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Review

Utilizing in vitro transporter data in IVIVE-PBPK: an overview

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Abstract

In vitro-in vivo extrapolation (IVIVE) integrated in physiologically-based pharmacokinetic (PBPK) models have been increasingly used during drug discovery and development processes to predict human pharmacokinetic (PK) parameters. Drug transporters can influence drug pharmacokinetics and are key aspects contributing to the development of a successful drug. This review provides a snapshot of challenges or shortcomings of in vitro and in vivo techniques for understanding the contribution of drug transporters to a drug's pharmacokinetics. The paper also describes the potential of IVIVE-PBPK models as prospective approaches to predict the role of drug transporters in drug discovery and development.

Keywords

Pgp; Bile acid transporter; Drug transporters; Drug metabolizing enzymes; Drug induced liver injury

Introduction

The role of drug transporters in drug efficacy and safety has been of much interest; drug transports play key roles in drug absorption, distribution, and excretion [1-3]. In drug discovery and development, drug transporters expressed in the intestine, liver, kidneys, and brain have received considerable attention [4-7]. Many of the drug transporters that have been cloned and characterized belong to two major superfamilies, ATP-binding cassette (ABC) and solute carrier (SLC). Major efflux transporters of the ABC family include MDR1 (P-glycoprotein, ABCB1), BCRP (ABCG2), and MRP2 (ABCC2), and are localized to barrier tissues in the body, such as the intestine, liver, kidneys, blood-brain barrier, and placenta [8-11]. Absorption of a drug is a key component to achieve good bioavailability, and for oral drugs, the majority of drug absorption occurs in the small intestine where the presence of villi and microvilli greatly increases the surface area for optimal absorption. Thus, drug-transporter interaction in the intestine often contributes to poor absorption, resulting in low bioavailability. Given that oral delivery is the preferred route of administration for drugs, intestinal transporters are potential targets to achieve optimal clinical oral plasma exposure for hydrophilic and polar drugs [12]. Although the significance of drug transporters in absorption, distribution, metabolism, and elimination (ADME) of drugs is well recognized, a knowledge gap exists in terms of change in drug transporter functionality (their amount and activity) in response to disease, and how pathophysiological pathways change transporter expressions [13]. Because of the central role of the liver in drug metabolism, the role of transporters in the liver in drug interactions and drug responses, as well as drug-induced liver injury, has been in focus of late.

Despite a steady growth in the field of transporter biology, which has bolstered transporter-mediated therapeutics, the physiological functions of many transporters are still largely unknown. These growing trends bring challenges for drug development, as well as for regulatory agencies, with regard to identification of membrane transporters that influence the disposition and safety of drugs, and also optimization of membrane permeability to improve oral absorption of new chemical entities. Thus, utilization of *in vitro* transporter assays has become a critical tool for assessing a drug's potential *in vivo* absorption properties and drug-drug interactions [14]. Use of *in vitro–in vivo* extrapolation (IVIVE) integrated in physiological-based pharmacokinetic (PBPK) models, together known as IVIVE-PBPK models, has gained recent momentum in the pharmaceutical industry. Since variation in protein expression of drug metabolizing enzymes and transporters is known to be a major complicating factor in IVIVE, the accurate quantification of these proteins in complex biological systems is essential for improving IVIVE-based pharmacokinetic predictions [15]. This review focuses on the use and shortcomings of qualitative and quantitative methods used to create drug transporter-based IVIVE-PBPK models in the liver, intestine, and brain.

Pitfalls of *in vitro* and *in vivo* systems used in drug transporter studies

In vitro cell-based systems are used routinely to elucidate passive and active transporter processes acting to influence drug permeability. Different *in vitro* systems include use of membrane vesicles or transfected cell lines for drug transporter studies; however, there are several pitfalls in estimating transporter kinetics using these conventional approaches [16]. Considering that one drug can be transported by more than one uptake or efflux transporter, a limitation of these systems is the presence of a single transporter of interest. Further, given that there exists an interplay between drug metabolizing enzymes and transporters, cell lines lacking metabolism machinery pose another limitation to their use. *In vitro* experiments can lead to biased estimation of apparent - K_m, the biochemical constant in experiments that assumes similar K_m for both apical and basal transport directions [17-20]**.** Another critical factor whether intracellular concentrations that drive efflux transport are accounted for [21]. Drug concentrations within the donor and acceptor compartments are dynamic; thus, the assumption that unbound drug concentrations on either side of a membrane are in thermodynamic equilibrium [22, 23] is not applicable for drugs that are poorly permeable, actively transported, or extensively metabolized.

