ELSEVIER

Teaser Used in combination, physiologically based pharmacokinetics, quantitative systems pharmacology and quantitative systems toxicology have the potential to improve prediction of the effective drug concentration and the associated beneficial and adverse effects, improving the chances of success in biopharma R&D.

Better prediction of the local concentration–effect relationship: the role of physiologically based pharmacokinetics and quantitative

Sebastian Polak[1,2], Zofia Tylutki[1,2], Mark Holbrook[2] and Barbara Wiśniowska[1]

[1] Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9 Street, 30-688 Kraków, Poland
[2] Certara-Simcyp, Level 2-Acero, 1 Concourse Way, Sheffield, S1 2BJ, UK

Model-informed drug discovery and development (MID3) is an umbrella term under which sit several computational approaches: quantitative systems pharmacology (QSP), quantitative systems toxicology (QST) and physiologically based pharmacokinetics (PBPK). QSP models are built using mechanistic knowledge of the pharmacological pathway focusing on the putative mechanism of drug efficacy; whereas QST models focus on safety and toxicity issues and the molecular pathways and networks that drive these adverse effects. These can be mediated through exaggerated on-target or off-target pharmacology, immunogenicity or the physiochemical nature of the compound. PBPK models provide a mechanistic description of individual organs and tissues to allow the prediction of the intra- and extra-cellular concentration of the parent drug and metabolites under different conditions. Information on biophase concentration enables the prediction of a drug effect in different organs and assessment of the potential for drug–drug interactions. Together, these modelling approaches can inform the exposure–response relationship and hence support hypothesis generation and testing, compound selection, hazard identification and risk assessment through to clinical proof of concept (POC) and beyond to the market.

Dr Sebastian Polak holds a tenured position at the Faculty of Pharmacy Jagiellonian University Medical College, Cracow, Poland (Associate Professor in Biopharmacy), where he leads a multidisciplinary team of scientists and engineers working on applying various modelling and simulation approaches in drug development. He is also a Senior Principal Scientist and Certara's Practice Lead for Cardiac Safety. He leads the development of the cardiac safety modelling and simulation systems based on the biophysically detailed models of human cardiac myocytes operating at the population level (Cardiac Safety Simulator; CSS). Among various projects, he has led transdermal absorption module development and co-led mechanistic IVIVC module development. He has published >50 peer-reviewed articles and has given a number of invited presentations at national and international scientific meetings. He earned an MSc in pharmacy from the Faculty of Pharmacy Jagiellonian University where he also received his PhD and DSc (biopharmacy).

Introduction

The concentration–response relationship is a fundamental principal in the study of how a compound interacts with a test system, whether it be *in vitro*, *in vivo* or *ex vivo*. However, at least historically, this basic principal has often been underappreciated or overly simplified and has been associated with a high degree of compound failure in the clinic [1]. In fact, these authors, in reviewing the reasons for failure in the Pfizer portfolio, proposed three key elements that need to be met to adequately test clinical proof of concept (POC): (i) exposure at the target site of action over a desired period of time; (ii) binding to the pharmacological target as expected for its mode of

Corresponding author: Polak, S. (sebastian.polak@uj.edu.pl)

https://doi.org/10.1016/j.drudis.2019.05.016

action; and (iii) expression of pharmacological activity commensurate with the demonstrated target exposure and target binding [1]. Although these three elements are arguably intuitive, there are technical challenges in their measurement in the experimental situation and subsequent integration for accurate interpretation. This has been a driving force in the evolution of quantitative systems biology (QSB), which can be defined as 'an approach to biology that seeks to understand and predict the quantitative features of a multicomponent biological system' [2]. QSB is a broad area made up of the related and overlapping subdisciplines of quantitative systems pharmacology (QSP) and quantitative systems toxicology (QST), along with physiologically based pharmacokinetics (PBPK). When used effectively in an integrated fashion these techniques are considered major components of model-informed drug discovery and development (MID3) [3,4]. It is important to emphasise the term 'informed' recognising that no model is perfect and data from such computational models need to be used by the scientist in conjunction with other clinical and nonclinical data in an integrated fashion to support interpretation. Together, these modelling approaches can improve mechanistic understanding of the exposure–response relationship and hence support hypothesis testing, compound selection, hazard identification and risk assessment through to and beyond clinical POC to the market. Ultimately, this should lead to more-successful and -efficient delivery of valuable medicines to patients. In fact, MID3 supports the exciting concept of personalised medicine through recognition of differences in drugs and individual patients. Computational models can accommodate inter- and to some degree intra-individual variability assessment such that consideration can be made at the population and individual level [5]. The scope of this review does not allow a detailed description of this aspect of modelling but variability assessment is an important consideration that is required by the current regulatory guidelines on the reporting of PBPK-based simulation results [6]. Further, we have not included the important topics of uncertainty and parameter identifiability analysis but direct the reader to these guidelines and other recent publications [7,8].

PBPK models

What are they and how are they utilised?

PBPK models are mathematical representations of cells, tissues and organs, with associated arterial and venous blood systems. They are built using parameters defining specific tissue physiology including cell volumes, expression of relevant proteins (e.g., transporters) and blood-flow rates. Briefly, each biological system model is composed of a series of compartments, representing cells, tissues and organs. The number of compartments differs between various models depending on the degree of complexity and the context of use for the model. Basic models lack detailed description of the biological structure and assume perfusion-limited drug distribution and well-stirred character resulting in homogeneity within and equilibria between compartments. Thus, description of the cellular and subcellular distribution of the drug of interest is not possible with such basic models. Obtaining a quantitative prediction of the disposition of a drug in the intra- and extracellular spaces requires a more complex permeability-limited model, where passive diffusion and/or active transport at the level of biological membranes is a regulating factor [9]. In practice, both types of model have utility for efficacy, toxicity and safety assessment with the type employed depending upon the context of use, objective, throughput and accuracy.

What are the potential benefits of PBPK models?

An appropriately designed PBPK model provides a fit-for-purpose prediction of the intra- and extra-cellular concentration of compounds (parent drug and metabolites) in selected tissues and organs, something that is not possible or practical to be routinely determined experimentally, especially in humans. Each model is initially built from experimentally measured and estimated parameters using a training dataset and undergoes an iterative learn–confirm cycle with verification through the use of a selected test set of experimental data that was not used for the initial generation of the model. The objective is to mitigate for any bias and quantify the predictive power of the model. This approach allows a critical judgement of the accuracy and the utility of the model with any assumptions in the model recognised and reported. Arguably this transparency, with any assumptions clearly stated, means that a more objective view can be formed on a PBPK model as compared with experimentally generated data where there can be greater unknowns, potential for operator bias and variance.

Owing to tissue differences in blood flow, transporter expression and metabolic activity, a well-constructed PBPK model enables the prediction of tissue-specific exposure to the drug and metabolites against time to depict the PK (or TK) profile. Further, the model supports the testing of hypotheses associated with (i) the drug (e.g., drug–drug interactions), (ii) the test system (e.g., different species, disease state, sex, stage of development) and (iii) extrinsic factors (e.g., diet, co-medications). This allows prediction of the clinical situation with consideration of potentially high-risk populations.

In addition, PBPK modelling has the potential to refine, replace and reduce the use of animals in medical research addressing the 3Rs initiative [10]. Potential benefits afforded by a well-designed PBPK model include:

- supporting interpretation, interpolation and extrapolation between nonclinical and clinical scenarios;
- prediction of clinical exposure;
- prediction of drug–drug interaction;
- prediction of local tissue concentration;
- 'what-if' scenarios testing for various theoretical clinical situations;
- utilisation in various industry sectors – pharmaceuticals, agrochemicals, cosmetics;
- supporting the 3R objectives.

