in vitro data to determine toxicity parameters, DILIsym accurately predicted CKA hepatotoxicity in rats and not in humans, consistent with observed preclinical and clinical data.

INTRODUCTION

- CKA is an antirheumatic drug intended as a treatment for rheumatoid arthritis and chronic obstructive pulmonary disorder.
- CKA produced dose-dependent hepatotoxicity signals in preclinical species (rats), where an increased -- but reversible -- level of liver enzymes was observed in single, supratherapeutic dose studies. Hepatotoxicity was not observed in human trials.
- *In vitro* experiments determined CKA to inhibit bile acid transporters, induce production of reactive oxygen species, and inhibit the mitochondrial electron transport chain activity.
- DILIsym is a systems pharmacology model of DILI that incorporates drug/metabolite disposition, bile acid physiology and pathophysiology, the hepatocyte life cycle, and liver injury biomarkers.

RESULTS

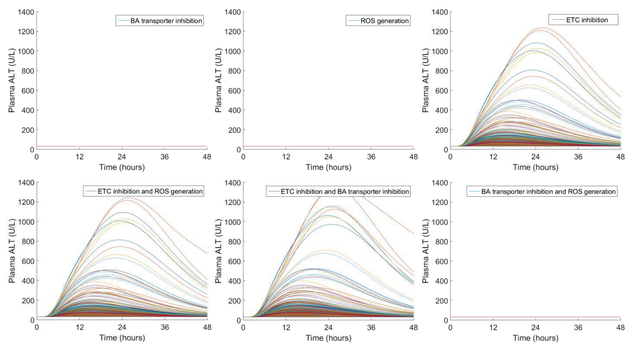
CKA toxicity mechanisms

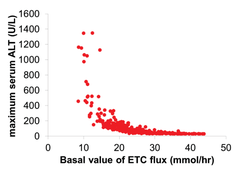


Toxicity parameters			
Bile acid transporter inhibition constant (μM)			
	CKA		CKA gluc
	Human	Rat	Rat
BSEP	94	129.7	-
MRP3	11.2	11.2	-
MRP4	12.3	12.3	-
NTCP	19.5	19.5	-
Bilirubin transporter inhibition constants (μM)			
	CKA		CKA gluc
	Human	Rat	Rat
MRP2	68.5	68.5	2.9
OATP1B1	0.84	0.84	-
Mitochondrial toxicity constant (mM)			
	CKA		CKA gluc
	Human	Rat	Rat
ETC inhibition constant	14.2	1.42	-
ROS production (mL/mol/hr)			
	CKA		CKA gluc
	Human	Rat	Rat
ROS production constant	7278	9705	-

Observed and simulated percentage changes in OCR (left figure) and oxidative stress (right figure) in response to CKA. Transporter inhibition constants for CKA pertaining to bile acid and bilirubin were used as Ki values in simulating CKA-mediated hepatotoxicity. The ETC inhibition constant and ROS production constant parameters were optimized to data using MITOsym and DILIsym, respectively.

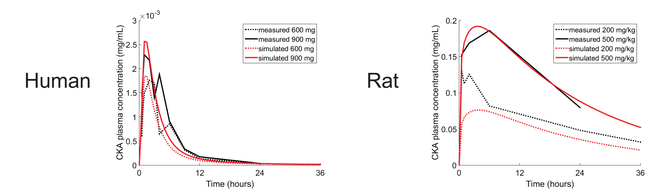
Multiple regression analysis





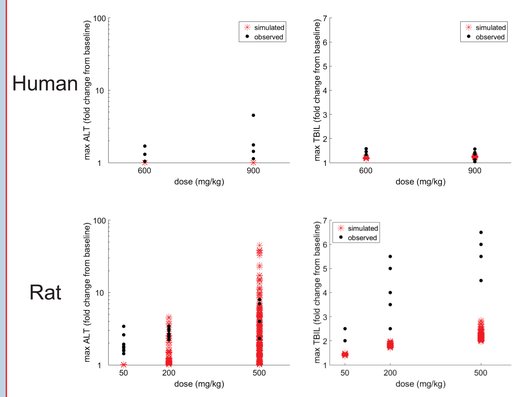
Each mechanism was modeled as the sole mechanism (left figure, top row) to study its individual effect on toxicity. ETC inhibition appears to be the main contributor to CKA-mediated rat hepatotoxicity. Two mechanisms are modeled at a time (left figure, bottom row). ETC inhibition and BA transporter inhibition together produce a greater peak plasma ALT, suggesting BA transporter inhibition as the secondary contributor to hepatotoxicity. The multiple regression analysis found the basal value of ETC flux, a parameter in the mitochondrial toxicity submodel, to be most closely correlated with simulated ALT elevations (right).