A host of *in vitro* assays have been developed to mimic the complexity of biological systems; however, many questions remain regarding the translation of *in vitro* information to *in vivo*. Therefore, *in vivo* systems provide the ultimate determination of the effect of drug transporters on disposition of drugs. Transgenic and mutant animal models have provided an important tool for assessing drug efflux transporter activity. To this end, the efflux transporters P-glycoprotein (P-gp) and multidrug resistance-associated protein (Mrp1) have been the most frequently investigated in transgenic animals [24-26]**.** Like *in vitro* systems, *in vivo* systems also have limitations. Mice generally have two genes to encode transporter proteins, and silencing one of these genes may not sufficiently suppress the expression of the protein [25]. Moreover, efflux transporters may work through a compensatory mechanism, where absence of one gene might be compensated for by another transporter gene [27]. Furthermore, due to the ubiquitous expression of drug efflux transporters throughout the body, the removal of a gene or genes affects tissues other than the organ of interest. This can lead to undesirable outcomes such as altered pharmacokinetics, lack of viability, or systemic toxicity. To get around these limitations, specific transporters can be modulated with various agents [28]. The advantage of using drug transporter modulators is that these studies are generally performed with wild-type animals, and thus, the effect of a compensatory mechanism

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is not so important. However, due to overlap in substrate specificities, and lack of specificity, drug efflux transporter modulation is not as effective as gene knockout models [28]. Further, if drug transporter modulators are dosed orally, it is often difficult to get an effective dose to the targeted organ; this is likely be due to first pass metabolism. Even with intraperitoneal dosing, the limited effective dose of the modulator could be because of physiochemical properties of the modulator, such as protein binding and/or unfavorable tissue distribution [29]. Importantly, the effects of modulators on drug transporters are transient and concentration dependent; therefore, proper dosing and study time points becomes critical variables in these studies. There exists potential species-specific differences in drug transporter activities and contributions, as exhibited in MDR1 functional studies, in which some compounds showed affinity for MDR1A/B activity in mouse MDR1 cells but not in human cells [30].

Currently available *in vitro* and *in vivo* models have a limited capability to quantitatively predict the impact of transporters on intracellular drug concentrations. As PBPK modeling approaches consider physicochemical properties of drugs, *in vitro* cell-based passive permeability, and cellular uptake/efflux, as well as *in vivo* animal and human pharmacokinetic data to estimate unbound intracellular drug concentrations in different tissues, this approach provides an effective platform over currently available *in vitro* and *in vivo* models. However, these models involve a number of assumptions. Use of *in vitro* models, such as three-dimensional cultured-hepatocytes that retain the activity of both apical and basolateral hepatic drug transporters, drug metabolizing enzymes, or *in vivo* multiple gene/tissue-selective knockouts, serves as an improved medium to bolster refinement of PBPK models for better prediction in humans.

IVIVE-PBPK

PBPK models offer a quantitative mechanistic platform for predicting drug pharmacokinetics. When modeled for pharmacodynamics, these models can be used to predict clinical efficacy and safety with varying drug doses. The IVIVE approach is well established for many drug metabolizing enzymes [31, 32]; however, the integration of drug transporters into PBPK models is lacking, with few positive examples reported [33-36]. IVIVE-PBPK models link *in vitro* systems to the *in vivo* system through algorithms and scaling factors [37, 38]. Therefore, for an effective IVIVE-PBPK model to predict the behavior of a drug *in vivo*, accurate and relevant *in vitro* data in conjunction with the model system parameters of passive permeability and transporter-mediated flux are necessary.

Static models used alongside PBPK models in drug development are simple and require limited input data; however, due to simplification, these often result in conservative estimates, a lack of ability to capture parallel clearance and elimination pathways, are not suitable for non-linear kinetics, and lack physiology inputs. Although PBPK models require extensive *in vitro* and *in vivo* data, they can capture transporter-mediated disposition, describe physiology, and can be applied for special populations such as pediatric patients, in which data is generally very limited.

