14 Pharmacokinetics and Toxicokinetics in Drug Discovery and Development

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14.1 INTRODUCTION

Pharmacokinetics (PK) is the science that describes the time course of drug concentration in the body, resulting from administration of a certain drug dose. In comparison, pharmacodynamics (PD) is the science that describes the relationship of the time course of drug concentration and the drug effects in the body [1]. Similarly, toxicokinetics (TK) reveals the toxicity effects as a function of drug exposure. Therefore, PK and TK can be considered biomarkers of drug exposure as well as markers of efficacy and safety. Key determinants of the PK/TK of a drug include absorption, distribution, metabolism, and elimination (ADME) [2]. The current practice of drug discovery and development requires PK and TK analyses from the earliest stages of the program. This assessment is helpful in (i) selection of dose for preclinical toxicity testing and dosage route, (ii) understanding drug exposure, and (iii) identifying dose for first-in-human (FIH) studies and the follow-up clinical trials. Accurate quantitation of drugs in biological media such as blood (usually plasma or serum) and tissues has become more or less a routine with much improved sensitivity via the bioanalytical tools. However,

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significant challenges to bioanalysis of potential new chemical entities (NCEs) still lurk in the background, with analytical chemists innovating to address issues.

The purpose of this chapter is to introduce to the fundamentals of PK and TK and their applications in drug discovery and development. It also presents the fundamentals of computational analysis of the data derived from the estimated concentrations in the biological matrices such as plasma. Finally, it discusses the implications of species differences, genomics, and exposure of the metabolites in determining the safe dose in the FIH clinical trials and further identifying clinical dosage regimen.

14.2 PHARMACOKINETIC (PK) ASSESSMENT IN DISCOVERY AND DEVELOPMENT

It is well established that poor drug PK is one of the leading causes of compounds failure in preclinical and clinical drug development [3]. Compounds with poor PK profile tend to have low oral systemic plasma exposure and high interindividual variability, which limits their therapeutic utility [4]. Therefore, a better understanding of the PK profile early on enables the discovery of compounds with druglike properties [5]. In drug discovery settings, the main outcomes of PK/TK assessments are to

- select compounds with the maximum potential of reaching the target
- determine the appropriate route of administration to deliver the drug (typically oral)
- understand how the drug blood levels relate to efficacy or toxicity in order to choose efficacious and safe doses
- facilitate appropriate dose sections for rodent and/or nonrodent species in toxicology testing and drug safety evaluation
- decide on the frequency and duration of dosing in order to maintain adequate drug concentration at target for disease modification
- accurately predict the PK in humans' profile before clinical studies.

A PK/TK study involves dosing animals or humans with NCE and collecting blood samples at predefined time points. After sample preparation and quantification, a concentration–time profile is generated (Fig. 14.1). In drug discovery, preliminary PK studies are usually conducted in rodents to evaluate the extent of drug exposure *in vivo* . This is commonly followed by studies in nonrodents, such as dogs or monkeys, to better characterize the PK profile of the compound and to support safety risk assessment studies. PK scaling, also known as *allometry*, is a discipline that is used to predict human PK profile using preclinical data and is widely used in predicting the drug's half-life, dose, and extent of absorption in humans. This approach is based on empirical observations that various physiological parameters are a function of body size. The allometric methods assume that the same metabolic and disposition processes in the species evaluated are correlated with those observed in humans. However, it has become increasingly realized that, for example, the cytochrome P450 enzymes in the rat are not the same as those in humans and thus may exhibit altered disposition of the compound or even produce different metabolite patterns (Section 14.7). Similarly, uptake and efflux transporters in the animal species may differ in substrate specificity or rate,

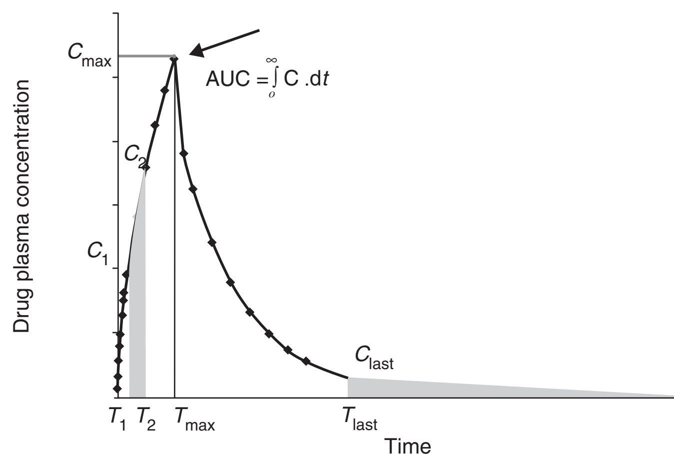

Figure 14.1 Estimation of the area under the plasma concentration–time curve (AUC).

as compared to humans, and thus may confound predictions of human PK. Accurate prediction of human PK profile is imperative to minimize drug failure in development because of the PK liability. More detailed description of methods in predicting human PK is beyond the scope of this chapter but can be found in many excellent reviews [6]. An in-depth discussion of various PK concepts and their application can be found in various references [7].

