BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **35**: 15–32 (2014)
Published online 10 December 2013 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/bdd.1879

Invited Review

Computational approaches to analyse and predict small molecule transport and distribution at cellular and subcellular levels

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ABSTRACT: Quantitative structure–activity relationship (QSAR) studies and mechanistic mathematical modeling approaches have been independently employed for analysing and predicting the transport and distribution of small molecule chemical agents in living organisms. Both of these computational approaches have been useful for interpreting experiments measuring the transport properties of small molecule chemical agents, *in vitro* and *in vivo*. Nevertheless, mechanistic cell-based pharmacokinetic models have been especially useful to guide the design of experiments probing the molecular pathways underlying small molecule transport phenomena. Unlike QSAR models, mechanistic models can be integrated from microscopic to macroscopic levels, to analyse the spatiotemporal dynamics of small molecule chemical agents from intracellular organelles to whole organs, well beyond the experiments and training data sets upon which the models are based. Based on differential equations, mechanistic models can also be integrated with other differential equations-based systems biology models of biochemical networks or signaling pathways. Although the origin and evolution of mathematical modeling approaches aimed at predicting drug transport and distribution has occurred independently from systems biology, we propose that the incorporation of mechanistic cell-based computational models of drug transport and distribution into a systems biology modeling framework is a logical next step for the advancement of systems pharmacology research. Copyright © 2013 John Wiley & Sons, Ltd.

Key words: cellular pharmacokinetics; computational modeling; drug transport; systems pharmacology

Impact of Cellular Pharmacokinetics on Pharmaceutical Research

Cell-based computational modeling of drug transport and distribution is an active area of research in pharmaceutical sciences, because the subcellular location of drug targets within the three dimensional architecture of cells, tissues and organs ultimately defines the site of action of drug molecules in the body. In multicellular organisms, cell monolayers constitute physical boundaries that separate one tissue or anatomical compartment from another, and determine the movement of molecules between compartments. For example, in the gastrointestinal tract, the monolayer of epithelial cells that lines the lumen of the gut also controls the absorption of nutrients and xenobiotics into the body (Figure 1A). After absorption, nutrients and xenobiotics also have to make their

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Received 29 June 2013
Revised 15 October 2013
Accepted 1 November 2013

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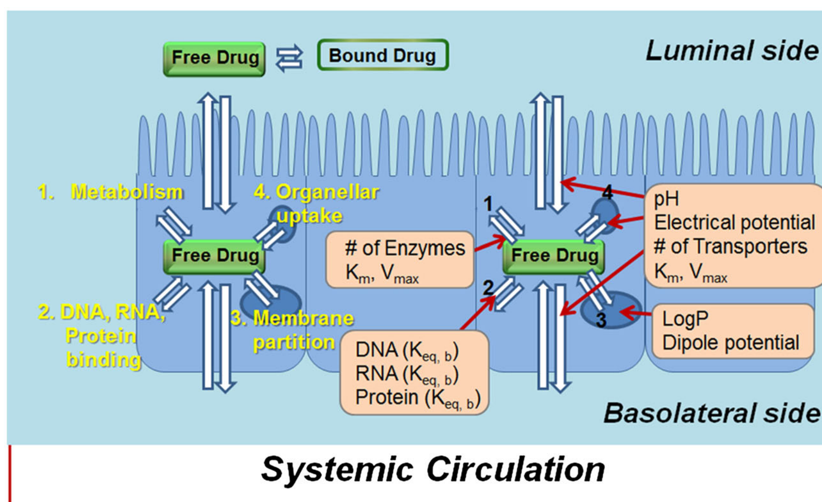

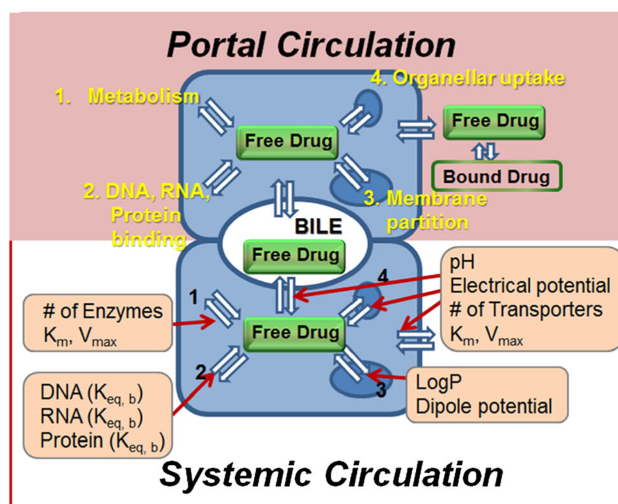

Figure 1. Cellular pharmacokinetic models can be used to study the transport and distribution of small molecules in the presence of a transcellular concentration gradient. As drugs are absorbed across the lining of the gastrointestinal tract (A) or as they are metabolized in the hepatocytes (B), cells normally experience a transcellular drug concentration gradient. Outside the cells, drug molecules can exist in free or bound forms. Only freely soluble molecules can diffuse across the membranes. Inside the cells, drug molecules can be metabolized, partition into cellular lipids, bind to cellular macromolecules, or become sequestered in organelles. All these mechanisms are influenced by chemical parameters such as the lipophilicity (as reflected in the octanol:water partition coefficient); the ionization states of the molecules (pK_a); physiological parameters such as the pH of the different compartments and the electrical potentials across their bound membranes; and, biological parameters such as the volume and number of organelles, member surface areas and the presence of binding, sorption and active transport mechanisms, as well as chemical transformations (K_m and V_{max}) catalysed by metabolic enzymes

way through hepatocytes in the liver where specialized efflux mechanisms and metabolizing enzymes act to keep xenobiotics from reaching the systemic circulation [1] (Figure 1B). Therefore, the ability to model, analyse and predict the subcellular transport properties of drug-like molecules is an increasingly important area of research in pharmaceutical sciences. Similarly, to the extent

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DOI: 10.1002/bdd

that systems pharmacology intends to analyse and predict the spatiotemporal dynamics of the effects of drugs on living systems, modeling the transport and distribution phenomena affecting the concentrations of drug molecules in different sites of an organism may be as important as modeling the effects of drug molecules on biochemical reactions, signal transduction pathways or other regulatory molecular control networks that are currently the subject of systems biology research.

