OXFORD

SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 167(2), 2019, 458–467

doi: 10.1093/toxsci/kfy253
Advance Access Publication Date: October 5, 2018
Research Article

Quantitative Systems Toxicology Analysis of *In Vitro* Mechanistic Assays Reveals Importance of Bile Acid Accumulation and Mitochondrial Dysfunction in TAK-875-Induced Liver Injury

Diane M. Longo,[*,1] Jeffrey L. Woodhead,[*] Paul Walker,[†] Krisztina Herédi-Szabó,[‡] Károly Mogyorósi,[‡] Francis S. Wolenski,[§] Yvonne P. Dragan,[§] Merrie Mosedale,[¶,‖] Scott Q. Siler,[*] Paul B. Watkins,[*,¶,‖] and Brett A. Howell[*]

[*]DILIsym Services, Inc., Research Triangle Park, North Carolina 27709; [†]Cyprotex, Inc., Macclesfield SK10 4TG, UK; [‡]SOLVO Biotechnology, Szeged 6728, Hungary; [§]Takeda Pharmaceuticals International, Inc., Cambridge, Massachusetts 02139; [¶]UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599; and [‖]UNC Institute for Drug Safety Sciences, University of North Carolina at Chapel Hill, Research Triangle Park, North Carolina 27709

[1]To whom correspondence should be addressed at 6 Davis Drive, PO Box 12317, Research Triangle Park, NC 27709. E-mail: dlongo@dilisym.com.

ABSTRACT

TAK-875 (fasiglifam), a GPR40 agonist in development for the treatment of type 2 diabetes (T2D), was voluntarily terminated in Phase III trials due to adverse liver effects. The potential mechanisms of TAK-875 toxicity were explored by combining *in vitro* experiments with quantitative systems toxicology (QST) using DILIsym, a mathematical representation of drug-induced liver injury. *In vitro* assays revealed that bile acid transporters were inhibited by both TAK-875 and its metabolite, TAK-875-Glu. Experimental data indicated that human bile salt export pump (BSEP) inhibition by TAK-875 was mixed whereas sodium taurocholate co-transporting polypeptide (NTCP) inhibition by TAK-875 was competitive. Furthermore, experimental data demonstrated that both TAK-875 and TAK-875-Glu inhibit mitochondrial electron transport chain (ETC) enzymes. These mechanistic data were combined with a physiologically based pharmacokinetic (PBPK) model constructed within DILIsym to estimate liver exposure of TAK-875 and TAK-875-Glu. In a simulated population (SimPops) constructed to reflect T2D patients, 16/245 (6.5%) simulated individuals developed alanine aminotransferase (ALT) elevations, an incidence similar to that observed with 200 mg daily dosing in clinical trials. Determining the mode of bile acid transporter inhibition (K_i) was critical to accurate predictions. In addition, simulations conducted on a sensitive subset of individuals (SimCohorts) revealed that when either BSEP or ETC inhibition was inactive, ALT elevations were not predicted to occur, suggesting that the two mechanisms operate synergistically to produce the observed clinical response. These results demonstrate how utilizing QST methods to interpret *in vitro* experimental results can lead to an improved understanding of the clinically relevant mechanisms underlying drug-induced toxicity.

Key words: fasiglifam; bile acids; DILI; DILIsym; quantitative systems pharmacology modeling.

TAK-875 (fasiglifam) is a G protein-coupled receptor 40 (GPR40) agonist that was developed for the treatment of type 2 diabetes (T2D) (Kaku *et al.*, 2015). In Phase II clinical trials, TAK-875 treatment lowered blood glucose levels and glycated hemoglobin (HbA1c) in patients with T2D (Kaku *et al.*, 2015). However, development of TAK-875 was voluntarily terminated in Phase III trials due to liver safety concerns (Kaku *et al.*, 2016).

Multiple mechanisms have been implicated in the liver injury associated with TAK-875. These include alterations in bile acid homeostasis, the formation of reactive metabolites, and inhibition of mitochondrial respiration (Kaku *et al.*, 2015; Li *et al.*, 2015; Otieno *et al.*, 2018; Wolenski *et al.*, 2017). The relative contribution of each of these to the clinical response is unknown.

DILIsym is a mathematical representation of drug-induced liver injury (DILI) in pre-clinical species and in humans which includes multiple hepatotoxicity mechanisms (ie, bile acid accumulation, mitochondrial dysfunction, and oxidative stress) (Longo *et al.*, 2016; Shoda *et al.*, 2014; Woodhead *et al.*, 2014; Yang *et al.*, 2017). Quantitative systems toxicology (QST) approaches like DILIsym, can integrate experimental data and physiologically based pharmacokinetic (PBPK) modeling to identify clinically relevant mechanisms of DILI. In this study, DILIsym was used to integrate pharmacokinetic data and *in vitro* toxicity data to simulate the *in vivo* response to TAK-875 in humans.

MATERIALS AND METHODS

DILIsym overview. DILIsym (http://www.dilisym.com; last accessed October 18, 2018) is a mathematical representation of drug-induced liver injury (Bhattacharya *et al.*, 2012; Shoda *et al.*, 2014; Woodhead *et al.*, 2012). Briefly, DILIsym consists of several smaller sub-models that are mathematically integrated to simulate an organism-level response. This work utilized sub-models representing drug distribution, mitochondrial dysfunction, and toxicity, bile acid physiology and pathophysiology, hepatocyte life cycle, and liver injury biomarkers. DILIsym is developed and maintained through the DILI-sim Initiative, a public-private partnership involving scientists in academia, industry, and the U.S. Food and Drug Administration. Simulations for this study were conducted in the baseline simulated human, and in SimPops as previously described in Longo *et al.* (2016) and Yang *et al.* (2015); TAK-875-specific adjustments are described in detail below.

Bile acid transporter input data. The DILIsym bile acid sub-model represents bile acid enterohepatic circulation via bile acid transporters. Specifically, hepatocyte uptake of bile acids from blood occurs by the NTCP transporter. Bile acid efflux occurs via BSEP-mediated canalicular transport and MRP3- and MRP4-mediated basolateral transport. Drug-mediated inhibition of efflux transporters can result in hepatocellular bile acid accumulation, and drug-mediated NTCP inhibition can mitigate this effect. Elevated intracellular bile acid concentrations can alter mitochondrial function, leading to hepatocyte death (Rolo *et al.*, 2000; Schulz *et al.*, 2013). Drug-mediated inhibition of transporters can be assessed in laboratory experiments using cells or membrane vesicles expressing the transporter of interest. The hepatotoxic potential of transporter inhibition can be influenced by the type of inhibition (eg, competitive vs non-competitive), as well as the strength of inhibition (Woodhead *et al.*, 2014). Inhibition type can be determined from experimental K_i data. For the parameterization of TAK-875, half maximal inhibitory concentration (IC_{50}) values for TAK-875 and TAK-875-Glu were available for BSEP, MRP3, MRP4, and NTCP (Wolenski *et al.*, 2017). In this study, experimental K_i data were collected to further characterize inhibition of BSEP and NTCP by TAK-875. Experimental details are provided in Supplementary material 1.

Mitochondrial dysfunction input data. The DILIsym mitochondria sub-model represents mitochondrial bioenergetics leading to adenosine triphosphate (ATP) production in hepatocytes. Compounds may induce mitochondrial dysfunction by inhibiting the ETC, by inhibiting the mitochondrial F_1F_O ATPase, or by uncoupling mitochondrial respiration from mitochondrial ATP synthesis. Compound effects on hepatocyte mitochondrial function can be assessed in laboratory experiments by measuring hepatocyte respiration following culture with the compound in a Seahorse XF Analyzer (Seahorse Bioscience, Massachusetts) (Eakins *et al.*, 2016; Nadanaciva *et al.*, 2012). For the parameterization of TAK-875, *in vitro* respiration data were collected in HepG2 cells to characterize the inhibition of mitochondrial respiration by TAK-875. Importantly, the compound concentration driving an intracellular response (ie, mitochondrial respiration) may not be equivalent to the nominal media concentration reported in the assay protocol (Groothuis *et al.*, 2015), where the nominal media concentration is defined as the reported (but not measured) concentration of compound in the media. To more closely describe the relationship between concentration at the site of action and effect, the intracellular concentrations of TAK-875 in the HepG2 cells were also assed via LC/MS/MS. Experimental details are provided in Supplementary material 2. TAK-875-Glu parameters were based on *in vitro* respiration data in rat hepatocytes (unpublished data). Because initial simulations in DILIsym indicated that the predicted *in vivo* hepatotoxicity is highly sensitive to the ETC inhibition parameter value for TAK-875 and relatively insensitive to the ETC inhibition parameter value for TAK-875-Glu (data not shown), additional *in vitro* respiration data in HepG2 cells was collected as part of this study for the parameterization of TAK-875, while the existing data in rat hepatocytes was used for the parameterization of TAK-875-Glu.

