USE OF SYSTEMS TOXICOLOGY MODELING TO INVESTIGATE MECHANISMS OF LIVER ENZYME ELEVATIONS MEDIATED BY SOLITHROMYCIN AND OTHER MACROLIDES

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ABSTRACT

BACKGROUND: Solithromycin, a 4th generation macrolide developed for the treatment of community acquired pneumonia, caused serum liver enzyme (ALT) elevations in clinical studies. A quantitative systems toxicology (QST) tool, DILIsym, was used to predict the occurrence and mechanisms of ALT elevations for solithromycin, erythromycin, clarithromycin, and telithromycin.

METHODS: In vitro assays were performed to assess effects of the macrolides on bile acid transport, mitochondrial function, and oxidative stress. These data were integrated with in vivo exposure using DILIsym; serum ALT responses were predicted in a simulated human population.

RESULTS: DILIsym reasonably predicted the incidence of ALT elevations observed for solithromycin, erythromycin, and clarithromycin; the predominant mechanism was reversible mitochondrial electron transport chain (ETC) inhibition for solithromycin and clarithromycin, and bile acid transport inhibition for erythromycin. ALT elevations by Telithromycin were only predicted at the highest observed exposure combined with noncompetitive inhibition of bile acid transporters.

CONCLUSION: Mechanisms for ALT elevations vary among macrolides, and solithromycin is similar to clarithromycin in this regard. The simulation results were presented to the FDA Advisory Committee for solithromycin on 11/4/16, a first to our knowledge for QST.

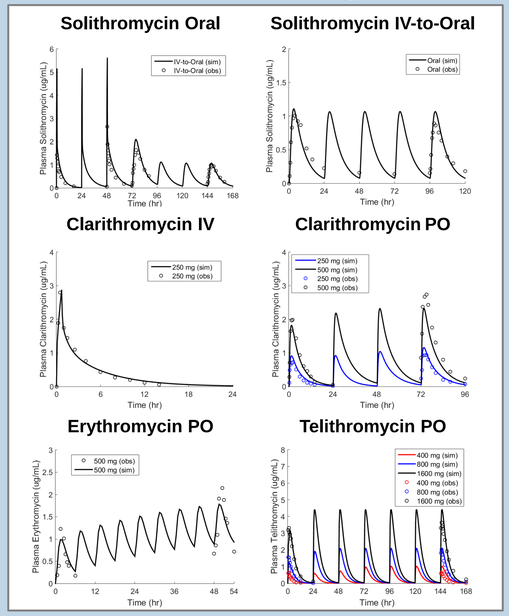
INTRODUCTION

- Solithromycin is a 4th generation macrolide and the first fluoroketolide, which was developed to treat moderate to moderately-severe community acquired bacterial pneumonia and gonococcal urethritis. ALT elevations > 3X ULN were observed in 5–9% of treated community acquired pneumonia patients administered solithromycin in phase 3 clinical trials.
- Erythromycin and clarithromycin are currently marketed, but are associated with liver enzyme elevations. The labeling for telithromycin was restricted for a number of indications after rare idiosyncratic severe liver injury was observed. [1,2]
- DILIsym is a quantitative systems toxicology model which integrates drug-specific data from multiple biological systems, dynamic drug disposition, and patient characteristics. DILIsym predicts whether new drug candidates will cause drug-induced liver injury (DILI) in patients and provides an enhanced understanding of the mechanisms underlying compounds that generate liver signals in the clinic. [3-6]

DILI-sim Initiative

RESULTS

PBPK Modeling



Simulated (lines) and observed (circles) plasma concentrations of macrolides

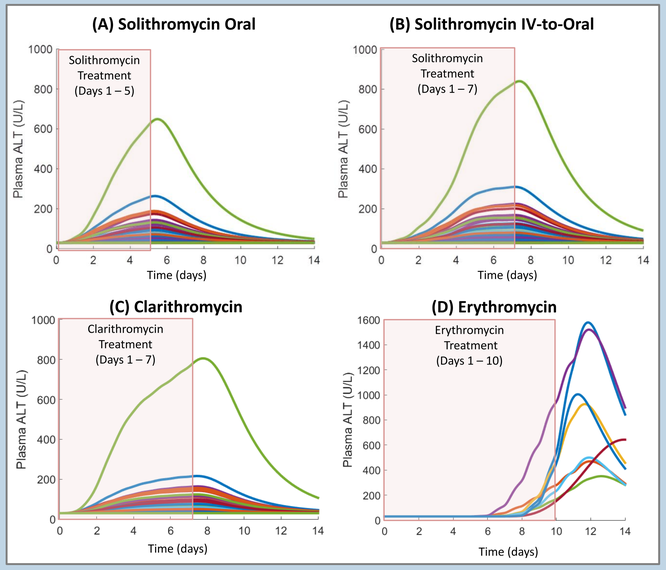
Comparison of DILIsym Input Parameters

Mechanism	Parameter	Unit	Value			
			Solithromycin	Clarithromycin	Erythromycin	Telithromycin
Mitochondrial Dysfunction	Coefficient for ETC inhibition 1[a]	mol/mL	4×10^{-5}	2.5×10^{-6}	No inhibition	1.77×10^{-4}
	Coefficient for ETC Inhibition 3[b]	mol/mL	1×10^{-10}	1×10^{-10}	No inhibition	No inhibition
	Max inhibitory effect for ETC inhibition 3[c]	Dimension -less	0.35	0.3	No inhibition	No inhibition
Oxidative Stress	RNS/ROS production rate constant 1[d]	mL/mol/hr	100,000	24,400	11,000	53,700
Bile Acid Transporter Inhibition	BSEP inhibition constant[e]	µM	28.2[7]	59[8]	13[7]	5[7]
	NTCP inhibition constant[e]	µM	No inhibition	No inhibition	No inhibition	No inhibition
	MRP4 inhibition constant[e]	µM	42.2[7]	No data[7]	No inhibition[7]	7.1[7]

