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Physiologically based pharmacokinetics (PBPK)

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Abstract

Allometric scaling is widely used to predict human pharmacokinetic parameters from preclinical species, and many different approaches have been proposed over the years to improve its predictive performance. Nevertheless, prediction errors are commonly observed in the practical application of simple allometry, for example, in cases where the hepatic metabolic clearance is mainly determined by enzyme activities, which do not scale allometrically across species. Therefore, if good correlation was noted for some drugs, poor correlation was observed for others, highlighting the need for other conceptual approaches. Physiologically based pharmacokinetic (PBPK) models are now a well-established approach to conduct extrapolations across species and to generate simulations of pharmacokinetic profiles under various physiological conditions. While conventional pharmacokinetic models are defined by drug-related data themselves, PBPK models have richer information content and integrate information from various sources, including drug-dependent, physiological, and biological parameters as they vary in between species, subjects, or with age and disease state. Therefore, the biological and mechanistic bases of PBPK models allow the extrapolation of the kinetic behavior of drugs with regard to dose, route, and species. In addition, by providing a link between tissue concentrations and toxicological or pharmacological effects, PBPK modeling represents a framework for mechanistic pharmacokinetic-pharmacodynamic models.

Keywords: *Physiologically based pharmacokinetic; interspecies extrapolation; human prediction; pharmacokinetic/pharmacodynamic; allometry; physiology; discovery; development; population*

Basics of interspecies scaling and limitations

Allometric scaling of animal data is historically the first, and still widely used, tool for the prediction of human pharmacokinetic parameters during drug development. This mainly applies to systemic clearance, but also to volume of distribution, half-life, mean residence time, and absolute bioavailability.

The relationship between physiological parameters and body size or body weight has long been studied. Already in 1838, Sarrus and Rameaux postulated that in order to maintain constant internal temperature, mammals must produce energy at a rate proportional to body-surface area. They demonstrated also that the surface area of an animal is proportional to two thirds the power of the body mass. More generally, it was shown that many physiological parameters (Y) are related to the body weight (BW) by the mathematical relationship shown in Equation 1:

$$Y = a \cdot W^{b} \quad (1)$$

with a and b being the allometric coefficient and exponent, respectively. Chappell and Mordenti (1989, 1991) have classified the allometric exponent b into the following five categories:

- When $b < 0$, then Y decreases with the increase in BW (e.g., heart rate).
- When $b = 0$, then Y is independent of BW (e.g., body temperature, hematocrit).
- When $0 < b < 1$, then Y does not increase as fast as BW (e.g., heart beat).

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- When b = 1, then Y increases proportionally with BW (e.g., blood volume).
- When b > 1, then Y increases faster than BW (e.g., skeleton weight of mammals).

The empirical allometric scaling of pharmacokinetic parameters is based on Equation 1, assuming that there are anatomical, physiological, and biochemical similarities among animals (Dedrick, 1973; Mordenti, 1986).

Systemic clearance is the most important pharmacokinetic parameter in drug discovery due to its link to exposure in the systemic circulation and bioavailability. Therefore, allometric scaling is widely used to predict clearance in human from animal data (Figure 1) by using simple allometry (i.e., using Equation 1). Clearance and physiological flow rates tend to have an exponent of 0.75, indicating that clearance increases as species get larger, but not as rapidly as body weight. Some reservations were made about the use of simple allometry for the scaling of hepatic metabolic clearance between species when the metabolism is not limited by liver blood flow. In such cases, the hepatic metabolic clearance is mainly determined by enzyme activities, which do not scale allometrically across species (Weiss et al., 1977). On the contrary, for drugs with high extraction ratios, the hepatic clearance is limited by the liver blood flow, and since liver blood flow is correlated with body weight, the hepatic clearance for high-extraction drugs is expected to scale allometrically across species (Lavé et al., 1999).

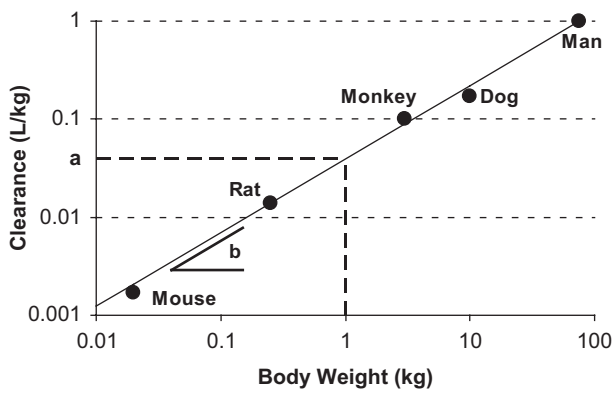

Figure 1. A typical log-log plot of clearance vs. body weight for mouse, rat, monkey, dog and man. The dotted line that originates at a body weight of one kg gives the constant **a** (on the clearance axis) while the slope gives the constant **b** of Equation (1) in the text.

There are many examples of applications of simple allometry to predict the clearance of drugs in human, but only a few examples of application in the antiallergic field (McGinnity et al., 2007; Lavé et al., 1999). For example, projected plasma clearance in humans for the antihistamine, cetirizine, using preclinical species data (rat, dog) was 0.2 mL/min/kg, while the actual value was 0.7 (McGinnity et al., 2007). Table 1 summarizes predicted and observed values for hepatic metabolic clearance in humans for theophylline and caffeine, using simple allometry (data compiled from Lavé et al., 1999). Table 2 summarizes the predicted and observed clearance of some drugs given by the intravenous route to animals and humans (Mahmood, 2005a) and illustrates also the range of values that could be obtained for the allometric exponent "b." Allometric scaling was performed by using at least three animal species without using human data.

Simple allometry alone is often not adequate to predict clearance in humans from animal data. On the basis of a diverse set of 22 metabolized drugs, Zuegge et al. (2001) showed that the prediction accuracy of the allometric approaches was at the lower end of all the methods they used for the prediction of hepatic metabolic clearance. Using 61 sets of clearance values in animal species for predicting human clearance, Tang and Mayersohn (2005) highlighted that using simple allometry, exponent b was not relatively constant around a typical value close to 0.75 (Boxenbaum, 1982), but rather ranging from 0.349 to 1.196, and that prediction errors are commonly observed in the practical application of simple allometry. Many different approaches have been proposed over the years to improve the predictive performance of allometry for clearance. These modifications include correction by 1) either maximum lifespan potential (MLP) (Boxenbaum, 1982) or the brain weight (BrW) (Mahmood and Balian, 1996a, 1996b), 2) *in vitro* metabolic data (Lavé, 1997), 3) the "rule of exponents" (ROE) (Mahmood and Balian, 1996b), and 4) scaling the unbound clearance (CL) (Feng et al., 2000). Other modifications of simple allometry, taking

Table 1. Simple allometry: predicted and observed values for hepatic metabolic clearance in humans for theophylline and caffeine (compiled from data in Lavé *et al.*, 1999).

Compound	Species	Parameter [a]	Predicted clearance	Observed clearance
Theophylline	Rat, rabbit, dog	CL	109 [b]	54.5 [b]
	Rat, rabbit, dog	$CL_{u,int}$	153 [b]	96.3 [b]
	Not known	CL	42 [b]	46 [b]
	Rat, rabbit, dog	CL; CL_u; CL_{int}	1.4; 1.2; 1.2 [c]	0.61 [c]
Caffeine	Not known	CL	153 [b]	98 [b]
	Rat, rabbit, dog	CL; CL_u; CL_{int}	1.4; 1.5; 1.4 [c]	2.0 [c]

[a]CL: clearance, CL_{int}: intrinsic clearance, CL_u: unbound clearance, $CL_{u,int}$: intrinsic clearance of unbound drug. [b]mL/min. [c]mL/min/kg.

Table 2. Simple allometry: observed and predicted clearance of some drugs in human following intravenous administration (compiled from data in Mahmood, 2005a).