At the level of the whole organism, the development and application of computational models to predict drug transport phenomena has been an active area of pharmacokinetics research for many years. For example, physiologically based pharmacokinetic (PBPK) models are mechanism-based mathematical models that have been used to analyse the concentration and distribution of drugs from the circulation to the different organs, based on the perfusion and permeability characteristics of each organ. In drug development, PBPK models allow the prediction of drug distribution from one species of organism to another [2–4]. Since their origin in the 1970s, a variety of PBPK modeling approaches have been developed to analyse the spatiotemporal dynamics of a wide range of drug absorption, distribution, metabolism and elimination phenomena affecting drug activity and toxicity [5–13]. Nevertheless, local concentrations of drug molecules at the microscopic sites where drug molecules interact with their intended (or unintended) biological targets may not be necessarily the same as the concentrations of drug molecules in macroscopic bulk compartments. This is because cells and subcellular organelles are delimited by membranes, and drug transport across these membranes is determined by active and passive transport mechanisms that can facilitate the accumulation of drug molecules on one side of the membrane relative to the other. Beyond PBPK modeling, molecular mechanisms affecting the active transport of drugs into subcellular compartments could be the subject of systems pharmacology research. These mechanisms include protein ‘pumps’ that translocate drug molecules from one side of the plasma membrane to the other by using energy derived from ATP hydrolysis. In addition, the passive distribution of drugs across plasma and organelle membranes can be influenced by differences in the local microenvironment of the two compartments separated by membranes. These differences lead to transmembrane pH gradients and electrical potentials affecting the transport and distribution of molecules possessing charged or ionizable functional groups. From the perspective of systems pharmacology, microscopic transmembrane transport phenomena can be considered as the key determinants of whether or not small molecule chemical agents can interact with the molecular circuitry that establishes and controls the structure and function of the organism. If the site of the action of a drug molecule is located in a specific organelle inside a specific cell type, the rate and extent to which the drug may accumulate at that target site can be influenced by active and passive transport mechanisms that may facilitate or prevent a drug from reaching that site. Similarly, if the drug target is localized on an extracellular membrane surface, mechanisms that drive the sequestration of drug molecules inside cells or that facilitate their excretion from the organism can effectively reduce extracellular drug concentrations to the point where the drug may be unable to influence the function of an extracellular target [14–19].

Nevertheless, at this microscopic level, pharmaceutical researchers have also been elaborating computational models to predict the transcellular permeability of small molecule chemical agents and to develop small molecule chemical agents targeted to specific sites of action inside cells [5,7,8,20–23]. In general, there have been two kinds of mathematical approaches applied towards predicting the subcellular transport and distribution properties of small drug-like molecules: statistics-based QSAR studies and differential equations-based mechanistic modeling. QSAR involves the application of correlation analyses to determine associations between the chemical structures and the subcellular transport and distribution properties of small molecules [8,21–28]. Mechanistic modeling involves using differential equations to represent the transport of molecules as a change in the concentration of molecules between compartments separated by cellular membranes [5,7,8]. The following sections discuss how these two different computational approaches originated and evolved, and evaluate their potential impact on the field of systems

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pharmacology. Furthermore, references are given of relevant research studies analysing drug–drug interactions and the interplay between efflux transporters, metabolic enzymes and drug targets while integrating pharmacokinetics and pharmacodynamics at cellular and subcellular levels.

QSAR Studies of Drug Transport and Distribution in Single Cells

The QSAR approach to predictive cellular pharmacokinetics can be considered an extension of classical QSAR studies which have been used widely in pharmaceutical and medicinal chemistry research since the 1960s [29]. For classical QSAR studies, multivariate, statistical regression techniques are used to link the results of experimental assays to the chemical structures of large sets of chemical agents tested with these assays. The regression techniques yield an equation that specifies the relationship between a physicochemical or biological property of interest (the dependent variable of the equation) to different input parameters that represent the chemical structures, atomic composition or physicochemical properties of the assayed molecules (the independent variables of the equation). Using a training set of molecules, the resulting regression equations can be used to predict the behavior of untested sets of molecules using the molecule's chemical structure or physicochemical properties as input [29]. Examples of QSAR studies range from the prediction of *in vitro* solubility [30–34] to the prediction of complex biological properties, such as *in vivo* bioavailability [35–37].

While several other different QSAR approaches have been developed to link the chemical structure with the physicochemical properties or pharmacological activities of small molecules [38–47], there are fewer examples of QSAR approaches linking the structure of small molecules with their cellular transport and distribution properties (Table 1) [8,25–27,48–50]. Interestingly, some of the first QSAR models ever published were aimed at predicting drug concentrations in cells. These QSAR models date all the way back to the 1960s. Indeed some of the original QSAR models were elaborated to describe the relationship between hydrophobicity and the biological activity of chemical agents, related to the ability of lipophilic molecules to cross cellular membranes but without partitioning into those membranes [29,51,52]. Subsequently, these regression-based QSAR models were elaborated and applied in an increasingly sophisticated manner, to study drug disposition and activity in multicompartment (aqueous and lipid) biosystems [28,53,54] and to analyse concentration–time profiles in bacterial or mammalian cells [55–59].

To predict the distribution of small molecules in different organelles of eukaryotic cells, a different kind of QSAR modeling approach was introduced in the 1990s, based on the construction of nested if/then rules [60]. With this approach, a simple decision tree-like model, named the Chinese box model, was used to describe the distribution of small drug-like molecules inside cells [27,48].