Mitochondrial dysfunction and oxidative stress signals were detected in HepG2 with cellular respiration assays (Seahorse XFe96 Flux Analyzer) and high content screening (probe: dihydroethidium), respectively. Toxicity parameters were determined by simulating the experimental data (intracellular concentrations and toxicity signals) in DILIsym; [a] the inhibition constant for complete ETC inhibition, [b] the inhibition constant for partial ETC inhibition, [c] the maximal inhibitory effect for partial ETC inhibition, [d] the first order rate constant for the production of reactive nitrogen/oxygen species.
Bile acid transport inhibition assays were performed using membrane vesicles or cell lines overexpressing a specific transporter to determine the inhibition constant (e.g., IC_{50}).

- DILIsym parameter values for toxicity mechanisms have been identified based on in vitro assay results
- Solithromycin and clarithromycin showed mitochondrial signals at clinically relevant concentrations
- All of the macrolides investigated induced oxidative stress signals and inhibited bile acid efflux transporters with varying potency.

Simulated Liver Enzyme Profiles



Simulated ALT in the human SimPops treated with (a) solithromycin oral protocol, (b) solithromycin IV-to-oral protocol, (c) erythromycin, and (d) clarithromycin. Liver enzyme elevations were predicted in a simulated human population (n=285) administered three different macrolides by combining PBPK-predicted exposure and mechanistic toxicity data. Each line represent simulated individuals.

Simulated vs. Observed Liver Enzyme Elevations And Underlying Mechanisms

Compound	Protocol	Peak ALT > 3X ULN		Predicted Hy's Law cases	Mechanism: Predominant (Minor)
		Observed	Simulated		
Solithromycin	Oral*	5.4% (2.8%†)	3.9%	0%	ETCi (BAi)
	IV-to-Oral**	9.1% (6.6%†)	6.0%	0%	
Clarithromycin	500mg BID 7 days	1-2%	2.8%	0%	ETCi (BAi)
Erythromycin	500mg QID 10 days	1-2%	2.8%	0%	BAi (OS)
Telithromycin	800mg QD 10 days	~0.5%	0%	0%	- (BAi)

ETCi: electron transport chain inhibition, BAi: bile acid transport inhibition, OS: oxidative stress
* PO 800 mg QD on day 1, PO 400 mg QD on days 2-5.
** IV 400 mg on days 1-3, PO 800 mg QD on day 4, PO 400 mg QD on days 5-7
† Among patients with normal baseline ALT

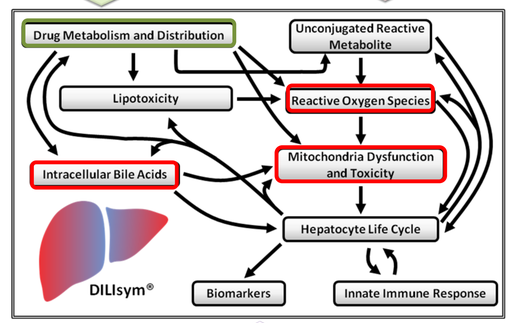
- ALT elevations from solithromycin, erythromycin, and clarithromycin were well predicted by DILIsym.
- ALT elevations from telithromycin were not well predicted by DILIsym based on the three principal mechanistic pathways. A mechanism not currently included in DILIsym (as of version 5A), possibly immunity-mediated, may contribute to the liver injury observed in the clinic with telithromycin.
- The simulations suggest that the mechanisms for ALT elevations from solithromycin are most closely related to those of clarithromycin. Telithromycin appears to be distinct from solithromycin, clarithromycin, and erythromycin in terms of the pathways responsible for the DILI signals.

METHODS

DILIsym Mechanism-Based Modeling

PBPK Input
- Physicochemical properties
- Absorption
- Tissue distribution
- In vitro metabolism and transport
- Renal and biliary clearance

DILIsym Input Panel
Assays performed to determine quantitative aspects of DILI mechanisms
- Oxidative stress
- Mitochondrial dysfunction
- Bile acid transporter inhibition



Population Variability (SimPops)
- Collections of simulated individuals with parameter variability
- Designed to reflect appropriate biochemical and anthropometric ranges
- A standard human SimPops represents variability in body weight, mitochondrial function, caspase activation (apoptosis), bile acid disposition, and oxidative stress (ROS/RNS) susceptibility

CONCLUSION

- Quantitative systems toxicology modeling using DILIsym reasonably predicted the incidence of ALT elevations observed for solithromycin, erythromycin, and clarithromycin.
- The hepatocyte effects of solithromycin are quite distinct from those of telithromycin and similar to those of clarithromycin.
- The mechanisms underlying the severe liver injuries due to telithromycin are not predicted by the three mechanisms examined

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DILIsym® Services
USING MECHANISTIC MODELING TO SOLVE LIVER TOXICITY PROBLEMS