PBPK for the prediction of transporter-mediated drug induced liver toxicity

Membrane transporters expressed on hepatocytes and enterocytes play a critical role in homeostasis of endobiotics, e.g., bile acids. Homeostasis of bile acid is maintained through enterohepatic circulation, which allows movement of bile acid molecules from the liver to the small intestine and back to the liver. Bile acid transporter, also known as apical sodium-dependent bile acid transporter, is considered a major determinant of bile acid homeostasis in the body, and is an essential regulator of lipid and cholesterol homeostasis [39-41]. Impaired bile acid export may lead to increased concentrations of liver bile acids, causing hepatocellular apoptosis and/or necrosis. Thus, drugs with the potential to inhibit these

transporters can disturb the disposition of both co-administered drugs and bile acid, contributing to the development of cholestatic drug-induced liver injury (DILI) [42, 43]. *In vitro* studies with isolated membrane vesicles have demonstrated an association between DILI and the inhibitory effects of drugs on bile acid efflux transporters [43-45]. *In vitro* findings may not translate directly to *in vivo* hepatotoxicity risk for various reasons, including complexity of bile acid homeostasis, feedback regulation of bile acid synthesis and transport, and dynamic drug/metabolite concentrations in the system [46, 47]. *In vivo* studies report that preclinical animals are less sensitive to DILI caused by bile acid transporter inhibition, as compared to humans [48, 49]. Therefore, mechanistic models that integrate physiological information and *in vitro* experimental data to evaluate DILI mechanisms may be useful to prospectively predict hepatotoxic potential of new drug candidates [50]. The development of a mechanistic model to predict hepatic bile acid transport mediated DILI of troglitazone (TGZ) [51] demonstrated the significant contribution of PBPK modeling to the prediction of hepatotoxicity due to the inhibition of drug transporters, and the potential of a transporter-mediated PBPK model to aid drug development. The mechanistic PBPK model used to study the involvement of bile acid homeostasis in TGZ hepatotoxicity incorporated drug/metabolite disposition, bile acid physiology/pathophysiology, and hepatocyte life cycle. Large population variability in bile acid exposure makes prediction of bile acid-mediated hepatotoxicity challenging; hence, the model included population-based analysis to overcome population variability and low incidence of hepatotoxicity. In a rat PBPK model, TGZ was not hepatotoxic; however, in a stimulated human PBPK model, TGZ resulted in a delayed increase in serum alanine aminotransferase (ALT) (marker of liver injury). Thus, the variability added to the parameters describing drug disposition, body weight, and sensitivity of ATP synthesis to hepatic bile acid accumulation, allowed prediction of the incidence of TGZ hepatotoxicity and the species specific bile acid-mediated DILI mechanism.

Intestinal transporters linked to IVIVE-PBPK model for oral absorption

The bioavailability of an orally administered drug is primarily dependent on the transporters expressed on the apical/basolateral side of enterocytes that influence both the fraction of drug absorbed [52, 53] and the fraction escaping gut metabolism. It is difficult to reproduce the complexity of the human gut within a laboratory setting; therefore, efforts are being made to incorporate drug absorption into PBPK models by taking into account several physiological elements of the gastro-intestinal tract, e.g., pH, active transporters, and metabolizing enzymes [54]. To mechanistically develop a PBPK model, the intestine essentially accounts for the region-specific anatomical and physiological differences. Advanced models such as the advanced dissolution, absorption, and metabolism (ADAM) model and the advanced compartmental absorption and transit model (ACAT) are based on adaptations of the original compartmental absorption and transit (CAT) model which accounts for small intestinal transit time (SI), permeability, and radii. Most importantly, these advanced models incorporate gastrointestinal transporter–metabolism interplay [55, 56]. A study by Bruyere and co-workers has highlighted the importance of obtaining regional intestinal transporter expression data for incorporation in PBPK models [57]. This study estimated the scaling factor based on protein estimation of both drug metabolizing enzymes and transporters across the small intestine; these were used for predicting intestinal clearance and for the development of an IVIVE-PBPK model. The results demonstrated that accounting for the MDR1 distributions enhanced PBPK predictions of bioavailability for the development of the compound under study. Likewise, a study by Darwich and co-workers [56], which incorporated regional MDR1 and CYP3A expression into an ADAM model, demonstrated two points: non-uniform regional distribution of MDR1 in the small intestine and colon, and disparity between mRNA and protein expression. This highlights that protein and mRNA expression together with drug parameter data leads to successful capture of drug disposition. The mechanistic PBPK