Table 1. Examples of empirical QSAR models for predicting subcellular distribution

Method	Localization	Descriptors	Number of compounds	References
QSAR	Mitochondria	logP, Z	41	
QSAR	Lysosome	logP, pK_a, CBN, Z	50	[27]
QSAR	Nuclei	logP, pK_a, Z, CBN, AI, LCF, LCF/CBN ratio	44	[25]
QSAR	ER	logP, pK_a, Z, CBN, AI, LCF	37	[26]
QSAR	Mitochondria/non-mitochondria	logP, pK_a, Z, CBN, AI, LCF	109	[60]
MLR (multiple linear regression)	Mitochondria	logD, Z, α, MW, PSA	20	[50]
Descriptor	Mitochondria/lysosome/nuclei/cytosol/ ER/Golgi body/plasma membrane/ multiple localization	483 2D and 3D descriptors	967	[66]

logP, logarithm of the octanol/water partition coefficient; logD, logarithm of the octanol/water partition coefficient at pH 7.4; pK_a, negative logarithm of the acidic associate constant; Z, electrical charge; CBN, conjugated bond number; AI, amphilicity index; LCF, the largest conjugated fragment; α, polarizability; MW, molecular weight; PSA, polar surface area.

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Initially, this model was applied to 41 cationic probes to study their mitochondrial localization as a function of the logarithm of the octanol/water partition coefficient (logP) [48]. Based on this model, cationic molecules with logP between 0 and 5 were expected to accumulate in the mitochondrial inner membrane. Cationic molecules with a $\log P < 0$ would be excluded outside cells, and with a $\log P > +5$ would accumulate preferentially in the plasma membrane [48]. A few years later, a similar decision tree-like model was built to analyse the accumulation of small molecules in lysosomes [27]. Based on this model, probes were classified into three major categories: (1) those that had $0 < \log P < 5$ were able to cross the plasma membrane; (2) those that had $\log P > 15$ could also enter the cell and accumulate in lysosomes by adsorptive pinocytosis; and (3) those that had $\log P < 0$, $z < 0$ could also enter cells and accumulate in lysosomes by fluid phase pinocytosis. Probes in group (1) were classified into two sub-categories: (1) Probes accumulating in lysosomes by ion trapping mechanisms; and, (2) probes comprising hydrolysable lipophilic esters, usually weak acids, which were metabolized into free acids by lysosomal esterases and trapped in lysosomes by precipitation in the low pH microenvironment [27]. To further extend this decision-tree based approach, additional studies with molecules localizing to other organelles (endoplasmic reticulum, nuclear chromatin and plasma membrane) have been performed [26,60,61].

More recently, an additive factorial logistic regression modeling approach was developed to analyse the subcellular localization of a combinatorial library of organelle-targeting cationic styryl dyes [21,62]. Each styryl dye was synthesized using a chemical conjugation reaction to link two chemical building blocks: an aldehyde building block combined with a pyridinium or quinolinium building block. By combining 168 aldehyde with eight pyridinium or quinolinium building blocks, the contributions of each building block to the peak excitation and emission wavelength of 1344 molecules in the library was calculated using least squares to minimize an additive, multivariate regression function over all compounds having experimental data [21,62]. Most importantly, using a factorial logistic regression approach, the binary (mitochondrial vs nonmitochondrial) localization data obtained from live cells incubated with these compounds were also related to the quantitative contributions of each aldehyde and pyridinium or quinolinium building blocks. Cross-validation was carried out for both spectral and localization data, to obtain unbiased estimates of prediction performance.

While most QSAR studies have focused on analysing the steady state distribution of small molecule chemical agents inside cells (for recent reviews, see [63–65]), complementary QSAR approaches have been developed to study the cellular and subcellular transport kinetics of small molecules. In one study, the time of exposure was integrated with a QSAR model to capture the kinetics of the drug–receptor interaction based on the law of mass action [28]. This 'QSTAR' model was developed by applying non-linear regression to a data set of 36 compounds [28]. Subsequently, this QSTAR modeling approach was further elaborated by incorporating enzymatic activity, membrane accumulation, non-covalent protein binding and excretion [56]. In a different study, using a combinatorial library of fluorescently tagged cell-permeant small molecule chemical agents, a nested multi-compartment model was used to analyse the subcellular transport and accumulation kinetics of the compounds between extracellular medium, cytosol and intracellular vesicles [22]. Using kinetic image data acquired from cells incubated with the fluorescent compounds at different times after the probes were added to the cells, changes in the total intensity and coefficients of variation of pixel intensities were linked to changes in the intracellular concentration of the probes. Using this approach, the partition coefficients from the extracellular medium to the cytosol, and from the cytosol to the intracellular vesicles was inferred and related to the chemical structures of the compounds [22].

Most recently, a database of 967 molecules with published subcellular localization features derived from a survey of the scientific literature was used as a starting point for identifying relationships between the chemical space occupied by small molecules and their reported intracellular localization features [66]. Because this is a diverse group of compounds whose localization was determined by a large number of different investigators using a wide variety of different

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experimental techniques, a rigorous, quantitative structure–localization relationship study was not performed. Nevertheless, by studying the localization of compounds to multiple different organelles, this study identified many interesting, candidate chemical property–subcellular localization relationships that had not been noted previously in QSAR studies focusing on a single target organelle. Amongst the most interesting trends, the molecular weight of the compounds was identified as a key variable associated with differences in the subcellular distribution properties of small molecules. More specifically, the chemical property–subcellular localization relationships of the compounds was very different for molecules > 500 Daltons compared with those that were < 500 Daltons.

