Prediction of the Liver Toxicity of the Endothelin Receptor Antagonists Sitaxsentan and Ambrisentan for the Treatment of Pulmonary Arterial Hypertension with a Quantitative Systems Toxicology Tool (DILIsym)

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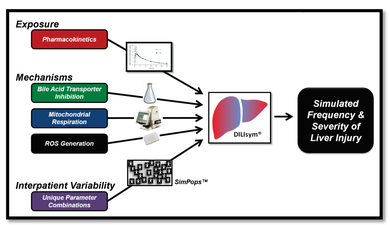
INTRODUCTION

- Sitaxsentan and ambrisentan are highly selective endothelin-1 type A receptor antagonists which were developed for the treatment of pulmonary arterial hypertension
- Sitaxsentan was voluntarily withdrawn from the market due to concerns about liver toxicity, whereas ambrisentan is currently on the market
- DILIsym, a mathematical framework of drug-induced liver toxicity, was used for quantitative system toxicology (QST) studies of the compounds
- It is important to ensure that QST tools are capable of distinguishing between toxic and non-toxic compounds

METHODS

- Oxygen consumption rate (OCR) was measured in HepG2 cells incubated with various concentrations of sitaxsentan and ambrisentan for 1 hr and 24 hr with the Seahorse XF Analyzer to assess effects on mitochondrial function
  - Intracellular concentrations were measured using cellular lysate mass spec data
  - Electron Transport Inhibition (ETC) parameter values were determined using MITOsym
- High content screening was used to measure oxidative stress (ROS) in HepG2 cells incubated with various concentrations of sitaxsentan and ambrisentan for 6 hr and 24 hr
  - Intracellular concentrations were measured using cellular lysate mass spec data
- Inhibition assays for BSEP and other bile acid transporters were available via publications for both compounds [1-3]
- The *in vitro* data were converted into DILIsym input parameters
- PBPK models were constructed using the PBPK sub-model in DILIsym in order to predict potential liver exposure
- Simulations were conducted in the DILIsym v4A-1 human SimPops consisting of 285 normal healthy volunteers



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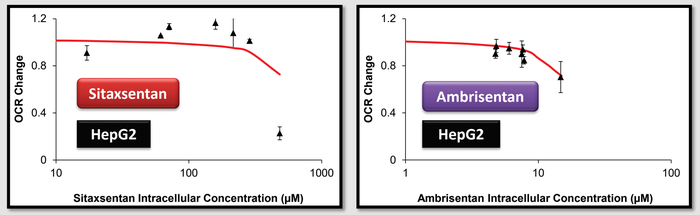
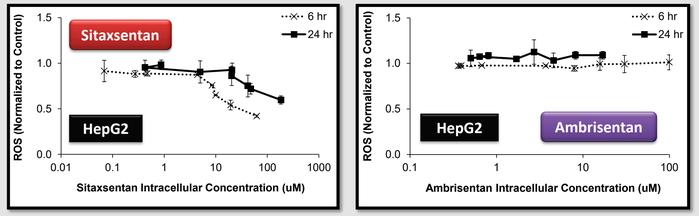
RESULTS

Mitochondrial Function



The Seahorse XF Analyzer measured the change in oxygen consumption rate (OCR) in HepG2 cells due to varying doses of each compound after 1 hour of exposure. A parallel cell culture was set up and incubated with the compounds; the lysate from these cells was analyzed using mass spectrometry in order to determine the intracellular concentrations. ETC inhibition due to each compound was then simulated in MITOsym; the ETC inhibition parameter was optimized to fit the Seahorse data. The MITOsym parameter values were translated into DILIsym values using established conversion factors derived from data with exemplar compounds.

Oxidative Stress

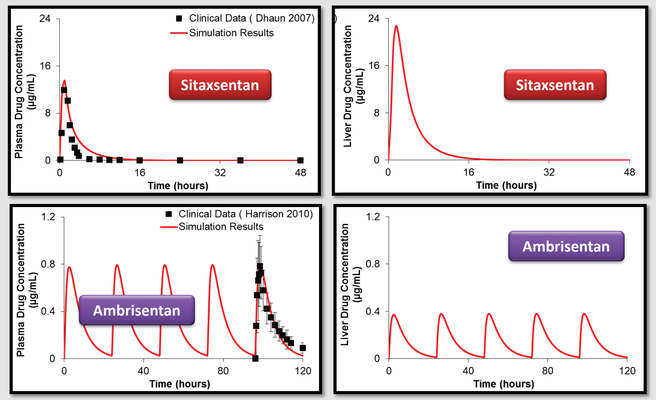


High content imaging measured the change in oxidative stress (ROS) in HepG2 cells due to varying doses of each compound after 6 and 24 hours of exposure. No compound-mediated increases in ROS were observed.