14.3 TOXICITY ASSESSMENT IN DRUG DISCOVERY AND DEVELOPMENT

Several toxicology studies are conducted during early drug discovery and all the way to the late stages of drug development before a New Drug Application (NDA) filing is made. In spite of comprehensive toxicity assessment in early- and late-stage discovery, attrition of NCEs in clinical studies is not uncommon owing to disconnect in predictions of risk in humans on the basis of preclinical data obtained from cell culture and animal models. Nevertheless, extensive preclinical assessment and appropriate scaling and modeling tools will improve predictions. In general, the correlation between human and animal toxicities is good for conditions such as cardiovascular, hematological, and gastrointestinal diseases and the poorest correlation for adverse drug reactions such as, idiosyncratic reactions, skin rash, hypersensitivity, and hepatotoxicity. Toxicology testing in drug discovery is initiated by the high throughput screening, which is followed up by definitive tests. Screening refers to the methods that yield rapid and comprehensive data often using *in vitro* tools. The origin of any toxicological or safety outcome is multifactorial and complex and thus demands for use of sophisticated systems for definitive assessment. Thus, many pharmaceutical companies are also introducing *in vivo* (i.e., animals) toxicology studies as early as possible, quite often in the lead optimization (LO) stage. Extensive and appropriate toxicology studies of varying duration ranging from acute, single dose to chronic, repeat dose in rodent and nonrodent species are needed to establish safe human clinical trials. Acute

toxicity (single dose ranging) studies in preclinical species are performed to support selection of a drug candidate for potential advancement to repeat-dose toxicology studies and ultimately to enable initial FIH clinical trials. The objective of such studies is to identify a dose at which the major adverse effects are observed. These studies are usually carried out in rodents, following a single dose up to a limit of 2000 mg/kg. The information obtained may be translated to select the dose levels for the FIH studies and also to give an indication of potential effects of acute overdose in humans.

Early drug development starts with candidate compound selection. Repeat-dose toxicity studies (7–14 days in duration) in both rodent and nonrodent species are used to better refine safety margins and PK/PD modeling, as well as set appropriate dosages for the subsequent good laboratory practice (GLP) one-month general toxicology and safety pharmacology (i.e., cardiovascular testing in a nonrodent; CNS and respiratory function tests in a rodent) studies that proceed the Investigational New Drug (IND) application before starting FIH clinical trials. TK assessment is based on the multiple samples obtained throughout the duration of the study along with the PK data. Such data are critical in defining a margin of safety between the no observed adverse effect level (NOAEL) and the projected plasma concentrations achieved in human. It is generally considered that a 100-fold safety factor (rodent-to-human exposure ratio) from the most sensitive species NOAEL provides good safety margin in clinical studies. However, our enhanced capability of understanding interspecies sensitivity and detecting more and more subtle effects may warrant a more flexible approach. The toxicology assessment profile includes, for example, the maximum tolerated dose (MTD), safety margins and therapeutic index, target organ toxicities, most sensitive preclinical species, and reversibility of an effect/toxicity. Biomarkers characterization and preclinical to clinical translation can also be investigated in these GLP toxicology studies.

Later drug development includes phases I–IV. Phase I (FIH) starts with a single dose escalation and then multiple dosing in normal healthy subjects. It is used to establish human safety profile and MTD. Phase II defines the efficacy/safety of candidate profile in target patient population (e.g., rheumatoid arthritis), drug–drug interactions, and proof of concept (POC) before proceeding into phase III. Several repeat-dose toxicology studies (general toxicology, embryo-fetal and developmental, fertility, juvenile, carcinogenecity) of longer duration (three months and up to two years) in both rodent and nonrodent species are conducted to support clinical trials of longer duration in patients.

14.4 PARAMETERS THAT DEFINE PK/TK PROFILE

14.4.1 Area Under the Curve (AUC)

AUC is a primary measure of the extent of drug availability to the systemic circulation, that is, it reflects the total amount of unchanged drug that reaches the systemic circulation following intravenous or extravascular administration. Mathematically, area under the plasma (or blood) concentration–time curve (AUC) can be calculated from the obtained concentration–time profile:

$$\mathrm{AUC} = \int_0^\infty C \cdot \mathrm{d}t \tag{14.1}$$

The unit for AUC is concentration per unit time (e.g., ng h/mL). AUC is determined using simple integration method as shown in Equation (14.1) or often by linear trapezoidal method (Fig. 14.1).

The area of each trapezoid is calculated using the following equation:

$$\mathrm{AUC}_{t_1 \to t_2} = \frac{(C_2 + C_1)}{2} \times (t_2 - t_1) \tag{14.2}$$

The extrapolated area from t_{last} to ∞, is estimated as

$$\mathrm{AUC}_{t_{\mathrm{last}} \to \infty} = \frac{C_{\mathrm{last}}}{K_e} \tag{14.3}$$

where C_{last} is the last observed concentration at t_{last} and K_e is the slope obtained from the terminal portion of the curve, representing the terminal elimination rate constant. The total AUC ($\mathrm{AUC}_{0 \to \infty}$) is determined as

$$\mathrm{AUC}_{0 \to \infty} = \mathrm{AUC}_{0 \to t_{\mathrm{last}}} + \mathrm{AUC}_{t_{\mathrm{last}} \to \infty} \tag{14.4}$$

AUC is used in the calculation of clearance (CL), apparent volume of distribution, and bioavailability (see following sections) and reflects the general extent of exposure over time.

