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DILIsym: Quantitative systems toxicology impacting drug development

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Abstract

DILIsym®, a quantitative systems toxicology model developed over the last decade by the drug-induced liver injury (DILI)-sim Initiative, has provided novel insights regarding mechanisms underlying drug-induced liver injury and why animal models sometimes fail to accurately assess the liver safety liability of new drug candidates. For example, DILIsym, but not routine preclinical testing, predicted the human hepatotoxicity of the migraine drugs telcagepant and MK3207 that terminated their clinical development. DILIsym also predicted that the next in-class drug, ubrogepant, would be relatively safe for the liver; this prediction was prospectively confirmed in phase-3 clinical trials leading to FDA approval without liver safety warnings. DILIsym also identifies mechanisms underlying liver toxicity, and this information can identify patient-specific risk factors for drug-induced liver injury including drug–drug interactions and certain disease states, improving risk management and pharmacovigilance. DILIsym provides an example of how increased application of quantitative systems toxicology modeling should lead to more efficient development of new drugs.

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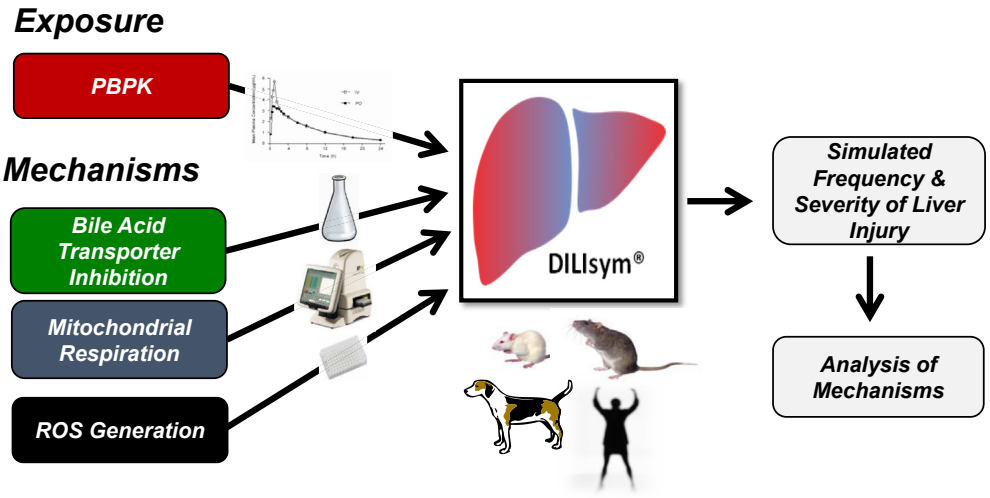
QST, DILI, Hepatoxicity, Modeling, Simulation.

Introduction

Hepatotoxicity remains a major adverse event that limits drug development [1]. Current nonclinical methods to identify liver safety liabilities in new drug candidates are not completely successful, and advanced clinical development programs continue to encounter unexpected liver toxicity with serious regulatory implications [2]. The drug-induced liver injury (DILI)-sim Initiative is a public–private partnership that is applying quantitative systems toxicology to understand and predict liver safety liability in new drug candidates [3]. Over the last decade, the Initiative has involved scientists from academia, the FDA, and 19 major pharmaceutical companies and produced successive versions of software termed DILIsym®. Liver pathways generally recognized to underlie hepatotoxicity [4,5] have been represented by differential equations in submodels, including oxidative stress, interference in mitochondrial function, and accumulation of bile acids due to inhibition of efflux transporters. The submodels in DILIsym are linked together such that the aggregate effect of a drug or metabolite on each pathway can contribute to hepatocyte death resulting in the predicted time-dependent release of serum biomarkers into circulation (e.g. alanine aminotransferase [ALT]). Regeneration of hepatocytes is built into DILIsym and net liver function (viable hepatocyte mass) is reflected in the predicted level of serum bilirubin. The quantitative ability of a drug or metabolite to affect each submodel pathway (such as half maximal inhibitory concentration (IC50) for expressed efflux transporters) can be assessed in the laboratory, and the results entered into DILIsym together with estimates of drug/metabolite exposure outside and within the hepatocyte based on selected dosing regimens (Figure 1).

