Assessing Effects of BHV-0223 40 mg Zydis Sublingual Formulation and Riluzole 50 mg Oral Tablet on Liver Function Test Parameters Utilizing DILIsym

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ABSTRACT

For patients with amyotrophic lateral sclerosis who take oral riluzole tablets, approximately 50% experience alanine transaminase (ALT) levels above upper limit of normal (ULN), 8% above $3\times$ ULN, and 2% above $5\times$ ULN. BHV-0223 is a novel 40 mg rapidly sublingually disintegrating (Zydis) formulation of riluzole, bioequivalent to conventional riluzole 50 mg oral tablets, that averts the need for swallowing tablets and mitigates first-pass hepatic metabolism, thereby potentially reducing risk of liver toxicity. DILIsym is a validated multiscale computational model that supports evaluation of liver toxicity risks. DILIsym was used to compare the hepatotoxicity potential of oral riluzole tablets (50 mg BID) versus BHV-0223 (40 mg BID) by integrating clinical data and *in vitro* toxicity data. In a simulated population (SimPops), ALT levels $> 3\times$ ULN were predicted in 3.9% (11/285) versus 1.4% (4/285) of individuals with oral riluzole tablets and sublingual BHV-0223, respectively. This represents a relative risk reduction of 64% associated with BHV-0223 versus conventional riluzole tablets. Mechanistic investigations revealed that oxidative stress was responsible for the predicted ALT elevations. The validity of the DILIsym representation of riluzole and assumptions is supported by its ability to predict rates of ALT elevations for riluzole oral tablets comparable with that observed in clinical data. Combining a mechanistic, quantitative representation of hepatotoxicity with interindividual variability in both susceptibility and liver exposure suggests that sublingual BHV-0223 confers diminished rates of liver toxicity compared with oral tablets of riluzole, consistent with having a lower overall dose of riluzole and bypassing first-pass liver metabolism.

Key words: BHV-0223; DILI; DILIsym; quantitative systems pharmacology modeling.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the death of motor neurons that leads to progressive muscle weakness and difficulties in speaking, breathing, and swallowing. The median survival time from onset to death ranges from approximately 2 to 3 years (Bensimon and Doble, 2004). The precise cause of the disease is unknown.

Riluzole is a neuroprotective drug that is thought to act by blocking glutamatergic neurotransmission in the central nervous system (Doble, 1996). Clinical trials have demonstrated that riluzole prolongs survival and time to tracheostomy in patients with ALS (Bensimon et al., 1994; Lacomblez et al., 1996). The original pivotal studies that led to the approval of riluzole for the treatment of ALS demonstrated an extended median survival time of 2–3 months and a 43% reduction in death in favor of riluzole over the trial period of 18 months. More recent studies suggest that riluzole can extend survival to 6–18 months with a longer follow up time (Brooks and Sanjak, 2004; Georgoulopoulou et al., 2013; Mitchell et al., 2006). Mandrioli et al. demonstrated that strict adherence (> 90% of days treated from time of diagnosis) to riluzole treatment is important for better
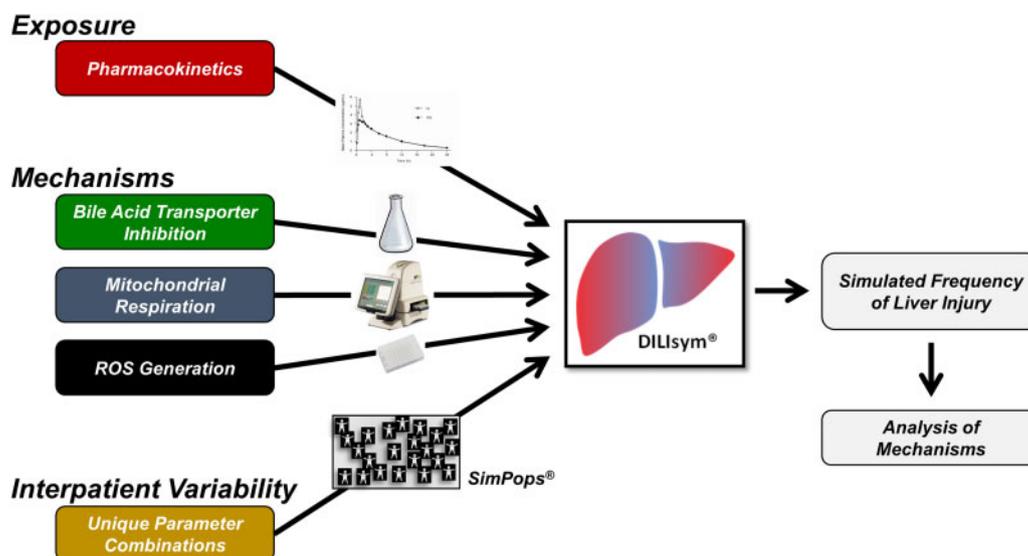
Figure 1. Diagram of the overall workflow for this study, including the processes of determining compound exposure, determining toxicity parameters from *in vitro* data, incorporating interpatient variability to simulate the frequency of liver injury, and analyzing mechanisms underlying simulated hepatotoxic injury.

survival benefit (Mandrioli *et al.*, 2018). Challenges to riluzole tablet adherence in ALS patients include the inability to swallow, gastrointestinal (GI) complications, and drug-induced abnormalities in liver function tests. Serum alanine aminotransferase levels greater than 3 times the upper limit of normal (ULN) have been observed in 10%–15% of patients receiving riluzole oral tablets (Bensimon and Doble, 2004).

