Abstract #1258

Quantitative Systems Toxicology Analysis of In Vitro Mechanistic Assays Reveals Importance of Bile Acid Accumulation in TAK-875-induced Liver Injury

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ABSTRACT

TAK-875 (fasiglifam), a GPR40 agonist in development for treatment of type 2 diabetes, was voluntarily terminated in phase 3 due to adverse liver effects. Alanine aminotransferase elevations were observed in approximately 2% of TAK-875 treated subjects. The potential mechanisms of TAK-875 toxicity were explored using in vitro experiments and quantitative systems toxicology (QST) analysis using DILIsym®, a mathematical model of drug-induced liver injury. *In vitro* assays revealed that human Bile Salt Export Protein (BSEP) was inhibited by both TAK-875 (K_i = 17.2 uM) and TAK-875-Glu (IC_{50} = 41.6 uM). The mode of BSEP inhibition by TAK-875 was found to be mixed with alpha = 2.172. Furthermore, *in vitro* assays demonstrated that both TAK-875 and TAK-875-Glu inhibit the mitochondrial electron transport chain (ETC). These mechanistic data were combined with a physiologically-based pharmacokinetic (PBPK) model constructed within DILIsym® used to estimate liver exposure of TAK-875 and TAK-875-Glu. 17 out of 245 (6.91%) individuals in a simulated population (SimPops™) constructed to reflect Type 2 diabetes patients developed ALT elevations. This generally recapitulates, though mildly overpredicts, the actual toxicity. In addition, simulations conducted on a sensitive sub-population of individuals (SimCohorts™) revealed that when either BSEP inhibition or ETC inhibition was removed from the simulation, ALT elevations did not occur. This suggests that both BSEP inhibition and ETC inhibition are necessary to explain the observed toxicity, and in this model the two mechanisms operate synergistically to produce the observed clinical response. These results demonstrate how combining in vitro experimental methods with QST methods can lead to improved predictions about the underlying mechanisms behind drug-induced toxicity than either method can provide alone.

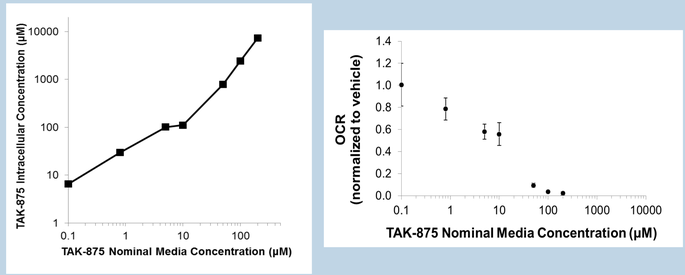
INTRODUCTION

- TAK-875, an experimental treatment for type 2 diabetes mellitus, led to rare (2-4%) transaminase elevations in Phase III clinical trials. Development of the drug was halted due to observed cases of serious DILI.
- Bile acid homeostasis disruption has been observed in pre-clinical species and has been suggested as a potential mechanism for DILI in humans (Wolenski 2017).
- The effects of bile acid accumulation have been shown to be exacerbated by drug-induced mitochondrial dysfunction, especially electron transport chain (ETC) inhibition (Woodhead 2016; Aleo 2014).
- *In vitro* experiments describing transporter inhibition and mitochondrial dysfunction effected by TAK-875 were used as inputs into DILIsym; modeling could provide mechanistic insight into the etiology of TAK-875-induced DILI.

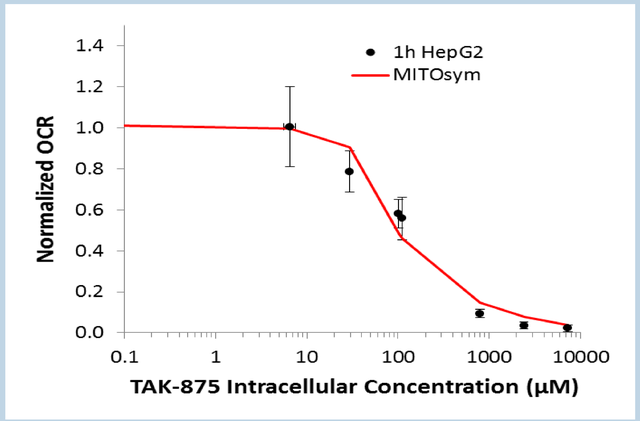
DILI-sim Initiative

RESULTS

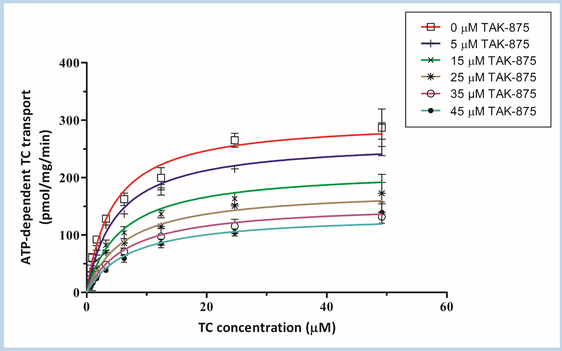
Mitochondrial Respiration Assay



The Seahorse XF Analyzer measured the change in oxygen consumption rate (OCR) in HepG2 cells due to varying doses of TAK-875 after 1 hour of exposure (right). A parallel cell culture was set up and incubated with TAK-875; the lysate from these cells was analyzed using mass spectrometry in order to determine the intracellular TAK-875 concentration that corresponds to each nominal media concentration. ETC inhibition due to TAK-875 was then simulated in MITOsym®; the ETC inhibition parameter was calibrated to fit the Seahorse data.