Considerations for PBPK model development and utilisation

While offering major practical advantages over the traditional approach (i.e., measurement of local tissue concentration), the development of PBPK models has several challenges that could potentially constrain their practical application.

i PBPK models are mechanistic in their nature, requiring a good understanding of the underlying pathways, constituents and processes affecting drug exposure. Despite the development and application of novel technologies such as proteomics, transcriptomics and metabolomics resulting in significant

progress with the elucidation of several biological pathways and systems, there are still several gaps that restrict a mechanistic description of the biological phenomena and in some cases reduce practical application of a model. One such area is transporter-mediated drug diffusion which is the subject of significant research owing to the key role that these proteins take in drug disposition. In time, this research will lead to the further elucidation of these transporter mechanisms and support the development of better models in an incremental fashion.

ii The practical application of PBPK models requires significant quantities of good quality and relevant data generated primarily from *in vitro* and *in vivo* human clinical studies and, to a lesser degree, animal studies. In general, owing to the large number of parameters required for PBPK modelling and the limited availability of *in vivo* data to verify individual parameters, model predictions can carry a degree of uncertainty associated with the level of confidence in the accuracy of individual parameters. For example, for drugs that have not been administered intravenously to humans, distribution and absorption parameters cannot be confirmed or verified experimentally, which introduces uncertainty into model parameters and consequently the simulated output.

iii Practical application of PBPK models requires combining and merging various modelling techniques, beginning from empirical heuristic algorithms, through classical statistical models, to the differential calculus at various level of complexity (0D–3D models, and therefore ordinary and partial differential equations). As with all modelling and experimental techniques, with increased complexity comes the risk of increased error, hence the desire is always to minimise the complexity while ensuring relevance to the context of use.

iv With the advancement in any technology and its application comes the need for continuous communication, education, review and acceptance. In the world of drug discovery and development this includes key stakeholders such as regulatory agencies; and sponsors need to be comfortable submitting data to regulatory agencies generated with such techniques [11].

v Some critics of the technique cite experience leading to lack of confidence with model extrapolation [11]. This is understandable and such experiences need to be addressed. In our view these issues are often the result of inadequate model verification and quality assessment procedures. As mentioned previously, every model, computational or experimental, has limitations and these need to be understood and confirmation made that the model is fit-for-purpose.

vi Defining the model structure, access to relevant parameterisation data and quality input data can be challenging yet it is crucial to produce a meaningful model. This drives the predictive power for the intended use and hence its value [12].

QSP models

Definition of QSP model

QSP is a rapidly emerging discipline combining wide and detailed biological knowledge of a biological pathway at the cell, tissue, organ and organ system level, with the PK of a compound and relevant metabolites. Defined as a translational medicine approach, it combines computational and experimental methods to elucidate the pharmacological pathway associated with the mechanism of action [13]. As a discipline, QSP aims to provide a quantitative description of the mechanisms lying behind established and novel chemical entities (NCEs) and novel biological entities (NBEs) offering the opportunity to optimise therapy for the individual patient by maximising therapeutic effect and minimising potential toxic reactions. The main difference between the traditional empirical pharmacodynamic model (PD)-based approach and QSP lies in the broader use of drug-independent information describing the biological system of interest – ultimately the human body [14].

The evolution of QSP has led to the development of QST

QST has been defined as 'an approach to quantitatively understand the toxic effects of a chemical on a living organism, from molecular alterations to phenotypical observations, through the integration of computational and experimental methods' [15]. In essence, QST could be viewed as a distinct but overlapping discipline with QSP. QST with its specific focus on the safety and toxicity of a compound presents some unique challenges. Although the modelling of efficacy focuses on the primary 'on-target' pharmacology associated with the intended modulation of this system, safety and toxicity consider the exaggerated modulation of this pathway as well as other pharmacology associated with secondary 'off-target' effects of the molecule and the physicochemical nature of the molecule. QST therefore needs to account for multiple effects and pathways that might be compound specific. Hence, consideration of compound 'promiscuity' with on-target and off-target binding and function adds complexity with the need to understand multiple pathways [16].

With the increased utilisation and verification comes confidence regarding the application and usefulness of computational models. Such models can be utilised not only to generate hypotheses but also to increase understanding and test virtual scenarios [17]. In the nonclinical arena, safety pharmacology and toxicology studies are designed for hazard identification, risk assessment and risk management – requiring a robust objective means to support translation into the clinic. Further, although the initial focus might be on healthy volunteers, it is important to extrapolate through to patient populations with consideration of their demographics, comorbidities and potential for drug–drug interaction. In addition, what constitutes a safety issue could vary with the intended therapeutic indication ranging from a toleration issue such as nausea to a safety issue such as an increase in heart rate or to a toxicity issue such as hepatotoxicity. To achieve this aim, QST models need to be supported with drug PK information and refined through the use of PBPK models to predict the tissue and cell concentration of the compound and metabolites of interests [18]. With that, the QST models can be further extended to quantitative systems toxicology and safety (QSTS) models, combining these two related fields [19].

Knowledge about the local concentration of compounds: why is it needed in the context of the QSP models?

Many drugs exert their intended or unintended functional effects following absorption or transport into cells and subsequent binding to intracellular targets. Therefore, knowledge about the

intracellular concentration of drugs, and in some cases metabolites, is necessary to accurately interpret their pharmacological and toxicological effects and their potential for interaction with other drugs [20]. Owing to the fact that the intra- and extra-cellular environments differ (e.g., the presence and concentration of proteins and the role taken by membrane transporters in controlling intracellular drug concentration) means that the plasma concentration might not be an accurate representation of the intracellular effective concentration.

QSP/QST is a holistic approach to translational medicine with the purpose of providing the understanding of disease mechanisms and therapeutic or toxic effects that span multiple biological scales. To analyse the dynamic interactions between drugs within a biological system requires a detailed understanding of physiological and disease processes at the level of cell, tissue, organ and patient with mathematical and computational models based on this. The QSP/QST approach builds upon recent advances in pharmacology, medicinal chemistry, molecular and cellular biology, biochemistry, molecular genetics, genomics, transcriptomics, pathology, mathematical methods and computational modelling. A comprehensive and meticulous description of a system targeted by a drug is one of the two elements required for simulation and prediction of *in vivo* effects at the patient level. The second is the knowledge about the amount of a compound that can interact with the target to exert the pharmacological response (i.e., the local drug concentration at the site of its interaction with the system).

Drug-target proteins can be located either in the cell membrane or inside the cell, thus binding takes place from extracellular fluid or a drug has to permeate or be transported through the cell membrane and sequester into a certain subcellular location to reach a target [1,21,22]. Basic models consider an organ to be one, well-stirred compartment and assume that unbound drug concentration within the tissue is in thermodynamic equilibrium with free-drug concentration in the circulatory system. Thus, the unbound blood or plasma concentration is used as a surrogate for tissue concentration. However, this assumption might not be valid for many drugs that bind to intracellular targets owing to active transportation across the cell membrane, the poor permeability of a compound, intracellular protein binding or rapid metabolism [23,24]. Consequently, the actual drug concentration at the effective site differs significantly from that observed in plasma. There can also be a hysteresis between the concentration–time profiles for plasma and tissues leading to an apparent delay in the maximum PD effect compared with the maximum plasma concentration of drug. Hence, the objective of the PBPK model is to provide a prediction of the concentration vs time profile of drug at the effective site which can be associated with the PD effect predicted by the QSP/QST model for the adequate assessment of the exposure–response relationship.