BHV-0223 is a novel 40 mg sublingually dissolving Zydis formulation of riluzole that is bioequivalent to the riluzole 50 mg oral tablet formulation. Sublingual administration of riluzole may improve adherence in patients with dysphagia (difficulty swallowing). In addition, because of its sublingual route of administration, the ability of BHV-0223 to bypass first-pass metabolism, while achieving adequate systemic drug concentrations, will diminish overall hepatic drug burden and potentially lower the risk of liver toxicity.

Quantitative systems toxicology (QST) is an approach that integrates computational and experimental methods to understand and predict the toxicity of drugs throughout their development (Bloomingdale *et al.*, 2017). DILIsym is a QST model of drug-induced liver injury (DILI) which includes multiple hepatotoxicity mechanisms (ie, bile acid accumulation, mitochondrial dysfunction, and oxidative stress) (Battista *et al.*, 2018; Longo *et al.*, 2019; Shoda *et al.*, 2014; Woodhead *et al.*, 2019). In this study, DILIsym was used to quantitatively and mechanistically compare the liver toxicity potential of oral riluzole tablets versus BHV-0223 by integrating clinical data and *in vitro* toxicity data (Figure 1). Responses to conventional oral riluzole tablets and BHV-0223 were analyzed in a simulated population (SimPops) which included variability to account for potential interpatient differences in key biochemical areas related to hepatotoxicity.

MATERIALS AND METHODS

Simulation platform. DILIsym version 6A was used to conduct the simulations in this article. DILIsym (http://www.dilisym.com, last accessed February 11, 2020) is a mathematical representation of DILI. DILIsym has been described previously, and many of the underlying equations have been made available in prior publications (Battista *et al.*, 2018; Bhattacharya *et al.*, 2012; Longo *et al.*, 2017, 2019; Shoda *et al.*, 2017, 2014; Woodhead *et al.*, 2012, 2019; Yang *et al.*, 2017). Briefly, DILIsym consists of several smaller submodels that are mathematically integrated to simulate an organism-level response. This work utilized submodels representing drug distribution, mitochondrial dysfunction and toxicity, bile acid physiology and pathophysiology, hepatocyte life cycle, and liver injury biomarkers.

SimPops. SimPops are a collection of simulated individuals including parameter variability that reflects anthropometric and biochemical ranges. This study utilized an $n = 285$ normal healthy volunteer SimPops (Human_ROS_apop_mito_BA_v4A_1 SimPops) included in DILIsym v6A. This SimPops represents variability in parameters related to bile acid homeostasis, mitochondrial function, oxidative stress, apoptosis, and regeneration. A list of parameters varied in the human v4A-1 SimPops, as well as the sources used in the construction of the SimPops are shown in Supplementary Table S1.

SimCohorts. SimCohorts are relatively small populations consisting of a subset of simulated individuals from existing SimPops in DILIsym. This work employed the human SimCohorts v4A-1-Multi16, which includes the baseline human as well as 15 individuals from the $n = 285$ human SimPops v4A-1. SimCohorts are computationally less expensive than the larger SimPops. For example, the simulation time required for simulations performed in the $n = 16$ SimCohorts for this study was approximately an order of magnitude lower than the time required for simulations performed in the $n = 285$ SimPops.

Development of a physiologically based pharmacokinetic model. A physiologically based pharmacokinetic (PBPK) representation of riluzole was constructed within DILIsym to describe liver exposure upon conventional oral tablet and sublingual administration. The DILIsym PBPK model framework used for riluzole consists of compartments for liver, blood, muscle, gut, and other tissues. The structure of the DILIsym PBPK submodel has been discussed in detail elsewhere (Howell *et al.*, 2012; Woodhead *et al.*, 2012, 2014). Riluzole metabolism was

Table 1. Parameters Used in the DILIsym PBPK Submodel for Riluzole

Parameter	Unit	Value	Source
Riluzole blood to plasma	Dimensionless	1.1	Optimization[a]
Riluzole gut to blood	Dimensionless	0.86	Optimization[a]
Riluzole liver to blood	Dimensionless	2.0	Optimization[a]
Riluzole muscle to blood	Dimensionless	0.3	Optimization[a]
Riluzole other tissue to blood	Dimensionless	6.16	Optimization[a]
Riluzole fraction unbound plasma	Dimensionless	0.04	96% protein-binding reported (BHV-0223 IB)
Riluzole molecular weight	g/mol	234.2	BHV-0223 IB
Riluzole renal clearance	ml/h/kĝ0.75	371.9	Calculated from renal clearance reported in Le Liboux et al. (1997) ($<$ 6 ml/min); validated with reported urinary excretion of unchanged parent
Riluzole gastric emptying rate, $k(ge)$	1/h	1.7	Optimization[a]
Riluzole absorption rate, $k(ab)$	1/h	9.99	Optimization[a]
Riluzole rate of elimination in feces	1/h	0.556	Set the first-order constant for gut absorption and fecal elimination from gut 9:1 (assuming approximately 90% absorption in humans)
Riluzole time for IV dose to become well-mixed in blood, $k(IV)$	1/h	6	Optimization[a]
K_m (Riluzole metabolite A)[b]	µmol/l	50	Optimization[a]
V_{max} (Riluzole metabolite A)[b]	nmol/h/kg^0.75	2 500 000	Optimization[a]

[a]Optimized using plasma riluzole concentrations reported in Le Liboux et al. (1997).
[b]Metabolite A represents sink pathway.

represented by 1 metabolic pathway, representing the aggregate of all riluzole metabolic pathways. Riluzole metabolites were assumed not to contribute to liver toxicity (due to the lack of any *in vitro* hepatotoxicity data for riluzole metabolites), and thus were not tracked. The tissue distribution of riluzole was assumed to be perfusion-limited and was represented by partition coefficients. Parameters used in the PBPK submodel for riluzole are shown in Table 1. Details of the PBPK model are provided in Supplementary A.