A companion mechanistic mathematical model, MITOsym, which simulates *in vitro* hepatocellular respiration was designed to reproduce data obtained via the Seahorse assay for the purposes of deriving parameters characterizing compound induced mitochondrial dysfunction (Yang *et al.*, 2014). MITOsym was used to determine parameter values for TAK-875- and TAK-875-Glu-mediated ETC inhibition, and MITOsym parameters were subsequently translated to *in vivo* DILIsym parameters.

ROS input data. The DILIsym oxidative stress sub-model represents the generation of ROS in response to compound exposure. ROS accumulation can lead to hepatocyte apoptosis or necrosis, depending on the extent of oxidative stress. Oxidative stress was not observed in HepG2 cells following exposure to TAK-875 as indicated by the dihydroethidium fluorescence assay. Experimental details are provided in Supplementary material 3. In addition, in previously published work characterizing both TAK-875 and TAK-875-Glu (Wolenski *et al.*, 2017), no evidence of compound-induced oxidative stress was detected. Because no ROS production was observed experimentally for either TAK-875 or TAK-875-Glu, the DILIsym parameter values for TAK-875 did not include ROS production.

PBPK modeling. A PBPK representation was constructed within DILIsym to describe the dynamics of TAK-875 and TAK-875-Glu in humans in both liver and blood. The DILIsym PBPK sub-model contains compartments for liver, muscle, gut tissue, and other tissue; distribution to the tissues was assumed to be

perfusion-limited for TAK-875. Metabolism of TAK-875 was represented by three pathways: one to TAK-875-Glu, one to TAK-875-M-1, and one lumped pathway for other minor metabolites.

A broad range of clinical doses of TAK-875 (Naik *et al.*, 2012) were used to construct the PBPK model for TAK-875 and its metabolites. Liver concentrations were constrained by *in vitro* data collected for this work and by measurements of TAK-875 in rat liver and plasma that indicated that the concentration of parent compound in the liver and the plasma were roughly equivalent (data not shown).

SimPops. SimPops, collections of simulated individuals with parameter variability designed to reflect appropriate biochemical and anthropometric ranges, were used to understand the role of inter-individual variability in simulated TAK-875-mediated hepatotoxicity. This study utilized two existing SimPops within DILIsym. Human_ROS_apop_mito_BA_v4A_1 ($n = 285$) was used to simulated responses in normal healthy volunteers (NHVs) and includes variability in mitochondrial function, caspase activation (apoptosis), bile acid transporter expression, and oxidative stress (ROS/RNS) susceptibility (Supplementary material 4). The NHV SimPops is designed to represent healthy human subjects typically included in early stage clinical trials. Human_T2D_ROS_apop_mito_BA_v6A_2 ($n = 285$) was used to simulate responses in T2D patients and includes disease-related variability in body mass, plasma glucose, plasma free fatty acid, liver glutathione (GSH), mitochondrial function, lipogenesis, and lipotoxicity as well as variability in caspase activation (apoptosis) and bile acid transporter expression (Supplementary material 5). The T2D SimPops was designed to represent T2D patients typically included in late stage clinical trials (such as TAK-875 Phase 2/3 studies) and therefore includes the pathological characteristics of T2D relevant to DILI mechanisms. The 40 simulated individuals in the T2D SimPops with underlying alanine aminotransferase (ALT) or bilirubin elevations were excluded from analysis to be consistent with clinical trial exclusion criteria that were in place during the TAK-875 clinical studies.

Human SimCohorts. SimCohorts are relatively small populations consisting of a subset of simulated individuals from existing SimPops in DILIsym. For sensitivity analysis simulation purposes, this work employed the human SimCohorts v4A-1-Multi16, which includes the baseline human as well as 15 individuals from the human SimPops v4A-1. The 15 individuals from the SimPops consist of 13 sensitive individuals as well as 2 individuals with low sensitivity in the areas of oxidative stress, mitochondrial dysfunction, bile acid transport inhibition, and combined bile acid transport inhibition and mitochondrial dysfunction.

Simulation protocols. TAK-875 dosing was simulated at 200 mg once daily (q. d.), the highest dose in Phase II clinical trials, for 12 weeks in the baseline human, in the NHV SimPops, and in the T2D SimPops. The 200 mg daily dosage regimen was also simulated in the human SimCohorts v4A-1-Multi16 for four different scenarios, sequentially omitting one potential mechanistic contributor to toxicity: bile acid transporter inhibition effects, mitochondrial dysfunction effects, all effects due to the parent drug (TAK-875), and all effects due to TAK-875-Glu. These mechanistic investigation simulations, listed in Table 1, evaluated the importance of each toxicity element to the overall DILI behavior of drug treatment. The 200 mg daily dosage regimen was simulated in the human SimCohorts v4A-1-Multi16 for the parameter sensitivity analyses. In addition, simulations were performed with 100 mg once daily dosing to explore the sensitivity of the simulation results to the dosing level.

Table 1. Mechanistic Investigation Simulations and the Mechanisms That Were Turned on and Off in DILIsym for Each Simulation of TAK-875 Administered 200 mg Daily for 12 Weeks

Mechanistic Investigation Simulation Name	Mechanisms On in DILIsym[a]	Mechanism(s) Off in DILIsym
All	TAK-875: BAi, ETCi TAK-875-Glu: BAi, ETCi	None
ETCi-Off	TAK-875: BAi TAK-875-Glu: BAi	ETCi
BAi-Off	TAK-875: ETCi TAK-875-Glu: ETCi	BA[b]
TAK-875-Off	TAK-875-Glu: BAi, ETCi	TAK-875
TAK-875-Glu-Off	TAK-875: BAi, ETCi	TAK-875-Glu

[a]BAi is inhibition of bile acid transport; ETCi is inhibition of ETC.
[b]BA is bile acids; turning this mechanism off means removing BSEP, basolateral (MRP3/MRP4), and NTCP inhibition.

Susceptibility factor analysis. Multiple linear regression analysis was performed with the simulation results from the NHV SimPops and the T2D SimPops (200 mg daily dosing for 12 weeks). Parameters that were varied to create the simulated individuals within the NHV SimPops and T2D SimPops (Supplementary materials 4 and 5, respectively) were used as independent variables, and simulated ALT elevations were used as dependent variables in multiple linear regression analyses (separate analyses performed for NHV SimPops and T2D SimPops). Parameters that were statistically significant predictors of serum ALT levels at a $p < .05$ threshold were identified as potential susceptibility factors. Multiple linear regression analyses were performed using R software (http://www.r-project.org/).

RESULTS

PBPK Modeling

Simulation results from the PBPK model for TAK-875 are shown in Figure 1. Figure 1A shows the fit to the plasma time course for parent TAK-875 after single ascending doses of TAK-875. The figure demonstrates that the fit to the plasma data (Naik *et al.*, 2012) is reasonable across the range of therapeutic doses (ie, observed/simulated plasma AUC and plasma $C_{max} \leq 1.5$).