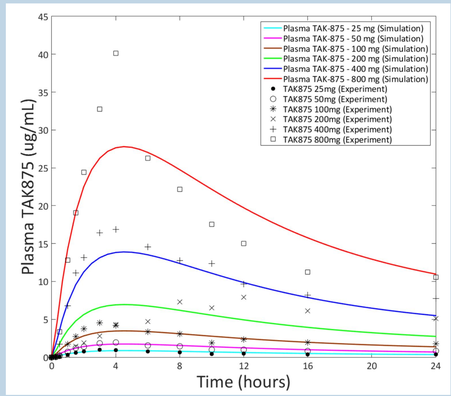


Transporter Inhibition Assay



TAK-875-mediated inhibition of taurocholate transport into BSEP-expressing HEK293 membrane vesicles was assayed at eight different concentrations of taurocholate. These data sets were then used to determine the K_i and mode of inhibition of TAK-875 on human BSEP.

PBPK Model



Above: Selected results for the PBPK model of TAK-875 constructed within DILIsym.

DILIsym Parameters Calculated

Parameter	Chemical Species	Value (units)
BSEP inhibition K_i	TAK-875	17.2 µM
BSEP inhibition α	TAK-875	2.172 (dimensionless)
Basolateral inhibition K_i*	TAK-875	11.7 µM
NTCP inhibition K_i**	TAK-875	4.3 µM
ETC inhibition coefficient	TAK-875	347.2 µM
BSEP inhibition K_i*	TAK-875-glu	41.6 µM
Basolateral inhibition K_i*	TAK-875-glu	3.36 µM
NTCP inhibition K_i**	TAK-875-glu	2.4 µM
ETC inhibition coefficient	TAK-875-glu	15800 µM

*Mode of inhibition was not calculated for basolateral (MRP3/4) inhibition or for TAK-875-glu BSEP inhibition; mode of inhibition was assumed to be same as TAK-875 BSEP inhibition
**NTCP inhibition was calculated to be competitive.

Simulation Results - Full Population

Simulated Population (SimPops)	Clinically Observed ALT >3x ULN Frequency	Simulated ALT >3x ULN Frequency
Normal healthy volunteers	1.8%-3.2%	14/285 (4.91%)
Type 2 diabetes patients		17/245 (6.91%)***

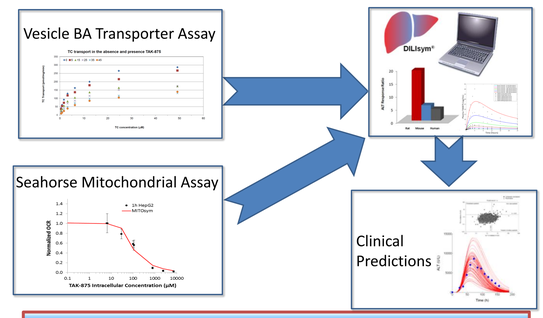
Simulation Results - SimCohorts

Mechanisms Off	Chemical Species Off	ALT >3x ULN
None	None	7/16
BAi	None	0/16
ETCi	None	0/16
None	TAK-875-glu	5/16
None	TAK-875	0/16

"Mechanisms Off" refers to mechanisms that were disabled during the simulation; "Chemical Species Off" refers to drug/metabolite species whose toxicity was disabled during the simulation.

METHODS

- Seahorse XF Analyzer experiments were performed on TAK-875 in HepG2 cells in order to assess the magnitude of mitochondrial dysfunction induced by TAK-875.
- Inhibition assays for BSEP and NTCP were conducted using inverted BSEP human membrane vesicles or stable NTCP-overexpressing cells, respectively. K_i values were determined by measuring the K_M of taurocholate in each assay system in the presence of various concentrations of TAK-875.
- The *in vitro* data were converted into input parameters for use in DILIsym.
- A PBPK model of TAK-875 was constructed using the PBPK sub-model in DILIsym in order to predict potential liver exposure of the compound.
- Simulations were conducted on two simulated populations (SimPops™): one of 285 normal healthy volunteers and one of 245 individuals with type 2 diabetes and initial ALT <2.5x ULN, both given 200 mg QD TAK-875 for 12 weeks.
- Further simulations were performed using a 16-individual subset (SimCohorts™) including sensitive individuals from the main population. One mechanism was disabled in each simulation in order to determine the relative importance of bile acid accumulation and mitochondrial ETC inhibition to the observed toxicity.



CONCLUSION

Simulations performed using the parameters for ETC inhibition and bile acid transporter inhibition derived from the novel *in vitro* experiments generally recapitulate, though mildly overpredict, the frequency of rare ALT excursions observed in the clinic. A mechanistic analysis of the results demonstrated that both ETC inhibition and bile acid transport inhibition were necessary to explain the observed toxicity; the two mechanisms were predicted to work synergistically in order to produce toxicity. The simulated Type 2 diabetes population was predicted to be somewhat more sensitive to TAK-875 toxicity than the normal population. Differing mitochondrial parameters likely explains the different outcomes of between the populations.

ACKNOWLEDGEMENTS

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cyprotex AN EVOTEC COMPANY
Takeda
DILIsym® Services USING MECHANISTIC MODELING TO SOLVE LIVER TOXICITY PROBLEMS
SOLVO BIOTECHNOLOGY THE TRANSPORTER COMPANY

References available upon request