The PBPK representation for riluzole was based on available data for BHV-0223 and published studies of riluzole. Specifically, data on plasma riluzole exposure from a published PK study of riluzole (single 50 mg IV dose and single 100 mg oral tablet dose in healthy volunteers, Le Liboux et al., 1997) were used to optimize the PBPK model parameters (Table 1). The PBPK model was evaluated against clinical data from a completed phase 1 trial and previously published trials in healthy volunteers (Chandu et al., 2010; Le Liboux et al., 1997), including the PK study of ascending doses of riluzole (25, 50, or 100 mg dose BID).

Riluzole oral tablet PBPK parameters were used as a starting point for the representation of sublingual riluzole (BHV-0223) in DILIsym. For compounds administered sublingually, a portion of the dose is absorbed from the oral mucosa and a portion is swallowed and passes through the GI tract (Bartlett and van der Voort Maarschalk, 2012; Xia et al., 2015). Because the fraction swallowed for BHV-0223 is unknown, BHV-0223 PK data (ie, plasma riluzole concentrations after a single 35 mg BHV-0223 sublingual riluzole dose) were used to estimate the portion of sublingual riluzole that is absorbed from the oral mucosa and the portion that passes through the GI tract. Simulations were conducted assuming either 0%, 25%, or 50% of the sublingual dose is absorbed via the oral mucosa, and simulated plasma riluzole concentrations after a single 35 mg sublingual dose were compared with measured concentrations after a single 35 mg BHV-0223 dose.

In vitro mechanistic hepatotoxicity assays. Riluzole was assessed in *in vitro* assays for the 3 main hepatotoxicity mechanisms

represented in DILIsym: mitochondrial dysfunction, oxidative stress, and bile acid transporter inhibition. To assess potential mitochondrial dysfunction signals for riluzole, cellular respiration assays were conducted using a Seahorse XFe96 Flux Analyzer in HepG2 cells incubated with various concentrations of riluzole for 1 or 24 h. The potential for riluzole to induce oxidative stress was assessed by high content screening using a fluorescent probe, dihydroethidium (DHE), in HepG2 cells incubated with various concentrations of riluzole for 6 or 24 h. In these whole cell-based assays, intracellular concentrations of riluzole were determined by LC/MS/MS analysis in parallel HepG2 cultures. Inhibitory effects of riluzole for bile acid transporters were assessed experimentally using membrane vesicles overexpressing a bile acid efflux transporter (ie, BSEP, MRP3, or MRP4) and CHO cells overexpressing NTCP. Detailed experimental methods are described in Supplementary B. Mitochondrial dysfunction and oxidative stress assays were performed by Cyprotex, Inc (Macclesfield, UK). Transporter inhibition assays were performed by Solvo Biotechnology (Budaors, Hungary).

Translation into DILIsym parameters. For each of the *in vitro* assays conducted, the results were translated into DILIsym parameters for use in the simulations. For the bile acid transporter parameters, the estimated IC_{50} values for riluzole were used directly as the inhibition constants in DILIsym. Mode of inhibition was assumed to be mixed inhibition with $\alpha = 5$. Although competitive and noncompetitive inhibition types may result in low and high extremes of potential bile acid accumulation, respectively, mixed inhibition with $\alpha = 5$ leads to a median impact on bile acid accumulation. In addition, mixed inhibitors are more common compared with pure competitive or noncompetitive inhibitors (Howell et al., 2016; Longo et al., 2019; Watkins, 2019; Woodhead et al., 2014, 2017). For riluzole-mediated mitochondrial dysfunction, the assay results comparing intracellular concentrations and oxygen consumption rate (OCR) were recapitulated in MITOsym; the resulting parameters were translated into DILIsym parameters using translation factors involving exemplar compounds, a process which has been reported elsewhere (Yang et al., 2015). For riluzole-mediated oxidative

Table 2. *In Vitro* Data, *In Silico* Calculations, and Rat Distribution Data Used as a Basis for the Default Liver K_b Value in the DILIsym Representation of Riluzole and for the Increased Liver K_b Value in DILIsym Used in the Sensitivity Analysis Simulations for Riluzole

	Liver:Blood Partition Coefficient (K_b)					L:B Value at 0.5 h After Dosing
	In vitro data HepG2 (seahorse media)[a]	*In vitro* data HepG2 (HepG2 media)[a]	*In vitro* data human HC (HC media)[a]	*In vitro* data human HC (HC media)[b]	*In silico* calculations (Rodgers and Rowland)[c]	
Measured or calculated	9.8	1.7	6.5	35	0.6–2	4.4 (in rats)
Default DILIsym value			2			7.3 (ie, simulated value with $K_b = 2$ in DILIsym)
Increased DILIsym value for sensitivity analyses			10			43 (ie, simulated value with $K_b = 10$ in DILIsym)

Media concentrations were either nominal[a] or measured[b] depending on the system.
[c]*In silico* calculations were based on physiochemical properties including logP. A range of values is listed for the *in silico* calculations, because a range of logP values were identified for riluzole (drugbank).
Abbreviation: HC, hepatocyte.