Several methods have been established to measure the amount of a drug within a tissue [22,25]. Post-mortem tissue biopsies can provide regional concentration data; however, such data should be interpreted with caution owing to post-mortem redistribution phenomenon [26]. The disadvantage of this method is that the characterisation of a time-course of drug concentration requires many animals to be sacrificed and tissue concentrations are usually determined from tissue homogenates or lysates. This ignores compartmental cell and tissue structure and possible heterogeneous drug distribution; a drug in a tissue could be located in a subcompartment rendering it unavailable for biological activity. Further, it does not account for differing degrees of binding and hence free fractions in different locations. Moreover, animal data need to be extrapolated to a human situation, which can introduce assumptions into the QSP/QST model. Information on the amount of drug retained in, or transported into, a tissue or fluid concentrations can also be obtained *in vivo*, in humans and animals, by serial tissue biopsies, for example during heart surgery [26], spinal or synovial fluid sampling or noninvasive techniques, including sampling of urine, saliva, faeces or exhaled air [22]. Relatively novel methods enabling assessment of drug levels at the site of action including microdialysis and imaging techniques (e.g., magnetic resonance spectroscopy and positron emission tomography) are valuable [27–31]. Microdialysis can be employed for sampling *in vivo* allowing direct measurement of the unbound fraction of a drug in the extracellular fluid of tissues of interest (e.g., adipose tissue, brain, heart, lung, solid tumours). It offers the opportunity for multiple measurements and allows continuous monitoring of local tissue concentrations of drugs and metabolites. Imaging techniques are powerful tools for noninvasive *in vitro* studies of drug distribution giving the possibility of continuous drug concentration measurements in virtually any organ. Knowledge of intracellular concentration or subcellular drug distribution could further improve efficacy and toxicity prediction for drugs; therefore, a number of sampling and analytical methods have been developed to enable their measurement [21,32–37]. These are useful techniques especially if used to support the development of PBPK models and so through progression reduce the reliance on the actual measurement.

Multicompartmental PBPK models: what is available?

Much has been said about the role of the PBPK models in the drug development process, including their influence on drug labelling and increasing regulatory acceptance [23]. In the current review, this element is just briefly mentioned while we focus on the detailed description of the available mechanistic, local tissue level PBPK models. PBPK models are becoming established tools for basic research as well as for regulatory submission [38–40]. In most cases the surrogate of the active fraction is the plasma concentration, which is utilised as input data in the model to predict the effective intracellular concentration. Models can be parameterised to predict the situation in special populations, or predictions of drug interaction. The local tissue concentration can be divided into subphases (intra- and extra-cellular) constituting individual components that together represent the driving force for complex PK models.

Lung

The development of a multicompartment lung model requires consideration of the different routes for exposure to compounds. The respiratory tract and lungs are served by two different blood circulatory systems; the pulmonary system serves the lungs to facilitate gas exchange whereas the tracheobronchial system serves the respiratory tract facilitating exposure to systemically available compounds. In addition, inhalation provides a further route for drug exposure with inhaled drugs being designed to be deposited

at targeted levels in the respiratory tree using specifically designed delivery devices. To develop an understanding of the complexities of lung physiology and pathophysiology, the development of new medicines and design of delivery devices has required experimentation with animals and extrapolation to humans. Ramsey and Anderson described one of the first ventilation–perfusion PBPK models using styrene inhalation in the rat [41]. The model was then extrapolated to humans. The authors assumed the rapid equilibrium of styrene between alveolar air and capillary blood and allowed for prediction of styrene kinetics in arterial blood, exhaled air and alveolar air of humans after inhalation exposure to styrene. However, the lung tissue was not considered as a separate compartment and the exchange of the substance was considered only in the alveolar region. Later, Johanson proposed a PBPK lung model consisting of 18 compartments (nine pairs of central and peripheral compartments) that represented nine regions of the respiratory tree [42]. These were connected serially from the trachea to alveoli by central compartments representing air and the outermost layer of the mucus, which enabled the modelling of the transfer of vapour through the lower respiratory tract. Sarangapani *et al*. described the lung as four compartments [i.e., the upper airways (nasal cavity), the conducting airways, the transitional airways and the pulmonary airways] to predict styrene and its metabolite concentration in the lung as a target organ [43]. The compartments that represented the nasal cavity, conducting airways and transitional airways were further divided into three compartments: lumen, epithelial cell layer and submucosal tissue. The unidirectional ventilation was assumed to occur in the lumen and perfusion at the submucosal tissue layer. The model accounted for pulmonary metabolism. Gloede *et al*. described a structure that linked a computational fluid dynamic approach with a PBPK model for the lower and upper respiratory tract [44]. The model was applied to predict inhaled diacetyl dosimetry. These PBPK models and others were applied to describe the process of vapour absorption via the respiratory tract rather than pulmonary drug disposition following inhalation [45]. Based on this background several lung PBPK models [46] have evolved including those in the Simcyp® Simulator [47], PK-Sim® [48] and GastroPlus® ADRM [46] platforms. The latter describes the respiratory tract by dividing it into four compartments: large conducting airways, small conducting airways, alveolar interstitium and thoracic, each of which is further divided into an airway liquid compartment, and an epithelial/lung tissue compartment. Pulmonary absorption from lung into the systemic circulation is assumed to be diffusion limited. Modelling of the inhaled administration of ciprofloxacin and its deposition in the oral cavity, trachea and bronchi, and the deep alveolar space in PK-Sim® [48] was performed empirically rather than mechanistically and, hence, has limitations. PK-Sim® together with MoBi® software was also used to simulate solithromycin concentration in epithelial lining fluid after oral administration of that antibiotic [49]. In that study, the concentration in the epithelial lining fluid compartment created in MoBi® software was described as a function of plasma concentration. Gaouha *et al*. [47] applied a mechanistic multicompartment permeability-limited model of the lung implemented in the Simcyp® Simulator to simulate the concentrations of orally administered antituberculosis drugs in the epithelial lining fluid, as well as different lobes of the lung tissue. The model was developed in such a way as to reflect lung anatomy and physiology; it distinguished two segments reflecting upper and lower airways, and five segments representing the lobes of the lung – three for the right lung and two for the left lung. Further division of each segment into four subcompartments (i.e., pulmonary capillary blood, tissue mass, fluid and alveoli air). The model is permeability-limited within the compartments in the same segment, allowing drug metabolism in the tissue mass and the active transport within the lung to be taken into account.

Liver

Liver has many functions including those related to nutrient processing and storage, detoxification and immunity. It also plays a crucial part in the metabolism of drugs; thus, it is not surprising that much attention has been given to development of PBPK liver models. The review by Lerapetritou *et al*. [50] summarises different mathematical approaches to describe the liver. The well-stirred, parallel-tube and dispersion models are currently the main PK liver models in use. The simplest, as in the case for all organs, assumes spatial homogeneity of the liver and 'lumped' kinetics of metabolism. In the well-stirred model the liver is considered as a single well-mixed compartment with the drug concentration uniform throughout the organ, and an influence of blood flow, drug binding and hepatic enzymes activity on the hepatic clearance in a steady-state conditions is described by Equation (1)1.

$$CL_H = \frac{Q_H * f_{ub} * CL_{int\,H}}{Q_H + f_{ub} * CL_{int\,H}} \quad (1)$$

Where Q_H is the hepatic blood flow; f_{ub} is fraction unbound in blood; $CL_{int\,H}$ is the intrinsic hepatic clearance [51–53]. It assumes instantaneous equilibration of the drug in the liver and in the emergent blood, no membrane transport barrier and that only a fraction of drug which is unbound to blood proteins is subject to elimination.