Mechanistic Models of Drug Transport and Distribution in Single Cells

The development of mechanistic models of drug transport and distribution at the single cell level began in the 1970s, with the incorporation of permeability as a parameter variable in the physiologically based pharmacokinetic (PBPK) model. In these models, permeability was used to capture differences in the rate of accumulation of drug molecules in the different organs. However, it was not until the mid-1990s that attempts were made to relate the abstract, pharmacokinetic permeability parameter to the physical cell permeability. The realization of the importance of cell permeability in pharmaceutical discovery was spurred by the development of the advanced compartmental absorption and transit model (ACAT) [67]. The development of the ACAT model effectively connected the results of *in vitro* cell-based assays measuring the transport rates of small molecule drugs across cell monolayers to the fraction of an oral dose of drug absorbed in the gastrointestinal tract.

To analyse the results of *in vitro* cell-based transport assays, compartmental cellular pharmacokinetic models began to be constructed to analyse cellular transport and metabolism properties of small molecules across cell monolayers, in the presence of a transcellular concentration gradient [6–8,11,54,68]. By incorporating the Goldman–Hodgkin–Katz equation, differential equation-based cellular pharmacokinetic models have been converted into predictive cell-based transport and distribution models. The Goldman-Hodgkin-Katz equation is derived from the Nernst–Planck equation (Equation (1)).

$$J = -D\left[\frac{dC(x)}{dx} + C(x)\frac{zF}{RT}\frac{dV(x)}{dx}\right] \quad (1)$$

The first term corresponds to the flux of the neutral species captured by Fick's law of diffusion. The second term corresponds to the flux of the ionized species which is influenced by the transmembrane electrical potential. D is the diffusion coefficient (area per time unit). F, R, T and z are the Faraday's constant, molar gas constant, temperature (in Kelvin) and electric charge, respectively. Assuming the transport direction (denoted by x) being perpendicular to the membrane, then $dC(x)/dx$ reflects the concentration change along the membrane, and $dV(x)/dx$ reflects the voltage change along the membrane. $C(x)$ indicates the concentration at point x. If the transmembrane electrical potential is assumed to be constant along the membrane, and the membrane thickness is d, Equation (1) is rewritten as Equation (2).

$$J = -D\left[\frac{dC(x)}{dx} + C(x)\frac{zF}{RT}\frac{V}{d}\right] \quad (2)$$

Rearranging the terms in Equation (2) leads to Equation (3):

$$1 = \frac{dC(x)/dx}{-\frac{J}{D} - C(x)\frac{zF}{RT}\frac{V}{d}} \quad (3)$$

Letting $N = zFV/RT$, and integrating from $x = 0$ to d yields Equation (4):

$$\int_0^d dx = \int_0^d -\frac{dC(x)/dx}{J/D + NC(x)/d}dx$$

$$d = -\frac{d}{N}\left[\ln\left|\frac{N}{d}C(d) + \frac{J}{D}\right| - \ln\left|\frac{N}{d}C(0) + \frac{J}{D}\right|\right] \quad (4)$$

Accordingly, the flux of a charged molecule across a biomembrane with a transmembrane

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electrical potential and concentration gradient is captured by Equation (5):

$$J = \frac{D}{d}\frac{N}{e^{N}-1}\left(C(0) - e^{N}C(d)\right) \quad (5)$$

where *D/d* captures the permeability of the molecule across the membrane (length per time units). *C(0)* is the concentration at the outer membrane surface, and *C(d)* is the concentration at the inner membrane surface.

To facilitate predictions, the flux of a molecule across a membrane can be expressed with Fick's equation (Equation (6)).

$$J_n = P_n\left(C_{o,n} - C_{i,n}\right) \quad (6)$$

where P_n is the membrane permeability which can be estimated as a function of the octanol:water partition coefficient of the neutral form of the molecule, C_n is the concentration of neutral form of the molecule with the subscripts *o* and *i* indicating the directions of the flux, *J*, from outside to inside compartments. Similarly, for charged molecules, the net fluxes of passive diffusion across membranes can be expressed using P_d as the membrane permeability of the ionized form of the molecule which can also be estimated as a function of the octanol:water partition coefficient, with Equation (7) (derived from Equation (5))

$$J_d = P_d\frac{N}{e^{N}-1}\left(C_{o,d} - C_{i,d}e^{N}\right) \quad (7)$$

For Equation (7), subscript *d* indicates the ionized form of the molecule. $C_{o,d}$ and $C_{i,d}$ are the concentration of ionized forms of the molecule outside and inside, respectively. Combining Equations (6) and (7) (Fick's and Goldman–Hodkin–Katz equation) and considering as the thermodynamic activity (*a*) of the different protonated states of a molecule may differ from their concentration depending on the pH, ionic strength, and binding to macromolecules and lipids present in the local microenvironment, the net fluxes of molecule weakly basic or weakly acidic molecule across lipid membranes delimiting cells and the various intracellular organelles can be described with Equation (8).

$$J = P_n\left(a_{o,n} - a_{i,n}\right) + P_d\frac{N}{e^{N}-1}\left(a_{o,d} - a_{i,d}e^{N}\right) \quad (8)$$

This equation directly captures the transmembrane fluxes of neutral and ionized species of monovalent weak acids and bases and can be elaborated further to capture dibasic, diacidic and zwitterionic small molecules [5,66,69]. For simulating subcellular transport phenomena with this equation, input parameters can be systematically varied, to capture the behavior of molecules with varying physicochemical properties, as well as to study the effect of variations in cell morphology and physiology on cellular pharmacokinetics. This equation has been parameterized to simulate the subcellular transport and disposition properties of small molecules in single cells suspended in a homogeneous extracellular drug concentration (Figure 2A) as well as in attached cell monolayers surrounded by a transcellular concentration gradient (Figure 2B) [5,7,8,69,70].