A liver:blood partition coefficient of 3.4, derived from the *in vitro* data collected for this work, was used to predict the liver concentration of TAK-875. Rat data indicated that the concentration of TAK-875 was roughly equivalent in liver and plasma (unpublished data). Figure 1B shows the simulated liver and plasma concentrations of TAK-875 after 12 weeks of 200 mg q. d. dosing; the simulated liver:plasma concentration ratio at steady state is 0.8, which corresponds well with the rat data. These results provide confidence that the prediction of TAK-875 liver concentration effectively approximates the physiological concentration in the patient population.

Toxicity Parameters

Experimental K_i data were collected as part of this study to assess the mode of BSEP and NTCP inhibition. Data indicated that BSEP inhibition by TAK-875 (K_i 17.2 μM) was mixed with α value

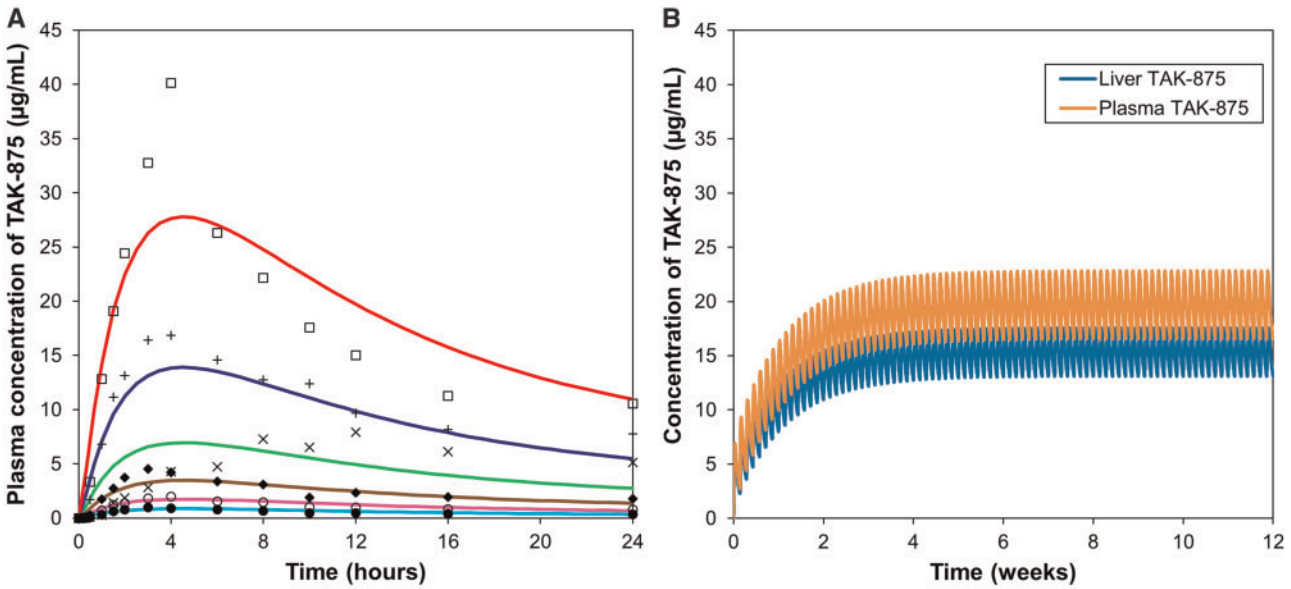

Figure 1. Physiologically based pharmacokinetic (PBPK) modeling results. A, The results are for simulations of TAK-875 after a single ascending dose (25, 50, 100, 200, 400, or 800 mg) compared with data from the literature (Naik *et al.*, 2012). B, Simulated liver concentration and the simulated plasma concentration of TAK-875 after 12 weeks of 200 mg q. d. dosing.

of 2.172 (Figure 2A) whereas NTCP inhibition by TAK-875 (K_i 4.30 µM) was competitive (Figure 2B). The K_i values determined here were combined with transporter inhibition constants (IC_{50} values) determined previously (Wolenski *et al.*, 2017) to parameterize the BA sub-model in DILIsym. To allow for the greatest possible inhibition of basolateral efflux transport (ie, the most toxicity), the lower of the two IC_{50} values for MRP3 and MRP4 were used for the basolateral efflux transport inhibition constants for TAK-875 and TAK-875-Glu. Because only IC_{50} data were available for basolateral inhibition and for TAK-875-Glu BSEP inhibition, the mode of inhibition for all efflux transporters was assumed to be the same as TAK-875 BSEP inhibition. Likewise, because only IC_{50} data were available for TAK-875-Glu NTCP inhibition, the mode of inhibition for NTCP was assumed to be the same as TAK-875 NTCP inhibition. The bile acid transport inhibition parameter values for TAK-875 and TAK-875-Glu used in DILIsym are shown in Table 2.

Previous studies also demonstrated the potential of TAK-875 to induce mitochondrial dysfunction (Otieno *et al.*, 2018; Wolenski *et al.*, 2017). In primary human hepatocytes, both TAK-875 and TAK-875-Glu were found to reduce the oxygen consumption rate (OCR) in a dose-dependent manner (Wolenski *et al.*, 2017), suggesting that both compounds can act as mitochondrial ETC inhibitors. In HepG2 cells, TAK-875 caused a dose-dependent reduction in OCR, whereas TAK-875-Glu had no effect on basal respiration (Otieno *et al.*, 2018). Initial simulations in DILIsym indicated that the predicted *in vivo* hepatotoxicity is highly sensitive to the potency of the ETC inhibition induced by TAK-875. Therefore, additional *in vitro* respiration data was collected as part of this study in an effort to more closely define the relationship between intracellular TAK-875 exposure and ETC inhibition. The changes in OCR associated with increasing intracellular concentration of TAK-875 in HepG2 cells are shown in Figure 3. The OCR values for TAK-875 determined here were combined with OCR data collected previously for TAK-875-Glu in rat hepatocytes to optimize the MITOsym ETC inhibition parameters for both species (Figure 4). Because intracellular TAK-875-Glu concentrations were not assessed experimentally, TAK-875-Glu intracellular concentrations were predicted based on PBPK simulation results. The MITOsym ETC inhibition parameter values for TAK-875 and TAK-875-Glu were transformed to DILIsym parameters (Table 2) and used for the toxicity simulations.

Finally, in previous research, TAK-875 or TAK-875-Glu were not found to be inducers of oxidative stress (Wolenski *et al.*, 2017). Consistent with the previous findings, additional data collected as part of this study showed no evidence for TAK-875-induced oxidative stress (Supplementary material 3). Because no ROS production has been observed experimentally for either TAK-875 or TAK-875-Glu, the DILIsym parameter values for TAK-875 did not include ROS production.

Toxicity Investigations

When the 200 mg TAK-875 daily dosage regimen was simulated for 12 weeks, 4.9% of simulated individuals in the NHV SimPops and 6.5% of simulated individuals in the T2D SimPops developed ALT elevations > 3× ULN. The 6.5% of simulated individuals in the T2D SimPops following administration of 200 mg TAK-875 daily dosing can be compared against the clinical observation of 2.8% of TAK-875-treated patients (for daily doses ranging from 25 to 50 mg) with ALT elevations > 3× ULN (Marcinak *et al.*, 2018) and 4.0% of TAK-875-treated patients (for daily doses of 200 mg) with ALT elevations > 3× ULN (unpublished data). The predicted incidence of Hy's Law cases (ALT > 3× ULN and total bilirubin > 2× ULN) in the T2D SimPops was 5.3%. In comparison, serious liver injury was rare in TAK-875-treated patients; only 3 cases of serious liver injury were identified in 9139 TAK-875-treated patients (one of the three cases was deemed to be a Hy's Law case and the other two cases were considered to closely approximate Hy's Law cases) (Marcinak *et al.*, 2018). The average simulated time to ALT elevations > 3× ULN in the T2D SimPops was 3.7 weeks which is consistent with the delayed onset of the occurrence of DILI observed clinically for TAK-875 (Marcinak *et al.*, 2018).