Drugs	Allometric exponent "**b**"	Observed clearance (mL/min)	Predicted clearance (mL/min)
Metoprolol	0.43	1050	826
Ofloxacin	0.54	219	85
Nicardipine	0.55	630	790
Antipyrine	0.59	43	55
Methotrexate	0.65	147	156
Theophylline	0.66	46	42
Propranolol	0.66	1050	840
Cefazolin	0.78	61	150
Furosemide	1.00	154	381
Cyclosporine	1.15	273	718

into account interspecies differences, involve correction factors for protein binding (Obach et al., 1997) and renally secreted (Mahmood, 2005b) or biliary excreted (Mahmood and Sahajwalla, 2002) drugs. Proposed corrections for simple allometry and how to apply them for the estimation of clearance are summarized in Table 3 (Poggesi, 2004).

Correction by *in vitro* metabolic data improved significantly the prediction of human CL of 10 extensively metabolized drugs (Lavé et al., 1997), as shown in Equation 2:

$$CL_{\text{in vivo, human}} = CL_{\text{in vivo, animal}} \times CL_{\text{in vitro, human}} / CL_{\text{in vitro, animal}} \quad (2)$$

Based on a data analysis of 16 drugs, however, Mahmood (2002) concluded that the use of *in vitro* data obtained from liver microsomes to predict hepatic CL in humans did not provide reliable predictions. In addition, *in vitro* metabolic corrections cannot be applied for compounds eliminated by excretion. Mahmood (2000b) also suggested that unbound clearance cannot be predicted any better than total clearance.

Corrections by MLP or BrW have also been shown to be inappropriate if used indiscriminately, which led to the idea of the ROE (Table 3). This rule provides selection criteria for the use of MLP or BrW, based on the values of the exponents obtained from simple allometry (Mahmood and Balian, 1996b). However, recent investigations (Nagilla and Ward, 2004) found that using MLP or BrW or the ROE did not result in significant improvements for the prediction of human clearance. Further, they proposed that the monkey liver blood flow approach was superior to the ROE.

Based upon the analysis of 61 drugs, Tang and Mayersohn (2005) have observed that the ratio of unbound fraction (fu) in plasma between rats and humans (Rfu, i.e., fu rat/fu human) may provide simple rules for anticipating the occurrence of large vertical

Table 3. Simple allometric equations and modifications commonly applied for the estimation of clearance (compiled from Poggesi, 2004).

Methods and proposed corrections	Equations
Simple allometry	$CL = a \times W^b$
Correction for maximum life span	$CL \times MLP = a \times W^b$ $MLP = 185.4 \times BrW^{0.636} \times W^{0.225}$
Correction for brain weight	$CL \times BrW = a \times W^b$
For biliary excreted drug	$CL^*/CF = a \times W^b$ CF = bile flow/W, or CF = bile flow/LiW
For renally secreted drugs	$CL_R^*/CF = a \times W^b$ $CF = GFR \times Q_{Ki}/W \times KiW$
Rule of exponents (RoE) (using simple allometry)	b < 0.55: underprediction of CL 0.55 > b > 0.7: $CL = a \times W^b$ 0.71 > b > 1: $CL \times BrW = a \times W^b$ 1 > b > 1.3: $CL \times MLP = a \times W^b$ b > 1.3: doubtful

Symbols used. a: allometric coefficient, b: allometric exponent, CL: clearance, W: body weight, MLP: maximum life span, BrW: brain weight, CL*: clearance calculated using the appropriate correction from the rule of exponents (RoE), CF: correction factor, LiW: liver weight, CL_R^*: renal clearance calculated using the appropriate correction from the rule of exponents, GFR: glomerular filtration rate, Q_{Ki}: kidney blood flow, KiW: kidney weight.

allometry (i.e., bias >10-fold in the predictions). Based upon these findings, they developed a new model for predicting human clearance, as shown in Equation 3:

$$CL_{\text{human}} = 33.35(\text{mL/min}) \times (a/Rfu)^{0.77} \quad (3)$$

where a is the coefficient obtained from simple allometric scaling. The researchers suggest that this new model provided better predictability for human values of clearance than did the ROE.

Using clearance values for rats and dogs and some molecular structural descriptors, such as molecular weight, clogP, and the number of hydrogen-bond acceptors obtained from 68 drugs from the literature, Wajima et al. (2002) demonstrated the ability of a multiple linear regression (MLR) analysis to predict human clearance better than allometric methods.

Like clearance, volume of distribution is also an important pharmacokinetic parameter, and there is a generally a good correlation between body weight and volume of distribution among species (Mahmood, 2005c). Often, exponent b in Equation 1 is equal to 1.0, indicating that body weight and volume of distribution are directly proportional. However, as for clearance, some corrections were proposed for allometric scaling of volume of distribution. In particular, the role of the differences in plasma-protein binding across species should be considered (Obach et al., 1997).

Unlike clearance and volume of distribution, the correlation between body weight and half-life ($t_{1/2}$) has been generally found to be poor. Conceptually, it is

difficult to establish a relationship between body weight and half-life, which may be due to the fact that half-life is not directly related to any physiological function of the body, but is rather a hybrid parameter of clearance and volume of distribution.

Mahmood (1998a) noticed that when simple allometry is applied to half-life, exponents of half-life vary widely. In order to improve the prediction of half-life, indirect approaches have been proposed. Among them, the most popular (Bachman, 1989; Mahmood and Balian, 1996a; Obach et al., 1997) is to estimate clearance and volume of distribution from allometric scaling and then calculate the half-life as shown in Equation 4:

$$t_{1/2} = 0.693 \times \text{Volume of Distribution} / \text{Clearance} \qquad (4)$$

Due to differences in the anatomical and physiological features of the gastrointestinal tract, dietary habits, blood flow through the gut and the liver, and enzymatic activity of the drug-metabolizing enzymes and transporters, the oral absorption of drugs varies among species. Therefore, the rate and extent of absorption (i.e., absolute bioavailability) varies from species to species. Further, it is conceptually difficult to justify that an allometric relationship may exist between body weight and absolute bioavailability. Therefore, if good correlation was noted for some drugs, poor correlation was observed for others (Mahmood, 2000a), highlighting the need for other conceptual approaches.

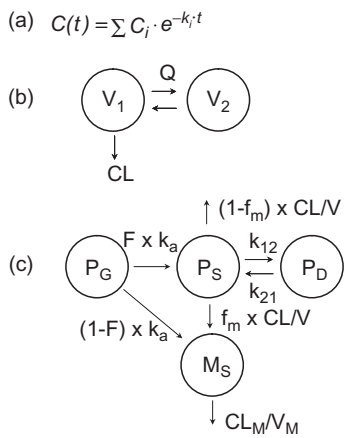

Figure 2. (a) A typical empirical pharmacokinetic model described by a sum of exponential terms, (b) a two-compartment model: V_1 , V_2 refers to the volume of compartment 1 and 2, respectively; CL is the clearance from compartment 1; Q is the inter-compartmental clearance, (c) a semiphysiological model: P and M, refers to parent drug and metabolite; subscripts G, S and D refers to gut, systemic circulation and distribution compartment; F is the bioavailability; k_a is the absorption rate constant; CL and CL_M are the clearances of the parent drug and metabolite, respectively; V and V_M are the volume of distribution of the parent drug and the metabolite, respectively; k_{12} and k_{21} are the distribution rate constants for the parent drug between systemic circulation and the distribution compartment (Adapted from Aarons, 2005).

Current PBPK methodologies with the identification of their limitations and outstanding issues

Introduction

Pharmacokinetic models can be classified in order of increasing complexity (Aarons, 2005): from empirical and semi-mechanistic models (described by a sum of exponential terms; see Figure 2A) to compartmental models (Figure 2B). Although useful for data description and interpolation, they are very poor for extrapolation. Because they do not have a physiological interpretation, it is difficult to predict kinetic profiles when the underlying physiology changes. When adopting a compartmental approach, some physiological parameterization can be attributed (Figure 2C). Nevertheless, compartmental models do not represent real physiological spaces, making these models only semi-mechanistic models. On the contrary, with physiologically based pharmacokinetic (PBPK) models (Figure 3), compartments are actual tissue and organs spaces and their volumes are the physical volumes of those organs and tissues.

PBPK models aim to describe the pharmacokinetics of drugs within the body in relation to blood flows, tissue volumes (Brown et al., 1997), routes of administration, biotransformation pathways, and interactions with the tissue or organ. These biological and mechanistic bases of PBPK models allow the extrapolation of the kinetic behavior of drugs with regard to dose, route, and species.