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absorption model of oxybutynin (OXY) was based on a CAT model that predicts the fraction absorbed based on permeability data in order to predict OXY's oral bioavailability, for two different formulations: immediate-release (IR) and modified-release (MR) [58]. As the primary objective is to predict the fraction absorbed, this multi-compartmental absorption model was a simplified version of a CAT model used to divide the small intestine into three compartments instead of seven, including the duodenum, jejunum, and ileum. The model allowed the drug amount to be modelled, either in solid or dissolved state, for each segment. Assumptions made included: well mixed GI compartments, no drug degradation in the GI tract, only the dissolved drug to be absorbed, and absorption to be a non-saturable process. Transfer of mass between adjacent segments was considered to follow first-order kinetics, controlled by a rate constant of gastric emptying. The dissolution rate of OXY was modeled based on spherical particles dissolving over time [55]. Further, OXY's regional intestinal metabolism was modeled through an enterocyte compartment, with the assumptions that the metabolism in the liver and intestine was equal, and there was no binding to enterocytes. The model showed that the fraction absorbed from the formulation was reduced compared to IR, despite higher intestinal availability of the formulation. Considering the assumption that the fraction remaining in the liver and intestine is the same, the model prediction supported the hypothesis that higher bioavailability with the OXY formulation is due to increased intestinal availability.

IVIVE-PBPK model for the central nervous system

Given the high failure rate of drugs developed for central nervous system (CNS) indications [59], early and accurate prediction of drug penetration across the blood–brain barrier (BBB) is vital during drug development. Further, the prediction of brain penetration for non-CNS drugs is important during drug development, in order to avoid unwanted neurotoxicity. Human pharmacokinetic data from the brain is highly restricted; thus, PBPK modeling is of significant value as it can be used to predict the target site concentrations in inter-species and inter-disease situations [60-62]. However, until recently, there has been relatively few published IVIVE-PBPK models to predict BBB permeability, mostly due to limitations in the current *in vitro* systems used to represent the complex and transporter-rich structure of the *in vivo* BBB, and due to difficulty in obtaining the appropriate *in vitro–in vivo* scaling factors [63]. A generic multi-compartmental CNS distribution model structure proposed by Yamamoto et al [64], based on the compounds acetaminophen, atenolol, methotrexate, morphine, paliperidone, phenytoin, quinidine, remoxipride, and risperidone, successfully described the pharmacokinetics in plasma and different CNS compartments [64]. Prediction of this multi-compartmental brain PK model matched well with the observed concentration-time profiles, adequately describing the data for all compounds studied. The model structure comprised physiologically relevant brain compartments including brain extracellular fraction (brainECF), brain intracellular fluid compartment (brainICF), compartment of cerebrospinal fluid (CSF) in lateral ventricle (CSFLV), compartment of CSF in third and fourth ventricle (CSFTFV), compartment of CSF in cisterna magna (CSFCM), and CSF subarachnoid space (CSFSAS). Thus, it could successfully describe the PK in plasma and different CNS compartments using microdialysis data. Recently, there has been an increasing focus on translational modelling approaches compared to the traditional practice of fitting model parameters to preclinical *in vivo* data [65, 66]. IVIVE-PBPK models reported by Fenneteau et al [67] and Ball et al [68] demonstrate promising examples. This review has addressed imperative differences among various PBPK models in use, including details on brain compartmentalization and parameterization. The general approach of using *in vivo* data to obtain drug specific parameters and physiological parameter estimates by fitting to *in vivo* data has poor predictivity. Similar to empirical PBPK models, the IVIVE-PBPK model uses a bottom-up approach through IVIVE, with the use of appropriate physiological scaling factors. Fenneteau and colleagues scaled *in vitro* passive and efflux permeabilities of domperidone to *in vivo*

intrinsic permeabilities, and then using literature-based physiological values for *in vivo* membrane surface area, they further scaled the permeabilities to whole organ permeabilities [67]. The *in vitro* intrinsic P-gp efflux permeability determined in Caco-2 cells was corrected using the relative fraction of MDR1A/1B messenger RNA expression measured in the brain compared to that in the intestine.