The relative importance of mitochondrial versus bile acid toxicity and parent versus metabolite were investigated by

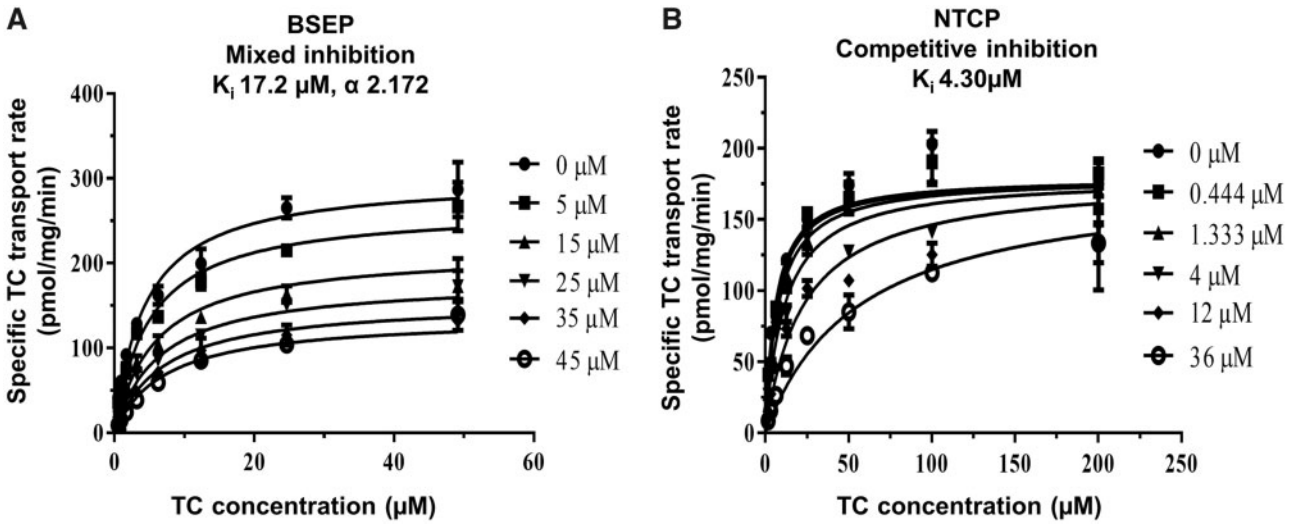

Figure 2. Results of transport inhibition K_i determination assays for TAK-875. A, Transporter specific accumulation of taurocholate (TC) at different TAK-875 concentrations in BSEP vesicles in the VT K_i determination assay. The data indicated that bile salt export pump (BSEP) inhibition by TAK-875 (K_i 17.2 μM) was mixed with α value of 2.172. B, Transporter specific accumulation of taurocholate at different TAK-875 concentrations in sodium taurocholate co-transporting polypeptide (NTCP)-expressing Chinese hamster ovary (CHO) cells. The data indicated that NTCP inhibition by TAK-875 (K_i 4.30 μM) was competitive.

Table 2. DILIsym Toxicity Parameter Values for TAK-875 Toxicity Simulations

Compound	DILI Mechanism	DILIsym Parameter	Parameter Description	Parameter Value
TAK-875	BSEP inhibition	Ki_BSEP_CompW[a]	Compound W BSEP inhibition constant	17.2 μM
TAK-875	BSEP inhibition	canal_alpha_CompW	Compound W BSEP alpha constant for inhibition	2.172
TAK-875	NTCP inhibition	Ki_NTCP_CompW[b]	Compound W NTCP inhibition constant	4.3 μM
TAK-875	Basolateral inhibition	Ki_baso_CompW	Compound W basolateral inhibition constant	11.7 μM
TAK-875	Basolateral inhibition	baso_alpha_CompW	Compound W basolateral alpha constant for inhibition	2.172
TAK-875	ETC inhibition	MitoS_ETC_Inhib	Coefficient to quantify ETC inhibition based on compound/metabolite levels in the liver	347.2 μM
TAK-875-Glu	BSEP inhibition	Ki_BSEP_CompW_MetA[a]	Compound W metabolite A BSEP inhibition constant	41.6 μM
TAK-875-Glu	BSEP inhibition	canal_alpha_CompW_MetA	Compound W metabolite A BSEP alpha constant for inhibition	2.172
TAK-875-Glu	NTCP inhibition	Ki_NTCP_CompW_MetA[b]	NTCP competitive inhibition constant for Compound W metabolite A	2.4 μM
TAK-875-Glu	Basolateral inhibition	Ki_baso_CompW_MetA	Compound W metabolite A basolateral inhibition constant	3.36 μM
TAK-875-Glu	Basolateral inhibition	baso_alpha_CompW_MetA	Compound W met. A basolateral alpha constant for inhibition	2.172
TAK-875-Glu	ETC inhibition	MitoS_ETC_Inhib_2	Coefficient to quantify ETC inhibition based on compound/metabolite levels in the liver	15 800 μM

[a]For mixed inhibition of BSEP and basolateral transport by TAK-875 and TAK-875-Glu, the 'switch_canal_CompW', 'switch_canal_CompW_MetA', 'switch_baso_CompW', and 'switch_baso_CompW_MetA' parameters were set to 0.
[b]For competitive inhibition of NTCP by TAK-875 and TAK-875-Glu, the 'Compound W NTCP switch' and 'Compound W metabolite A NTCP switch' parameters were set to 1.

conducting mechanistic investigation simulations in the human SimCohorts v4A-1-Multi16 (see Materials and Methods section). The exploratory simulations suggested that both mechanisms of hepatotoxicity and both molecular species may have been involved in the observed toxicity for TAK-875 (Table 3). Whereas the simulations with all mechanisms active and both molecular species active yielded 5/16 simulated patients with liver injury, inactivating mitochondrial toxicity (Simulation ETCi-Off) or bile acid-mediated toxicity (Simulation BA-Off) eliminated the simulated hepatotoxicity altogether (0/16 simulated individuals with liver injury); this suggests that both hepatotoxicity mechanisms are major contributors to simulated TAK-875-mediated liver injury. Removing parent simulated TAK-875-mediated toxicity (Simulation TAK-875-Off) also eliminated all simulated hepatotoxicity (0/16 simulated individuals with liver injury), while removing toxicity mediated by the TAK-875-Glu metabolite (Simulation TAK-875-Glu-Off) only attenuated the simulated toxicity (2/16 simulated individuals with liver injury); this suggests that parent TAK-875 is the primary molecular species contributing to liver injury whereas the

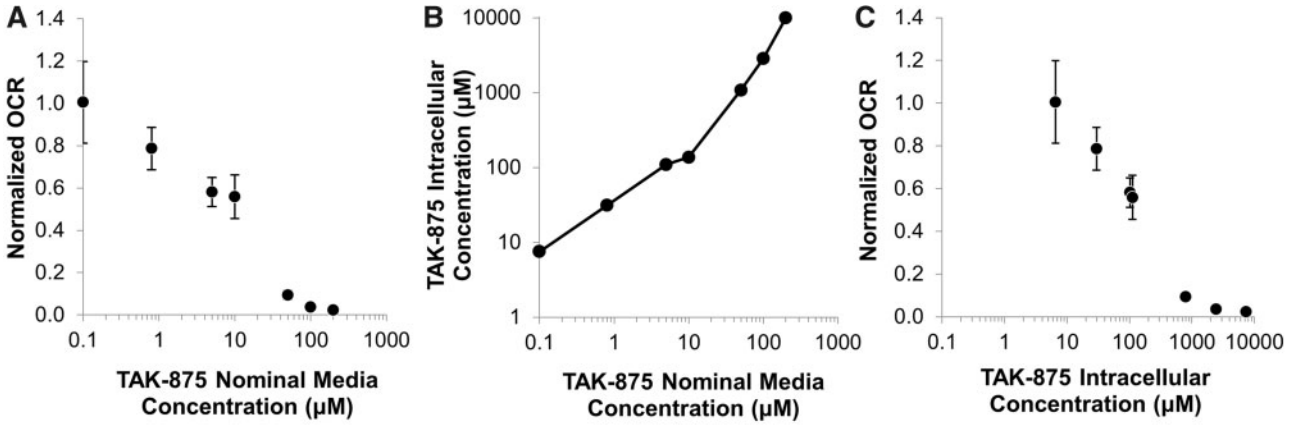

Figure 3. The effect of 1 h treatment of TAK-875 on oxygen consumption rate (OCR) in HepG2 cells. A, OCR as a function of the nominal media TAK-875 concentration. B, The relationship between the nominal media TAK-875 concentration and the intracellular TAK-875 concentration determined via mass spectrometry. C, OCR as a function of the intracellular TAK-875 concentration.