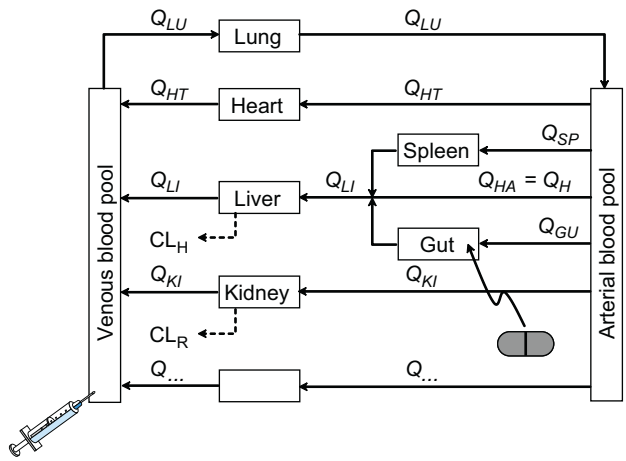

Figure 3. An example of a physiologically-based pharmacokinetic model where compartments represent actual tissues and organs arranged anatomically. Connecting arrows represent blood supplies and elimination processes can be attributed to some organs (*e.g.* liver and kidney, dotted arrows). Q and CL, refers to blood flows and clearance processes, respectively. Subscripts LU, HT, SP, LI, HA, H, GU, and KI refer to lung, heart, spleen, hepatic artery, hepatic, gut and kidney, respectively. An intravenous administration and an oral administration are illustrated. The model depicted here could be extended (empty box at the bottom) to incorporate additional organs like brain, bone, muscle, fat, skin, fat, *etc*...

While conventional PK models are defined by drug-related data themselves, PBPK models were derived from the anatomical and physiological structure of the organism studied. In whole-body PBPK models (Figure 3), the drug enters the compartment in the arterial blood and returns to the heart in the venous blood compartment. Elimination processes (e.g., biotransformation) can occur in specific organs, such as kidney and liver.

While empirical or compartmental models poorly address such questions as "How do we predict likely exposure profiles in various target tissues and organs in humans?" or "How can we predict events in human from animal data?", PBPK models are mathematical models to conduct extrapolations for dose-response and exposure assessments and to generate simulations of PK profiles under various physiological conditions (e.g., age, disease) (Theil et al., 2003; Rowland et al., 2004). As seen previously, allometric scaling may include some physiological parameters (e.g., maximum lifespan, BrW, enzymatic activity, bile flow, creatine clearance, and glomerular filtration rate) or incorporate a correction for plasma-protein binding. As such, allometric scaling has demonstrated some utility in predicting PK parameters (e.g., clearance, volume of distribution) in human from PK profiles in animal species or differences between children and adults and between genders, but they cannot alone accommodate the effect of disease states or interpret the differences between neonates and adults (Björkman, 2004).

Building PBPK models

Parameters for incorporation into PBPK models are either drug dependent (e.g., binding to blood, fu_b; tissue-to-plasma distribution coefficient, Kp_T; tissue permeability–surface area product, PS_T; enzymatic activity, V_m/K_m) or drug independent (e.g., blood flows, Q_T; tissue volumes, V_T; tissue composition).

PBPK modeling refers to the development of mathematical descriptions of the uptake and disposition of drugs, based on quantitative interrelations among the critical biological determinants of these processes, that is, 1) interaction of the drug with the components of the tissues, which is driving the partitioning into tissues (Kp_T, the partition coefficient, i.e., a measure of the differential solubility of a compound in two "solvents"); 2) rates of biological reactions due to enzymes or transporters (functionality of the organ), and 3) physiological characteristics of species (Q_T, V_T, tissue composition) (Gerlowski and Jain, 1983).

Four different steps could be identified in developing a whole-body PBPK model (WBPBPK) (Haddad et al.,1996; Chiu et al., 2006): 1) the model representation, which consists of a mathematical description of the relevant compartments (or organs) incorporating exposure and metabolic pathways of the drug; 2) the model parameterization, that is, inclusion of mechanistic determinants in the model equations (i.e., physiological, physicochemical, and biological parameters); 3) the model simulation, which consists of the prediction of the uptake and disposition of a chemical for a defined exposure scenario by solving the set of mass balance differential equation using a numerical integration algorithm (e.g., the fourth-order Runge-Kutta method, which is an iterative method of numerical analysis for the approximation of solutions of ordinary differential equation); and finally, 4) the model validation, where predicted outcomes are compared with experimental data, including a parameter sensitivity analysis and an uncertainty analysis.

Model representation (whole-body PBPK)

The first step consists in selecting the relevant organs or tissues to incorporate in the model. The "core" tissues/organs pool may include the blood compartment(s) (arterial and/or venous), the eliminating organs (generally liver and kidney), and adipose tissue. Other "drug-dependent" tissues/organs could be envisaged, such as the site of administration (gut, skin, and lung) or the site of action (brain, heart). Those organs expected to contribute significantly to mass balance could also be incorporated (skin, muscle, and bone). When a drug and its metabolite(s) are studied, the model implies to develop a sub-PBPK structure for each compound and link the models through the site of metabolism compartment (Clewell et al., 2001). This compartment becomes the input process to the sub-PBPK model of the metabolite. In addition, when a compound is subjected to enterohepatic cycling, appropriately modified PBPK models can be derived (Ploeger et al., 2000).

In a second step (mathematical description), each tissue compartment is described by a mass balance differential equation, which includes a series of CL terms (biotransformation reaction, V_m/K_m; intrinsic clearance; permeability–surface area product). The general principle of such equations is shown in Equation 5:

$$\begin{aligned}\text{Rate of change of mass A in the system} = {} & \text{Rate of gain of A} \\ & - \text{Rate of loss of A} \\ & \pm \text{Rate of gain or loss of A by reaction}\end{aligned} \tag{5}$$

At this stage, each tissue or organ can be considered as 1) a perfusion rate-limited tissue or blood flow-limited organ (Figure 4A), or as 2) a permeability rate-limited tissue or membrane-limited organ (Figure 4B) (Nasu et al., 2005). In the former situation, the drug distributes

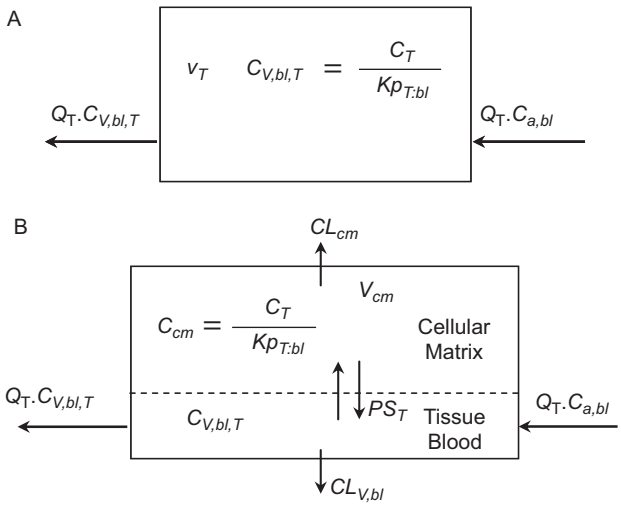

Figure 4. (A) Representation of a perfusion rate limited tissue or blood flow (Q_T) limited organ. The tissue or organ is considered homogenous (well stirred) and concentrations in tissue T (C_T) are in equilibrium with the concentrations in the venous blood leaving the tissue ($C_{v,bl,T}$). (B) A permeability rate limited tissue or membrane limited organ. The movement of the drug from the tissue blood into cellular matrix is proportional to a mass transfer coefficient i.e. the permeability surface area product (PS_T) for the tissue. Tissue uptake is diffusion-limited when $PS_T << Q_T$. At this stage two subcompartments are to be considered. A_T: amount in tissue T (=V_T. C_T, where V_T is the volume of tissue T), $Kp_{T:bl}$: the partition coefficient of the drug between tissue and blood (total concentration in tissue/total concentration in blood), $C_{a,bl}$, concentration in the arterial blood entering the tissue. A_{cm}, C_{cm} and V_{cm} refer to amount and concentration in, and volume of the cellular matrix, respectively. $CL_{v,bl}$ and CL_{cm} refer to clearance process from tissue blood and cellular matrix.

instantaneously within the physiological space (moves freely from capillaries into interstitial fluid and tissue cells so that the rate-limiting step controling the movement of drug into and out of the organ is the perfusion or blood flow to the organ) and no concentration gradients are likely within this physiological compartment (well-stirred or venous equilibrium model). In the latter situation, membrane permeability (P, a measure of the ability of the membrane to permit the passage of a compound) limits the distribution within the tissue/organ and the tissue could be divided in 2 or 3 well-stirred subcompartments with a permeability-rate–limited transfer between them (Nestorov, 2003). Practically, the use of a membrane-limited organ model is indicated if tissue-drug concentrations do not decline in parallel with drug concentrations in blood/plasma. A formal criterion has been found by Dedrick and Bischoff (1968). By approximation, if $Q_T/PS_T << 1$ (perfusion of the tissue by the blood is the rate limiting step, not the permeation through membranes), the organ can be represented as blood-flow limited.