14.4.2 Maximum Plasma Concentration (C_{max}) and Time of Maximum Concentration (T_{max})

C_{max} is defined as the maximum observed drug concentration in the plasma concentration–time profile following intravenous or oral dosing. Most commonly, C_{max} is obtained by direct observation of the plasma concentration–time profile (Fig. 14.1). For some drugs, the pharmacological effect is dependent on the C_{max}; for example, aminoglycosides, which are widely used antibiotics, need to achieve a C_{max} that is at least 8- to 10-fold higher than that of the minimum inhibitory concentration (MIC) to obtain a clinical response $>90\%$ [1]. The unit of C_{max} is concentration unit (e.g., ng/mL).

T_{max} is the time required to reach C_{max}. As with C_{max}, T_{max} is usually determined from direct observation of the plasma concentration–time profile. The unit of T_{max} is time (e.g., h).

14.4.3 Clearance (CL)

CL is a primary parameter that describes the process of irreversible elimination of a drug from the systemic circulation. It is defined as the volume of blood or plasma that is totally cleared of its content of drug per unit time. Thus, it measures the removal of drug from blood or plasma. However, CL does not indicate the extent of drug is being removed but represents the rate, and thus the units are given as mL/min or mL/min/kg (normalized to body weight).

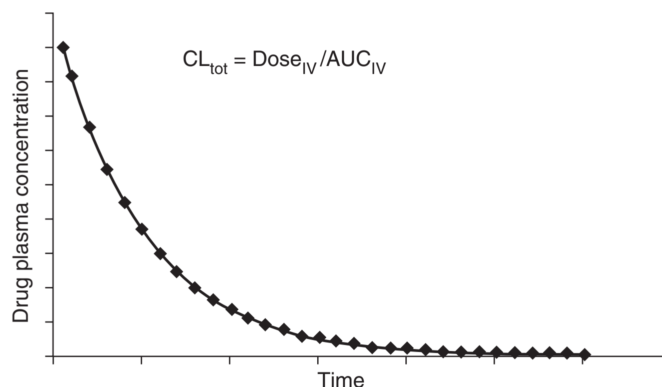

Figure 14.2 Plasma concentration–time profile following IV dosing.

The most widely used approach to evaluate plasma (total) CL involves intravenous administration of a single dose and measuring its plasma concentration at different time points (Fig. 14.2).

$$CL_{tot} = \frac{Dose_{IV}}{AUC_{IV}} \quad (14.5)$$

In general, a drug is either eliminated unchanged through excretion in the urine and/or bile or by metabolic conversion into more polar metabolite(s) that can be readily excreted in urine and/or bile. Since all these CL mechanisms operate independently, total body CL is a sum of all CLs by individual mechanisms and can be expressed as

$$CL_{tot} = CL_{hep} + CL_{ren} + CL_{bil} \quad (14.6)$$

where CL_{tot} is the total body CL from all different organs and mechanisms, CL_{hep} is the hepatic CL, CL_{ren} is the renal CL, and CL_{bil} is the biliary CL [7].

It is interesting to note that around three-quarters of the top 200 prescribed drugs in the United States are primarily cleared by hepatic metabolism [8]. The hepatic extraction ratio (E_h) is a PK parameter that is widely used to assess the liver's ability to extract drug from the systemic circulation [7]. E_h is defined as the fraction of a drug in the blood that is cleared (extracted) on each passage through the liver and is a function of hepatic blood CL (CL_{hep}) and the hepatic blood flow (Q) [7].

$$E_h = \frac{CL_{hep}}{Q} \quad (14.7)$$

If the predominant CL mechanism for a compound is via hepatic metabolism, then it is reasonable to assume that the CL_{tot} is equal to CL_{hep}. Thus

$$E_h = \frac{CL_{hep}}{Q} = \frac{CL_{tot}}{Q} \quad (14.8)$$

Compounds that undergo hepatic metabolism can be categorized according to their E_h. Compounds with $E_h < 0.7$ are considered high extraction drugs, whereas

compounds with $E_h < 0.3$ are considered low extraction drugs. E_h has a major impact on oral drug bioavailability (see below).

Renal CL (CL_{ren}) can be obtained from the unchanged amount excreted (Ae_{IV}) and plasma AUC (AUC_{IV}) following intravenous dosing [9].

$$CL_{ren} = \frac{Ae_{IV}}{AUC_{IV}} = \frac{Ae_{Oral}}{AUC_{Oral}} \tag{14.9}$$

Similarly, the unchanged amount excreted in urine (Ae_{Oral}) and plasma AUC (AUC_{Oral}) following oral administration can also be used for estimating CL_{ren}.

14.4.4 Apparent Volume of Distribution (V_d)

Volume of distribution (V_d) is a proportionality factor that relates the amount of a drug in the body to its blood or plasma concentrations:

$$\text{Amount of drug in the body at time } t = V_d \times Ct \tag{14.10}$$

Similar to CL, V_d is a primary PK parameter and is represented as volume by unit body weight (e.g., L/kg). V_d is used to assess the extent of drug distribution in the body. This is usually achieved by comparing the drug V_d to the total body water. If the drug has a V_d smaller than the total body water (human total body water = 42 L per 70 kg human body weight), the drug has limited tissue distribution (e.g., naproxen has a $V_d = 11$ L per 70 kg human body weight) [7]. Being proportionality constant, V_d ranges from 3 to more than 40,000 L per 70 kg human body weight and therefore is usually referred to as *apparent volume of distribution*.