Parameters in DILIsym have been varied to create simulated patient populations (SimPops®) reflecting genetic and nongenetic factors that can potentially account for interpatient heterogeneity in susceptibility to DILI. The successive DILIsym versions have been developed by adjusting the model parameters to reproduce the known incidence and severity of liver toxicity observed with multiple ‘exemplar’ drugs. The needed data, which are often provided by the Initiative partners, are for drugs with or without preclinical liver safety signals and with or without liver safety signals in the clinic. Although the human DILIsym model is most developed, parameters have been changed in the model to create virtual populations of rats or dogs to examine species differences in susceptibility of DILI. More detailed description of the Initiative and DILIsym model can be found the manuscripts by Howell et al. [3] and Watkins et al. [6] were reviews not studies.

Figure 1



Using DILIsym. Hepatic concentrations of drug/metabolite resulting from a specified dosing regimen are estimated using physiologically based pharmacokinetic modeling and other available data (e.g. preclinical tissue distribution data and the role of transporters). Next assessed is the concentration-dependent ability of the drug/metabolite to 1) inhibit bile acid transporters and thereby raise hepatocyte bile acid concentration, 2) inhibit mitochondrial respiration, and 3) cause oxidative stress (reactive oxygen species [ROS] generation). The model will then predict the time-dependent death of hepatocytes, and hence the time-dependent release and level of certain biomarkers into blood, first in a baseline (normal) simulated patient, and then in a simulated patient population where variables in DILIsym have been varied to account for interpatient heterogeneity. Anticipated or observed variation in pharmacokinetics can also be incorporated. Typical outputs from the model are time-dependent serum levels of alanine aminotransferase (ALT) reflecting rate of hepatocyte death and serum bilirubin reflecting global liver function. Modifications in DILIsym provide modeling predictions in rat and dog. More detailed discussion of DILIsym input data and data outputs is available [32].

As covered in a recent review [7], the DILI-sim Initiative has provided many novel insights into mechanisms underlying DILI (some of which are summarized in Table 1).

DILIsym applications in drug development

A frequent application of DILIsym has been after treatment emergent elevations in serum ALT have been observed in a clinical trial of a new molecular entity. The first question is whether ALT elevations (generally > 3 X upper limits of normal) are predicted with the new molecular entity when the process outlined in Figure 1 is undertaken. In the growing validation cohort, DILIsym has correctly predicted the liver safety liability of 80% of the predicted cases (out of around 70 drugs predicted) (Brett Howell, personal communication February, 2020 based on the DILIsym Performance Review, which is compiled for the DILI-sim members each year).

Improving nonclinical assessment of liver safety

DILIsym has also been applied to understand why animal models sometimes fail to predict hepatotoxicity in humans. It is well known that species differences in drug metabolism exist and this can be modeled in DILIsym if the relevant metabolites have undergone the process outlined in Figure 1. DILIsym modeling has also provided additional explanations for species differences in susceptibility. The rat profile of bile acids is inherently less toxic than is the case in humans [8]. Just on the basis of drug effects on bile acid homeostasis, DILIsym has been able to account for liver safety liability observed in humans despite clean rat studies [6,9–12]. In addition, a recent study indicated that rats were more sensitive to inhibition of mitochondrial respiration from a chemokine receptor antagonist, and that this contributed liver toxicity was observed in rats but not in humans [13]. These observations support the idea that DILIsym modeling may improve lead candidate selection even when hepatotoxicity is observed in preclinical species.