Role of Mechanistic Cellular Pharmacokinetic Modeling in Pharmacology

From an empirical pharmacological perspective, cellular pharmacokinetic models have been mostly used to analyse the interaction of small molecule chemical agents with specific intracellular drug targets, in the context of passive and active transport mechanisms affecting the transport of the molecules between extracellular and intracellular compartments (Table 2). One excellent example of such an application involved studying the effect of P-glycoprotein (P-gp) on the intracellular binding of paclitaxel to microtubules [20,71,72]. The model took into account saturable binding to extracellular proteins, saturable and nonsaturable binding to intracellular components, cell density variation and changes in tubulin concentration as a function of paclitaxel concentration. First, the model was validated in human breast MCF7 tumor cells, which had negligible P-glycoprotein expression, after which the effect of P-glycoprotein mediated efflux was added into

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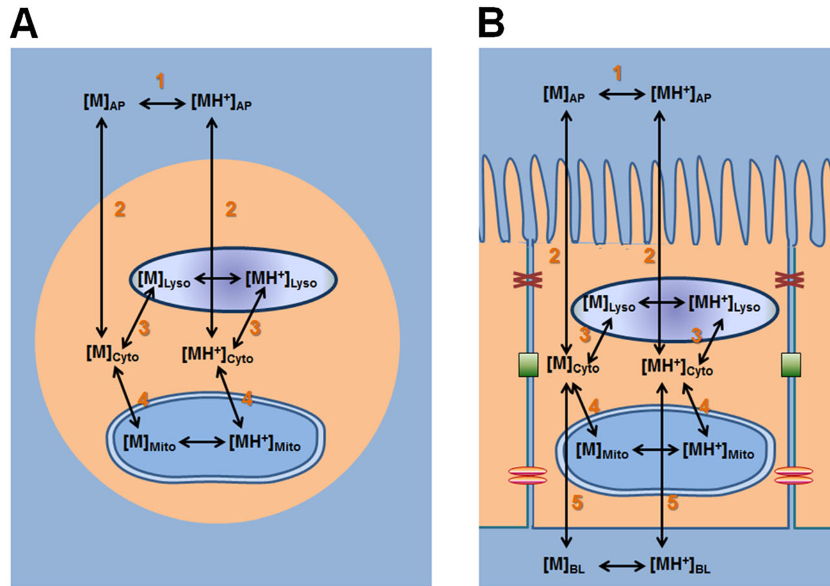

Figure 2. Passive transport mechanisms can be modeled to predict the accumulation and distribution of the weakly basic small molecule inside cells surrounded by a homogeneous drug concentration (A) or in the presence of a transcellular concentration gradient (B). For mechanistic modeling, the different ionization states of the molecule is calculated using the Henderson-Hasselbalch equation (1). Transport of the neutral or charged (ionized) species of the molecule from the surrounding medium or the apical compartment into the cells (2) can be modeled with the Fick and Goldman–Hodgkin–Katz equations. From the cytosol, transport into lysosomes (3) or mitochondria (4), as well as transport from the cytosol into the basolateral compartment can also be modeled with the Goldman–Hodgkin–Katz equation

Table 2. Examples of mechanistic cellular pharmacokinetic models

Drugs/molecules	Model components	Cell type	Relation to systemic PK/PD	References
Monovalent small molecules	Passive transcellular transport and subcellular organelles	Epithelial and round shaped non-polarized cells	Absorption, tissue distribution	[5,7,8,69]
Paclitaxel	P-gp efflux, extracellular/ intracellular binding	Human breast cancer cell lines MCF7 and BC19	Uptake to cancer cells	[20,71,72]
Substrates of multiple transporters	Active uptake, passive diffusion, nonspecific binding	Chinese hamster ovary (CHO) cells overexpressing OATP1A1 or OATP1B1 and rat hepatocytes	Liver clearance	[74]
Ranitidine	Uptake and efflux transporters, paracellular and transcellular transport	Caco-2	Absorption	[75]
Baicalein	Passive diffusion, cellular binding, transporters and enzymes	Caco-2 or other similar *in vitro* system	Absorption, metabolism	[6]
GCSF	Endosomal trafficking, PK/PD	GCSF-dependent human suspension cell line: OCI/AML1	Cell-mediated clearance, link with PD modeling	[138]

the model and validated in a human breast carcinoma cell line derived from MCF7 cells transfected with *mdr1* [72]. As a follow-up study, a parametric analysis was performed to study the differential effects of the extracellular drug concentration, intracellular drug binding capacity and affinity, and P-glycoprotein expression level on the intracellular drug accumulation [20]. The study showed that the four biological factors determined paclitaxel intracellular concentration

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interdependently. Among the four factors, the extracellular concentration was the most sensitive factor, followed by intracellular binding capacity and affinity. The effect of P-glycoprotein expression was relatively minor, suggesting that to improve clinical efficacy, effective delivery of paclitaxel to tumor cells was more important than other factors, such as inhibition of P-glycoprotein efflux.

Another important application of empirical, mechanistic cellular pharmacokinetic modeling involved the determination of V_{max} and K_m values of drug transporters and enzymes with an intracellular site of action, using the results of experiments performed with intact cells [72–75]. Based on extracellular drug concentrations, the mechanism-based empirical modeling approach can facilitate an estimation of intracellular V_{max} and K_m values, or elementary rate constants of enzymes including drug transporters in their natural, intracellular microenvironments [76–78]. Using a mechanistic cell-based transport and distribution model to guide interpretation of experimental measurements, parameters associated with the effect of an enzyme substrate or inhibitor can be estimated by fitting the experimental data with a model, using nonlinear least-squares regression. For modeling drug metabolism, a catenary model based on the compartmental analysis was developed to analyse the activity of intracellular, drug metabolizing enzymes [6]. This model captured the mechanisms of passive diffusion, cellular binding, carrier-mediated and efflux transporter-mediated transport, in addition to metabolic activity. The model was applied to study the transport and metabolism of baicalein inside cells [6]. This empirical, compartmental modeling approach has also been applied to facilitate the design and interpretation of experiments exploring the role of transporters and metabolic enzymes in limiting drug absorption and has helped to explore the molecular mechanisms responsible for drug–drug interactions [11,79–84]. Empirical, compartmental, cellular pharmacokinetic modeling approaches are increasingly being used to help to interpret the interplay between drug molecules, drug transporters and metabolic enzymes, from a molecular, mechanistic perspective [85–87].