Figure 4A illustrates a perfusion-limited uptake where the tissue is considered homogenous (well-stirred) and where the concentration in tissue T (C_T) is in equilibrium with the concentration in the venous blood leaving the tissue. The rate of change of the drug in the whole tissue is described by the following differential equation (Equation 6) (for the signification of all symbols, see Figure 4A) (MacDonald et al., 2004; Parrott et al., 2005a; Jones et al., 2006a):

$$\frac{dA_T}{dt} = V_T \frac{dC_T}{dt} = Q_T \left(C_{a,bl} - C_{v,bl,T}\right) = Q_T \left(C_{a,bl} - \frac{C_T}{Kp_{T:bl}}\right) \quad (6)$$

where $C_{a,bl}$ and $C_{v,bl,T}$ refer to the concentration in the arterial blood and the concentration in the venous blood leaving the tissue, respectively.

As mentioned previously, loss of drug by metabolism (dA_{met}/dt) can be incorporated in the mass balance equation of the organ/tissue, as shown in Equations 7–9:

$$V_T \frac{dC_T}{dt} = Q_T \left(C_{a,bl} - C_{v,bl,T}\right) - \frac{dA_{met}}{dt} \quad (7)$$

$$V_T \frac{dC_T}{dt} = Q_T \left(C_{a,bl} - C_{v,bl,T}\right) - CL_{int,vivo,u} \cdot C_{u,T} \quad (8)$$

with,

$$C_{u,T} = C_{u,v,bl,T} = C_{v,bl,T} \cdot f_{u,bl} \quad (9)$$

where dA_{met}/dt accounts for the rate of loss due to metabolism, $CL_{int,vivo,u}$ is the intrinsic unbound *in vivo* metabolic clearance, $C_{u,T}$ is the unbound concentration in tissue, and $f_{u,bl}$ is the drug unbound fraction to blood.

Figure 4B illustrates a diffusion-limited uptake. In this situation, the movement of the drug from tissue blood into cellular matrix is proportional to a mass transfer coefficient, that is, the permeability surface area product (PS_T) for the tissue. Tissue uptake is diffusion limited when $PS_T << Q_T$. At this stage, two subcompartments are to be considered. The rate of change in the amount of drug in the cellular matrix is described by the following differential equation (Equation 10) (for the signification of all symbols, see Figure 4B):

$$\begin{aligned} \frac{dA_{cm}}{dt} &= V_{cm} \frac{dC_{cm}}{dt} = PS_T \cdot C_{v,bl,T} \\ &\quad - PS_T \cdot \frac{C_T}{Kp_{T:bl}} = PS_T \cdot \left(C_{v,bl,T} - \frac{C_T}{Kp_{T:bl}}\right) \end{aligned} \quad (10)$$

where ($C_{v,bl,T} - C_T / Kp_{T:bl}$) is the net flux from tissue blood. If a clearance (CL) from the cellular matrix occurs, the equation becomes as shown in Equation 11:

$$V_{cm} \frac{dC_{cm}}{dt} = PS_T \cdot C_{v,bl,T} - PS_T \cdot \frac{C_T}{Kp_{T:bl}} - CL_{cm} \cdot \frac{C_T}{Kp_{T:bl}} \quad (11)$$

The rate of change of the drug in the tissue blood subcompartment is given by Equation 12:

$$\frac{dA_{T,bl}}{dt} = V_{T,bl}\frac{dC_{T,bl}}{dt} = Q_T\left(C_{a,bl} - C_{v,bl,T}\right) + PS_T\left(\frac{C_T}{Kp_{T:bl}} - C_{v,bl,T}\right) \quad (12)$$

where ($C_{a,bl}$ – $C_{v,bl,T}$) and ($C_T/Kp_{T:bl}$ – $C_{v,bl,T}$) represents net retention from blood flow and net flux from cellular matrix, respectively. If clearance from tissue blood ($CL_{v,bl}$) occurs, the result is as shown in Equation 13:

$$V_{T,bl}\frac{dC_{T,bl}}{dt} = Q_T\left(C_{a,bl} - C_{v,bl,T}\right) + PS_T\left(\frac{C_T}{Kp_{T:bl}} - C_{v,bl,T}\right) - CL_{v,bl}\cdot C_{v,bl,T} \quad (13)$$

In Figure 3 (whole-body PBPK model), the resulting "mixed" venous blood concentration ($C_{v,bl}$) of the drug is made of all venous blood concentrations leaving the organs considered in the model, as shown in Equation 14:

$$C_{v,bl} = \frac{\sum_i^n Q_{Ti}\cdot C_{v,bl,Ti}}{Q_C} \quad (14)$$

where Q_{Ti} is the blood-flow rate to organ/tissue i, $C_{v,bl,Ti}$ is the concentration in the venous blood leaving organ/tissue i, and Q_c is the cardiac blood flow, that is, the cardiac output (identical to Q_{LUNG}).

In Figure 3, we have illustrated part of a whole-body PBPK model with an intravenous and an oral administration. To take into account drug delivery into the venous compartment and the gut lumen, the mass balance of the venous blood pool and the gut should be modified, as shown in Equations 15 and 16:

$$V_{v,bl}\cdot\frac{dC_{v,bl}}{dt} = \left[\sum_i^n Q_{Ti}\cdot C_{v,Ti}\right] - Q_{LU}\cdot C_{v,bl} + K_{IV} \quad (15)$$

$$V_{GU}\cdot\frac{dC_{GU}}{dt} = R_{abs} + Q_{GU}\cdot C_{a,bl} - Q_{GU}\cdot C_{v,bl,GU} \quad (16)$$

where K_{iv} and R_{abs} account for the rate of intravenous infusion and the rate of absorption (mg/h), respectively.

Model parameterization

As illustrated in the sections before mass balance, differential equations are incorporating three different classes of parameters: physiological, physicochemical, and biochemical. Their respective values must be specified.

Physiological parameters include body weight, tissue volumes (V_T), cardiac output (Q_C), and tissue perfusion (Q_T). This type of information as been compiled for many species (Brown et al., 1997) and many are also available for humans. This list is not exhaustive and could be extended to other kinds of physiological parameters, where needed, by the model. As an example, for the administration of inhaled drugs, the alveolar ventilation rate (the volume of gas per unit time that reaches the alveoli) has to be taken into account. In relatively simple PBPK models for inhalation, this, together with cardiac output, and the blood:air partition coefficient, is the most important parameter for modeling lung uptake. If the rate of distribution into blood cells is rate limiting (i.e., transfer between plasma and blood and binding to blood-cell constituents), the nature, distribution, and capacity of the binding sites (i.e., intra- and extracellular) are to be incorporated.

Biochemical parameters include rate of absorption, rate of metabolism, rate of binding to specific proteins (e.g., albumin, lipoproteins, etc.), and excretion. Binding to drug transporters or receptors involved in the pharmacodynamic response could also be incorporated. In the context of the drug-drug interactions, parameters for competitive, non-, un- or mixed-competitive inhibition and mechanism-based inactivation have been incorporated into physiological models (Ito et al., 1998; Kanamitsu et al., 2000). In these models, the substrate and inhibitor share the same model structure and only the biorelevant compartments were modeled (e.g., the liver, the systemic blood, and the portal vein). Most of these biochemical parameters could be obtained *in vitro* and eventually scaled up to the *in vivo* situation before incorporation into the models.