Identifying DILI potential of next in class

DILIsym has been increasingly used to compare liver safety across members of the same class — particularly when others in class have had liver safety concerns. DILIsym has successfully predicted the relative liver safety profile of pairs of drugs in the same class when their liver safety profiles have been discordant (e.g. tolcapone vs entacapone [14] and troglitazone vs pioglitazone [10]). In these cases, the predictions were retrospective as the discordant liver safety profiles were already established.

An exciting recent development has been DILIsym's successful prospective prediction of the liver safety of ubrogepant, a small molecule antagonist of calcitonin

gene-related peptide. Merck developed the first in-class drug, telcagepant, but had to abandon it late in phase-three clinical trials when potentially serious liver toxicity was observed. Merck's next in class molecule, MK-3207, was also abandoned during clinical development because of liver safety concerns. Ubrogepant was Merck's third in class molecule, and this was acquired by Allergan before any human studies had been undertaken. All three molecules did not have significant liver safety alerts in traditional preclinical studies. Both telcagepant and MK-3207 were modeled in DILIsym, and significant liver safety liability was correctly predicted for both compounds [15]. Ubrogepant was then modeled in DILIsym, and no simulated patients experienced elevations in serum ALT >3 X upper limits of normal (ULN), even at dosing exposures 10-fold anticipated to be necessary to achieve efficacy [15]. In the clinical trials of ubrogepant, there was no difference in incidence of serum ALT elevations >3 X ULN between those receiving active drug and those receiving placebo [16], as had been prospectively predicted by the DILIsym. At the end of December 2019, the FDA approved sale of ubrogepant without any liver safety warnings (https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-adults-migraine).

There are now several clinical trials being undertaken with new drug candidates predicted by DILIsym to have an improved liver safety profile over others in their class. For example, the only approved treatment for autosomal dominant polycystic kidney disease (ADPKD) , tolvaptan, was successfully predicted by DILIsym to be hepatotoxic [9] whereas a next in class drug, lixivaptan, has been predicted by DILIsym to be safe for the liver in the dosing proposed for this population [17]. A clinical trial of lixivaptan in ADPKD patients is now underway.

Identification of patient risk factors

When DILIsym is successful in predicting the observed elevations in serum ALT, it is possible to examine the subset of simulated individuals who experienced the hepatotoxicity to determine which specific variables in DILIsym contributed to this sensitivity. This approach with troglitazone DILI suggested a number of potentially important susceptibility factors, including inter-patient variation in the colonic microbiome [10]. A simpler approach to identifying risk factors is to determine which of the three toxicity mechanisms chiefly account for the predicted elevations. This can be easily assessed by turning off each of the three mechanisms in succession in DILIsym to determine the effect on the predicted incidence of ALT elevations. In some cases, identifying the dominant mechanism has revealed specific patient risk factors for DILI. For example, in clinical trials of two drugs in different therapeutic classes, it was noted that patients concomitantly receiving treatment with metformin experienced an increased incidence of ALT elevations versus those patients not receiving metformin, and this was not due to a pharmacokinetic interaction. DILIsym modeling revealed that for both drugs, interference in mitochondrial respiration was the dominant mechanism accounting for the observed ALT elevations. Metformin then underwent DILIsym modeling confirming an effect on mitochondrial respiration. Interestingly, DILIsym did not predict that metformin would cause ALT elevations at usual therapeutic doses, and this is consistent with the excellent liver safety profile of this drug. However, modeling the combined effect of each drug administered together with metformin successfully predicted the observed increased incidence of ALT elevations (Brett Howell, personal communication, February 2020). For one of the drugs, continued drug development is planned adding metformin treatment as a protocol exclusion criterion. An important question was what other drugs in usual therapeutic doses might have a mitochondrial effect similar to that of metformin and should, therefore, also be excluded. Continued application of DILIsym should generate such a list.