14.4.5 Apparent Volume of Distribution at Steady State (V_{dss})

V_{dss} is the volume of distribution that is determined when plasma concentrations are measured at steady state and in equilibrium with the drug concentration in the tissue compartment.

$$V_{dss} = \frac{\text{Amount of drug in the body at equilibrium conditions}}{\text{Steady - state plasma concentrations } (C_{ss})} \tag{14.11}$$

Although V_{dss} is a steady-state parameter, it can be calculated using non-steady-state data as

$$V_{dss} = CL \times MRT \tag{14.12}$$

where mean residence time (MRT) is the average time for all drug molecules to exist in the body. Following intravenous dosing, MRT is calculated as

$$MRT = \frac{AUMC}{AUC} = \frac{\int_0^\infty C \cdot t \cdot dt}{\int_0^\infty C \cdot dt} \tag{14.13}$$

where AUMC is the area under the first moment versus time curve from time $t = 0$ to ∞ and calculated using trapezoidal rule similar to AUC.

14.4.6 Half-Life ($t_{1/2}$)

Half-life, usually expressed in hours, is the time that is required for the amount (or concentration) of a drug in the plasma to decrease by one half.

$$t_{1/2} = \frac{0.693 \times V_d}{\mathrm{CL}} \tag{14.14}$$

Half-life is a dependent PK parameter that is determined by the independent PK parameters, CL and V_d. It is the most widely reported PK parameter since it may constitute a major determinant of the duration of action after single and multiple dosing. In addition, half-life plays a key role in determining the time that is required to reach steady state following multiple dosing and the frequency with which doses can be given.

14.4.7 Bioavailability ($F\%$)

According to the European Medicines Evaluation Agency (EMEA), bioavailability ($F\%$) is "the rate and extent to which an active moiety is absorbed from a pharmaceutical form, and becomes available in the systemic circulation." It is usually determined by calculating the respective AUC after oral and intravenous administrations as

$$\text{Absolute bioavailability} = \frac{\mathrm{AUC_{PO}}}{\mathrm{AUC_{IV}}} \times \frac{\mathrm{Dose_{IV}}}{\mathrm{Dose_{PO}}} \tag{14.15}$$

Oral bioavailability is determined by the fraction of dose absorbed (F_a) in the gastrointestinal tract and fraction of dose that does not undergo metabolism in the intestinal tract (F_g) and liver (F_h) (Fig. 14.3) and is expressed as

$$F = F_a \cdot F_g \cdot F_h \tag{14.16}$$

F_h is calculated using the following equation:

$$F_h = 1 - E_h = 1 - \frac{\mathrm{CL_h}}{Q} \tag{14.17}$$

Thus, if a drug has a high hepatic extraction ($E_h > 0.7$), then its bioavailability will be low when it is given orally ($F < 0.3$). On the other hand, if a drug has low hepatic extraction ($E_h < 0.3$), then the extent of bioavailability will be high, provided it is completely absorbed and not significantly metabolized by the intestine.

14.5 PK/TK MODELING IN PREDICTING CLINICAL DOSE

PK/TK is an area of science dealing with the exposure of test compound and metabolites, which is determined by the kinetics of exposure, absorption, distribution, metabolism, and excretion. Generally, the extent and duration of exposure are related to the pharmacological or toxicological effects, and thus, the change of the occurrence of the observed effects can be optimized by altering the dose or exposure period.

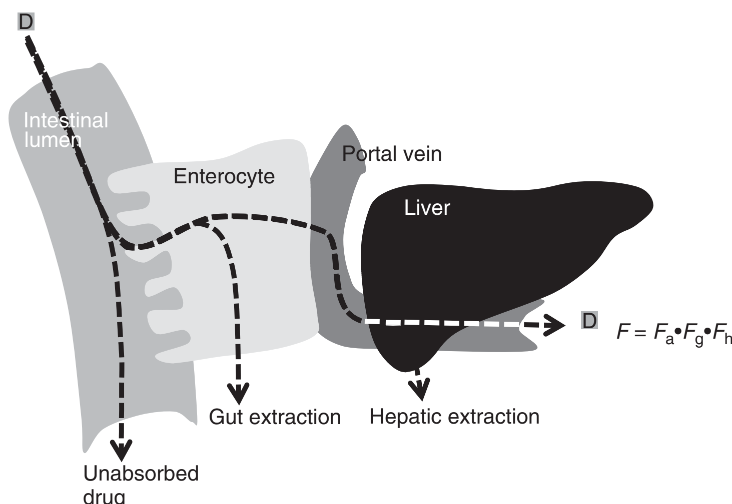

Figure 14.3 Oral bioavailability is a function of intestinal absorption (F_a), the fraction escaping intestinal metabolism (F_g), and the fraction escaping hepatic extraction (F_h), which occur in series. (See color insert.)

A basis for toxicity assessment is the NOAEL or no observed effect level (NOEL) or low observed effect level. NOAEL represents the highest dose at and below which no significant adverse effects are seen. Owing to ethical and practical reasons, assessment of the NOAEL is derived only from animal toxicity data and extrapolated to identify a clinical dose that is significantly lower than the NOAEL. The same strain and species used in the toxicology studies should be used in the TK studies. Initial studies may involve one sex of each species; however, the use of multiple species is relevant to build confidence in models predicting human effects. Using dose–response relationship, the statistical confidence limits of dose at which the incidence or frequency of a toxic effect is established.