Arguably, for the future development of systems pharmacology, the application of differential equations-based, mechanistic mathematical models will become increasingly important, because many biochemical and signaling networks are localized at specific organelles inside the cell. For example, mitochondria are involved in the regulation of apoptosis, and thus are considered as important target sites for anticancer agents [88–90]. Mitochondria are also the sites of energy production, with the electron transport chain located in the mitochondrial inner membrane generating a marked electrical potential and pH gradient [91,92]. Many lipophilic cations have been observed accumulating in mitochondria as a function of the transmembrane electrical potential, which can be predicted with a compartmental, cellular pharmacokinetic model using the Goldman–Hodgkin–Katz equation [21,91,93–95]. Examples of these molecules include rhodamine 123 [91,93,96,97], F16 [88,89] and styryl molecules [98–101]. Differences in mitochondrial membrane potential and pH gradient explain differences in the toxicity of cationic compounds on different cell lines of different origins [91].

Like mitochondria, lysosomes are another example of an organelle that is interesting from a systems pharmacology perspective. Biologically, mutations in genes affecting lysosomal function lead to a range of protein and membrane accumulation defects that are characteristics of lysosomal storage diseases [102]. Unlike mitochondria, the intralumenal pH of lysosomes is acidic [103] while the cytosolic pH is near neutrality [18]. The low pH of lysosomes is caused by the activity of a proton ATPase [103]. As a result, weakly basic molecules tend to accumulate inside lysosomes by a pH-dependent ion-trapping mechanism [104]: for weakly basic molecules with a pK_a close to physiological pH, they exist predominantly as neutral species in cytosol (pH ~ 7.2). After neutral molecules enter the acidic subcellular organelles, they become protonated due to the acidic environment. Generally, the lipophilicity may differ greatly between neutral species and ionized species [8]. Therefore, after entering the acidic compartment, the transmembrane permeability of the molecules is reduced due to the protonation, and accumulation is induced. Because of the ion-trapping mechanism, many clinically useful drugs accumulate in lysosomes, including the antimalarial drug chloroquine [105,106], as well as many antidepressant drugs [107].

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Furthermore, while mechanistic cellular pharmacokinetic models have been useful to describe organelle targeting, drug bioaccumulation inside cells, and also to predict the effect of microenvironments on drug transport and distribution inside cells [5,7,8,69,70], these models can also be used as building blocks to study drug transport in higher order cellular organizations, from the tissue to the organ level [108,109]. The ability to model the transport properties of small molecules at the level of single cells, and to predict the pharmacokinetics and biodistribution properties of drug from organelles to cells to tissues to organisms could be used to predict systemic pharmacokinetic parameters, such as the volume of distribution. Indeed, correlative *in vitro* and *in vivo* studies suggest that the volume of distribution and the intracellular accumulation of small molecules are related to each other [110–114]: propranolol, a drug with a high volume of distribution, mostly accumulates in association with mitochondria [110]. Mefloquine, another drug with a very large volume of distribution, extensively accumulates in lysosomes [114]. Other basic drugs with large volumes of distribution that accumulate in lysosomes are chlorpromazine, imipramine and biperiden [111,112,115]. Extensive distribution in the lung has been observed for many lipophilic bases