Table 3. Simulated Frequency of ALT Elevations in the v4A-1 SimPops Administered Riluzole

Riluzole Dose and Duration	DILIsym Parameter Settings	Simulated Peak ALT > 3× ULN[a]	Simulated Peak ALT > 5× ULN[a]
Conventional oral tablets 50 mg BID for 12 weeks	Median PK, liver K_b 2	0/285	0/285
	High PK, liver K_b 2	0/285	0/285
	Median PK, liver K_b 10	0/285	0/285
	High PK, liver K_b 10	11/285	3/285
Sublingual 40 mg BID for 12 weeks	Median PK, liver K_b 2	0/285	0/285
	High PK, liver K_b 2	0/285	0/285
	Median PK, liver K_b 10	0/285	0/285
	High PK, liver K_b 10	4/285	2/285

[a]ULN in DILIsym is 40 U/l.

stress, the assay results were reproduced using DILIsym by mimicking *in vitro* conditions; appropriate parameter values for the oxidative stress effects were identified by comparing simulation results with the measured data. Further details on the translation of the experimental data into DILIsym parameters are provided in Supplementary B.

Simulations conducted. DILIsym v6A was used to perform simulations for comparison of oral tablet and sublingual riluzole. The clinical protocols simulated were as follows:

- BHV-0223 (sublingual riluzole) protocol: SL 40 mg BID (taken 12 h apart) for 12 weeks
- Riluzole oral tablet protocol: PO 50 mg BID (taken 12 h apart) for 12 weeks

For both sublingual and oral riluzole tablet clinical protocols, the following simulation types were run:

- SimPops simulations: These simulations were conducted in the $n = 285$ human v4A_1 SimPops described above.
- Sensitivity analysis simulations: Sensitivity analyses were performed to explore the hepatotoxic potential of riluzole under alternate plausible scenarios. The simulation response evaluated in the sensitivity analyses was the simulated frequency of alanine transaminase (ALT) elevations greater than 3× ULN and the simulated frequency of ALT elevations greater than 5× ULN in the human v4A-1 SimPops. Simulations were performed with a "High PK" parameterization to represent individuals with high plasma riluzole exposure. In the "High PK" parameter set, the

metabolism V_{max} parameter value was decreased by approximately 4-fold. Mechanistically, a decrease in riluzole metabolism may contribute to higher plasma riluzole exposure. The "High PK" exposure levels were based on the variability observed in the completed BHV-223 phase 1 study and were consistent with exposures approximately 1 standard deviation above the median exposure level.

- Simulations were also performed to explore the sensitivity to an increase in the value of the riluzole liver to blood partition coefficient (liver K_b), given uncertainty in the liver K_b value. A liver K_b value of 2 was chosen as the default value based on available *in vitro* data, *in silico* calculations, and rat distribution data (Table 2). Simulations were also performed with an increased liver K_b value of 10, which was also within the range of the available data (Table 2).
- The different scenarios simulated (ie, High PK or Median PK with either liver K_b value of 2 or liver K_b value of 10 for either conventional oral riluzole tablets or sublingual riluzole) are listed in Table 3.