Developing mathematical models is of value only when the mechanisms of toxicity are understood and/or the parameters that are being used in model building have established relationships with the observed toxicity effect. While it is important to understand whether the parent molecule or its metabolite is responsible for toxicity, an estimate of whether humans are of less, equal, or greater sensitivity in comparison to the test species is needed for translating the preclinical data to predict human effects. To establish relationship between exposure and dosimetry, a PK/TK model should incorporate the rate and extent of absorption, compound and/or metabolites distributed in the body, metabolism and kinetics of metabolites (if appropriate), elimination rate and elimination route(s), and the influence of dose on all the above processes (dose dependency).

14.5.1 Noncompartmental Analysis

Various PK parameters such as CL, V_d, $F\%$, MRT, and $t_{1/2}$ can be determined using noncompartmental methods. These methods are based on the empirical determination of AUC and AUMC described above. Unlike compartmental models (see following

section), these calculation methods can be applied to any other models, provided the drug follows linear PK. However, a limitation of the noncompartmental method is that it cannot be used for the simulation of different plasma concentration–time profiles when there are alterations in dosing regimen or when multiple dosing regimens are used.

14.5.2 Compartmental Analysis

Compartmental models of PK analysis are widely used to describe drug distribution and disposition. In these models, the body is assumed to be composed of one compartment or more and the drug kinetics can be defined by differential equations generally of first-order process. These compartments do not have any physiological significance. However, they may represent a group of tissues or organs with similar distribution characteristics. For example, highly blood-perfused body organs such as liver, lungs, and kidney often have different drug distribution than fat tissue.

14.5.2.1 One-Compartment Open Model. One-compartment model, which has gained wide acceptance in TK because of its simplicity, describes the whole body as a single compartment in which the compound is homogeneously distributed following administration (Fig. 14.4). Here, the body is assumed to be a homogeneous unit where the compound is rapidly distributed throughout the body and follows a monoexponential decline. Following intravenous dosing, the plasma drug concentration can be calculated as

$$C = \frac{\text{Dose}}{V_\text{c}} \cdot \text{e}^{-K_e t} = C^0 \cdot \text{e}^{-K_e t} \qquad (14.18)$$

where V_c is the volume of compartment, K_e is the rate of elimination, t is time, and C^0 is concentration at time zero.

However, following extravascular administration, the peak plasma concentration is a function of the rate and extent of the absorption as well.

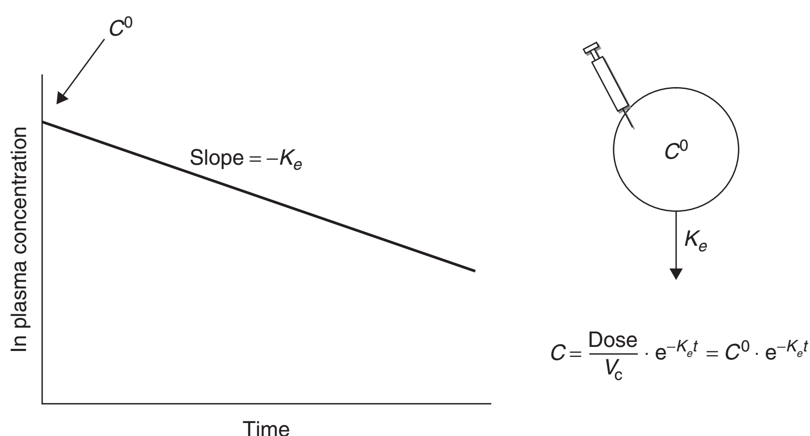

Figure 14.4 One-compartment model.

14.5.2.2 Two-Compartment Open Model. In the two-compartment model, the drug is assumed to distribute into two compartments, the central and the tissue compartments. The central compartment represents the highly blood-perfused body organs where the drug distributes rapidly and uniformly. On the other hand, in the tissue compartment, the drug distributes more slowly (Fig. 14.5).

When the drug concentration versus time profile demonstrates a biexponential decline following intravenous dosing, a two-compartment model that is the sum of two first-order processes (distribution and elimination) will better describe the data (Fig. 14.5). For a drug that follows the two-compartment model, the rate of drug plasma concentration change following intravenous dose can be determined as

$$C = A\mathrm{e}^{-\alpha \cdot t} + B\mathrm{e}^{-\beta \cdot t} \tag{14.19}$$

where A and B are functions of the administered dose and α and β are the first-order constants for the distribution and elimination phases, respectively.

In this chapter, only the one- and two-compartment models following intravenous dosing were described. Other models with extravascular dosing have an additional compartment with an absorption rate constant describing input into the central compartment. Models with three or greater compartments may be used if the drug concentration versus time may be described better with additional exponential terms. However, these models have greater complexity.