The parallel-tube model considers liver as a series of identical cylindrical tubes, arranged in parallel, representing sinusoids. The liver enzymes are assumed to be distributed evenly around the tubes and the drug concentration gradient is produced along the tubes [51,54,55]. The assumptions made for this model are the same as for the well-stirred model with the exception of equal drug concentration along the organ. The parallel-tube model combines the blood flow, drug binding, hepatic enzyme activity with the hepatic clearance (Equation (2)2).

$$CL_H = Q_H * \left(1 - e^{-\frac{f_{ub} * CL_{int\,H}}{Q_H}}\right) \quad (2)$$

Both models have proved to be useful in simulation and prediction of xenobiotic apparent kinetics and the well-stirred model, owing to its mathematical simplicity, is particularly widely used as a standard in PBPK modelling [56].

The dispersion model, proposed by Roberts and Rowland [53], lies between the two above-mentioned examples, representing the extremes (ideal mixing and no mixing). It uses the axial dispersion model with the dispersion number (parameter defining extent of blood mixing) to describe the blood flow and spreading of a drug within the liver. Although the consideration of drug transit time adds complexity to the model, it more accurately reflects the liver physiology, although its solution is much more complex mathematically compared with the well-stirred and parallel-tube models.

Being one-compartment, all the afore-mentioned models assume uniform metabolic and biochemical properties and are simplified representations of the heterogenic organ. They are based on the assumption that the overall rate of drug loss reflects the sum of all metabolic and excretion pathways (e.g., CYPs, UGTs, SULTs, GSTs, P-gp, AOTP). However, this is a gross oversimplification because some pharmacological and toxicological processes might target specific cell types leading, for example, to enzyme- or transporter-mediated drug–drug interactions that are not detectable in a simple homogeneous model. For this reason, several other, more-sophisticated liver models that can capture heterogeneities of blood flow, enzymes and transporter distribution or protein binding have been developed. One of these, the zonal model, splits the liver into three metabolic zones (compartments) corresponding to periportal, midzonal and perivenous regions displaying diverse abundances of enzymes and transporters [57–60]. Transport of a drug across the sinusoidal membrane is characterised by influx and efflux intrinsic clearances. Once in the tissue, a drug is subject to metabolism with the capacity defined by intrinsic clearance specific to each zone. The zonation and resulting concentration differences throughout the liver tissue could be crucial for simulation of liver physiology and pathophysiology, including sequential metabolism of a drug, cirrhosis or hepatic neoplasms. Other attempts to model the liver with consideration of its heterogeneous structure and metabolic properties include combining a series of well-stirred compartments and distribution of tubes with different metabolic capacity and specific blood flow: distributed model and its extension which includes intermixing between sinusoids with uniform flow; interconnected-tubes model or intermittent mixing; circulatory and fractal models; agent-based models; or 2D and 3D models [50,57,61–65].

Heart

Cardioactive drugs can trigger their effects by binding to receptors, enzymes, ion channels or other targets from either the extracellular or intracellular side of the sarcolemma. Therefore, knowledge of a drug concentration at its site of action, in the exact compartment of the heart, is needed to comprehend the exposure–response relationship. One of the approaches to calculate drug myocardial uptake under *in vivo* conditions requires knowledge of arterial (i.e., heart tissue input concentration) and coronary sinus (i.e., heart tissue output concentration). This approach assumes a well-stirred heart compartment with perfusion-limited kinetics. This has been applied to evaluate myocardial uptake of some drugs in humans [66–69]. More recently, to enable the prediction of drug distribution within cardiac tissue, a semi-mechanistic four-compartment heart model nested in the full-PBPK model was proposed [70]. The added compartments represented the epicardium, midmyocardium, endocardium and pericardial fluid. The model accounted for cardiac metabolism. Despite some limitations, the model reflected a simplified description of human cardiac anatomy and physiology and the ratios of predicted concentrations of the drug amitriptyline in the heart tissue and venous compartment were within the Kp (tissue to plasma partition coefficient) values reported in post-mortem human and animal studies. Subsequently, this model was expanded into a five-compartment structure [70]. The new model structure consists of compartments representing the pericardial fluid, heart extracellular compartment, epicardial intracellular, midmyocardial intracellular and endocardial intracellular compartments, which are linked under the assumption of permeability-limited kinetics. The model accounts for cardiac metabolism, passive diffusion and active transport as the routes for drug distribution within heart tissue. Interindividual variability was implemented into the model parameterisation. The model was verified in terms of PK and the usefulness of predicted cardiac concentrations for modelling of amitriptyline-related electrophysiological effect [71].

Kidney

Renal clearance is an important contributor of drug total clearance. The mechanistic insight into the processes that drugs undergo in the kidneys enables the quantitative prediction of renal elimination. Tang-Liu *et al*. developed a model that described the dependence of renal clearance of passively reabsorbed drugs on urine flow [72]. However, the model did not account for active or pH-dependent secretion or reabsorption. Katayama *et al*. incorporated the processes of glomerular filtration and active secretion as a Michaelis–Menten function into the animal model [73]. Subsequently, this model was further adapted by Brightman *et al*. [74,75]. They subdivided the kidney into three diffusion-limited compartments representing the capillary bed, interstitial fluid and intracellular space. The physiology-based renal excretion model incorporated the renal tubule as a separate compartment. The renal clearance was described as influenced by glomerular filtration, urine flow, active secretion of compound and passive diffusion, which occurred among the model compartments. A new mechanistic kidney PBPK model: Mech KiM, was proposed by Neuhoff *et al*. [76]. Mech KiM is designed in such a way as to predict the integrated effects of intrarenal processes (i.e., glomerular filtration, active and passive secretion and reabsorption and intrarenal drug metabolism). The model consists of three compartments: tubular fluid, cell mass and blood space, subdivided into eight segments. The segments consist of glomerulus, proximal tubule and collecting ducts. The model was parameterised to reflect human renal physiology. Because the model provides the framework for active uptake and efflux in the kidney, it was applied effectively to predict transporter-mediated drug–drug interaction for drugs eliminated by the kidney including the interaction between nonsteroidal anti-inflammatory drugs and pemetrexed [77], cimetidine and metformin [78], or probenecid and oseltamivir carboxylate, cidofovir or cefuroxime [79]. Applying mechanistic kidney model parameterisation also allowed the prediction of renal clearance in a situation of renal impairment [79], and pregnancy which is known to affect renal secretion and filtration [80].

Gastrointestinal tract (GIT)

In addition to the liver, the intestine also plays a major part in first-pass extraction and, thus, influences the bioavailability and systemic concentration of orally administered drugs. In its simplest form, derived from the well-stirred liver model, the well-stirred gut model is not appropriate owing to the need to accommodate additional factors including the interplay between permeation, local blood flow and metabolism in the gut [76]. The Q_{gut} model corrects and further extends the well-stirred model by accounting

for the influence of permeability through enterocyte membrane and the villus blood flow on the exposure to the metabolic enzyme [81]. The segregated flow model (SFM) splits the total intestinal blood flow into the fraction perfusing the enterocyte region (≈0.05–0.3 × total intestinal flow) and the fraction that perfuses other, nonabsorbing, nonactive metabolically, regions [82].

Simple compartmental models, however, are not adequate to address effects of complex interplay of binding, passive permeability, segmental differences of transporters and enzyme abundance and activity, relevant blood flows and transit times. The segmental segregated flow model (SSFM) provides regional heterogeneity of metabolic functions and transport via division of the intestine into three segments of equal length and blood flow [83]. The compartmental and transit model (CAT) represents further refinement in oral absorption modelling. It replicates the intestinal tract as two series of seven compartments representing the duodenum (one compartment), jejunum (two compartments) and ileum (four compartments). Each series of compartments represents either dissolved or undissolved drug [84–86] so that the CAT model captures not only the spatial and temporal difference along the length of the GIT but also the influence of the drug dissolution process on the absorption. The advanced compartmental and transit model (ACAT), the CAT model extension, adds further compartments describing unreleased drug in formulation, undissolved drug and dissolved drug to allow assessment of formulation release rate on the absorption process. Supplementary compartments for stomach and colon are also incorporated, thus the additional heterogeneity of the GIT can be represented to better describe the *in vivo* situation. The advanced dissolution, absorption and metabolism model (ADAM) represents another example of oral absorption model improvement. The model is based mainly on the CAT model with advancements through consideration of the type of formulation (suspension, immediate release, enteric-coated tablets, etc.), the physicochemical and pharmaceutical processes in each GI compartment (disintegration, de-aggregation, dissolution, solubility and supersaturation/precipitation) and enterohepatic recirculation [87]. Recently, the ADAM model was expanded to incorporate an unstirred boundary layer for oral absorption from luminal fluid to enterocyte, a permeability-limited basolateral membrane between the enterocyte and the intestinal interstitial fluid and lymphatic absorption from the intestinal fluid to systemic circulation (multilayer gut wall within ADAM model; M-ADAM).