To illustrate this specific issue, let us take as an example a PBPK model for elimination through metabolic biotransformation. This example aims to illustrate how to integrate *in vitro* metabolism data into physiological models to predict *in vivo* kinetic profiles (Houston, 1994; De Buck and Mackie, 2007c). This is illustrated in Figure 5. Biotransformation rates can be obtained with microsomes or hepatocytes either through substrate disappearance or initial rate of metabolite formation. Using microsomes, Michaelis-Menten parameters (V_m, K_m) can be obtained to derive an *in vitro* intrinsic clearance ($CL_{int,vitro} = V_m/K_m$; in µL/min/mg mic. protein). An *in vitro* intrinsic clearance can also be obtained with incubated hepatocytes (µL/min/10^6 cells). The second stage of this strategy (from "*in vitro* intrinsic clearance" to "*in vivo* intrinsic clearance") involves scaling steps to quantify the full activity of the liver. Scaling factors include hepatocellularity, microsomal recovery, nonspecific binding to biological material, and liver weight. Stage 3 requires the use of a liver model to incorporate the estimated *in vivo* intrinsic clearance into the predicted

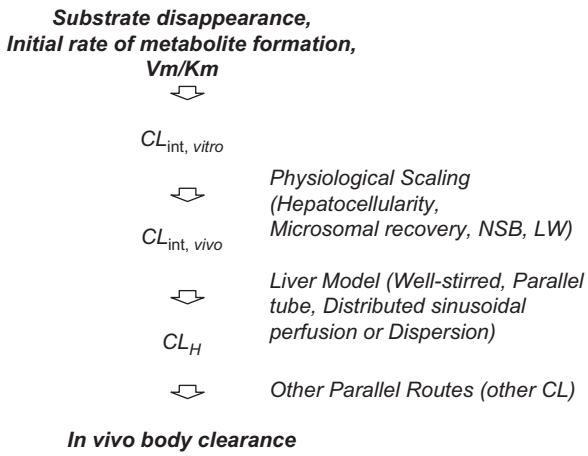

Figure 5. Liver metabolism and *in vitro* to *in vivo* scale up methodology (adapted from Houston, 1994).

hepatic clearance (CL_H). A hepatic blood-flow value and information on the binding of drug in the blood are required. The overall effect of a liver model is to express clearance in terms of the circulating blood drug concentration rather than the drug concentration within the liver. In the final stage, it is suggested to consider parallel nonhepatic routes, for example, renal elimination. Hence, if we consider the liver as an eliminating organ and not only as a distribution space, elimination by the liver (a drug-specific input parameter) is incorporated in the model, using Equation 17:

$$V_{LI}\cdot\frac{dC_{LI}}{dt}=Q_H\cdot C_{a,bl}+Q_{GU}\cdot C_{v,bl,GU}+Q_{SL}\cdot C_{v,bl,SP} -Q_{LI}\cdot C_{v,bl,LI}-CL_{int,vivo,u}\cdot C_{v,bl,LI,u} \quad (17)$$

For renally cleared drugs, other scaling methods are proposed: 1) the "glomerular filtration rate (GFR) ratio approach," if only minimal preclinical (e.g., rat) data are available, and 2) the allometric approach, if renal clearance (CL_R) information from three to four animal species is available. The first one (Lin, 1998) assumes that the ratio of $CL_{R,unbound}$ (expressed per kg of bodyweight) is approximately equal to the ratio of GFR between rats and humans (i.e., 4.8). Hence, the unbound human renal clearance can be estimated ($CL_{R,unbound,human} = CL_{R,unbound,rat} / 4.8$). This $CL_{R,u}$ can now be incorporated in the PBPK model considering the kidney as an eliminating organ, using Equation 18:

$$V_{KI}\cdot\frac{dC_{KI}}{dt}=Q_{KI}\cdot C_{a,bl}-Q_{KI}\cdot C_{v,bl,KI}-CL_{R,u}\cdot C_{v,bl,KI,u} \quad (18)$$

Model simulation and validation

It is beyond the scope of this section to cover exhaustively these two aspects of physiological modeling. What should be emphasized here is the availability of commercial softwares that are able to perform both simulation and parameter optimization and, for a couple of them, *in silico* predictions mainly for absorption and distribution processes. *In silico* approaches for the prediction of excretion is mostly based on allometric approaches across animal species. Physiologically based custom-designed software for WBPBPK modeling include GastroPlus™ (http:\\www.simulations-plus.com), PK-Sim™ (Accessed 28th April 2009) (http:\\www.Pk-Sim.com), and the population-based pharmacokinetic modeling and simulation software Simcyp™ (http:\\www.simcyp.com). Other high-level scientific computing softwares or biomathematical modeling softwares are available to scientists, but they require programming skills. They include Matlab-Simulink™ (http:\\www.mathworks.com), WinNonlin™ (http:\\www.pharsight.com), and acslX (http:\\www.acslX.com) for PBPK and PK/PD modeling. Let us introduce two of them, GastroPlus and Simcyp.

Gastroplus

GastroPlus simulates gastrointestinal absorption and pharmacokinetics for drugs administered orally and intravenously in humans and animals (Parrott and Lavé, 2002; Jones et al., 2006b). The simulation model imbedded in GastroPlus is known as the advanced compartmental absorption and transit (ACAT) model (Agoram et al., 2001; Yu et al., 1996; Yu and Amidon, 1998 and 1999). This gut-absorption model allows for the simulation of the effects of physicochemical, physiological, and formulation parameters on absorption. This dynamic model includes time dependency of absorption and is able to predict both the rate and extent of absorption. The gastrointestinal tract is viewed as a series of tanks ($n = 9$ in the latest release). The transit between compartments is described by a set of differential equations with a first-order kinetic rate constants process (k_t is the same for all compartments). In addition to kinetics of transit, dissolution and uptake processes are input parameters for these differential equations. This model (seven tanks) was validated by estimating k_a for 10 drugs from their effective permeabilities (P_{eff}), measured from the rate of disappearance of the drug from a section of the gastrointestinal tract (GIT) (Yu and Amidon, 1999). The simulations that can be run with GastroPlus include, 1) prediction of the fraction absorbed (Fa) using *in vitro* properties (solubility—as a function of pH, pKa(s), permeability, dose, dosage form, particle size and density, diffusion coefficient, etc.), 2) prediction of Fa using *in vivo* data in other species and *in vitro* data (Caco-2), 3) parameter sensitivity analysis (PSA), 4) controled release evaluation and design, and

5) dosage formulation evaluation and design, in relation with physiological parameters. Other modules are also available to perform PK and PD simulations as well as a metabolism and transporter module. For pure *in silico* predictions, GastroPlus has on board a companion product ADMET Predictor™ providing structure-property predictions and estimates for pKa, logP, solubility, permeability, plasma-protein binding, etc. Models based upon two- or three-dimensional (2D or 3D) structures are available. Then, partition coefficients (Kps) are estimated from logP and logD and tissue properties.

Simcyp

The Simcyp population-based ADME simulator (Jamei et al., 2009) is a typical example of integrating interindividual variability into PBPK modeling for the prediction of drug clearance and metabolic drug-drug interactions in virtual populations. By combining information on genetic, physiology, and demography/ethnicity with *in vitro* data, Simcyp performs extrapolation to *in vivo* situations and virtual populations. Parameters relevant to the IVIVE scaling process are obtained for all individuals and combined with *in vitro* metabolism data ($CL_{int,vitro}$) to obtain $CL_{int,vivo}$ and, subsequently, CL_H. Interindividual variability is also considered for renal clearance and first-pass gut metabolism (Howgate et al., 2006). Hence, by integrating interindividual variability into PBPK models, together with mechanistic pharmacodynamic modeling, provides a basis for understanding and predicting individual differences in drug response (Tucker et al., 2001; Rostami-Hodjegan and Tucker, 2007). A clear application of this is in the estimation of demographic and genetic differences in ethnic groups (Inoue et al., 2006), and in the evaluation of age-related changes in pediatrics (Björkman, 2004; Johnson et al., 2006). Also, the tool was particularly used for predicting the relevance of potential drug-drug interactions (Grime et al., 2009).

