Investigating the Potential Hepatotoxicity of ORM-48824 in a Quantitative Systems Toxicology Platform for Liver Safety, DILIsym®

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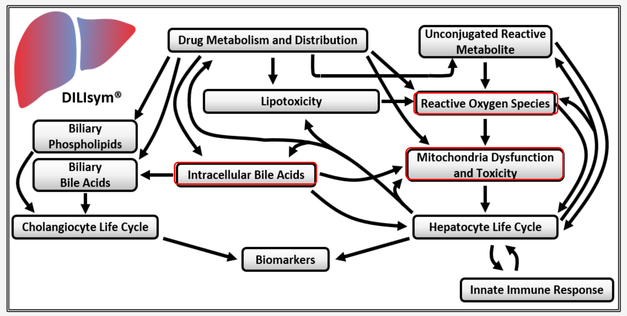
INTRODUCTION

ORM-48824 is a transient receptor potential Ankyrin-1 (TRPA1) antagonist and was initially being developed for patients with diabetic neuropathic pain, osteoarthritis, and other pathophysiological conditions to attenuate pain hypersensitivity. *In vitro* experiments suggested that ORM-48824 may inhibit bile acid transporters and induce mitochondrial dysfunction and oxidative stress, mechanisms that contribute to liver toxicity. DILIsym was utilized to prospectively predict clinical liver toxicity and the dominant toxicity mechanism potentially responsible for any signals predicted.

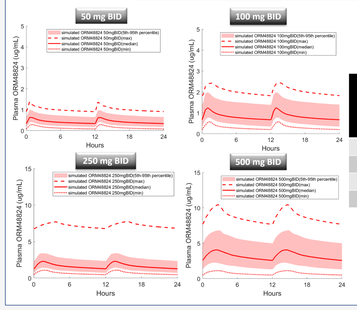


METHODS

- HepG2 cells were treated with doses of ORM-48824 ranging from 2 to 100 µM and mitochondrial respiration was measured using a Seahorse XFe96 Analyzer
- Reactive oxygen species (ROS) production was measured using high content screening to quantify dihydroethidium staining following ORM-48824 exposure (0.4-200 µM)
- ORM-48824 was administered in human ATP-binding cassette (ABC) efflux (BSEP, MRP3, and MRP4) transporters and in human solute carrier (uptake) transporter NTCP, where the accumulation of a probe substrate was measured.
- A PBPK model was built in GastroPlus 9.8.2® for ORM-48824 and variables with known dependency on species, gender, age, height, weight, or BMI had distributions defined by the built-in Population Estimates for Age-Related Physiology (PEAR Physiology) generator
- MITOsym® was used to parameterize ETC inhibition to *in vitro* mitochondrial respiration studies of ORM-48824 and ROS parameterization was performed in DILIsym
- Doses of 500 mg BID, 250 mg BID, 100 mg BID, and 50 mg BID were simulated for 12 weeks in the NHV SimPops (N=285)
- Mechanistic analysis was conducted by simulating 250 mg BID in a SimCohorts of 32 individuals

RESULTS

Simulated Exposure of ORM-48824



ORM-48824 Protocol for 12 weeks	Simulated NHV PK Population	
	AUC ± STD (µg/mL)	C_{max} ± SD (µg/mL)
500 mg BID	16588 ± 5956.1	4.36 ± 1.38
250 mg BID	8493.6± 3022.8	2.49 ± 0.69
100 mg BID	4426.8 ± 1571.3	1.34 ± 0.37
50 mg BID	2179.6 ± 742.1	0.68 ± 0.17

Simulation Scheme

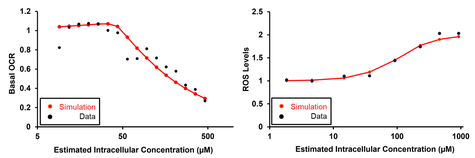
To predict ORM-48824 induced liver toxicity a PBPK model for ORM-48824 was built in GastroPlus 9.8.2 where 50 mg, 100 mg, 250 mg, and 500 mg were simulated. The pharmacokinetic profile and the toxicity parameters (ROS production, ETC inhibition, bile acid transporter inhibition constants) for ORM-48824 were imported into DILIsym. All doses were simulated for 12 weeks in a Normal Healthy Volunteer (NHV) SimPops (N=285). To determine the dominant mechanism of toxicity, simulations were conducted with 250 mg for 12 weeks in a SimCohorts of 32 individuals where each toxicity mechanism was turned off separately. Levels of ALT above the upper limit of normal (ULN) and bilirubin levels above the ULN were assessed and/or plotted in eDISH plots.

Summary of *In Vitro* Toxicity Study Signals

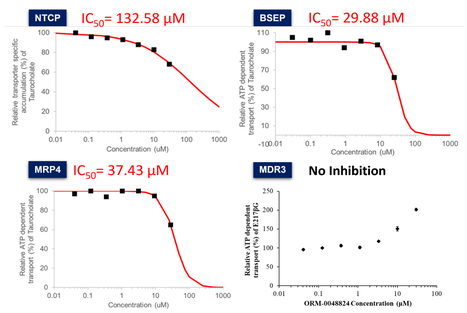
In vitro studies showed that ORM-48824 induced mitochondrial dysfunction, ROS elevation, and inhibition of bile acid transporters. ORM-48824-induced electron transport chain (ETC) inhibition was simulated in MITOsym, and ROS production in DILIsym, against observed mitochondrial respiration and ROS production from *in vitro* studies. IC_{50} values were calculated for bile acid transporter inhibition.