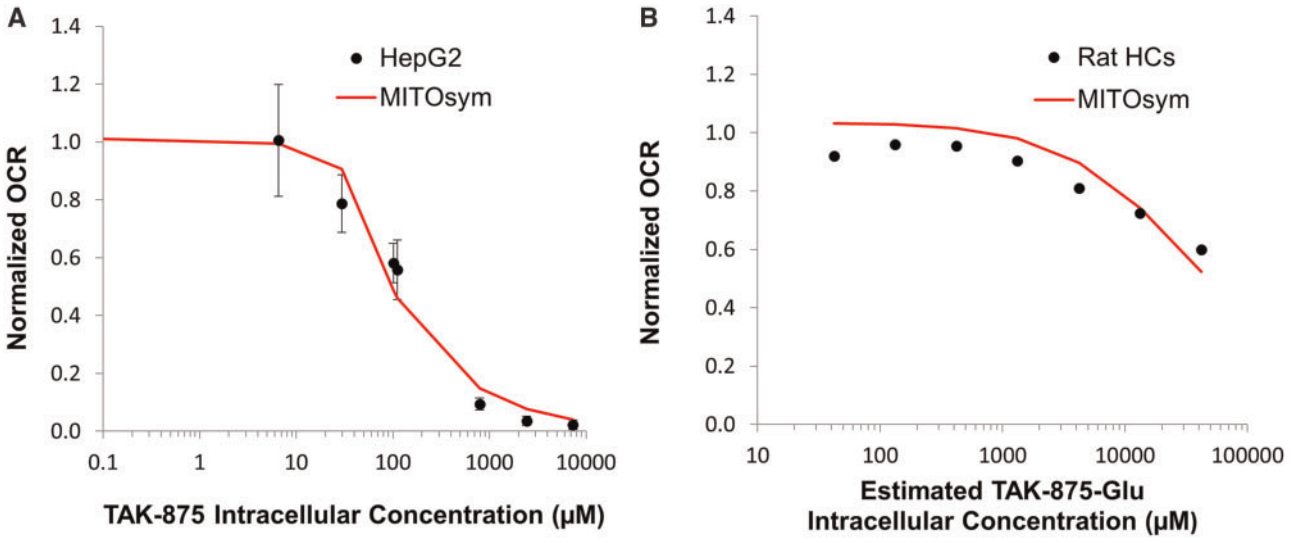

Figure 4. Observed and simulated oxygen consumption rate (OCR) in response to TAK-875 and TAK-875-Glu. A, *In vitro* respiration data for TAK-875 plotted against intracellular concentrations obtained from HepG2 cells. B, *In vitro* respiration data for TAK-875-Glu obtained from rat hepatocytes plotted against estimated intracellular concentrations based on simulations using the physiologically based pharmacokinetic (PBPK) sub-model for TAK-875. The results of simulated OCR responses for TAK-875 and TAK-875-Glu conducted in MITOsym are overlaid on both graphs.

Table 3. Frequency of Simulated Alanine Aminotransferase (ALT) Elevations in the SimCohort in the Mechanistic Investigation Simulations

Toxicity Mechanisms[a]	Simulated ALT > 3× ULN
All	7/16
ETCi-Off	0/16
BA-Off	0/16
TAK-875-Off	0/16
TAK-875-Glu-Off	5/16

[a]The simulation names refer to the mechanistic investigation simulation names listed in Table 1. Mechanisms present and not present for each simulation can be found in Table 1.

TAK-875-Glu metabolite contributes to a lesser extent (Table 3). Taken together, these mechanistic investigation simulations indicated that in DILIsym, simulated TAK-875-mediated toxicity is likely multifactorial in nature.

Susceptibility Factors

The TAK-875 simulations revealed that a subset of SimPops individuals were susceptible to simulated TAK-875-mediated hepatotoxicity. To identify the most important SimPops parameters in the context of simulated TAK-875-mediated DILI, multiple linear regression analyses were performed with maximum serum ALT as the dependent variable and the SimPops parameters as independent variables (separate analyses performed for NHV SimPops and T2D SimPops). The results of the analysis for the NHV SimPops are shown in Table 4. Of the 34 parameters varied in the NHV SimPops, 3 were statistically significant predictors of peak serum ALT levels. Two of these parameters are related to bile acid transport (uptake transporter regulation scaling factor and canalicular transporter regulation scaling factor), and the other is related to drug distribution (body mass).

The results for the T2D SimPops are shown in Table 5. For this SimPops, five parameters were statistically significant predictors of serum ALT: two bile acid transport parameters (uptake transporter regulation scaling factor and canalicular

Table 4. Results of Multiple Linear Regression Analysis for the Normal Healthy Volunteer (NHV) SimPops

Parameter Name[a]	Parameter Description	*p* Value[b]
Body_mass	Body mass	.009
uptake_reg_scale	Uptake transporter regulation scaling factor	.010
canal_reg_scale	Canalicular transporter regulation scaling factor	.012

[a]Only parameters meeting the statistical threshold of $p < .05$ are listed. Body mass is related to ADME. Uptake transporter regulation scaling factor and canalicular transporter regulation scaling factor are related to bile acid transport.
[b]Multiple linear regression was performed using R software (http://www.r-project.org/).

Table 5. Results of Multiple Linear Regression Analysis for the Type 2 Diabetes SimPops

Parameter Name[a]	Parameter Description	*p* Value[b]
uptake_reg_scale	Uptake transporter regulation scaling factor	< .001
canal_reg_scale	Canalicular transporter regulation scaling factor	< .001
Km_TGnegfeed	TG esterification negative feedback Km	.017
RNS_ROS_cl_Vmax	Liver RNS/ROS baseline clearance Vmax	.032
GSHo	GSH basal level	.044

[a]Only parameters meeting the statistical threshold of $p < .05$ are listed. Uptake transporter regulation scaling factor and canalicular transporter regulation scaling factor are related to bile acid transport. TG esterification negative feedback Km is related to liver triglyceride stores. Liver RNS/ROS baseline clearance Vmax is related to the RNS/ROS balance. GSH basal level is related to glutathione stores.
[b]Multiple linear regression was performed using R software (http://www.r-project.org/).

transporter regulation scaling factor), one parameter related to liver triglyceride stores (TG esterification negative feedback Km), one parameter related to the RNS/ROS balance (Liver RNS/ROS baseline clearance maximum velocity [Vmax]), and one parameter related to GSH (basal level). Notably, the two bile acid transport-related parameters (uptake transporter regulation scaling factor and canalicular transporter regulation scaling factor) that had the highest statistical significance in the multiple linear regression analysis for the T2D SimPops were also statistically significant parameters in the NHV SimPops. These results, along with the results of the mechanistic investigation simulations described in the previous section, suggest that bile acid toxicity may play an important role in simulated TAK-875-mediated hepatotoxicity.