- Mechanistic investigation simulations: These simulations were conducted using the human SimCohorts v4A-1-Multi16. The 50 mg BID oral tablet dosing protocol was simulated in the SimCohorts v4A-1-Multi16 with sequential omission of 1 potential mechanistic contributor to toxicity: bile acid transporter inhibition effects, mitochondrial dysfunction effects, or oxidative stress effects. These mechanistic investigation simulations evaluated the contribution of each toxicity element to the overall simulated toxicity.
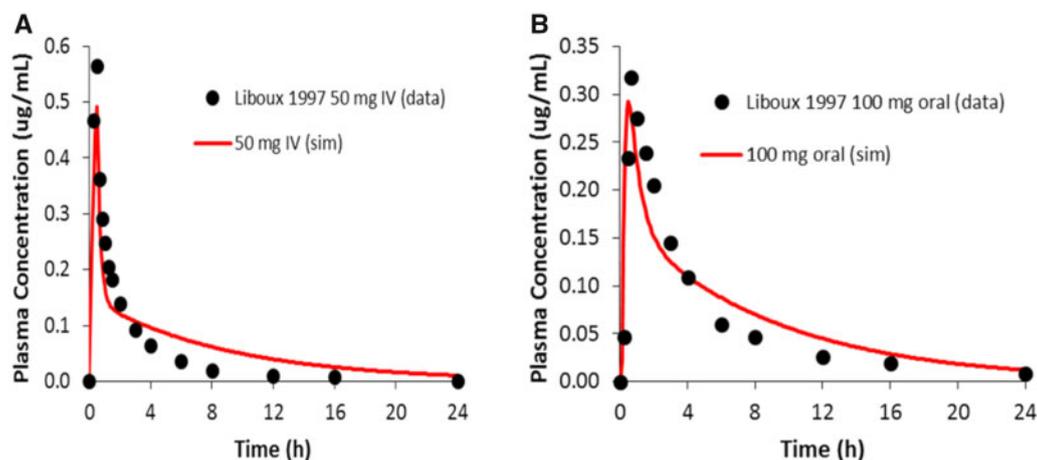
Figure 2. Simulated (lines) and observed (symbols) plasma concentrations of riluzole following a single 50 mg IV dose (A) and following a single 100 mg conventional oral tablet dose (B). Observed data are from Le Liboux *et al.* (1997). Lines represent simulated plasma concentrations in the baseline human.
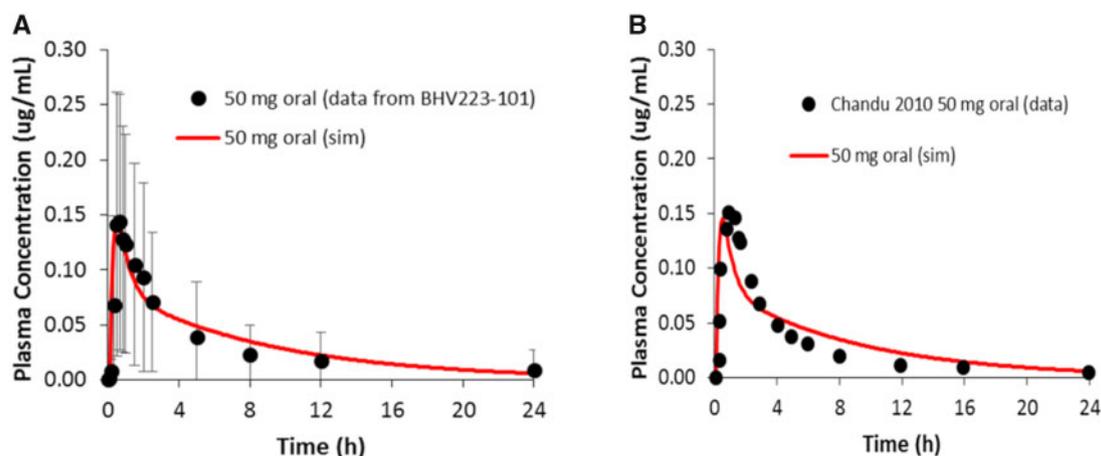

Figure 3. Simulated (lines) and observed (symbols) plasma concentrations of riluzole after a single 50 mg conventional oral tablet dose for observed data from the phase 1 study of BHV-0223 (BHV223-101) (training data) (A) and data reported in Chandu *et al.* (2010) (validation data) (B). Lines represent simulated plasma concentrations in the baseline human. Symbols and error bars represent mean and SD of the observed plasma concentrations.

RESULTS

PBPK Modeling

Simulation results from the PBPK representation of riluzole oral tablet and clinical plasma riluzole exposure data used for optimization of the PBPK model are shown in Figure 2. The simulated plasma area under curve (AUC) and plasma C_{max} values were within 1.5-fold of those observed in clinical trials (Chandu *et al.*, 2010; Le Liboux *et al.*, 1997). The simulations reasonably captured the plasma pharmacokinetics of riluzole, based on comparisons with training data (Figure 3A) and comparisons with data that were not used in the optimization (validation data set; Figure 3B). Simulation results from both the median PK riluzole parameterization and the "High PK" riluzole parameterization, representing individuals with increased plasma riluzole exposure, compared with clinical data, are shown in Figure 4.

PK data (ie, plasma concentrations of BHV-0023 after a single 35 mg sublingual dose) were used to estimate the portion of sublingual riluzole that is absorbed via the oral mucosa and the portion that is swallowed and passes through the GI tract. (Note that data for the 35 mg sublingual dose was used, rather than the 40 mg approved dose for BHV-0223, due to the availability of

PK data for the 35 mg sublingual dose at the time of this study.) Simulated plasma concentrations after a 35 mg sublingual dose were conducted, assuming 0%, 25%, and 50% of the dose is absorbed via the oral mucosa, respectively. Simulations with 0% of the sublingual dose absorbed via the oral mucosa and 100% passed through the GI tract underestimated observed plasma concentrations following a single 35 mg sublingual dose. Simulated plasma concentrations with 25% of the dose being absorbed from the oral mucosa and 75% of the dose passing through the GI tract reasonably approximated observed plasma riluzole concentrations following a single 35 mg sublingual dose (Figure 5).