Impact of drug related physicochemical properties on the tissue distribution in humans

Tissue distribution is dependent on a variety of processes, including passive diffusion, active transport, and cellular concentrations of lipids and binding proteins. Tissue-to-blood partition coefficients (Kps) describe the steady-state concentration of a drug in the tissue compared with blood. In addition to Kps, the volume of distribution at steady state (V_{ss}), which describes the extent of tissue distribution in the whole body, is another PK parameter commonly used to describe tissue distribution of a drug. Kps and V_{ss} are closely related (Sawada et al., 1984), as shown in Equation 19:

$$V_{ss} = V_{pl} + \sum\left(V_T \cdot K_{p,T}\right) + \left(V_E \cdot \frac{E}{pl}\right) \tag{19}$$

where E is erythrocyte and E/pl is the erythrocyte to plasma ratio, which can be estimated from the blood-to-plasma ratio and the hematocrit content in blood.

Because blood concentration-time profiles are the resultant of drug distribution in extravascular tissues, Kps are *the* central parameters of PBPK models (Kawai et al., 1994, 1998).

Kps may be estimated *in vitro* (using radioactive or nonradioactive material) in ultrafiltration, equilibrium dialysis, or vial equilibration procedures (Lin et al. 1982; Ballard et al., 2000; Daniel and Wojcikowski, 1997). The partition coefficients estimated by these *in vitro* methods are acceptable, provided equilibrium is attained during the experimental conditions.

Determination of Kps as input parameters for PBPK models has also been done by fitting model simulations to *in vivo* data describing blood and tissue concentrations. In such cases, pharmacokinetic data collected following a single bolus dose or repeated doses (leading to steady state) are analyzed with the PBPK model to estimate the Kps (Chen and Gross, 1979; Gabrielsson and Bondesson, 1987; Gallo et al., 1987). Steady-state data provide the most straightforward data; however, they require correction for tissues in which there are significant specific binding or metabolic processes. In these tissues, the calculation of an apparent Kp must account for the amount of drug consumed by such processes (Chen and Gross, 1979).

From an experimental point of view, these *in vitro* and *in vivo* approaches are labor intensive, time-consuming, and some of them are limited to animal species. In addition, some *in vivo* estimates require knowledge of modeling and nonlinear regression. With regard to these "difficulties" and limitations, more *in silico* and mechanistic methods were recently developed and algorithms based on the consideration of physicochemical characteristics of the drug and the biological composition of the tissue were proposed. These approaches are based on the consideration of solubility and binding of drugs in biological matrices components. They relate tissue distribution to tissue composition and use information on tissue composition and distribute the drug into the different phases of the biological matrices.

Predicted values of Kps could be derived from the mechanistic tissue-composition–based equation developed by Poulin and coworkers (Poulin and Theil,

2000, 2002a, 2002b; Poulin et al., 2001), as shown in Equation 20:

$$Kp_{T:pl} = \frac{\left[\left(f_{NL,T} + 0.3 \cdot f_{PL,T}\right) \cdot P + \left(f_{water,T} + 0.7 \cdot f_{PL,T}\right)\right] \cdot f_{u,pl}}{\left[\left(f_{NL,pl} + 0.3 \cdot f_{PL,pl}\right) \cdot P + \left(f_{water,pl} + 0.7 \cdot f_{PL,pl}\right)\right] \cdot f_{u,T}} \quad (20)$$

where P is the antilog value of log $P_{octanol:water}$ for nonadipose tissues or is the vegetable oil/buffer partition coefficient for both the ionized and nonionized species at pH 7.4 ($D_{vo:water}$) for adipose tissue. $D_{vo:w}$ is calculated from log $P_{oc:w}$ by using the Henderson-Hasselbach equations and the following relationship: log $P_{vo:w}$ = 1.115 x log $P_{oc:w}$ – 1.35 (Leo et al., 1971), as shown in Equations 21 and 22.

For monoprotic acids:

$$\log D_{vo:w} = \log P_{vo:w} - \log\left(1 + 10^{pH\text{-}pKa}\right) \quad (21)$$

For monoprotic bases:

$$\log D_{vo:w} = \log P_{vo:w} - \log\left(1 + 10^{pKa\text{-}pH}\right) \quad (22)$$

f is the fractional tissue volume content of neutral lipids (NL), phospholipids (PL), or water in tissue (T) and plasma (pl). The physiological data on human and rat values used for $f_{NL,T}$, f_{NLpl}, $f_{PL,T}$, $f_{PL,pl}$, $f_{water,T}$, and $f_{water,pl}$ have been described in the literature (Poulin and Theil, 2002a, 2002b). The fraction unbound in tissue ($f_{u,T}$) was estimated as shown in Equation 23:

$$f_{u,T} = \frac{1}{11\left[\frac{12\, f_{u,pl}}{f_{u,pl}}\right]RA} \quad (23)$$

where RA is the ratio of albumin concentration found in tissue over plasma. For lipophilic and highly protein-bound compounds, it has been assumed that for adipose tissue, RA equals 0.15, whereas for nonadipose tissue, RA equals 0.5 (Ellmerer et al., 2000; Poulin and Theil, 2002a, 2002b). Poulin and Theil validated their equations with *in vivo* data of about 140 drugs. Overall, 80% of all predicted V_{ss} were within a factor of two of the experimental values (Poulin and Theil, 2002a, 2002b). Follow-up studies indicated that when applied to different data sets, the overall V_{ss} prediction accuracy of these equations was reduced (Jones et al., 2006a; Parrott et al., 2005a, 2005b). These discrepancies could be explained by different physicochemical properties or distribution processes (active transport, specific macromolecular binding, limitation for membrane permeation, and ionic interactions (Poulin and Theil, 2002a, 2002b; De Buck and Mackie, 2007a, 2007b, 2007c).

Using the Poulin and Theil equations led to some underprediction for Kps in lung and intestine and also for the V_{ss} of moderate-to-strong bases. Rodgers and coworkers (Rodgers et al., 2005) demonstrated that electrostatic interactions with acidic membrane phospholipids will predominate for basic drugs positively charged within tissues. They have developed a new tissue-composition–based approach and improved the predictability of Kps. More recently, De Buck et al. (2007a, 2007b) compared both approaches on a data set mainly comprised of moderate-to-strong basic compounds. In both rats and humans, the approach by Rodgers yielded more accurate predictions of V_{ss} (for rats ca. 80% within 2-fold, with human ca. 80% within 3-fold). For strong bases ($pK_a > 7.0$), the mechanistic equation of Rodgers for $Kp_{T:plasma,unbound}$s ($Kp_{T:pl,u}$) is shown in Equation 24:

$$Kp_{T:pl,u} = \frac{c_{T,ss}}{c_{u,pl,ss}} = f_{EW} + \left(\frac{1 + 10^{pKa\text{-}pH_{IW}}}{1 + 10^{pKa\text{-}pH_{pl}}} \cdot f_{IW}\right) + \left(\frac{K_{a,AP^-} \cdot \left[AP^-\right]_T \cdot 10^{pKa\text{-}pH_{IW}}}{1 + 10^{pKa\text{-}pH_{pl}}}\right) + \left(\frac{P \cdot f_{NL} + \left(0.3 \cdot P + 0.7\right) \cdot f_{NP}}{1 + 10^{pKa\text{-}pH_{pl}}}\right) \quad (24)$$

where f is the fractional tissue volume of neutral lipids (NL), neutral phospholipids (NP), extracellular water (EW), and intracellular water (IW), and $[AP]_T$ is the concentration of acidic phospholipids in tissue. All physiological data on f_{EW}, f_{IW}, f_{NL}, f_{NP}, and $[AP]_T$ for both adipose and nonadipose tissue have been described in the literature (Rodgers et al., 2005), pKa represents the dissociation constant of the protonated base, and P is the anti-log value of log $P_{vo:w}$ (calculated from $P_{oc:w}$ as described above). $K_{a,AP-}$ is the association constant of the compound with the acidic phospholipids, and was estimated by applying the equation to red blood cells (where $f_{EW} = 0$) and by solving for $K_{a,RBC}$ with,

$$Kp_{u,RBC} = \frac{c_{RBC}}{c_{u,pl}} = \frac{Ht - 1 + B/P}{Ht \cdot f_{u,pl}} \quad (25)$$

where Ht and B/P are the hematocrit and blood-to-plasma ratio.