It should also be noted that DILIsym may be useful in identifying susceptibility to DILI related to specific diseases. An example is the increased susceptibility to tolvaptan DILI observed in patients with ADPKD [18]. A mechanism underlying this increased susceptibility was suggested by the demonstration of reduced activity of the canalicular efflux transporter MRP2 in a rat model of ADPKD [19]. Additional studies demonstrated that the reduction in MRP2 expression was associated with decreased biliary secretion of tolvaptan and its major metabolite of tolvaptan, DN-4103 [20]. DILIsym successfully modeled liver toxicity due to tolvaptan in humans [9], and because altered bile acid homeostasis was a prominent underlying mechanism, rodents would not be a good model to study the liver toxicity of tolvaptan [8]. Instead, the available DILIsym model for tolvaptan [9], which included the parent drug and its major metabolite DM-4103, was used to assess the toxic outcome accompanying reduced biliary efflux. The modeling demonstrated that reduced efflux of DM-4103 results in increased hepatotoxicity but reduced efflux of parent tolvaptan had little effect [21]. The modeling therefore supported reduced biliary efflux of DM-4103, likely through reduced activity of MRP2, as a basis for increased DILI susceptibility to tolvaptan DILI in the ADPKD patients. Progression of disease in the patients with ADPKD resulting in progressive loss of biliary efflux of DM-1403 could therefore also account for the sudden development of DILI in patients treated with tolvaptan for more than one year [18] — an unusual observation for drugs causing DILI [2]. As more diseases are associated with altered expression of relevant liver transporters (e.g. fatty liver disease [22]), DILIsym is likely to be able to predict increases in DILI risk for certain drugs in these patient populations.

Table 1 Some mechanistic insights from the DILI-sim initiative.

	Insight	Comments	References
1).	Effects on just three processes account for the majority of dose-dependent DILI in patients	The data inputs in Figure 1 can predict ~80% of liver safety liabilities in a validation cohort of drugs.	[7]
2).	Dominant DILI mechanisms can vary among drugs that are closely related in structure	This was best shown for macrolide antibiotics	[33]
3).	Importance of bile acids in DILI	Bile acid accumulation has emerged as the most frequent contributor to DILI predictions	[7,11,29]
4).	Importance of mechanisms of BSEP inhibition	Although not typically assessed, mechanism of BSEP inhibition (competitive vesus noncompetitive) can have large effects on DILI potential	[11,29]
5).	Weak inhibition of BSEP can substantially contribute to DILI potential.	Although a recent consensus considered a BSEP IC50 > 25 uM as not a DILI risk factor, modeling has predicted a DILI risk contribution with IC 50 > 100 uM for some drugs (when one or both of the other mechanisms are involved).	[9,12,29]
6).	Species differences in DILI susceptibility	In addition to variation in toxic potential of bile acids, different effects on mitochondrial respiration can contribute	[6,9–11,13,15,29]
7).	DILIsym results may be relevant to prediction of delay idiosyncratic DILI	DILIsym has predicted DILI liability for troglitazone, tolcapone, TAK-875, and tolvaptan	[9,10,14,29]
8).	DILIsym can optimize interpretation of serum biomarkers	DILIsym provides estimates of hepatocyte loss and global liver function and has been used to refine interpretation of 'Hy's law cases'.	[27]
9).	Disease-associated changes in efflux transporter function could account increased susceptibility to DILI in patients	Alternations in biliary efflux of a major metabolite of tolvaptan (likely due to reduced MRP2 activity) could account for increased DILI susceptibility noted in patients with autosomal dominant polycystic kidney disease	[21]

DILI, drug-induced liver injury; BSEP, bile salt excretory protein.

Optimizing clinical trial protocols and biomarker interpretation

If DILIsym predicts ALT elevations for a new drug candidate, it is also possible to use DILIsym to vary the exposure parameters to predict dosing regimens to reduce or even eliminate serum ALT elevations [23]. If elimination of ALT elevations is not achieved at doses needed to be effective, the model can predict the frequency of liver chemistry monitoring and appropriate stopping criteria based on the ALT value to avoid serious liver injury. The use of DILIsym has been applied in this way to drugs in development [24].