Simulated Plasma and Hepatic Exposure for Oral Tablet and Sublingual Riluzole

Simulated plasma concentrations and simulated liver concentrations for a single 50 mg oral tablet dose of riluzole and for a single 40 mg sublingual dose (ie, the sublingual dose corresponding to Zydis formulation) are shown in Figure 6. Notably, whereas plasma concentrations for the 2 dosing regimens (40 mg sublingual vs 50 mg oral tablet) are quite similar (ratio of 0.97 for predicted plasma AUC following 40 mg sublingual vs 50 mg oral tablet), the predicted hepatic exposure for the 40 mg
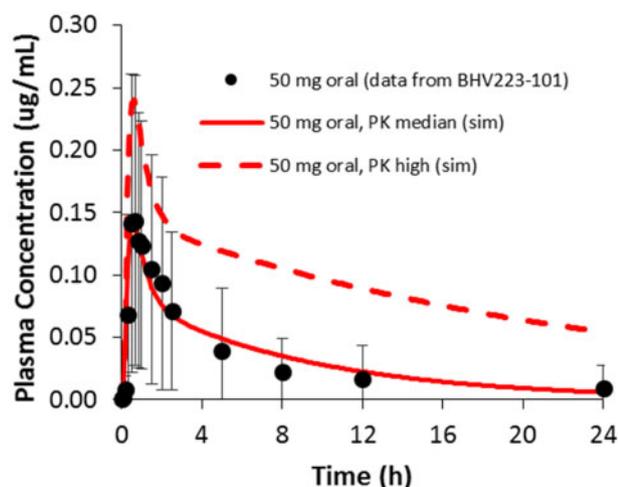
Figure 4. Simulated (lines) and observed (symbols) plasma concentrations of riluzole after a single conventional 50 mg oral tablet dose for observed data from the phase 1 study of BHV-0223 (BHV223-101) ($n = 9$ subjects). The solid line represents simulated plasma concentrations in the baseline human with the Median PK riluzole parameterization. The dashed line represents simulated plasma concentrations in the baseline human with the High PK riluzole parameterization. Symbols and error bars represent mean and SD of the observed plasma concentrations.
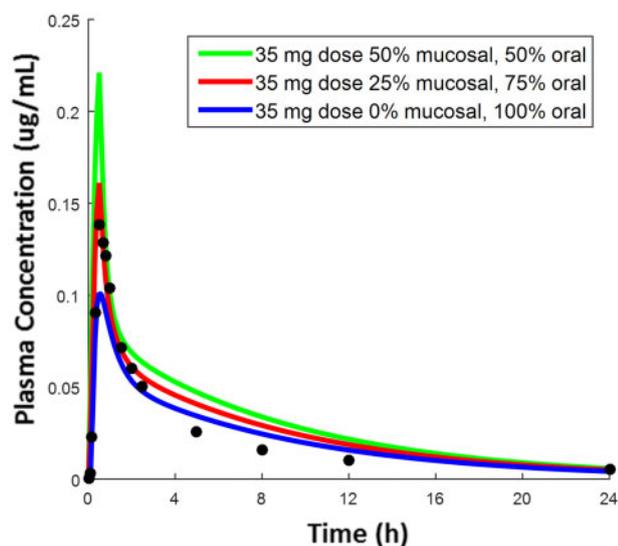

Figure 5. Simulated (lines) and observed (symbols) plasma concentrations of riluzole after a single 35 mg sublingual dose. The solid line represents simulated plasma concentrations under 3 different assumptions: 50% of the sublingual dose absorbed through the oral mucosa and 50% passing through the GI tract (green), 25% of the sublingual dose absorbed through the oral mucosa and 75% passing through the GI tract (red), or 100% of the sublingual dose passing through the GI tract (blue).

sublingual dose is lower than the predicted hepatic exposure for the 50 mg oral tablet dose (ratio of 0.80 for predicted liver AUC following 40 mg sublingual vs 50 mg oral tablet).

In Vitro *Mitochondrial Toxicity Assay Results*
In the mitochondrial respiration assay, riluzole decreased the OCR in a concentration-dependent manner after 1 and 24 h incubation. These data suggest that riluzole is a mitochondrial electron transport chain (ETC) inhibitor. To define the DILIsym parameters for riluzole-mediated mitochondrial ETC inhibition,
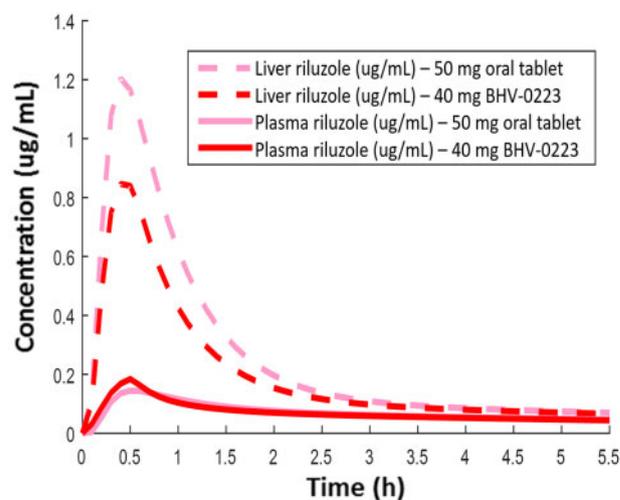

Figure 6. Simulated plasma concentrations (solid lines) and simulated liver concentrations (dashed lines) in the baseline human following a single 50 mg conventional oral tablet dose of riluzole (pink) or following a single 40 mg sublingual dose of BHV-0223 (red).

the 1 h *in vitro* data were simulated within MITOsym and subsequently translated into DILIsym values as described in the Materials and Methods section. The optimized ETC inhibition parameter value listed in Table 4 reasonably recapitulated riluzole effects on the OCR as shown in Figure 7.

In Vitro *Oxidative Stress Assay Results*
Riluzole increased reactive nitrogen and oxygen species (RNS/ROS) in a concentration-dependent manner after 24 h incubation, but not following 6 h incubation (Figure 8; 6 h data not shown). These data suggest that riluzole can elicit oxidative stress. DILIsym parameters for riluzole-induced production of RNS/ROS were optimized to recapitulate intracellular concentrations versus cellular RNS/ROS data by simulating *in vitro*-like conditions within DILIsym (Figure 8, Table 4).

In Vitro *Bile Acid Transporter Inhibition Assay Results*
Experimental data indicated that riluzole inhibited BSEP and MRP4; riluzole had no effect on MRP3 or NTCP. Inhibition constants for riluzole are presented in Table 4.