Using this tissue composition-based approach, Rodgers and Rowland where also able to derive equations to predict the tissue distribution (Kps) of acids, very weak bases, neutrals, and zwitterions (Rodgers and Rowland, 2006, 2007).

Potential contributions of PBPK in drug development

A systematic review of the literature indicates that over the past 10 years, the number of publications in

physiologically based pharmacokinetics has more than doubled. Of these publications, the vast majority are in the field of environmental risk assessment, due to the ability of PBPK modeling to predict the exposure to pollutants/toxics in remote and/or inaccessible compartments. However, PBPK is increasingly used in all phases of drug development, due to increased knowledge in physiological systems, to the encouragement for the use of *in-silico* models from the regulatory agencies for a more efficient drug selection/development, and thanks to the availability of user-friendly softwares and increased computational power.

Drug discovery

In the past, one of the reasons of the failure in the development of new drugs was their poor pharmacokinetic profile. To minimize this risk, and to streamline drug development, PBPK is more and more often used during the phase of drug discovery and development. Knowing the ideal pharmacokinetic characteristics for the new drug candidate, series of compounds can be screened not only for their pharmacological properties, but also with respect to their pharmacokinetic behavior, even before any animal testing, just by leveraging the information on physicochemical characteristics and *in vitro* tests. This results in a reduction of unnecessary animal testing and significant time savings (Germani et al., 2007).

The major issue for the use of PBPK models in this very early phase of drug development is the derivation of the input parameters. In the simplest case (intravenous administration), they are represented by the tissue-to-plasma (or blood) partition coefficients (Kps), providing information for the distribution of the drug and the *in vivo* clearance ($CL_{in\ vivo}$) for the elimination.

Kps in the past were derived experimentally by measuring drug levels in plasma and tissues, but this was time-consuming and unfeasible in humans. Therefore, Arundel (1997) proposed a method to relate, through a multicompartmental model, V_{dss} and Kps. However, although the method avoided the need for tissue sampling and analysis, it was still based on *in vivo* animal data. More recently, Poulin and Theil (2002a, 2002b) proposed a new model for deriving tissue-to-plasma ratios, based on the physicochemical properties of the drug and its plasma protein binding.

In vivo clearance was traditionally derived by using conventional allometric scaling of *in vivo* plasma clearance in animals, according to species body weight. Although reliable predictions was provided for compounds devoid of great interspecies differences in their pharmacokinetics (Sanwald-Ducray and Dow, 1997; Mahmood, 1998b; Leusch et al., 2000), allometric scaling failed for compounds with mixed elimination and/or active processes (Lavé et al., 1999).

Luttringer et al. (2003) provided an example of the application of PBPK modeling to predict the disposition of epiroprim, an antimicrobial agent, in humans, by comparing the aforementioned methods for deriving the input parameters. Human predictions for this compound were challenging, due to the large interspecies differences in its elimination pathways.

During the screening phase, the absorption characteristics are of primary importance to predict absorption/bioavailability in humans. Therefore, an extended PBPK model must also account for absorption processes, through the use of *in vitro* and/or *in silico* estimated biopharmaceutical properties. In the case of oral administration, the absorption is determined by subprocesses, such as the release of the drug into the intestinal lumen, uptake from the intestinal lumen to the portal vein, and metabolism during the first pass through the liver. These subprocesses will depend upon physiological factors (e.g., pH in the gastrointestinal tract, gastric emptying, intestinal transit, active transport, and gastrointestinal metabolism), drug-specific properties (e.g., lipophilicity, pKa, solubility, particle size, permeability, and metabolic stability), and formulation factors (e.g., release kinetics, dissolution kinetics). The interplay of these factors can be included in extended PBPK models. This is achieved by segmenting the GIT into different compartments, where the kinetics of transit, dissolution, and uptake are described by sets of differential equations. The input parameters needed for these absorption models are *in vitro* measured or calculated parameters, such as permeability, solubility, pKa, and dose. Sensitivity analyses are of valuable interest to define critical factors affecting the bioavailability of the drug to be further addressed and investigated for improving the drug formulation or to optimize the drug candidate.

Poulin and Theil (2002b) provided an example of a generic, integrative PBPK model of drug disposition, including absorption processes, on two lipophilic bases (diazepam and propranolol) and one neutral hydrophilic drug (ethoxybenzamide). Predicted concentration-time profiles in plasma and tissues were in reasonable agreement with the corresponding experimental data determined *in vivo* (less than a factor of 2). However, some deviations are noticed for specific tissues, such as brain, gut, liver, and lung. The researcher attributes these deviations to processes neglected in the PBPK model. If these preliminary integrated PBPK models are useful for compound prioritization, when moving to the lead candidate optimization the performance of the model has to be confirmed and possibly refined, based on acquired *in vivo* animal data.

Preclinical development

During the preclinical phase, the overall pharmacokinetics of potential drug candidates in animals and in humans (through a combination of scaling of physiological parameters and use of *in vitro* data) can be anticipated through the use of PBPK modeling. Accounting for differences in tissue-specific transporters and binding sites, PBPK models help to improve the design of preclinical and clinical studies. Further, the simulated concentration-time profiles in main organs, in addition to experimental plasma profiles, are valuable to identify critical accumulation effects or to allow target-specific pharmacokinetic-pharmacodynamic (PKPD) analyses. Such PBPK/PD evaluations provide crucial information on the potency and safety of drug candidates *in vivo* and bridge PKPD concepts established during the preclinical phase to clinical studies.

Jones et al. (2006a) compared the performance of PBPK models for predicting human pharmacokinetics with allometric methods (using Dedrick plots), and indicated a better accuracy for the PBPK models, with 70% of success on the 19 molecules considered. Mispredictions were attributed to processes not accounted for by the model, such as concurrent elimination processes (biliary, pulmonary elimination, enterohepatic recirculation) or active processes (hepatic uptake, biliary active efflux, etc.). The value of these predictions is raised by their potential use for mechanistic evaluations for generating research hypothesis to understand inaccurate simulations and finally better understand the candidate's PK properties.

Parrot et al. (2005b) demonstrated the utility of PBPK/PD modeling to select the best candidate for development, among five. The human EC_{90} could be estimated by accounting for the different affinities of each compound for the rat and human receptors and also for the different unbound fractions in rat and human. The output from the modeling was summarized as a series of charts comparing the predicted effective doses, associated exposures, and corresponding error ranges around the predictions.

Human predictions are prospectively and routinely applied at F.Hoffmann-La Roche Ltd, Basel, Switzerland. The experience within this company confirms the superiority of PBPK predictions over more empirical approaches. Lavé et al. (2007) illustrated the performances of the PBPK predictions for four projects, at high and low doses. The predicted data were in agreement with the observed data at the high doses. At the low dose, a significant overestimation was evidenced for one project, attributed to the binding to the target not accounted for in the model. This misprediction was negligible, as at the expected therapeutic dose, the predictions were in agreement with the observed data.

Clinical development

First, clinical trials are generally conducted in a very standard population, including healthy subjects. This is usually not the target population for the new drug. Demographic and physiological parameters (body weight and composition, renal, hepatic, and cardiovascular function) can vary in patients and can differ, according to various genetic profiles or following the administration of other drugs. Therefore, physiologically based models help in appreciating differences between the reference population represented by healthy subjects and subpopulations with different diseases, different demographic characteristics, and different ethnic origins.

A common application of PBPK modeling is in supporting pediatric trial designs by guiding dosing regimens, ensuring efficient blood sampling times, maximizing therapeutic effect, and potentially reducing the number of children required for the study.

Prediction of the exposure of neonates, infants, and children to the drug is likely to be more successful with using physiologically based pharmacokinetic models than simplistic allometric scaling, particularly in younger children. However, such models require comprehensive information on the ontogeny of anatomical, physiological, and biochemical variables. User-friendly softwares, including different special population characteristics, have been developed and validated (e.g., Simcyp™, http:\\www.simcyp.com or PK-Sim from Bayer Technology; Willmann et al., 2003, 2004, 2005).