All of these advances have led to the possibility of predicting drug concentration in various GI segments and their subcompartments, such as luminal fluid interfacing the enterocyte microvillus membrane or enterocytes, and capacity to consider spatial and temporal heterogeneity in pH, effective surface area, transporter and metabolic enzymes expression and transit time, as well as the role of metabolic and transporter-based drug–drug interactions.

Skin

The estimation of exposure following absorption of any drug from the skin is important, not only from the development of dermal pharmaceutical and cosmetic products but also knowing local and systemic concentrations for safety assessment. Potential toxic reactions after intended or unintended use of drugs, cosmetic products, environmental and occupational chemicals are generally concentration dependent. Regardless of the mechanism of action and clinical manifestation of the reaction, knowledge about the amount of xenobiotic in the biophase, in this case certain skin layer or in the blood, is essential. Skin is the largest organ in the body, covering ~7600 cm^2 in an average adult, and consisting of several types of tissues. The total thickness of the skin varies in different parts of the body, being on average 1.5 mm. Skin is made of two principal layers: the outer stratified epidermis and the inner dermis (D). The epidermis is the protective layer of the skin and is made of stratified squamous epithelial cells. The stratum corneum (SC) is the outermost layer of the epidermis and consists of several layers of completely keratinised, dead cells that are constantly desquamated. The rest of epidermis, which lies below the SC, is alive, has different characteristics (less lipophilic, more hydrophilic) and is referred to as viable epidermis (VE). The dermis has connective tissue, cells (fibroblasts), blood vessels, lymph, oil and sweat glands, nerves, hair follicles and other structures. It is made up of a thin upper layer called the papillary dermis and a thick lower layer called the reticular dermis. The dermis provides the important functions of thermoregulation and supports the vascular network to supply the avascular epidermis with nutrients, but also clears the dermis from xenobiotics [88].

Determination of compound toxicology requires prediction of the concentration in the viable skin layers [89]. Depending on the drug, or general chemical of interest, the mathematical models based on the PBPK approach can provide information about defined local or systemic absorption after topical application. They divide skin into compartments that are functional representations of the above-mentioned skin structures [90]. In addition, they allow investigation into the role of other system-specific parameters (i.e., appendages) and drug-formulation-specific information. In summary, PBPK models coupled with the databases of human anatomy and physiology data allow differentiation between various populations, including special (e.g., paediatric, pregnant women) and disease populations, there is however a need for a proper parameterisation with the use of data describing real variability in a population.

Examples of PBPK (local tissue concentration) and QSP/QST model combinations

In the case of drugs that exert their therapeutic or toxic effects in a specific tissue or organ, such as the central nervous system, liver, lung, skin, synovial fluid and tumours, the use of plasma concentration might lead to inadequate assessment of the exposure–response relationship. Thus, the coupling of QSP/QST models offering detailed description of the mechanism of action of a drug with an appropriate PBPK model able to compute relevant drug exposure data should enhance the accuracy of the prediction and so aid translation between experimental situations and hence interpretation. Although there are reports of linking PBPK and QSP/QST models, very few utilise models of local (i.e., at site of action) concentration with a mechanistic model of QSP/QST to predict exposure response. The majority either combine predictions of plasma concentration with a mechanistic QSP/QST model or combine the PBPK model of local concentration with an empirical PD model [91–93]. Examples of the more accurate, former case are discussed below.

Drug efficacy

A recent report by Thiel *et al*. provides a nice example of the effective combination of PBPK and QSP approaches to inform drug efficacy [94]. These authors employed a computational modelling approach to predict the efficacy of a set of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) inhibitors in humans and assess the impact of rifampicin pre-treatment on prostaglandin formation. They coupled drug-specific PBPK models with cellular network models of arachidonic acid metabolism and rifampicin-induced pregnane X receptor (PXR)-mediated cytochrome P450 (CYP) activation. Simulated drug concentrations (venous blood for COX-2 and 5-LOX inhibitors and intracellular hepatocytes for rifampicin) were used to inform the PD model to enable evaluation of drug efficacy and the impact of drug interactions on the PK behaviour in 11 therapeutic situations. Additionally, the model predicted a correlation between the decrease in prostaglandin formation and pain relief. This study might be considered as a POC and an illustration of the potential of the QSP approach and role of PBPK-simulated local drug concentration.

Drug triggered liver toxicity

The active metabolites of paracetamol deplete glutathione, which has protective functions within cells, and hence paracetamol can cause toxicity when given at high doses or in a situation when detoxification is inhibited. Therefore, the determination of hepatic glutathione status has potential value for therapeutic optimisation and of toxicity risk assessment in patients exposed to paracetamol. However, currently the correlation of glutathione hepatic content with the proposed plasma and urine biomarkers is unclear. Geenen *et al*. proposed a solution for this problem by combining a mechanistic model describing hepatic glutathione homeostasis with a PBPK model of metabolism and disposition of paracetamol [95]. The former correlates intracellular glutathione concentration with the exposure to 5-oxoproline and ophthalmic acid, which are biomarkers of human liver cell toxicity, whereas the PBPK model allows the prediction of not only the local dynamic changes of paracetamol in the liver but also the distribution of drug-induced liver injury (DILI) biomarkers. The final coupled model was verified against the experimentally measured data, showing good correlation with the simulated values. Despite its limitations, including the lack of dynamic, adaptive changes in liver cell metabolism, the model can be potentially useful for therapy optimisation. The same approach can also be used for other compounds that deplete hepatic glutathione stores.

Drug-induced atherosclerosis

In their review, Pichardo-Almarza and Diaz-Zuccarini gave an overview of the approaches combining PBPK models with semi-mechanistic and QSP models for safety assessment of cholesterol-lowering drugs [96]. By discussing models of various mathematical complexities these authors described the benefits from combining models describing drug kinetics (including PBPK models) and mechanistic computational models of cholesterol-lowering drugs [92,97].

Chemical-induced neurotoxicity

Brain-derived neurotropic factor (BDNF) plays an important part in the control of neuronal cell proliferation, differentiation and survivability and has been used as a biomarker linking chemical exposure to neuronal disorders [98]. In a study by Sharma *et al*. [99], a PBPK model predicting local target tissue concentration of a test compound was integrated with a mechanistic model of BDNF formation. The test compound readily passed through the blood–brain barrier and hence the model based on target tissue concentrations was selected to provide better brain exposure prediction than a simple plasma exposure–response model. The modelled brain PK profile of the compound was coupled to a mechanistic miRNA/BDNF pathway model and the output of the PBPK–PD model subsequently linked to neuronal survivability. The resultant QST model effectively described the observed changes in the concentration of endogenous molecules during and after discontinuation of test compound administration and was shown to be able to predict the adverse effect.