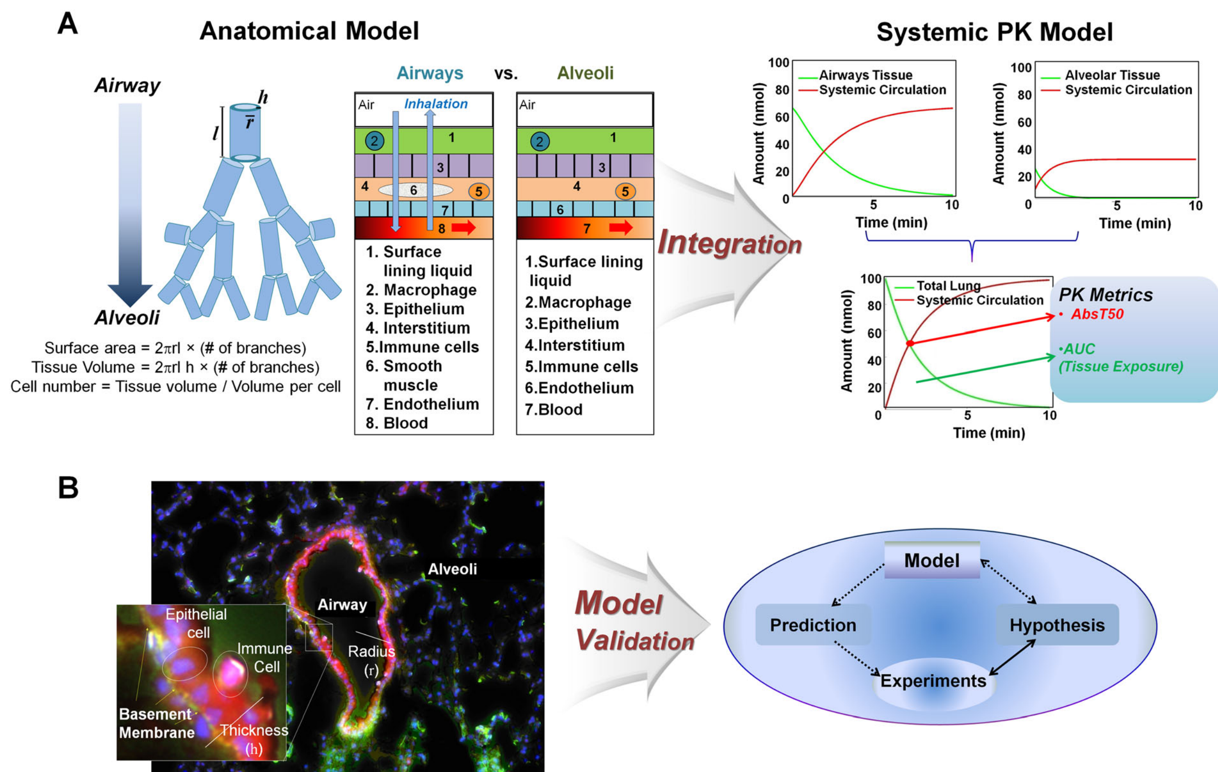

Figure 3. Mechanistic modeling facilitates predicting the transport and distribution properties of small molecules from the microscopic to the macroscopic level. At the macroscopic level (A) the anatomy of an organ can be modeled geometrically as surfaces comprise cylinders. Histologically, the walls of these cylinders can be modeled as concentric layers of different cell types. By integrating the transport of small molecules across these multilayered organizations, it is possible to calculate the changes in drug concentrations over time at both local and systemic levels. (B) Using fluorescent molecules as probes of transport phenomena, the cellular labeling patterns of the probes can be related to predictions based on the models. In this case, the micrograph shows the distribution of Mitotracker Red (red), Hoechst 33342 (blue) and Rhodamine 1,2,3 (green) in the lungs, after the dyes have been administered directly into the airways. Relative to the other two fluorescent probes, Mitotracker Red was retained inside the epithelial cells that line the airways, as predicted by its transmembrane transport properties. Hypotheses are used to guide the design of experiments and build models. The models are used to make predictions which may or may not be validated by experiments, leading to formulation of new hypotheses leading to further development of the models

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[115–120] and lysosomal ion trapping has been proposed as a mechanism contributing to high drug accumulation in the lung [113,115,119,120]. Interestingly, lysosomal volume changes have been independently implicated in various drug–drug interactions involving lysosomotropic compounds [121,122].

Illustrating the application of mechanistic cellular pharmacokinetic models for predicting small molecule transport and distribution from the microscopic to the macroscopic levels, the passive transport of small drug-like molecules in the lung was modeled based on differences in the physiological, anatomical and histological organization of airways and alveoli (Figure 3A). In this model, the transmembrane transport was modeled using the Fick and Goldman–Hodgkin–Katz equations to describe the transport of small molecules across the various membrane bound compartments separating the lumen of the airway from the