Sensitivity Analyses

To investigate the sensitivity of DILI responses to the potency of TAK-875- and TAK-875-Glu-mediated effects on bile acid transport inhibition and mitochondrial function, simulations were performed in the human SimCohorts v4A-1-Multi16 (see Materials and Methods section) with 10-fold smaller and larger K_i values for inhibition of each transporter by each molecular species (ie, TAK-875 and TAK-875-Glu) and with 10-fold smaller and larger values for the ETC inhibition parameter for each molecular species (Table 6). The simulated frequency of ALT elevations in the human SimCohorts v4A-1-Multi16 was most sensitive to 10-fold changes in the K_i value for inhibition of BSEP by TAK-875, with the simulated frequency shifting to 100% (16/16) with a 10-fold decrease in the K_i value and to 0% (0/16) with a 10-fold increase in the K_i value. The simulated TAK-875-mediated injury was also quite sensitive to changes in the ETC inhibition parameter value for TAK-875 (simulated incidence of 15/16 and 2/16 with a 10-fold decrease and 10-fold increase, respectively). Simulated injury was moderately sensitive to changes in the K_i value for inhibition of NTCP by TAK-875 (simulated incidence of 5/16 and 8/16 with a 10-fold decrease and 10-fold increase, respectively). In contrast, simulated TAK-875-mediated injury was relatively insensitive to changes in the K_i value for inhibition of basolateral transport by TAK-875 (simulated incidence of 7/16 and 6/16 with a 10-fold decrease and 10-fold increase, respectively). For TAK-875-Glu, 10-fold changes in the K_i value for inhibition of BSEP led to moderate changes in the simulated incidence of injury (simulated incidence of 9/16 and 5/16 with a 10-fold decrease and 10-fold increase, respectively). In contrast, for TAK-875-Glu, the simulated incidence of injury was not sensitive to changes in the K_i values for NTCP or basolateral transport. In addition, simulated TAK-875-mediated injury was not sensitive to 10-fold changes in the parameter value for TAK-875-Glu-mediated ETC inhibition (Table 6).

Next, the sensitivity of simulated TAK-875-mediated hepatotoxicity to the mode of inhibition for each transporter and each molecular species was investigated (Table 7). The frequency of simulated ALT elevations in the human SimCohorts v4A-1-Multi16 was very sensitive to the mode of inhibition of BSEP by TAK-875, with the incidence of injury increasing to 11/16 and decreasing to 0/16 with a change to non-competitive or competitive inhibition, respectively. TAK-875-mediated hepatotoxicity in the simulations was also very sensitive to a change in the mode of inhibition of NTCP by TAK-875 from competitive to either mixed (alpha = 2.2) or non-competitive; all simulated toxicity disappeared (ie, incidence of 0/16) in both of these simulated scenarios. In contrast, the incidence of ALT elevations in the simulations was relatively insensitive to a change in the mode of inhibition of basolateral transport by TAK-875 (Table 7). In addition, changes to the mode of inhibition for BSEP, NTCP, or basolateral transport by TAK-875-Glu had minimal impact on the simulated incidence of ALT elevations (Table 7).

The sensitivity of simulated TAK-875-mediated hepatotoxicity to the dosing level was examined by reducing the simulated dose from 200 mg daily dosing to 100 mg daily dosing. When the 100 mg TAK-875 daily dosage regimen was simulated for 12 weeks, none of the simulated individuals in the NHV SimPops and none of the simulated individuals in the T2D SimPops developed ALT elevations > 3× ULN. Whereas no ALT elevations were predicted for the 100 mg daily dosing regimen, reductions in simulated hepatic ATP levels were predicted; the simulated median (range) values of minimum postdose hepatic ATP concentrations were 4.13 mmol/l (3.79–4.19) and 4.08 mmol/l (3.74–4.17) in the NHV SimPops and in the T2D SimPops, respectively, compared with a baseline human hepatic ATP concentration of 4.2 mmol/l. The predicted reductions in simulated hepatic ATP levels at the 100 mg dosing level were less substantial than predicted reductions in simulated hepatic ATP levels for the 200 mg dosing regimen; the simulated median (range) values of minimum postdose hepatic ATP concentrations were 4.05 mmol/l (1.78–4.16) and 3.99 mmol/l (0.90–4.14) in the NHV SimPops and in the T2D SimPops, respectively.

DISCUSSION

DILIsym is a mathematical model of DILI that can be applied to predict hepatotoxicity based on preclinical *in vitro* and/or *in vivo*

Table 6. Frequency of Simulated Alanine Aminotransferase (ALT) Elevations in the SimCohorts in the Toxicity Parameter Sensitivity Analysis

Compound	DILI Mechanism	DILIsym Parameter[a]	Simulated ALT > 3× ULN		
			10× Decrease	1×	10× Increase
TAK-875	BSEP inhibition	Ki_BSEP_CompW	16/16	7/16	0/16
TAK-875	NTCP inhibition	Ki_NTCP_CompW	5/16	7/16	8/16
TAK-875	Basolateral inhibition	Ki_baso_CompW	7/16	7/16	6/16
TAK-875	ETC inhibition	MitoS_ETC_Inhib	15/16	7/16	2/16
TAK-875-Glu	BSEP inhibition	Ki_BSEP_CompW_MetA	9/16	7/16	5/16
TAK-875-Glu	NTCP inhibition	Ki_NTCP_CompW_MetA	7/16	7/16	7/16
TAK-875-Glu	Basolateral inhibition	Ki_baso_CompW_MetA	7/16	7/16	7/16
TAK-875-Glu	ETC inhibition	MitoS_ETC_Inhib_2	7/16	7/16	7/16

[a]Description of each parameter is included in Table 2.

Table 7. Frequency of Simulated Alanine Aminotransferase (ALT) Elevations in the SimCohorts in the Transporter Mode of Inhibition Sensitivity Analysis

		Simulated ALT > 3× ULN		
		Inhibition Type		
Compound	Transporter	Non-Competitive	Mixed (Alpha = 2.2)	Competitive
TAK-875	BSEP	11/16	7/16[a]	0/16
TAK-875	NTCP	0/16	0/16	7/16[a]
TAK-875	Basolateral	7/16	7/16[a]	6/16
TAK-875-Glu	BSEP	8/16	7/16[a]	5/16
TAK-875-Glu	NTCP	6/16	7/16	7/16[a]
TAK-875-Glu	Basolateral	7/16	7/16[a]	7/16

[a]Indicates default mode of inhibition for each transporter/molecular species.
BSEP, bile salt export pump; NTCP, sodium taurocholate co-transporting polypeptide.

data and can provide insight into the underlying mechanisms responsible for DILI. In this study, multiple integrated DILIsym sub-models representing drug distribution, mitochondrial dysfunction, bile acid physiology and pathophysiology, hepatocyte life cycle, and liver injury biomarkers were used to simulate the response to TAK-875. Inter-patient variation was taken into account by simulating treatment protocols in SimPops, simulated populations that include variability in parameters relevant to hepatotoxicity mechanisms.

Following treatment with 200 mg TAK-875 daily for 12 weeks, the simulated incidence of ALT elevations > 3× ULN observed in the T2D SimPops (6.5%) generally recapitulates, though mildly overpredicts, the frequency of ALT elevations observed in the clinic at the 200 mg daily dose (4.0%, unpublished data). The predicted incidence of Hy's Law cases in the T2D SimPops was 5.3%, whereas only 3 cases of serious liver injury (0.03%) relevant to TAK-875 treatment were reported in clinical trials (Marcinak *et al.*, 2018). The overprediction may be partially attributed to the absence of compensatory mechanisms, such as mitochondrial biogenesis, in the DILIsym simulations. In the NHV SimPops, the simulated incidence of ALT elevations > 3× ULN (4.9%) was lower than the simulated incidence in the T2D SimPops (6.5%); this difference demonstrates the impact of incorporating disease pathophysiology relevant to DILI on the predicted hepatotoxic responses in DILIsym.

Mechanistic investigation simulations indicated that parent TAK-875 is the primary molecular species contributing to liver injury whereas the TAK-875-Glu metabolite contributes to a lesser extent. Furthermore, mechanistic investigation simulations demonstrated that when either mitochondrial dysfunction or bile acid transport inhibition was removed from the TAK-875 simulations, toxicity did not occur. These results suggest that, in DILIsym, a synergistic effect between bile-acid mediated effects on the mitochondrial proton gradient and simulated TAK-875-mediated electron transport chain inhibition underlie the clinically observed toxicity for TAK-875. The multifactorial nature of TAK-875-mediated toxicity is supported by previous studies which indicated that the effects of bile acid accumulation can be exacerbated by drug-induced mitochondrial dysfunction, especially electron transport chain (ETC) inhibition (Aleo *et al.*, 2014; Woodhead *et al.*, 2017a). This synergy could be mediated by the fact that bile acid accumulation and subsequent mitochondrial effects of excess bile acids can interfere with compensatory mechanisms that can attenuate the effects of ETC inhibition; it could also be mediated by the fact that bile acid transporters require ATP to function (Adachi *et al.*, 1991; Nishida *et al.*, 1991), and ETC inhibition leads to declines in cellular ATP (Imaizumi *et al.*, 2015; Li *et al.*, 2003) which can further exacerbate the effect of bile acid transporter inhibition.