Two methods of IVIVE used for PBPK modelling of morphine and oxycodone describe different strategies for IVIVE of drug transporters at the BBB, which can be used in different phases of drug development [69]. The morphine IVIVE-PBPK model was based on data from *in vitro* Caco-2 permeability and *in vivo* total concentrations in brain homogenate. On the other hand, the oxycodone IVIVE-PBPK model used data from complicated experiments generally performed in later stage of drug development, which included *in vivo* unbound extracelluar fraction (ECF) concentrations obtained from microdialysis and *in vitro* uptake kinetics in rat brain microvessel endothelial cells. Relative activity factor (RAF) was used to account for the difference between *in vitro* and *in vivo* data. Since it is very difficult to obtain human brain homogenate or primary brain endothelial cells from isolated microvessels, determination of drug-specific parameters from *in vitro* systems of human origin is not always feasible. Brain microdialysis and cerebrospinal fluid (CSF) sampling is another approach to determine drug pharmacokinetics, but due to the invasive nature of these techniques, they are only carried out on diseased patients [70], which introduces significant challenges for the evaluation of model predictions with the human CNS PBPK model. Linking PBPK/PD models to biomarkers or observed *in vivo* pharmacodynamics are alternative approaches to evaluate human CNS PBPK predictions. However, the success of these approaches depends on selection of appropriate biomarkers and the establishment of a reliable PK/PD relationship. Thus, there is need for more IVIVE-PBPK models to fully explore and evaluate their applicability for the prediction of drug pharmacokinetics in the brain.

IVIVE-PBPK model for transporter mediated drug-drug interaction

Traditional pharmacokinetic modeling lacks good predictability for complex drug-drug interactions (DDIs) because in most cases, only *in vitro* and limited clinical data are available in early drug development. As IVIVE-PBPK enables scaling of early *in vitro* and animal data for human predictions, it can aid DDI risk assessment. The effect of transporter-enzyme interplay on drug bioavailability and hepatic disposition is well recognized [71, 72].There are examples of the involvement of transporter-enzyme interplay in DDIs. Lau et al reported that the inhibitory effect of rifampin on atorvastatin kinetics involves transporter-enzyme interplay [73]. Rifampin is a potent inhibitor of organic anion-transporting polypeptide (OATP) transporters and atorvastatin is primarily metabolized by CYP3A in the liver, via its lactone group. In the study by Lau et al [73], rifampin demonstrated inhibition of atorvastatin uptake into the liver and a change in the ratio of atorvastatin lactone to atorvastatin acid, thereby changing the amount of substrate available to the enzymes in the liver. Grillo et al [74] demonstrated the use of IVIVE-PBPK modeling to predict potential clinically relevant DDI between rivaroxaban and a combination of P-gp and a moderate CYP3A4 inhibitor in renal impaired patients. Thus, bottom-up PBPK models are a powerful tool for the prediction of transporter-mediated drug interactions, and accurate quantitation of protein concentrations in *in vitro* and *in vivo* systems will further enhance their accuracy. Given that *in vitro* systems do not completely reproduce the transport rates and affinities like *in vivo*, the middle out parameter optimization approach is a necessary step in model verification.

Conclusions

IVIVE requires information from numerous processes and organs. Each has its advantages and limitations in translating data to the clinic. IVIVE-PBPK modeling is a powerful approach when full kinetic

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characterization of transporters is implemented. Thus, understanding the influence of drug transporters on drug pharmacokinetics can be obtained through a combination of approaches. The future of drug transporter-based IVIVE-PBPK models lies in their potential to contribute to pharmacogenetic studies. Drug transporters and metabolizing enzymes differ significantly based on various factors, including gender, age, race/ethnicity, genetics (polymorphisms), disease state, diet, and lifestyle. The impact of polymorphisms in drug metabolizing enzymes on pharmacokinetic variation is largely studied and known; however, knowledge regarding the impact of polymorphisms in drug transporters is limited. Studies undertaken to date report significant changes in intracellular drug levels due to polymorphisms in drug transporters, which suggests potential alterations in the systemic pharmacokinetics of drugs. Most drug transporters (e.g., P-gp and MRP-1) follow a developmental ontogeny and like most drug metabolizing enzymes, the activities of membrane transporters are immature in neonates. Thus, future research is needed to drive system-dependent scaling factors for predicting transporter contributions of new molecular entities, in different disease states and among special populations, using IVIVE-PBPK modeling approaches.

Conflict of interest: None

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