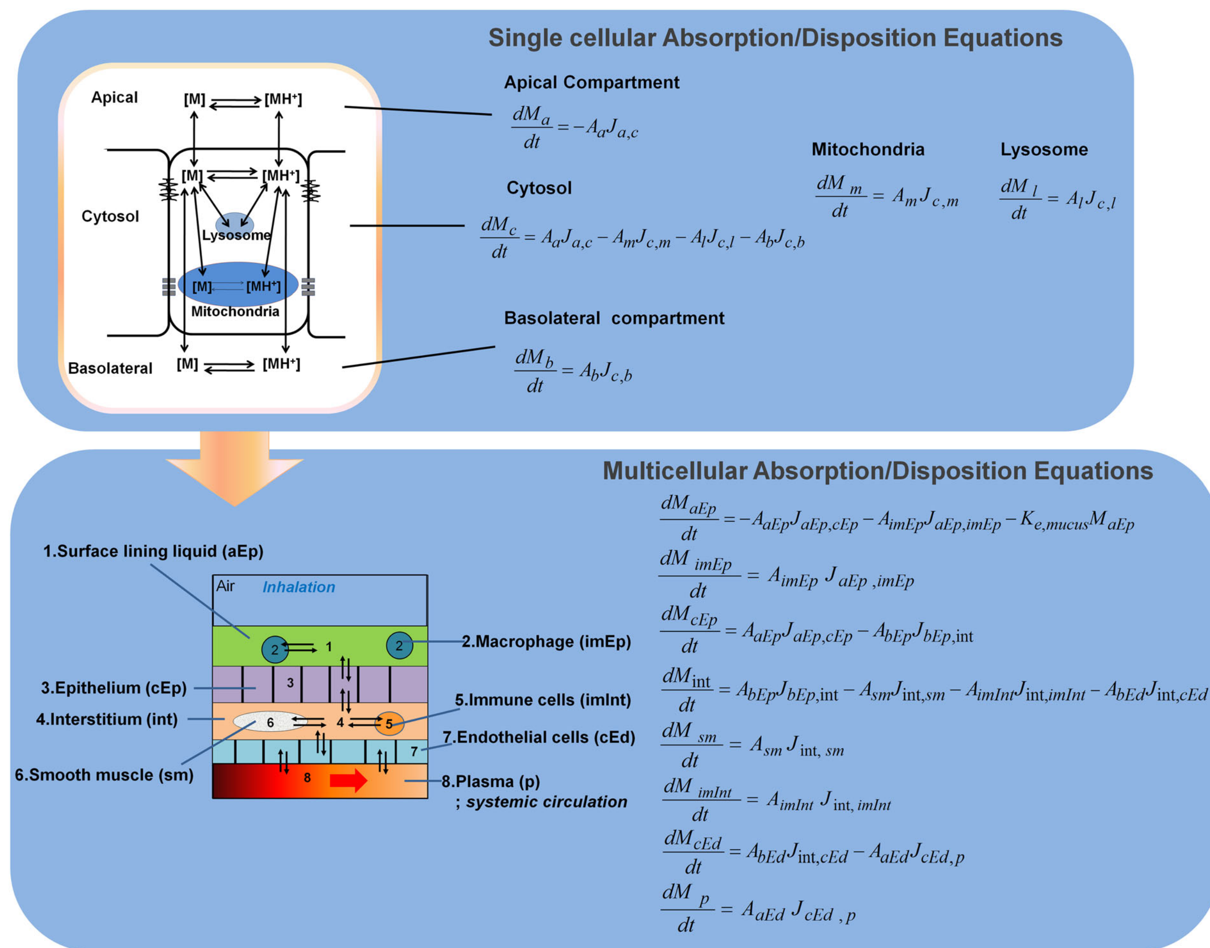

Figure 4. Transmembrane mass transport equations can be used to build increasingly complex models from single cells to multicellular organizations. The same equations can be used to capture the transport into single cells and intracellular, membrane-bound organelles, as can be used to capture the transport across multicellular tissue layers. In essence, the mass of drug that diffuses from one side of the membrane to the other over time is calculated by multiplying the flux of molecules across the membrane (J) times the area of the membrane (A). For passively diffusing molecules, the flux can be calculated based on the ionization states of the molecules, the transcellular concentration gradients of freely soluble molecule species, and the membrane permeability of each ionized or neutral molecular species. Membrane surface areas are biologically defined, measurable parameters that reflect the overall number, volume, shape, spatial arrangement and membrane organization of the cells, as well as the number, volume, shape spatial arrangement and membrane organization of organelles

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DOI: 10.1002/bdd

circulation. This model was used to predict the rate of absorption of passively diffusing molecules from the airway surface lining liquid, across the epithelial cells, the interstitium, the capillary endothelial cells and ultimately into the blood [123]. Furthermore, the predictions of the model were tested after intravenous vs intratracheal injection of fluorescent compounds [124] (Figure 3B), reflected in differences in the local distribution behavior and systemic absorption profiles of the fluorescent compounds with different physicochemical properties in airways and alveoli (Figure 4). These results illustrate how mechanistic cellular pharmacokinetic models can be used to analyse local differences in the transport and distribution properties of small molecule chemical agents from the individual organelles to a whole organ.

Conclusions and Future Outlook

An important consideration to evaluate the scientific value of a computational approach involves assessing the predictive accuracy of the approach. Comparing QSAR with differential equations-based mechanistic modeling approaches [60] in terms of predicting mitochondrial accumulation, both methods have been found to predict the mitochondrial localization of lipophilic cations and lipophilic weak acids with some accuracy [60]. However, for electrically neutral species, including zwitterions, predictions with the empirical QSAR model were better than with the mechanistic model [60]. For lipophilic cations of partially ionized bases, the mechanistic model failed to predict mitochondriotropic behavior in eight of nine cases, while the QSAR model successfully predicted all nine cases [60]. Thus, the QSAR approach may be advantageous when there is a good training set of molecules and a very specific target organelle. In a different study, the prediction performances of mechanistic and empirical QSAR models were compared using a dataset of toxicities against *Tetrahymena pyriformis* [125]. Based on this toxicity study, the mechanistic model had a slightly higher predictive accuracy than the empirical models (based on a leave-one-out cross-validation and two types of leave-several-out cross-validation approaches) [125]. More importantly, this second study suggests that the mechanism-based model performed better than the empirical models for compounds falling outside the parameters space of the training set [125].

Nevertheless, as a tool to aid in experimental design and data interpretation, differential equations-based cellular pharmacokinetic models can be more useful than statistical QSAR models. First, mechanistic predictive cellular pharmacokinetic models that are based on differential equations can be readily integrated with differential equation-based systems biology models capturing the interaction of biological components inside cells. Using a mechanistic cellular pharmacokinetic model, concentrations of small molecule drugs can be calculated inside cells, and these concentrations can be incorporated into a systems biology model to calculate the effect on a biochemical reaction or signal transduction pathway. Second, as we have argued before, mechanistic models can be integrated across multiple scales [126], to make predictions about differences in drug transport and drug action at different sites within the same organ. Lastly, from a biological perspective, mechanistic pharmacokinetic models can be integrated with systems biology models and used to frame quantitative hypotheses about the effects of exogenous, small molecule activators or inhibitors on cell structure and function from the local control of signal transduction pathways [127] to the global control of cell population dynamics [128–131]. Furthermore, the inability of a mechanistic model to capture a molecule's behavior is often an indication of an unknown mechanism affecting drug transport and distribution [132–136]. This can point to additional experimental studies to identify new biological phenomena affecting drug transport behavior [66,137].

To conclude, a variety of computational approaches have been developed to analyse, interpret and predict the transport and distribution properties of small molecule chemical agents inside cells. These approaches can be broadly classified either as statistics-based QSAR or as differential equations-based mechanistic models. While statistics-based, predictive QSAR models are simple and straightforward in terms of pointing to chemical modifications that may be useful for drug development purposes, mechanistic models based on differential equations have

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many additional advantages in the context of systems pharmacology. Mechanistic models that are based on differential equations can be readily integrated with other differential equation-based models of drug–target interactions. Furthermore, these integrated models can be subsequently extended by adding the new mechanism, and can be used to model compounds outside the training dataset, and can be used as a starting point for discovering new mechanisms. In line with one of the primary objectives of systems pharmacology, which is to predict the effects of exogenous chemical agents on living systems, many mechanistic cell-based pharmacokinetic models are already being used to help analyze and predict the transport, distribution, metabolism and excretion properties of exogenous chemical agents from the subcellular to the organism levels. Therefore, further integration of differential equations-based cellular pharmacokinetics with mechanistic systems biology modeling research seems like a natural next step in the advancement of systems pharmacology.

Acknowledgements

This work was supported by funding from the National Institute of General Medical Sciences (NIH grant R01 GM078200 to G.R.R.).

Conflict of Interest

The authors have declared that there is no conflict of interest.

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DOI: 10.1002/bdd

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