14.5.3 Physiology-Based Pharmacokinetic (PBPK) Modeling

The accuracy of human prediction will depend on the degree of physiological and biochemical fidelity captured by the model. The advent of structured physiology-based pharmacokinetic (PBPK) models had a dramatic influence on the ability to predict tissue exposure. Such modeling is governed by more fundamental physiological and biochemical processes and is especially useful in estimating tissue exposure, which may be vital in predicting the organ specific toxicity. The major advantages of such modeling are twofold. First, unlike the empirical models (e.g., allometry), the obtained PK parameters are physiologically relevant, and second, concentration–time profiles of

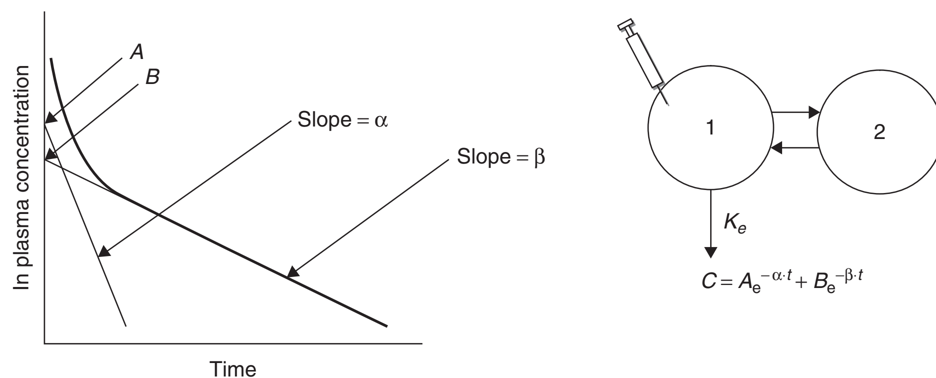

Figure 14.5 Two-compartment open model.

each tissue can be obtained simultaneously. With the exploratory relationships between tissue concentration–time profiles and the pharmacological or toxicological effects, PBPK modeling provides framework for mechanistic PK/PD modeling. Mathematically, the model constitutes of multiple compartments, each corresponding to different body organs or tissues and linked together based on their anatomical placement with respect to blood flow. The vital physiological information in building these models are the tissue volumes, blood flow to the tissue, and the tissue composition. Partition coefficients are measured by equilibrating with tissue homogenates [10]. PBPK models are also most reliable in dose–response and tissue exposure assessments under various physiological conditions (e.g., age, disease condition) [10]. Data collected during a preclinical TK study with focus on time–concentration profiles of compound and its metabolites in plasma and other tissues, facilitates development and validation of comprehensive PBPK models. Such models are useful in assessing the toxicological effects on repeated dose exposure, enzyme induction and inhibition, age- and sex-related physiological factors, and drug–drug interactions, as well as enhance our understanding of interspecies differences.

14.6 TOXICOGENOMICS AND BIOMARKERS

Toxicology safety biomarkers are functional or structural measurements that correlate with a morphologic histopathologic or clinical pathologic change in an organ system such as the liver or heart. Having a translatable biomarker is critical in drug discovery and development. Toxicogenomics is the integration of the *omics* technologies genomics, proteomics, and metabonomics, bioinformatics, and toxicology to better understand drug- or toxicant-induced alterations in biochemical networks (gene, protein, and metabolite) of drug candidate development in a pharmaceutical setting. Therefore, toxicogenomics data can be used as drug toxicity/exposure biomarkers or signatures that provide insights into the toxic mechanism of action of a drug candidate and support safety risk assessment. Such approach can be used in high throughput screening in discovery research. In fact, gene expression is used in the clinic to predict pathologic conditions (e.g., breast cancer), prognosis, and response to therapy. Issues with toxicogenomics experiments are variability in experimental design, strain, gender, age of experimental animals, husbandry, nutrition; interanimal variation; clinical health effects of test compounds; and organs heterogeneity. For example, although the liver appears heterogeneous, it is composed of ~60% hepatocytes, 20% endothelial cells, 15% Kupffer cells, 5% biliary epithelium, and 5% stellate cells.

A good example of the application of toxicogenomics was its use to identify a transcriptional biomarker of the histopathological liver change of oval cell-mediated bile duct hyperplasia (BDH). BDH is a histopathologic finding that occurs in both rodent and nonrodent species. BDH can progress to cholangiocarcinoma with low margins of safety, which can lead to costly, late-stage compound terminations and increased risk to patient safety. Thus, interpretation of the significance of BDH requires consideration of a number of variables, including duration of drug exposure, margins of safety, intended drug indication, and availability of biomarkers to monitor patient safety. The specificity and sensitivity of the discovered candidate biomarker, called *deleted in malignant brain tumor* (DMBT1), was evaluated in livers of rats treated with more than 30 different compounds comprising hepatotoxicities that ranged from BDH and

hepatocyte proliferation to phospholipidosis, hepatocellular vacuolation, apoptosis, and inflammation [11]. Multidisciplinary collaboration between toxicologic pathologists, toxicologists, biologists, personnel in toxicokinetics, and statisticians is needed for successful toxicogenomic efforts.

14.7 SPECIES DIFFERENCES IN DRUG DISPOSITION

Oral exposure of drug molecules is a product of their absorption and hepatic and intestinal first pass. Species differences were reported in the oral exposure of various drug molecules. Several investigators attributed these findings mainly to differences in anatomical and physiological factors such as hepatic blood flow, metabolizing enzyme type, and expression or extent of protein binding. There are significant species differences in bile flow rate and hepatic blood flow (Table 14.1), which may explain some of this variation. In addition, bile composition (acid, ions, electrolytes) also varies between species and may further explain reported species differences in drug biliary excretion rate and thus differences in PK disposition of various drugs [12].