Toxicity Investigations
Clinical dosage regimens of riluzole oral tablet (50 mg BID) and sublingual riluzole (40 mg BID) were simulated for 12 weeks in the v4A_1 SimPops. In the SimPops simulations, no ALT elevations > 3× ULN were predicted for either dosing protocol (oral tablet or sublingual) with Median PK and high or default liver exposure assumptions (Table 3). In the simulations with High PK and high liver exposure (liver K_b 10), the predicted incidence of ALT elevations was higher for oral tablet dosing (11 of 285 individuals) versus sublingual dosing (4 of 285 individuals).

Mechanistic Investigation Simulations were conducted to evaluate the contributions of each hepatotoxicity mechanism (ie, riluzole-mediated mitochondrial ETC inhibition, riluzole-mediated bile acid transport inhibition, and riluzole-mediated ROS) to the simulated ALT elevations, as described in the Materials and Methods section. To investigate the importance of each mechanism to predicted hepatotoxicity, the oral tablet dosing protocol with the High PK scenario and high liver K_b (ie, 10) was simulated in SimCohorts (v4A-1-Multi16) with sequential omission of 1 potential mechanistic contributor at a time.

Table 4. Toxicity Parameter Values Utilized in the Riluzole Simulations in DILIsym v6A

Mechanism	DILIsym Parameter Name	Unit	Value[b]
Mitochondrial dysfunction	Coefficient for ETC inhibition	μM	382
Oxidative stress	RNS/ROS production rate constant	ml/nmol/h	6×10^{-4}
Bile acid transporter inhibition	BSEP inhibition constant[a]	μM	200
	NTCP inhibition constant[a]	μM	No inhibition
	Inhibition constant for basolateral efflux[a]	μM	125

[a]IC_{50} values; default assumption is mixed inhibition type with $\alpha = 5$, based on the experience of the DSS team. For basolateral efflux, the more potent value between MRP3 and MRP4 was employed as a conservative approach.
[b]Values shown in the table for DILIsym input parameters should not be interpreted in isolation with respect to clinical implications, but rather, should be combined with exposure in DILIsym to produce simulations that have predictive and insightful value.
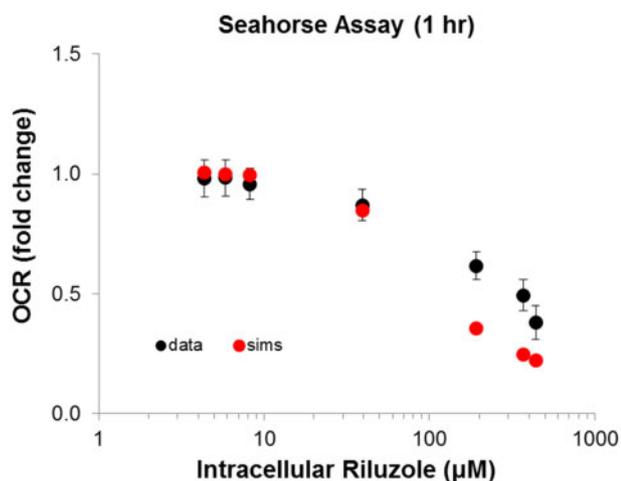

Figure 7. Comparison of simulation results and *in vitro* assay data to identify DILIsym parameter values that reproduce the riluzole intracellular concentration-mediated OCR response at 1 h.
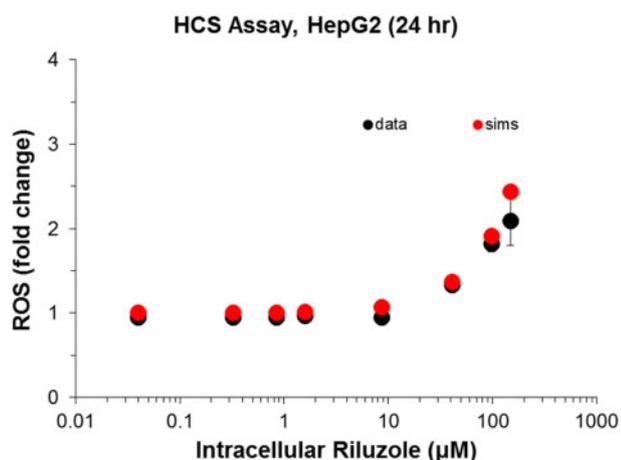

Figure 8. Comparison of simulation results and *in vitro* assay data to identify DILIsym parameter values that reproduce the riluzole intracellular concentration-mediated ROS response at 24 h.

With removal of the ROS mechanism, no ALT elevations were predicted, indicating the oxidative stress is required for simulated ALT elevations (Table 5). In contrast, there was no impact on the incidence of ALT elevations with removal of either mitochondrial ETC inhibition or removal of bile acid transport inhibition, indicating that these 2 mechanisms are not required for

simulated ALT elevations (Table 5). These results demonstrate that the primary driver of ALT elevations in the DILIsym riluzole simulations is oxidative stress.

DISCUSSION

Riluzole is a medication that has been developed to treat ALS. BHV-0223 is a novel 40 mg sublingually dissolving Zydis formulation of riluzole that is bioequivalent to the riluzole 50 mg oral tablet formulation. Because of its sublingual route of administration, BHV-0223 first-pass metabolism with BHV-0223 is mitigated, achieving adequate drug concentrations with reduced hepatic exposure. A sublingual formulation of riluzole may be particularly beneficial to ALS patients, because these patients often have difficulty swallowing.