PBPK Simulations



PBPK models reasonably predict plasma concentrations at multiple dose levels for humans (left) and rats (right) administered CKA. Simulated plasma profiles (red) are within one standard deviation of measured profiles (black).

DILI predictions



(Left) Maximum ALT fold change from baseline for increasing doses of CKA in human (top) and rat (bottom). (Right) Maximum total bilirubin (TBIL) fold change from baseline for increasing doses of CKA in human and rat. Simulations are from human (n=285) or rat (n=294) SimPops.

DILIsym qualitatively captures species differences in ALT and TBIL elevations but slightly underpredicts frequency of ALT elevations in rat population and severity of TBIL elevations.

	Simulations			Preclinical / Clinical Trials		
Species	rat	rat	human	rat	rat	human
Dose	200 mg/kg	500 mg/kg	900 mg	200 mg/kg	500 mg/kg	900 mg
Population size	n=294	n=294	n=285	n=8	n=4	n=8
ALT > 3x ULN (%)	2.4	36.4	0	25	75	16.7
ALT > 5x ULN (%)	0	20.1	0	0	50	0
ALT > 10x ULN (%)	0	7.8	0	0	25	0
TBIL > 1.5x ULN (%)	100	100	0	100	100	0
TBIL > 2x ULN (%)	0	83	0	100	100	0
Hy's Law cases (%)	-	-	0	-	-	0

METHODS

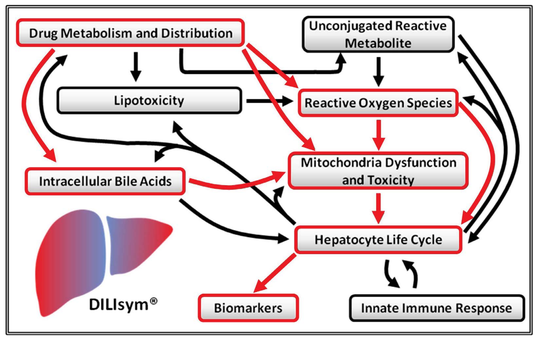
Determining CKA toxicity mechanism parameters from *in vitro* experiments MITOsym®, a mitochondrial modeling platform used to mimic in vitro experimental results[2], was used to determine parameter values for CKA-mediated mitochondrial ETC inhibition by fitting simulated results to Seahorse XF96 basal respiration data for HepG2 cells exposed to CKA. Conversion factors translated predicted results from HepG2 cells to human and rat hepatocytes. The ROS production constant parameter was found by sweeping through potential values until the DILIsym "*in vitro*-like" simulation yielded ROS effects in line with the data. The BSEP/Bsep IC_{50} for CKA was used to represent the inhibition constant (Ki) in DILIsym (Ulloa et al., provided by Astra Zeneca, unpublished). Further studies performed in transporter-overexpressing vesicles determined IC_{50} values for MRP3, MRP4, and NTCP which were used for both species.

Physiologically-based pharmacokinetic (PBPK) model development Human and rat PBPK models were developed in DILIsym using available *in vitro* and *in vivo* pharmacokinetic data.

Simulation of CKA responses Previously constructed SimPops that include variability in species-specific parameters and the BA, ROS, and mitochondrial submodels within DILIsym were used to predict the hepatotoxic effects of CKA at the population level. For humans, single doses of 600 and 900 mg were simulated for a period of 96 hours following the administration of CKA. Rat simulations were run for 72 hours after single doses of 200 or 500 mg/kg.

Multiple Regression Analysis To identify the most important parameters associated with DILI in rats, a multiple regression analysis was performed with maximum ALT as the dependent variable. The 34 parameters varied in the rat SimPops were normalized and used as the independent variables. Statistical analyses were performed using JMP 11 (SAS, Cary, NC).

DILIsym® Overview



A general overview of DILIsym and its submodels. Topics outlined in red indicate active submodels used in modeling CKA-mediated effects in human and rat populations. Mechanistic *in vitro* toxicity data were used in the intracellular bile acids, reactive oxygen species, and mitochondria dysfunction and toxicity submodels to predicitDILI outcomes.

CONCLUSION

- DILIsym modeling based on *in vitro* data for the aforementioned toxicity mechanisms qualitatively predicted species differences in CKA hepatotoxicity.
- ETC inhibition is statistically the most potent toxicity mechanism with bile acid transporter inhibition also contributing to ALT elevations. ROS generation has a minimal effect on CKA-mediated hepatotoxicity.
- Quantitative systems pharmacology models (QSP) can be used to evaluate DILI mechanisms and investigate observed species differences.

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1. Ulloa, J.L. et al. *NMR Biomed* 26(10): 1258—1270. 2013.
2. Yang, Y. et al. *Pharm Res* 32(6): 1975—1992. 2015.

DILI-sim Initiative

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DILIsym® Services
USING MECHANISTIC MODELING TO SOLVE LIVER TOXICITY PROBLEMS