Compound	Bile acid transporter inhibition signals	Mitochondrial dysfunction signals	Oxidative stress signals
ORM-48824	Yes	Yes	Yes

Parameterization of Mitochondrial Dysfunction and ROS Elevation



Inhibition of Bile Acid Transporters by ORM-48824



Toxicity Parameter	Value
Non-saturable Coefficient for ETC Inhibition	670.14 µmol/L
Saturable K_m for ETC Inhibition	18055.55 µmol/L
Saturable V_{max} for ETC Inhibition	0.0208
Uncoupler Effect K_m	576 µmol/L
ROS Production V_{max}	0.075 1/hr
ROS Production K_m	95 µmol/L
ROS Production Hill	1.4

Summary of DILIsym Simulation Results

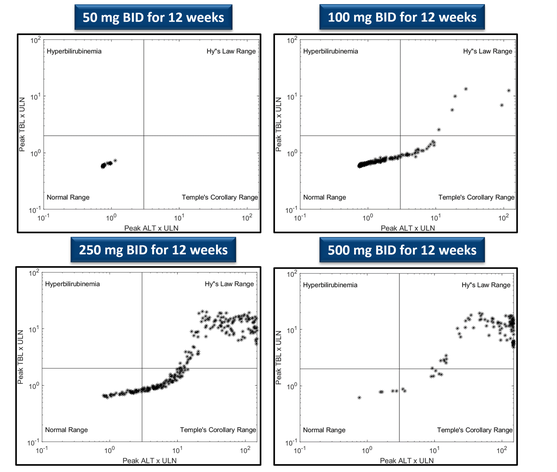
Dose-Dependent Simulation Results

Together with simulated exposure of ORM-48824 for 12 weeks at doses of 50, 100, 250, and 500 mg BID, liver toxicity of ORM-48824 was predicted in DILIsym in a Normal Healthy Volunteer (NHV) simulated population (SimPops, N=285). Dose-dependent toxicity was observed in the NHV SimPops, with no ALT elevations predicted during 50 mg BID dosing simulations.

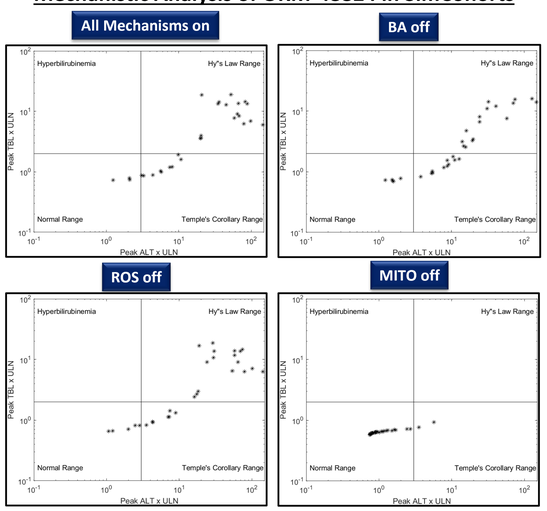
Mechanistic Analysis Results

A mechanistic analysis was performed with 250 mg for 12 weeks in a SimCohorts of 32 individuals. When contributions from mitochondrial dysfunction were not included in simulations, simulations predicted a significant decrease in individuals with ALT elevations suggesting this pathway as the dominant mechanism for ORM-48824-induced toxicity.

Dose-dependent Toxicity of ORM-48824 in NHV SimPops



Mechanistic Analysis of ORM-48824 in SimCohorts



Dosing Protocol	Mechanisms Off*	Simulated ALT > 3X ULN	Simulated Bilirubin > 2X ULN	Simulated Hy's Law Cases
ORM-48824 250 mg BID 12 weeks	All	0/32	0/32	0/32
	None	29/32	20/32	20/32
	BAi	27/32	16/32	16/32
	ETCi	2/32	0/32	0/32
	ROS	27/32	20/32	20/32

CONCLUSION

- DILIsym is comprised of submodels that interact with one another to predict liver injury outcomes. DILIsym combines data from in vitro toxicity studies for ORM-48824, predictions exposure from GastroPlus for ORM-48824, as well as inner workings of liver physiology to predict the potential for ORM-48824 to induce liver injury or cause liver toxicity
- In vitro studies showed that ORM-48824 induces mitochondrial dysfunction, elevation of ROS, and bile acid inhibition
- Simulating 50 mg BID, 100 mg BID, 250 mg BID, and 500 mg BID for 12 weeks in in the NHV SimPops in DILIsym showed dose-dependent toxicity
- No ALT elevations were predicted with 50 mg BID for 12 weeks
- Mechanistic analysis showed that the number of individuals with ALT elevations decrease significantly when the mitochondrial dysfunction mechanism is turned off, suggesting this is the dominant toxicity pathway
- Due to these results and other data gathered by Orion Corporation, ORM-48824 was discontinued due to potential liver toxicity, aiding in drug discovery and liver safety

CONFLICT OF INTEREST

Drs. Bhargava, Tallapaka and Howell are/were employees of Simulations Plus Inc., a company that licenses the DILIsym software for commercial use

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