DILIsym Input Parameters for Liver Toxicity Pathways

Toxicity Mechanism	DILIsym Toxicity Parameters	Units	Sitaxsentan	Ambrisentan	References
Mitochondrial dysfunction	Coefficient for ETC inhibition 2	µM	3,470	10.4	Cyprotex, MITOsym
Bile-acid transporter inhibition	Uptake (NTCP) inhibition constant	µM	1E10	100	[1]
	Canalicular efflux (BSEP) inhibition constant	µM	10.4	288.1	[2]
	Basolateral efflux (MRP3/4) inhibition constant	µM	28.4	1E10	[3]
Bilirubin transporter inhibition	OATP inhibition constant	µM	100	44.60	[1,4]
	MRP2 inhibition constant	µM	61	75	[1-2]
	MRP3 inhibition constant	µM	45.4	1E10	[3]

PBPK Models of Sitaxsentan and Ambrisentan



Above: Selected results for the PBPK models of each compound [5-6]. High, median, and low exposure models were subsequently developed for sitaxsentan.

Sitaxsentan Simulation Results

Sitaxsentan

Mode of transporter inhibition	Simulated DILI in SimPops with n = 285 (ALT>3x ULN)			Reported Clinical DILI [7-9]			
	Low	Mean	High	n	Dose	Time (weeks)	ALT> 3x ULN
Competitive NTCP Non-competitive BSEP Non-competitive basolateral	1.4%	5.96%	11.2%	55	100 mg QD	12	0%
Mixed NTCP (α=5) Mixed BSEP (α=5) Mixed basolateral (α=5)	0%	0%	0%	61		18	3%
Mixed NTCP (α=5) Mixed BSEP (α=2) Mixed basolateral (α=2)	0%	0.35%	1.75%	887		NA	7%

Simulations were performed with 100 mg QD oral sitaxsentan dosing for 18 weeks
n = Number of patients

Toxicity Mechanisms Included in Sensitivity Analysis	Simulated DILI (ALT>3 ULN)
Mitochondrial Toxicity + Bile Acid	5.96% (17/285)
Mitochondrial Toxicity	0% (0/285)
Bile Acid	3.86% (11/285)
Simulations were performed with 100 mg QD oral sitaxsentan dosing for 18 weeks	

Ambrisentan Simulation Results

Mode of transporter inhibition	Simulated DILI (ALT>3 ULN)	Reported Clinical DILI [10]			
		n	Dose	Time (weeks)	ALT> 3 ULN
Comp. NTCP Non-comp.BSEP Non-comp. MRP3/4	0%	67	10 mg QD	12	0% (0/67)
Mixed NTCP (α=5) Mixed BSEP (α=5) Mixed basolateral (α=5)	0%	61 67	5 mg QD	12	0% (0/61) 0% (0/67)
		63	2.5 mg QD	12	0% (0/67)

Simulations were performed with 10 mg QD oral ambrisentan dosing for 12 weeks

Ambrisentan

CONCLUSIONS

- Depending on the mode of transporter inhibition and PK variability, 0-11.2% of the simulated humans were predicted to have liver toxicity (plasma ALT > 3x ULN) after 18 weeks of 100 mg QD oral dosing of sitaxsentan
- Irrespective of the mode of transporter inhibition, 0% of the SimPops showed liver toxicity after 10 mg of ambrisentan
- These simulation results are comparable with the clinical data where 0-7% and 0% of patients experienced liver toxicity for sitaxsentan and ambrisentan, respectively
- Further mechanistic simulations showed that synergy between mitochondrial dysfunction and bile acid transporter inhibition was primarily responsible for sitaxsentan induced liver toxicity
- Although *in vitro* data for ambrisentan and sitaxsentan showed potential DILI signals, predicted DILI risk was also dependent upon compound exposure
- DILIsym was able to help elucidate the clinical relevance of the *in vitro* signals by putting them in the context of the predicted liver exposures

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