Johnson et al. (2006) and Edginton et al. (2006) have extended existing physiologically based pharmacokinetic models for adults to reflect the age-related physiological changes in children from birth (excluding premature neonates) to 18 years. They applied their extended models to eleven (midazolam, caffeine, carbamazepine, cisapride, theophylline, diclofenac, omeprazole, S-warfarin, phenytoin, gentamicin, and vancomycin) or five (paracetamol, alfentanil, morphine, theophylline, and levofloxacin) compounds, respectively. Predictions (including the corresponding variability) were in good agreement with the reported observed data in the different age groups (neonates, infants, children, and adolescents), and both models were superior to allometric scaling, especially in children aged < 2 years old.

Willmann et al. (2006) reported a PBPK model for ciprofloxacin in obese and renally impaired subjects. Predicted mean pharmacokinetic parameters were in agreement with the mean observed data, but the variability in the renally impaired population was underestimated. This effect was attributed to sources of variability not accounted for by the model, such as

the decrease in intracellular water consequent to the muscle atrophy or the increase in extracellular water in renal impairment.

Another interesting application in clinical development has been described by Jones et al. (2006b): GastroPlus was used to predict the food effect (under fasted, fed, and high-fat conditions) for six compounds developed at F. Hoffmann-La Roche Ltd, Basel, Switzerland, using permeability, solubility, metabolism, and distribution data at different dose levels, as input informations. The models were able to accurately distinguish between significant food effects (two compounds) and mild effects (four compounds). The simulations captured well the magnitude of the food effect in all cases and, for the majority of compounds, correctly predicted the observed plasma exposure in fasted, fed, and high-fat conditions.

Early clinical trials are usually performed in homogeneous ethnic groups. Therefore, a key issue for drug development is the potential for interethnic differences in pharmacokinetics and pharmacodynamics. Differences can derive from genetic, physiological, demographic, and environmental factors. In particular, the frequencies of particular genotypes for drug-metabolizing enzymes differ in the various ethnic groups. Simcyp is a software accounting for genetic and demographic ethnic differences. This software was used to compare the oral clearance and corresponding variability in Caucasians and Japanese for 11 drugs (alprazolam, caffeine, chloroxazone, cyclosporine, midazolam, omeprazole, sildenafil, tolbutamide, triazolam, S-warfarin, and zolpidem) (Inoue et al., 2006). However, in Japanese, the predictability was less than that observed for the same drugs in Caucasians. This was attributed to a lack of information on specific values of some parameters in Japanese populations, such as cardiac output, intestinal CYP3A4 abundance, and MPPGL (microsomal protein *per* gram liver).

Induction or inhibition of metabolic enzymes, primarily CYPs, are sometimes responsible for lack of efficacy or at origin of adverse events. It is, therefore, important to predict the extent of drug-drug interactions (DDIs) based on *in vitro* human metabolism and inhibition data. *In vitro* intrinsic clearance as well as K_m and K_i values are being used with *in vivo* parameters for both the victim and the perpetrator to simulate quantitative DDIs (Yang, 2001). A piece of software such as Simcyp allows one to simulate individuals with anatomic variations (differing on age, sex, liver weight, and blood flow). It is, therefore, possible to simulate extreme effects in populations. This approach fits well with recommendations from the FDA that highlight the desirability of "stressing the system" by conducting *in vivo* studies in the most sensitive individuals.

PBPK is also particularly useful in situations where there are some ethical issues to conduct any experimental work. An example is given by the evaluation of the safety or risk assessment of retinoic-acid derivatives (Clewell et al., 1997). The FDA requested to evaluate, through PBPK simulation, the potential fetal exposure to retinin-A applied topically in women of reproductive age. The PBPK model demonstrated that the topical exposure to tretinoin resulted in an internal exposure that was 4–5 orders of magnitude lower than a minimally teratogenic dose. This reassured the FDA during review and ensured subsequent approval.

Conclusions

The modern drug-development process needs an efficient, informed selection of compounds for development (Edginton et al., 2008). PBPK models are used in early evaluation phases for candidate prioritization, optimization, selection (Lüpfert and Reichel, 2005), and prediction to man by using preclinical data obtained in animal species (Luttringer et al., 2003; Germani et al., 2007) and *in vitro* with human material. PBPK modeling is a well-established approach in environmental and occupational toxicology and risk analysis. Its development and implementation in pharmacology and drug research and development is still narrow in comparison to the potential, but definitively growing within drug research teams. As opposed to empirical pharmacokinetic models and, to a certain extent, allometry, PBPK have richer information content and can integrate information from various sources, including drug-dependent parameters and physiological parameters as they vary in between subjects or with age and disease state. In this respect, the usefulness of PBPK has been demonstrated in guiding experimental efforts to obtain the pertinent and relevant information necessary to understand the compound's properties and behavior (plasma or organs concentration-time profiles) before entry into man (Tanaka et al., 2000; Brightman et al., 2006a, 2006b; Peters, 2008). In addition to allowing for prediction of human PK before first-in-human, expected variability, or for prediction of likely drug-drug interactions (Ito et al., 2003; Kato et al., 2008; Moghadamnia et al., 2003), they are also used in late-stage drug-development. The potential of PBPK modeling goes beyond predicting animal or human PK. It is primarily a mechanistic approach to gain insights into the intrinsic properties of drugs.

Blood concentration-time profiles are the resultant of drug distribution in extravascular tissues. If the site of action is outside the blood, blood concentration-time profiles are only a "surrogate marker" of concentration-time profiles at the site of action (and the

relationship may not be simple and straightforward). By providing a link between tissue concentrations and toxicological effects (risk assessment; see Leung and Paustenbach, 1995) or pharmacological effects, PBPK modeling represents the framework for mechanistic PK/PD models (PBPK/PD). It allows for the understanding of the possible/likely reasons for poor predictions, likely because in PBPK modeling, there is a clear and explicit distinction between properties of the organism and the drug.

While allometric scaling cannot (always) work (actually, allometric scaling of clearance assumes a constant liver blood flow vs. body weight, or assumes constant enzymatic activity with age, or liver volume to be a function of body weight raised to the power of 0.75, etc.), physiological clearance scaling accounts for growth and maturation in a rational mechanistic manner (Edginton et al., 2006). On the same line, pure *in vitro* to *in vivo* extrapolations (IVIVE), which are extrapolating drug-specific parameters (e.g., V_m, K_m) obtained from subcellular (microsomes, S9 fraction, etc.) or cellular (hepatocytes) components of organs, are sometimes misleading as they totally ignore the influence of physiological processes and interaction of the drug with intra- or extracellular components. This is of particular importance with regard to the data acquired in *in vitro* or animal models in allergy and asthma, which are intrinsically not always satisfying.

For example, *in vitro* models of human respiratory ciliary cells or fibroblasts (primary cultures) have a limited survival time and the passages influence deeply the cell behavior or reactivity. Further, even if some monocellular cultures could allow the exploration of inflammatory factors on cell behavior, there is actually no convincing full-tissue model able to mimic respiratory mucosa in inflammatory conditions.

On the other hand, allergy models in mice use a sensitization procedure (e.g., intraperitoneal injection of ovalbumine), which is not reflecting the natural sensitization process. As a consequence of this, some immune responses have been described in mice, but their correlation to human figures is uncomplete or, in some cases, inadequate. Finally, some pathogenic components, such as tissue damage or remodeling, are only poorly controlled in animal or *in vitro* models and are usually not appearing on the list influencing parameters in these applications.

In consequence, in the field of allergy and asthma, many extrapolations from drug absorption or metabolism in animals or *in vitro* models are hazardous and their respective projection to human beings should be considered with caution.

Although there are few applications in (Björkman, 2004) allergy and asthma, PBPK could facilitate the following processes: 1) interspecies comparisons (i.e., change in parameters such as Q, V, CL, fu, Kps, etc.); 2) dose extrapolation and exposure scenarios (i.e., concentration-dependent parameters CL, fu, etc.); 3) evaluating the impact of change in parameters due to pathological conditions or special populations (e.g., polymorphism); 4) evaluating different routes (i.e., add input functions to appropriate tissue compartment); 5) evaluating different dose forms (i.e., add dissolution characteristics to gut model); 6) predicting exposure to specific targets (e.g., organ/tissues, milk, fetus, etc.); and 7) sensitivity analysis (i.e., which parameters are important?). However, all that was made in the literature highlight the importance of an initial validation in animal species before a successful prediction to man (Jones et al., 2006a; De Buck et al., 2007a, 2007b).

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