Nelson *et al*. [13] reported that so far 14 CYP gene families have been identified in mammals with significant variations in the primary sequence of amino acids across species. However, these members of the superfamily had highly conserved regions of amino acid sequence. Similar findings were also reported with uridine diphosphoglucose transferases and carboxylesterases [14,15]. Overall, these small differences in the amino acid sequences can lead to significant differences in substrate affinity and specificity, which translate into differences in the metabolism rate and metabolism profiles. As a general rule, compounds with good passive absorption, high rat hepatic extraction ratio, and poor oral bioavailability tend to have better oral bioavailability in higher species such as dogs, monkeys, and humans. There are many cited examples that are consistent with this trend. For example, atomoxetine is a CYP2D6 substrate with an absolute human oral bioavailability of 94% and 63% in poor and extensive metabolizers of CYP2D6, respectively [16]. The moderate to high human oral bioavailability suggests nearly complete oral absorption of atomoxetine. However, preclinical evaluations showed that the absolute oral bioavailability of atomoxetine in rat was only 4% [17] but was 74% in dog [17]. Overall, the disposition of atomoxetine is similar in rats, dogs, and humans with a primary oxidative metabolite of 4-hydroxyatomoxetine that is subsequently conjugated to form 4-hydroxyatomoxetine-*O*-glucuronide. In a

TABLE 14.1 Mean Hepatic Blood Flow and Bile Flow in Selected Species[a]

Species	Hepatic Blood Flow (mL/min/Kg)	Bile Flow (mL/min/Kg)
Mouse	73	100
Rat	39	90
Monkey	33	25
Dog	23	12
Human	17	5

[a]Ref. 18.

radiolabeled study in rats administered ^{14}C-atomoxetine, atomoxetine AUC following oral administration accounted for only 2% of the total ^{14}C AUC as compared to 30% of the ^{14}C AUC following intravenous administration, indicating extensive first-pass metabolism in rats [17]. In a corresponding radiolabeled study in dogs, atomoxetine AUC following oral administration accounted for 33% of the total ^{14}C AUC as compared to 39% of the ^{14}C AUC following intravenous administration, indicating considerably less pronounced first-pass metabolism [17]. This example clearly illustrates the importance of understanding not only the species differences in a drug's metabolic fate but also the extent of species differences in the first-pass metabolism when utilizing preclinical data to project human oral bioavailability.

Indinavir, a CYP3A4 substrate, is an HIV protease inhibitor for which variable oral bioavailability have been observed in preclinical species, ranging from 72% in dogs to 19% in monkeys, and was 24% in rats [18]. This variability was mainly attributed to species differences in the extent of hepatic first-pass metabolism. Chemical and immunochemical inhibition studies indicated the potential involvement of CYP3A isoforms in the metabolism of indinavir in rats, dogs, and monkeys [19], which is consistent with the observation that CYP3A4 is the main isoform responsible for the oxidative metabolism of indinavir in human liver microsomes [19]. The *in vitro* profile of indinavir metabolism was qualitatively similar across species [19]. In addition, an *in vitro–in vivo* correlation was established in rats and dogs using the *in vivo* hepatic CL and hepatic first-pass extraction ratio obtained from *in vitro* rat and dog metabolic data, respectively. Based on the *in vitro–in vivo* correlation established in rats and dogs, the *in vitro* intrinsic CL of indinavir in human liver microsomes projected a small first-pass metabolism in humans ($E_h = 0.25$), which was consistent with indinavir's high oral bioavailability (60–65%) observed in humans at clinically relevant doses [20]. This example depicts the importance of establishing an *in vitro–in vivo* correlation in tested preclinical species so as to use it as a basis to project human CL and oral bioavailability. Overall, these successful medications would not be on the market if the discovery team solely depended on rat oral bioavailability to evaluate their metabolism in humans.

Species differences in the extent of protein binding of various xenobiotics were also reported. It is interesting to note that albeit their structural and functional homologies, there are minor differences in the amino acid sequence in plasma protein molecules such as albumin among various mammals. This may be another contributing factor to the differences in both the binding affinity and sites of drugs in protein molecules among different species [21].

14.8 MIST (METABOLITES IN SAFETY TESTING)

Guidance for Industry on Safety Testing of Drug Metabolites, MIST (METABOLITES IN SAFETY TESTING), was published in 2008 from the Center for Drug Evaluation and Research (CDER) defining the threshold for human metabolites that raise safety concerns as 10% of total drug exposure. Additional guidelines issued from the International Conference on Harmonization (ICH) on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, ICH M3[R2], define the threshold as 10% of total drug-related exposure, which supersedes when in conflict with the CDER guidance. Others have suggested that