DILIsym predicts the time-dependent death of hepatocytes reflected in the rise in serum of ALT and, by also predicting the rate of hepatocyte regeneration, the net functioning hepatocyte mass over time as reflected in the serum bilirubin level. If serial assessments of serum ALT are available in an actual patient experiencing DILI, it is possible to use DILIsym 'in reverse' to predict liver function based on the viable hepatocyte mass at any time point. Such modeling has pointed out that the peak serum ALT value, which is typically used as cutoffs for action in clinical trials or in product labeling, may be misleading in terms of degree of liver injury [25]. In several cases [26,27], DILIsym modeling has resulted in reinterpretation of liver events observed in clinical trials that fit the regulatory definition of a Hy's law case (i.e. hepatocellular injury due to study drug and serum ALT and total bilirubin rising above 3 and 2 X upper limits of normal, respectively [2]). DILIsym also incorporates newer biomarkers, such as the cytokeratin 18 and its caspase cleaved fragment enabling estimation of the relative apoptosis versus necrosis, which can affect the relationship between serum ALT and predicted percent hepatocyte loss [26]. DILIsym can also predict elevations in direct and/or indirect bilirubin due to drug/metabolite inhibition of bilirubin transporters and/or inhibition of UGT1A1 [28]. The use of DILIsym in biomarker interpretation has been recently reviewed [27].

Future applications of DILIsym

DILIsym modeling is increasingly used by pharmaceutical companies to contribute to the weight of evidence supporting drug development decisions. It is not known to what extent DILIsym modeling was considered in the recent FDA decision to approve marketing of ubrogepant and, in view of the in-class liver toxicity, to approve without an advisory committee meeting and without liver safety warnings. However, there is general recognition that drug development must become more efficient, and recent FDA demands for large clinical trials just to demonstrate liver safety [2] is not consistent with this goal. As pressure rises from the Congress and the public for reduced pricing of new drugs, it is likely that regulators will increasingly rely on DILIsym to support NDA approvals with smaller liver safety databases, perhaps with pharmacovigilance including increased scrutiny on specific patient populations predicted by DILIsym to be at the greatest DILI risk. It should be noted that a DILIsym license has been acquired by the Center for Evaluation of Drug Research (https://apnews.com/484d6cf5a1a845208b03289ad6eadc91), and some pharmacometric staff there have undergone training in the use of the software. There is no regulatory path for approval of models such as DILIsym, which like GastroPlus and Simcyp, can simply gain regulatory application as confidence in the modeling results grows.

It should be noted that it is currently unknown to what extent DILIsym modeling can reduce the risk of the very rare and typically delayed idiosyncratic DILI. Current data support that these events often result from an adaptive immune attack on the liver [4]. However, the current belief is that drug-induced stress to hepatocytes is an essential first step in the cascade of events leading to the adaptive immune attack [4]. If so, reducing or eliminating this stress should reduce the risk of idiosyncratic DILI. The fact that DILIsym has predicted liver safety liability of drugs capable of causing delayed idiosyncratic DILI [9,10,14,29] supports that the modeling is relevant. Whether using DILIsym in lead compound selection will reduce or eliminate the risk of delayed idiosyncratic DILI remains to be determined. It should be noted that components of the immune responses are now being incorporated into DILIsym [30], with the initial goal of understanding and predicting DILI associated with checkpoint inhibitors used in oncology [31].

Conclusion

DILIsym is being increasingly utilized in decision making in drug development and is likely in the future to help reduce the size of clinical trials needed to establish adequate liver safety for marketing. DILIsym provides an example of how development of quantitative systems toxicology models should improve the efficiency of drug development.

Disclosure

Dr. Watkins chairs the Scientific Advisory Board for the DILI-sim Initiative and receives compensation for this role.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Watkins chairs the

Scientific Advisory Committee for the DILI-sim Initiative and receives compensation for this role.

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