DILIsym is a QST model of DILI that can be applied to predict hepatotoxicity based on *in vitro* mechanistic data and *in vivo* clinical data and can provide insight into the underlying mechanisms responsible for DILI. DILIsym analyses performed in this study support the advantage of the sublingual administration of riluzole.

Specifically, PBPK representations of riluzole oral tablet and sublingual riluzole (BHV-0223) developed in DILIsym predicted similar plasma concentrations following a single 50 mg oral tablet dose of riluzole compared with a single 40 mg sublingual dose of riluzole. While plasma concentrations for the 2 dosing regimens were similar, the predicted hepatic exposure for the 40 mg sublingual dose was lower than the predicted hepatic exposure for the 50 mg oral tablet dose, (Figure 6), consistent with a subsequent study demonstrating that 40 mg BHV-0223 administered sublingually is bioequivalent to a 50 mg dose of a conventional riluzole oral tablet.

To compare the liver toxicity potential of the oral tablet and sublingual formulations of riluzole, DILIsym simulations were performed for both sublingual (40 mg BID sublingual riluzole) and oral tablet (50 mg BID PO riluzole) clinical protocols in a simulated population (SimPops) that includes variability in parameters relevant to hepatotoxicity mechanisms. Simulations were performed with Median PK and with a "High PK" parameterization (Figure 5) to represent individuals with high plasma riluzole exposure. In addition, given uncertainty in the liver to blood partition coefficient (liver K_b) value for riluzole, simulations were performed with either a liver K_b value of 2 (default value) or an increased liver K_b value of 10.

No ALT elevations > 3× ULN were predicted in the SimPops for either dosing protocol (oral tablet or sublingual) with Median PK and high or default liver exposure assumptions (Table 3). For simulations with High PK and high liver exposure (liver K_b value of 10), the predicted incidence of ALT elevations was higher for

Table 5. Mechanistic Investigation Simulation Results for SimCohorts Administered Riluzole

Riluzole Dose and Duration	DILIsym Parameter Settings	Mechanisms	Simulated Peak ALT > 3× ULN[a]	Simulated Peak ALT > 5× ULN[a]
Conventional oral tablets 50 mg BID for 12 weeks	High PK, liver K_b 10	All	3/16	1/16
		No ROS	0/16	0/16
		No mitochondrial toxicity	3/16	1/16
		No bile acid transport inhibition	3/16	1/16

[a]ULN in DILIsym is 40 U/l.

oral tablet dosing (11 of 285 individuals) versus sublingual dosing (4 of 285 individuals). This represents a relative risk reduction of 64% associated with sublingual administration of riluzole versus conventional riluzole tablets.

The low incidence of ALT elevations (ie, 11/285 or 4% of simulated individuals) in the SimPops simulations with High PK and high liver exposure for the riluzole oral tablet dosing protocol (Table 3) approximates the low incidence of ALT elevations reported for ALS patients treated with riluzole. Serum ALT elevations > 3× ULN have been observed in 10%–15% of patients with ALS taking riluzole (Bensimon and Doble, 2004). The slight underprediction of the incidence of ALT elevations may be due to the use of a normal healthy volunteer SimPops which does not capture any potential hepatic dysfunction in ALS patients prior to treatment. A high incidence of mild liver dysfunction in ALS patients has been reported (based on the evaluation of liver function tests in 37 ALS patients) (Nakano et al., 1987).

Mechanistic investigation simulations were performed to assess the contributions of each hepatotoxicity mechanism (ie, riluzole-mediated mitochondrial ETC inhibition, riluzole-mediated bile acid transport inhibition, and riluzole-mediated oxidative stress). The oral tablet dosing protocol with High PK and liver K_b value of 10 was simulated in an $n = 16$ SimCohorts with sequential removal of 1 potential hepatotoxicity mechanism at a time. With omission of the ROS mechanism, no ALT elevations were predicted, demonstrating that riluzole-mediated oxidative stress is required for simulated ALT elevations (Table 5). In contrast, removal of either riluzole-mediated mitochondrial ETC inhibition or removal of riluzole-mediated bile acid transport inhibition did not impact the incidence of ALT elevations (Table 5). These results indicate that oxidative stress is the primary driver of ALT elevations in the DILIsym riluzole simulations.

As noted above, 1 limitation of this study is that the normal healthy volunteer SimPops utilized for this study does not capture any disease characteristics of ALT which may contribute to susceptibility to liver injury. An additional limitation of this study is that, as described above, this study utilized a relatively simple approach (ie, median PK vs High PK parameterization) to assess the potential impact of pharmacokinetic variation. However, whereas the full range of potential riluzole exposure levels was not simulated, the approach utilized for this study accomplished the objective of assessing the sensitivity of the hepatotoxic response to different exposure levels.

In conclusion, using DILIsym to integrate clinical and mechanistic in vitro toxicity data for riluzole allowed for a quantitative comparison of the liver toxicity potential of the oral tablet and sublingual formulations of riluzole. DILIsym analyses suggest that sublingual BHV-0223 has reduced hepatic exposure and, consequently, less risk of liver toxicity compared with riluzole oral tablets.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

DECLARATION OF CONFLICTING INTERESTS

D.M.L., L.K.M.S., and B.A.H. were compensated employees of DILIsym Services (A Simulations Plus Company), and V.C., R.M.B., and I.A.Q. were compensated employees of Biohaven Pharmaceuticals when this research was conducted.

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