total metabolite burden that accounts for dose is a more relevant threshold [22,23]. Early clinical assessment of human metabolites in plasma or urine enables identification of significant human metabolites and whether they are present at equal or greater exposure levels (AUC) in any one of the preclinical safety species is critical for addressing MIST implications. While rare, this is exceedingly important if the metabolite is formed in humans only. Traditionally, preclinical measurement of circulation concentrations of metabolites only occurs to address specialized cases such as when extensive metabolism of parent drug is expected. When the human metabolite profile is expected to be qualitatively similar to at least one of the preclinical safety testing species, measurement of circulating parent in plasma is adequate in preclinical PK and TK studies. Drug development history has taught us that human drug metabolites can play significant roles in primary and secondary pharmacology, general toxicity, and potentially idiosyncratic drug toxicity (Table 14.2). Therefore, one must develop fit-for-purpose metabolite assessment strategy and use the knowledge of the chemical space of the drug candidate in question, structural activity relationship for both primary and related secondary pharmacology, metabolic CL pathways, and potential target organ toxicity to evaluate if measuring metabolites in preclinical PK/TK samples is warranted and would provide the discovery team with knowledge to design a best-in-class compound. Once a compound has progressed through early development, usually post POC, definitive radiolabeled ADME studies are conducted to identify metabolites and assess the need to monitor these in the clinic or qualify in toxicology studies. Realistically, successful drug discovery teams know well in advance the outcome of these studies.

14.8.1 Pharmacologically Active Metabolites

Metabolism of drug candidates can occur at sites of the molecule that do not alter the active pharmacophore, sometimes even resulting in metabolites that have greater pharmacological activity or longer PD duration. This is usually first suspected when existing PKPD relationships fail to predict the accurate dose required for the desired effect and thus may serve as a trigger for metabolite scouting activities. Prodrug strategies are the intentional design of active pharmacological species from an inactive precursor. Where prodrug approaches achieve quantitative conversion of inactive precursor to the active species, circulating metabolite analysis is the only manner to confirm that dose was administered.

14.8.2 Reactive Metabolites

The MIST guidance addresses a majority of direct or indirect conjugates; the latter class of metabolites is considered harmless, but toxicity continues to be a major challenge

TABLE 14.2 Examples of Human Drug Metabolites that are Known to be Responsible for General Toxicity and Potentially Idiosyncratic Drug Toxicity

Parent Drug	Metabolite	Suspected Metabolite Activity
Acetaminophen	*N*-acetyl-*p*-benzoquinone imine (NAPQI)	Toxicity
Troglitazone	GSH conjugation/ring scission	Idiosyncratic toxicity
Losartan	5-Carboxylic acid (10–40 × potency)	Pharmacologically active
Dexfenfluramine	D-Norfenfluramine	Pharmacologically active

leading to drug development failures. Reactive metabolites are frequently suspected when hepatotoxicity or idiosyncratic toxicity is observed. By the very nature of these species, circulating concentrations of reactive metabolites often occur at trace levels, making them difficult to detect and quantitate. Recent strategies to identify reactive metabolites as conjugates to biomolecules such as glutathione or cysteine offer an approach to confirming the formation of these species *in vivo* [24]. Confirming suspected reactive metabolites is informative, and also designing enzymology studies to understand pathways leading to their formation allows an understanding of the potential for species-specific reactions.

14.8.3 Enabling Technologies

Plasma pooling methods were compared to the traditional approaches of obtaining quantitative information on the levels of circulating metabolites in preclinical species. The exposure values obtained via sample pooling are comparable to those obtained by traditional methods of analyzing samples individually. When kinetic information is not needed, this approach allows metabolite identification and exposure burden estimates with a modest resource investment. While metabolite analysis without authentic standards can be accomplished using high resolution or accurate mass LCMS techniques, new techniques using chromatographic measures such as retention time shifting or background subtraction of vehicle-dosed samples offer methods to identify metabolites [25].

There are various approaches to assess the metabolite exposure margin between toxicology species and humans: either by direct or indirect comparison. The preferences in when and how to pursuit metabolite assessment is based on the overall development strategy. Therefore, it is important to understand the utility and limitations of analytical instruments in order to apply an appropriate analytical tool to address specific questions posed at different stages of drug development. The urgency of metabolite monitoring depends on the intrinsic nature of the compound, therapeutic intent, and objective of the clinical development; there is not a unified approach that provides efficient resource utilization.

14.9 CONCLUSIONS

PK/TK are important biomarkers of drug exposure, efficacy, and safety in early- and late-stage drug discovery and development. Determinants of a drug PK/TK include ADME. Several toxicity assessment studies (e.g., acute, single, repeat dose studies, GLP) in both rodent and nonrodent species are used to better refine safety margins and PK/PD modeling and set appropriate dosages before starting FIH clinical trials. Critical parameters that define PK/TK profile are AUC, $C_{\max}$, $T_{\max}$, V_{d}, V_{dss}, $t_{1/2}$, and F. Mathematical modeling can be of great value in the PK/TK evaluation. There is an emerging and increased utility of toxicogenomics to better define drug toxicity/exposure to support compound selection and safety risk assessment. There are significant species differences in the oral exposure to various drugs that must be taken into consideration. Such differences are related to various anatomical and physiological factors including hepatic and renal blood flow, metabolizing enzymes (i.e., CYPs), and protein binding. A valid strategy should be a fit-for-purpose metabolite assessment strategy that uses

the knowledge of the chemical space of a drug candidate being developed, structural activity relationship to pharmacology and toxicology and metabolism CL pathways. Ultimately, a well-designed PK/TK evaluation would provide the discovery team with knowledge to design and advance a best-in-class compound.

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