Drug-induced cardiac toxicity

Patel and colleagues proposed a multiscale mechanistic modelling framework consisting of PBPK simulations (Simcyp® Simulator) of clinically relevant drug exposures combined with QST models of cardiac electrophysiology to predict the risk of the cardiac arrhythmia Torsades de Pointes (TdP) [100]. The QST model used the QTc interval of the electrocardiogram (ECG) as a biomarker for the risk of TdP. Moxifloxacin, a widely used antibacterial agent, known to cause QTc prolongation, was used to exemplify the model and show the importance of accurate drug exposure assessment to combine with the mechanistic model and quantify the risk of drug-induced TdP. Systemic plasma moxifloxacin concentrations were obtained from the literature and used as verification for the developed PBPK model. Local heart tissue concentrations were further simulated. These exposure data were utilised as input for the QST model of cardiac electrophysiology, to simulate the cardiac-specific clinical endpoint; QTc interval was derived from a pseudo ECG signal. In combination with the *in vitro* measured inhibition of the ionic currents the authors were able to test multiple virtual scenarios and define the most predictive model, which was further verified against the clinically observed QTc prolongation values ($\Delta\Delta$QTc in milliseconds). Based on the results obtained, it was proposed that PBPK-simulated heart-tissue concentration is a better surrogate of the active drug fraction than plasma concentration. The authors recommended that PBPK–QST modelling should be considered throughout drug discovery and development, to assess cardiac safety liability and be a key tool in aiding decision making.

A similar study that extended this consideration to the situation of multiple drugs administered at the same time including the added complexity of metabolic interaction was published by Wiśniowska and Polak [101]. The Simcyp® simulator was used to develop a PBPK model to extrapolate *in vitro* terfenadine specific data to the *in vivo* situation in the presence of various metabolic inhibitors. Electrophysiological effects were simulated with the use of the Cardiac Safety Simulator™ (Simcyp, Certara UK), where the modification of ion channel activity was dependent on the inhibitory potential of each respective drug. The authors concluded that mechanistic *in-vitro–in-vivo* extrapolation can be applied to predict the clinical observed effects of drug–drug interactions through simulation of PK and PD.

Concluding remarks

The integration of PBPK modelling with quantitative systems approaches to improve efficacy and safety assessments is a much-needed step towards improving the drug discovery and development process. Success requires the effective partnership between scientific disciplines including chemistry, pharmacology, toxicology, drug metabolism, mathematics and computing. Computational models need to be fed with relevant data from *in vitro*, *in vivo* and *ex vivo* experiments and need to continually evolve in an iterative fashion. Their role is to inform the scientist to facilitate hypothesis testing and better decision making. Supporting evolution, MID3 justifies the significant efforts expended in the development of the major components [14].

There are multiple ways to apply and continually evolve these techniques, including, but not limited to:

- the addition and linkage of further relevant tissues, including the extension of models to the cellular and subcellular level to PBPK models;
- utilisation of additional sources of relevant data to improve model development, for example high throughput omics data, stem-cell-derived specialised models such as spheroid, organoid and other microphysiological systems;
- inclusion of the drug-related dynamic modification of the physiological parameters;
- development of the patient-specific models.

We look forward to seeing and actively participating in the continued evolution and impact of this exciting area of science in the future.

References

1 Morgan, P. *et al.* (2012) Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov. Today* 17, 419–424
2 Way, J.C. and Silver, P.A. (2007) Systems engineering without an engineer: why we need systems biology. *Complexity* 13, 22–29
3 Marshall, S.F. *et al.* (2016) Good practices in model-informed drug discovery and development: practice, application, and documentation. *CPT Pharmacometrics Syst. Pharmacol* 5, 93–122
4 FDA (2017) *FDA's Predictive Toxicology Roadmap*. 2017 Available at: https://www.fda.gov/media/109634/download
5 Rostami-Hodjegan, A. and Tucker, G.T. (2007) Simulation and prediction of *in vivo* drug metabolism in human populations from *in vitro* data. *Nat. Rev. Drug Discov.* 6, 140–148
6 EMA CHMP (2016) *Guideline on the qualification and reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation*. EMA/CHMP/458101/20162016
7 Hsieh, N.-H. *et al.* (2018) Applying a global sensitivity analysis workflow to improve the computational efficiencies in physiologically-based pharmacokinetic modeling. *Front. Pharmacol.* 9, 588
8 Garcia, R.I. *et al.* (2015) Identifiability of PBPK models with applications to dimethylarsinic acid exposure. *J. Pharmacokinet. Pharmacodyn.* 42, 591–609
9 Jones, H. and Rowland-Yeo, K. (2013) Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT Pharmacometrics Syst. Pharmacol.* 2, e63
10 Jaroch, K. *et al.* (2018) Cell cultures in drug discovery and development: the need of reliable *in vitro-in vivo* extrapolation for pharmacodynamics and pharmacokinetics assessment. *J. Pharm. Biomed. Anal.* 147, 297–312
11 Tan, Y.-M. *et al.* (2018) Challenges associated with applying physiologically based pharmacokinetic modeling for public health decision-making. *Toxicol. Sci.* 162, 341–348
12 Zineh, I. (2017) FDA Briefing Document. In *Pharmaceutical science and clinical pharmacology advisory committee meeting. Strategies, approaches, and challenges in model-informed drug development (MIDD)*. Available at: https://www.fda.gov/media/103858/download
13 Abernethy, D.R. *et al.* (2011) An NIH White Paper by the QSP Workshop Group. In *Quantitative and systems pharmacology in the post-genomic era: new approaches to discovering drugs and understanding therapeutic mechanisms*. https://www.nigms.nih.gov/training/documents/systemspharmawpsorger2011.pdf
14 Rostami-Hodjegan, A. (2018) Reverse translation in PBPK and QSP: going backwards in order to go forward with confidence. *Clin. Pharmacol. Ther.* 103, 224–232
15 Bloomingdale, P. *et al.* (2017) Quantitative systems toxicology. *Curr. Opin. Toxicol.* 4, 79–87
16 Bowes, J. *et al.* (2012) Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling. *Nat. Rev. Drug Discov.* 11, 909–922
17 Leil, T.A. and Bertz, R. (2014) Quantitative systems pharmacology can reduce attrition and improve productivity in pharmaceutical research and development. *Front. Pharmacol.* 5, 247
18 Bass, A.S. *et al.* (2015) A historical view and vision into the future of the field of safety pharmacology. *Handb. Exp. Pharmacol.* 229, 3–45
19 Patel, N. *et al.* (2018) Virtual thorough QT (TQT) trial-extrapolation of *in vitro* cardiac safety data to *in vivo* situation using multi-scale physiologically based ventricular cell-wall model exemplified with tolterodine and fesoterodine. *AAPS J.* 20, 83
20 Guo, Y. *et al.* (2018) Advancing predictions of tissue and intracellular drug concentrations using *in vitro*, imaging and PBPK modeling approaches. *Clin. Pharmacol. Ther.* . http://dx.doi.org/10.1002/cpt.1183
21 Dollery, C.T. (2013) Intracellular drug concentrations. *Clin. Pharmacol. Ther.* 93, 263–266
22 Rizk, M. *et al.* (2017) Importance of drug pharmacokinetics at the site of action. *Clin. Transl. Sci.* 10, 133–142
23 Shebley, M. *et al.* (2018) Physiologically based pharmacokinetic model qualification and reporting procedures for regulatory submissions: a consortium perspective. *Clin. Pharmacol. Ther.* . http://dx.doi.org/10.1002/cpt.1013
24 Chu, X. *et al.* (2013) Intracellular drug concentrations and transporters: measurement, modeling, and implications for the liver. *Clin. Pharmacol. Ther.* 94, 126–141
25 Upton, R.N. (1990) Regional pharmacokinetics. I. Physiological and physicochemical basis. *Biopharm. Drug Dispos.* 11, 647–662
26 Tylutki, Z. and Polak, S. (2015) Plasma vs heart tissue concentration in humans – literature data analysis of drugs distribution. *Biopharm. Drug Dispos.* 36, 337–351
27 Brunner, M. and Langer, O. (2006) Microdialysis versus other techniques for the clinical assessment of *in vivo* tissue drug distribution. *AAPS J.* 8, E263–E271
28 Pitz, M.W. *et al.* (2011) Tissue concentration of systemically administered antineoplastic agents in human brain tumors. *J. Neurooncol.* 104, 629–638
29 Mariappan, T.T. *et al.* (2013) Insight into tissue unbound concentration: utility in drug discovery and development. *Curr. Drug Metab.* 14, 324–340
30 Li, Y. *et al.* (2006) Microdialysis as a tool in local pharmacodynamics. *AAPS J.* 8, E222–E235
31 Fischman, A.J. *et al.* (2002) Pharmacokinetic imaging: a noninvasive method for determining drug distribution and action. *Clin. Pharmacokinetics* 41, 581–602
32 Pfeifer, N.D. *et al.* (2013) Determination of intracellular unbound concentrations and subcellular localization of drugs in rat sandwich-cultured hepatocytes compared with liver tissue. *Drug Metab. Dispos.* 41, 1949–1956
33 Zhou, F. *et al.* (2011) Toward a new age of cellular pharmacokinetics in drug discovery. *Drug Metab. Rev.* 43, 335–345
34 Mateus, A. *et al.* (2017) Intracellular drug bioavailability: a new predictor of system dependent drug disposition. *Sci. Rep.* 7, 43047
35 Chien, H.-C. *et al.* (2016) Rapid method to determine intracellular drug concentrations in cellular uptake assays: application to metformin in organic cation transporter 1-transfected human embryonic kidney 293 cells. *Drug Metab. Dispos* 44, 356–364
36 Gordon, L.J. *et al.* (2016) Direct measurement of intracellular compound concentration by RapidFire mass spectrometry offers insights into cell permeability. *J. Biomol. Screen.* 21, 156–164
37 Tansi, F.L. *et al.* (2017) A fast and effective determination of the biodistribution and subcellular localization of fluorescent immunoliposomes in freshly excised animal organs. *BMC Biotechnol.* 17, 8
38 Huang, S.-M. and Rowland, M. (2012) The role of physiologically based pharmacokinetic modeling in regulatory review. *Clin. Pharmacol. Ther.* 91, 542–549
39 Yoshida, K. *et al.* (2017) Impact of physiologically based pharmacokinetic models on regulatory reviews and product labels: frequent utilization in the field of oncology. *Clin. Pharmacol. Ther.* 101, 597–602