Susceptibility factor analysis revealed that parameters related to bile acid transport were particularly important in the context of simulated TAK-875-mediated DILI. Specifically, two bile acid transport-related parameters (uptake transporter regulation scaling factor and canalicular transporter regulation scaling factor) were statistically significant predictors of peak serum ALT levels in both the NHV SimPops and the T2D SimPops. The predicted importance of bile acid-mediated toxicity to simulated TAK-875-mediated liver injury is consistent with a recent study that reported that bile acid homeostasis is disrupted in pre-clinical species following treatment with TAK-875 (Wolenski *et al.*, 2017).

Sensitivity analyses demonstrated that simulated TAK-875-mediated ALT elevations in the human SimCohorts were most sensitive to changes in the K_i values for inhibition of BSEP and NTCP by TAK-875, the K_i value for inhibition of BSEP by TAK-875-Glu, and the ETC inhibition parameter value for TAK-875 (Table 6). These results indicate that both bile acid transport inhibition and mitochondrial effects mediated by TAK-875 play a critical role in the simulated TAK-875-mediated liver injury. TAK-875-Glu appears to play a lesser role in the injury, with TAK-875-Glu-mediated effects on bile acid transport and mitochondrial function contributing only minimally to the simulated toxicity. These findings are consistent with the conclusions of recent studies which suggest that disruption of bile acid transport (Otieno *et al.*, 2018; Wolenski *et al.*, 2017) and

TAK-875 effects on mitochondrial function (Otieno *et al.*, 2018) are involved in TAK-875-mediated DILI.

The simulated injury was very sensitive to a change in the mode of inhibition of BSEP by TAK-875. Specifically, non-competitive inhibition of BSEP by TAK-875 led to much greater potential toxicity than competitive inhibition (Table 7). A similar finding has been reported for the impact of the mode of BSEP inhibition on the potential hepatotoxicity of the terminated anti-cancer drug CP-724,714 (Woodhead *et al.*, 2014). Notably, the default mode of inhibition, mixed inhibition of BSEP by TAK-875 (based on experimental data collected as part of this study), led to a more moderate simulated hepatotoxic response than purely non-competitive inhibition and to a more potent simulated hepatotoxic response than purely competitive inhibition (Table 7). These results demonstrate the importance of measuring K_i values to assess the mode of inhibition. TAK-875-mediated hepatotoxicity in the simulations was also relatively sensitive to a change in the mode of inhibition of NTCP by TAK-875 (Table 7), whereas simulated hepatotoxicity for TAK-875 was less sensitive to the mode of inhibition of basolateral efflux transport by TAK-875 and to the mode of inhibition of BSEP, NTCP, or basolateral transport by TAK-875-Glu (Table 7). These findings indicate that parent TAK-875 plays a prominent role in the disruption of bile acid transport.

The simulated TAK-875-mediated injury was also sensitive to the dosing level. When the TAK-875 dosing was reduced from 200 mg daily dosing to 100 mg daily dosing, reductions in hepatic ATP levels were predicted but no ALT elevations $> 3\times$ ULN were predicted.

One limitation of this study is that intracellular TAK-875-Glu concentrations were not assessed experimentally in the previously collected OCR data set that was used for determining the ETC inhibition parameter value for TAK-875-Glu in DILIsym. Additional data measuring cell lysate concentrations (via LC/MS/MS analysis) would help to more closely define the relationship between the concentration of TAK-875-Glu at the site of action and ETC inhibitory effects. However, the sensitivity analysis indicated that the simulation results are relatively insensitive to mitochondrial effects of the TAK-875-Glu metabolite (Table 6). These findings are consistent with recent work which reported that TAK-875-Glu appears to have negligible effects on mitochondria (Otieno *et al.*, 2018). A second limitation is that only IC_{50} data were available for TAK-875 and TAK-875-Glu inhibition of basolateral transporters and for TAK-875-Glu inhibition of BSEP and NTCP. However, the sensitivity analyses performed as part of this study demonstrated that the simulated TAK-875-mediated injury was relatively insensitive to the mode of inhibition for TAK-875-mediated inhibition of basolateral efflux transport and TAK-875-Glu-mediated inhibition of BSEP and NTCP, and simulated injury showed no sensitivity to the mode of inhibition for TAK-875-Glu-mediated inhibition of basolateral transport (Table 7).

A further limitation of the study is that DILIsym does not incorporate the effects of the adaptive immune system, which has been proposed as a potential toxicity mechanism for TAK-875 (Otieno *et al.*, 2018). However, it is likely that a certain level of cellular stress is necessary to trigger an adaptive immune attack (Cho and Uetrecht, 2017; Mosedale and Watkins, 2017), and previous research with DILIsym has shown that underlying cellular stress caused by bile acid accumulation and ETC inhibition can explain the presence of toxicity that is generally thought to be immune-mediated (Woodhead *et al.*, 2017a). This research therefore lends further credence to the idea that bile acid accumulation and ETC inhibition can serve as necessary precursors to an immune attack (Woodhead *et al.*, 2017b).

In summary, this study illustrates the capability of QST modeling to integrate pharmacokinetic data, *in vitro* toxicity data, and inter-patient variability to provide an account of how multiple hepatotoxicity mechanisms may come together to cause simulated TAK-875-mediated liver injury. By combining the effects of both parent TAK-875 and TAK-875-Glu on mitochondrial function and bile acid transport inhibition, DILIsym reproduced a low frequency of liver injury in a simulated T2D population treated with 200 mg TAK-875 daily doses. DILIsym simulations suggested a synergistic role for bile acid accumulation and ETC inhibition in TAK-875-mediated liver injury. These findings suggest that QST modeling with DILIsym may allow for the prospective prediction of the hepatotoxic potential of new drugs, thus reducing the risk to patients and lowering drug development costs associated with late-stage attrition due to liver toxicity.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

ACKNOWLEDGMENTS

The authors would like to acknowledge the members of the DILI-sim Initiative for their support in the development of the DILIsym model. More information on the DILI-sim Initiative can be found at www.dilisym.com. D.M.L., J.L.W., S.Q.S., and B.A.H were compensated employees of DILIsym Services (A Simulations Plus Company), P.W. was a compensated employee of Cyprotex, K.H. and K.M were compensated employees of Solvo Biotechnology, and F.S.W. and Y.P.D. were compensated employees of Takeda Pharmaceutical Company Limited when this research was conducted. P.B.W. has served as a compensated consultant for Takeda Pharmaceutical Company Limited.

FUNDING

This work was supported by the members of the DILI-sim Initiative.

REFERENCES

Adachi, Y., Kobayashi, H., Kurumi, Y., Shouji, M., Kitano, M., and Yamamoto, T. (1991). ATP-dependent taurocholate transport by rat liver canalicular membrane vesicles. *Hepatology (Baltimore, MD)* **14**, 655–659.

Aleo, M., Luo, Y., Swiss, R., and Bonin, P. (2014). Human drug-induced liver injury severity is highly associated to dual inhibition of liver mitochondrial function and bile salt export pump. 1–33. *Hepatology* **60**, 1015–22.

Bhattacharya, S., Shoda, L. K. M., Zhang, Q., Woods, C. G., Howell, B. A., Siler, S. Q., Woodhead, J. L., Yang, Y., McMullen, P., Watkins, P. B., *et al.* (2012). Modeling drug- and chemical-induced hepatotoxicity with systems biology approaches. *Front. Physiol.* **3**, 462.

Cho, T., and Uetrecht, J. (2017). How reactive metabolites induce an immune response that sometimes leads to an idiosyncratic drug reaction. *Chem. Res. Toxicol.* **30**, 295–314.

Eakins, J., Bauch, C., Woodhouse, H., Park, B., Bevan, S., Dilworth, C., and Walker, P. (2016). A combined *in vitro* approach to improve the prediction of mitochondrial toxicants. *Toxicol. In Vitro* **34**, 161–170.

Groothuis, F. A., Heringa, M. B., Nicol, B., Hermens, J. L. M., Blaauboer, B. J., and Kramer, N. I. (2015). Dose metric considerations in *in vitro* assays to improve quantitative *in vitro-in vivo* dose extrapolations. *Toxicology* **332**, 30–40.

Imaizumi, N., Kwang Lee, K., Zhang, C., and Boelsterli, U. A. (2015). Mechanisms of cell death pathway activation following drug-induced inhibition of mitochondrial complex I. *Redox Biol.* **4**, 279–288.

Kaku, K., Enya, K., Nakaya, R., Ohira, T., and Matsuno, R. (2015). Efficacy and safety of fasiglifam (TAK-875), a G protein-coupled receptor 40 agonist, in Japanese patients with type 2 diabetes inadequately controlled by diet and exercise: A randomized, double-blind, placebo-controlled, phase III trial. *Diabetes Obes. Metab.* **17**, 675–681.

Kaku, K., Enya, K., Nakaya, R., Ohira, T., and Matsuno, R. (2016). Long-term safety and efficacy of fasiglifam (TAK-875), a G-protein-coupled receptor 40 agonist, as monotherapy and combination therapy in Japanese patients with type 2 diabetes: A 52-week open-label phase III study. *Diabetes Obes. Metab.* **18**, 925–929.

Li, N., Ragheb, K., Lawler, G., Sturgis, J., Rajwa, B., Melendez, J. A., and Robinson, J. P. (2003). Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J. Biol. Chem.* **278**, 8516–8525.

Li, X., Zhong, K., Guo, Z., Zhong, D., and Chen, X. (2015). Fasiglifam (TAK-875) inhibits hepatobiliary transporters: A possible factor contributing to fasiglifam-induced liver injury. *Drug Metab. Dispos. Biol. Fate Chem.* **43**, 1751–1759.

Longo, D. M., Yang, Y., Watkins, P. B., Howell, B. A., and Siler, S. Q. (2016). Elucidating differences in the hepatotoxic potential of tolcapone and entacapone with DILIsym(®), a mechanistic model of drug-induced liver injury. *CPT Pharmacomet. Syst. Pharmacol.* **5**, 31–39.

Marcinak, J. F., Munsaka, M. S., Watkins, P. B., Ohira, T., and Smith, N. (2018). Liver safety of fasiglifam (TAK-875) in patients with type 2 diabetes: Review of the global clinical trial experience. *Drug Saf.* **41**, 625–640.

Mosedale, M., and Watkins, P. B. (2017). Drug-induced liver injury: Advances in mechanistic understanding that will inform risk management. *Clin. Pharmacol. Ther.* **101**, 469–480.

Nadanaciva, S., Rana, P., Beeson, G. C., Chen, D., Ferrick, D. a., Beeson, C. C., and Will, Y. (2012). Assessment of drug-induced mitochondrial dysfunction via altered cellular respiration and acidification measured in a 96-well platform. *J. Bioenerg. Biomembr.* **44**, 421–437.

Naik, H., Vakilynejad, M., Wu, J., Viswanathan, P., Dote, N., Higuchi, T., and Leifke, E. (2012). Safety, tolerability, pharmacokinetics, and pharmacodynamic properties of the GPR40 agonist TAK-875: Results from a double-blind, placebo-controlled single oral dose rising study in healthy volunteers. *J. Clin. Pharmacol.* **52**, 1007–1016.

Nishida, T., Gatmaitan, Z., Che, M., and Arias, I. M. (1991). Rat liver canalicular membrane vesicles contain an ATP-dependent bile acid transport system. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6590–6594.

Otieno, M. A., Snoeys, J., Lam, W., Ghosh, A., Player, M. R., Pocai, A., Salter, R., Simic, D., Skaggs, H., Singh, B., *et al.* (2018). Fasiglifam (TAK-875): Mechanistic investigation and retrospective identification of hazards for drug induced liver injury. *Toxicol. Sci. Off. J. Soc. Toxicol.* **163**, 374–384.

Rolo, A. P., Oliveira, P. J., Moreno, A. J., and Palmeira, C. M. (2000). Bile acids affect liver mitochondrial bioenergetics: Possible relevance for cholestasis therapy. *Toxicol. Sci. Off. J. Soc. Toxicol.* **57**, 177–185.

Schulz, S., Schmitt, S., Wimmer, R., Aichler, M., Eisenhofer, S., Lichtmannegger, J., Eberhagen, C., Artmann, R., Tookos, F., Walch, A., *et al.* (2013). Progressive stages of mitochondrial destruction caused by cell toxic bile salts. *Biochim. Biophys. Acta* **1828**, 2121–2133.

Shoda, L. K. M., Woodhead, J. L., Siler, S. Q., Watkins, P. B., and Howell, B. A. (2014). Linking physiology to toxicity using DILIsym(®), a mechanistic mathematical model of drug-induced liver injury. *Biopharm. Drug Dispos.* **35**, 33–49.

Wolenski, F. S., Zhu, A. Z. X., Johnson, M., Yu, S., Moriya, Y., Ebihara, T., Csizmadia, V., Grieves, J., Paton, M., Liao, M., *et al.* (2017). Fasiglifam (TAK-875) alters bile acid homeostasis in rats and dogs: A potential cause of drug induced liver injury. *Toxicol. Sci. Off. J. Soc. Toxicol.* **157**, 50–61.

Woodhead, J. L., Brock, W. J., Roth, S. E., Shoaf, S. E., Brouwer, K. L. R., Church, R., Grammatopoulos, T. N., Stiles, L., Siler, S. Q., Howell, B. A., *et al.* (2017a). Application of a mechanistic model to evaluate putative mechanisms of tolvaptan drug-induced liver injury and identify patient susceptibility factors. *Toxicol. Sci.* **155**, 61–74.

Woodhead, J. L., Howell, B. A., Yang, Y., Harrill, A. H., Clewell, H. J., 3rd, Andersen, M. E., Siler, S. Q., and Watkins, P. B. (2012). An analysis of N-acetylcysteine treatment for acetaminophen overdose using a systems model of drug-induced liver injury. *J. Pharmacol. Exp. Ther.* **342**, 529–540.

Woodhead, J. L., Watkins, P. B., Howell, B. A., Siler, S. Q., and Shoda, L. K. M. (2017b). The role of quantitative systems pharmacology modeling in the prediction and explanation of idiosyncratic drug-induced liver injury. *Drug Metab. Pharmacokinet.* **32**, 40–45.

Woodhead, J. L., Yang, K., Siler, S. Q., Watkins, P. B., Brouwer, K. L. R., Barton, H. A., and Howell, B. A. (2014). Exploring BSEP inhibition-mediated toxicity with a mechanistic model of drug-induced liver injury. *Front. Pharmacol.* **5**, 240.

Yang, K., Battista, C., Woodhead, J. L., Stahl, S. H., Mettetal, J. T., Watkins, P. B., Siler, S. Q., and Howell, B. A. (2017). Systems pharmacology modeling of drug-induced hyperbilirubinemia: Differentiating hepatotoxicity and inhibition of enzymes/transporters. *Clin. Pharmacol. Ther.* **101**, 501–509.

Yang, Y., Nadanaciva, S., Will, Y., Woodhead, J. L., Howell, B. A., Watkins, P. B., and Siler, S. Q. (2014). MITOsym®: A mechanistic, mathematical model of hepatocellular respiration and bioenergetics. *Pharm. Res.* **32**, 1975–1992.

Yang, K., Pfeifer, N. D., Köck, K., and Brouwer, K. L. R. (2015). Species differences in hepatobiliary disposition of taurocholic acid in human and rat sandwich-cultured hepatocytes: Implications for drug-induced liver injury. *J. Pharmacol. Exp. Ther.* **353**, 415–423.