40 Paini, A. *et al.* (2017) Investigating the state of physiologically based kinetic modelling practices and challenges associated with gaining regulatory acceptance of model applications. *Regul. Toxicol. Pharmacol.* 90, 104–115
41 Ramsey, J.C. and Andersen, M.E. (1984) A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* 73, 159–175
42 Johanson, G. (1991) Modelling of respiratory exchange of polar solvents. *Ann. Occup. Hyg.* 35, 323–339
43 Sarangapani, R. *et al.* (2002) Physiologically based pharmacokinetic modeling of styrene and styrene oxide respiratory-tract dosimetry in rodents and humans. *Inhal. Toxicol.* 14, 789–834
44 Gloede, E. *et al.* (2011) A validated hybrid computational fluid dynamics-physiologically based pharmacokinetic model for respiratory tract vapor absorption in the human and rat and its application to inhalation dosimetry of diacetyl. *Toxicol. Sci.* 123, 231–246
45 Morris, J.B. (2012) Biologically-based modeling insights in inhaled vapor absorption and dosimetry. *Pharmacol. Ther.* 136, 401–413
46 Bäckman, P. *et al.* (2018) Advances in experimental and mechanistic computational models to understand pulmonary exposure to inhaled drugs. *Eur. J. Pharm. Sci.* 113, 41–52
47 Gaohua, L. *et al.* (2015) Development of a multicompartment permeability-limited lung PBPK model and its application in predicting pulmonary pharmacokinetics of antituberculosis drugs. *CPT Pharmacometrics Syst. Pharmacol.* 4, 605–613
48 Stass, H. *et al.* (2013) Inhalation of a dry powder ciprofloxacin formulation in healthy subjects: a Phase I study. *Clin. Drug Invest.* 33, 419–427
49 Salerno, S.N. *et al.* (2017) Development of an adult physiologically based pharmacokinetic model of solithromycin in plasma and epithelial lining fluid. *CPT Pharmacometrics Syst. Pharmacol.* 6, 814–822
50 Lerapetritou, M.G. *et al.* (2009) Tissue-level modeling of xenobiotic metabolism in liver: an emerging tool for enabling clinical translational research. *Clin. Transl. Sci.* 2, 228–237
51 Pang, K.S. and Rowland, M. (1977) Hepatic clearance of drugs. I. Theoretical considerations of a ''well-stirred'' model and a ''parallel tube'' model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J. Pharmacokinet. Biopharm.* 5, 625–653
52 Pang, K.S. and Rowland, M. (1977) Hepatic clearance of drugs. II. Experimental evidence for acceptance of the ''well-stirred'' model over the ''parallel tube'' model using lidocaine in the perfused rat liver in situ preparation. *J. Pharmacokinet. Biopharm.* 5, 655–680
53 Roberts, M.S. and Rowland, M. (1986) A dispersion model of hepatic elimination: 1. Formulation of the model and bolus considerations. *J. Pharmacokinet. Biopharm.* 14, 227–260
54 Bass, L. *et al.* (1976) Enzymatic elimination of substrates flowing through the intact liver. *J. Theor. Biol.* 61, 393–409
55 Bass, L. *et al.* (1978) Hepatic elimination of flowing substrates: the distributed model. *J. Theor. Biol.* 72, 161–184
56 Ito, K. and Houston, J.B. (2004) Comparison of the use of liver models for predicting drug clearance using *in vitro* kinetic data from hepatic microsomes and isolated hepatocytes. *Pharm. Res.* 21, 785–792
57 Rezania, V. *et al.* (2016) A physiologically-based flow network model for hepatic drug elimination III: 2D/3D DLA lobule models. *Theor. Biol. Med. Model.* . http://dx.doi.org/10.1186/s12976-016-0034-5
58 Abu-Zahra, T.N. and Pang, K.S. (2000) Effect of zonal transport and metabolism on hepatic removal: enalapril hydrolysis in zonal, isolated rat hepatocytes *in vitro* and correlation with perfusion data. *Drug Metab. Dispos.* 28, 807–813
59 Ohno, H. *et al.* (2008) Construction of a biological tissue model based on a single-cell model: a computer simulation of metabolic heterogeneity in the liver lobule. *Artif. Life* 14, 3–28
60 Liu, L. *et al.* (2005) Vascular binding, blood flow, transporter, and enzyme interactions on the processing of digoxin in rat liver. *J. Pharmacol. Exp. Ther.* 315, 433–448
61 Anissimov, Y.G. *et al.* (1997) Interconnected-tubes model of hepatic elimination. *J. Theoretical Biology* 188, 89–101
62 Pang, K.S. *et al.* (2007) Advanced pharmacokinetic models based on organ clearance, circulatory, and fractal concepts. *AAPS J.* 9, E268–E283
63 Rezania, V. *et al.* (2013) A physiologically-based flow network model for hepatic drug elimination I: regular lattice lobule model. *Theor. Biol. Med. Model.* 10, 52
64 White, D. *et al.* (2016) Building a 3D virtual liver: methods for simulating blood flow and hepatic clearance on 3D structures. *PLoS One* 11, e0162215
65 Holzhütter, H.-G. *et al.* (2012) The virtual liver: a multidisciplinary, multilevel challenge for systems biology. *Syst. Biol. Med.* 4, 221–235
66 Ritchie, R.H. *et al.* (1998) Myocardial effect compartment modeling of metoprolol and sotalol: importance of myocardial subsite drug concentration. *J. Pharm. Sci.* 87, 177–182
67 Zeitz, C.J. *et al.* (2003) Myocardial uptake and biochemical and hemodynamic effects of ACE inhibitors in humans. *Hypertension* 41, 482–487
68 Morgan, D.J. *et al.* (1989) Prediction of acute myocardial disposition of antiarrhythmic drugs. *J. Pharm. Sci.* 78, 384–388
69 Ritche, R.H. *et al.* (1998) Relationship between myocardial milrinone content and its acute hemodynamic and electrophysiologic effects. *J. Cardiovasc. Pharmacol.* 31, 885–893
70 Tylutki, Z. and Polak, S. (2017) A four-compartment PBPK heart model accounting for cardiac metabolism – model development and application. *Sci. Rep.* 7, 39494
71 Tylutki, Z. *et al.* (2018) Physiologically based pharmacokinetic-quantitative systems toxicology and safety (PBPK-QSTS) modeling approach applied to predict the variability of amitriptyline pharmacokinetics and cardiac safety in populations and in individuals. *J. Pharmacokinet. Pharmacodyn.* 45, 663–677
72 Tang-Liu, D.D.-S. *et al.* (1983) Dependence of renal clearance on urine flow: a mathematical model and its application. *J. Pharm. Sci.* 72, 154–158
73 Katayama, K. *et al.* (1990) Kinetic studies on drug disposition in rabbits. I. Renal excretion of iodopyracet and sulfamethizole. *J. Pharmacobio. Dyn.* 13, 97–107
74 Brightman, F.A. *et al.* (2006) Application of a generic physiologically based pharmacokinetic model to the estimation of xenobiotic levels in rat plasma. *Drug Metab. Dispos.* 34, 84
75 Brightman, F.A. *et al.* (2006) Application of a generic physiologically based pharmacokinetic model to the estimation of xenobiotic levels in human plasma. *Drug Metab. Dispos.* 34, 94
76 Neuhoff, S. *et al.* (2013) Accounting for transporters in renal clearance: towards a mechanistic kidney model (Mech KiM). In *Transporters in Drug Development* (Sugiyama, Y. and Steffansen, B., eds), pp. 155–177, Springer
77 Posada, M.M. *et al.* (2015) Prediction of renal transporter mediated drug–drug interactions for pemetrexed using physiologically based pharmacokinetic modeling. *Drug Metab. Dispos.* 43, 325
78 Burt, H.J. *et al.* (2016) Metformin and cimetidine: physiologically based pharmacokinetic modelling to investigate transporter mediated drug–drug interactions. *Eur. J. Pharm. Sci.* 88, 70–82
79 Hsu, V. *et al.* (2014) Towards quantitation of the effects of renal impairment and probenecid inhibition on kidney uptake and efflux transporters, using physiologically based pharmacokinetic modelling and simulations. *Clin. Pharmacokinet.* 53, 283–293
80 De Sousa Mendes, M. *et al.* (2015) Physiologically-based pharmacokinetic modeling of renally excreted antiretroviral drugs in pregnant women. *Br. J. Clin. Pharmacol.* 80, 1031–1041
81 Yang, J. *et al.* (2007) Misuse of the well-stirred model of hepatic drug clearance. *Drug Metab. Dispos.* 35, 501–502
82 Cong, D. *et al.* (2000) A new physiologically based, segregated-flow model to explain route-dependent intestinal metabolism. *Drug Metab. Dispos.* 28, 224–235
83 Tam, D. *et al.* (2003) Segmental intestinal transporters and metabolic enzymes on intestinal drug absorption. *Drug Metab. Dispos.* 31, 373–383
84 Yu, L.X. *et al.* (1996) Compartmental transit and dispersion model analysis of small intestinal transit flow in humans. *Int. J. Pharm.* 140, 111–118
85 Yu, L.X. (1999) An integrated model for determining causes of poor oral drug absorption. *Pharm. Res.* 16, 1883–1887
86 Yu, L.X. *et al.* (1996) Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Adv. Drug Deliv. Rev.* 19, 359–376
87 Jamei, M. *et al.* (2009) Population-based mechanistic prediction of oral drug absorption. *AAPS J.* 11, 225–237
88 Riviere, J.E., ed. (2005) *Dermal absorption models in toxicology and pharmacology*, Taylor & Francis
89 Dancik, Y. *et al.* (2013) Design and performance of a spreadsheet-based model for estimating bioavailability of chemicals from dermal exposure. *Adv. Drug Deliv. Rev* 65, 221–236
90 Polak, S. *et al.* (2012) Prediction of concentration-time profile and its inter-individual variability following the dermal drug absorption. *J. Pharm. Sci.* 101, 2584–2595
91 Boger, E. and Fridén, M. (2018) Physiologically based pharmacokinetic/pharmacodynamic modeling accurately predicts the better bronchodilatory effect of inhaled versus oral salbutamol dosage forms. *J. Aerosol. Med. Pulm. Drug Deliv.* . http://dx.doi.org/10.1089/jamp.2017.1436
92 Rose, R.H. *et al.* (2014) Application of a physiologically based pharmacokinetic model to predict OATP1B1-related variability in pharmacodynamics of rosuvastatin. *CPT Pharmacometrics Syst. Pharmacol.* 3, e124

93 Sadiq, M.W. *et al.* (2017) A whole-body physiologically based pharmacokinetic (WB-PBPK) model of ciprofloxacin: a step towards predicting bacterial killing at sites of infection. *J. Pharmacokinet. Pharmacodyn.* 44, 69–79
94 Thiel, C. *et al.* (2018) Using quantitative systems pharmacology to evaluate the drug efficacy of COX-2 and 5-LOX inhibitors in therapeutic situations. *NPJ Syst. Biol. Appl.* 4, 28
95 Geenen, S. *et al.* (2013) Multiscale modelling approach combining a kinetic model of glutathione metabolism with PBPK models of paracetamol and the potential glutathione-depletion biomarkers ophthalmic acid and 5-oxoproline in humans and rats. *Integr. Biol.* 5, 877–888
96 Pichardo-Almarza, C. and Diaz-Zuccarini, V. (2016) From PK/PD to QSP: understanding the dynamic effect of cholesterol-lowering drugs on atherosclerosis progression and stratified medicine. *Curr. Pharm. Des.* 22, 6903–6910
97 Lippert, J. *et al.* (2012) A mechanistic, model-based approach to safety assessment in clinical development. *CPT Pharmacometrics Syst. Pharmacol.* 1, e13
98 Boulle, F. *et al.* (2012) Epigenetic regulation of the BDNF gene: implications for psychiatric disorders. *Mol. Psych.* 17, 584–596
99 Sharma, R.P. *et al.* (2017) Developing integrated PBPK/PD coupled mechanistic pathway model (miRNA-BDNF): an approach towards system toxicology. *Toxicol. Lett.* 280, 79–91
100 Patel, N. *et al.* (2018) Towards bridging translational gap in cardiotoxicity prediction: an application of progressive cardiac risk assessment strategy in TdP risk assessment of moxifloxacin. *AAPS J.* 20, 47
101 Wiśniowska, B. and Polak, S. (2016) Virtual clinical trial toward polytherapy safety assessment: combination of physiologically based pharmacokinetic/pharmacodynamic-based modeling and simulation approach with drug–drug interactions involving terfenadine as an example. *J. Pharm. Sci.* 105, 3415–3424