

DRUG PHARMACOKINETICS AND TOXICOKINETICS

1.1 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. Similarly, toxicokinetics (TK) is the science that investigates how the body handles toxicants as illustrated by its plasma profile at various time points. In comparison, pharmacodynamics (PD) is the science that describes the relationship of the time-course of drug concentration and its effects in the body [1, 2].

PK is considered a biomarker of drug exposure as well as marker of efficacy and safety. Key determinants of the pharmacokinetics of a drug include absorption, distribution, metabolism, and elimination (ADME) [3]. Discovering novel therapeutic agents is an increasingly time-consuming and costly process. Most estimates indicate that it takes approximately 10–15 years and more than \$1.2 billion to discover and develop a successful drug product [4]. It is well established that poor drug PK is one of the leading causes of compounds failure in preclinical and clinical drug development [5]. For example, attrition due to poor pharmacokinetics contributed to 10% of the attrition reported for compounds developed by the pharmaceutical industry in 2001 (Figure 1.1) [6].

Compounds with poor PK profile tend to have low oral systemic plasma exposure and high interindividual variability, which limits their therapeutic utility (Figure 1.2) [7]. Therefore, a better understanding of the PK profile early on enables the discovery of compounds with drug-like properties [8]. 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; and
- accurately predict the PK in humans profile prior to clinical studies.

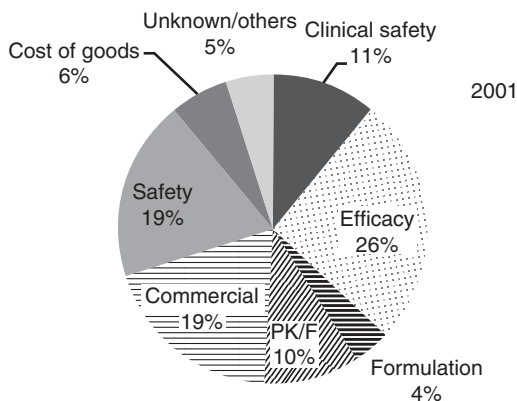


FIGURE 1.1 The contribution of various factors to the overall attrition of NCEs in year 2001. Kola and Landis 2004 [6]. Reproduced with permission of Nature Publishing Group.

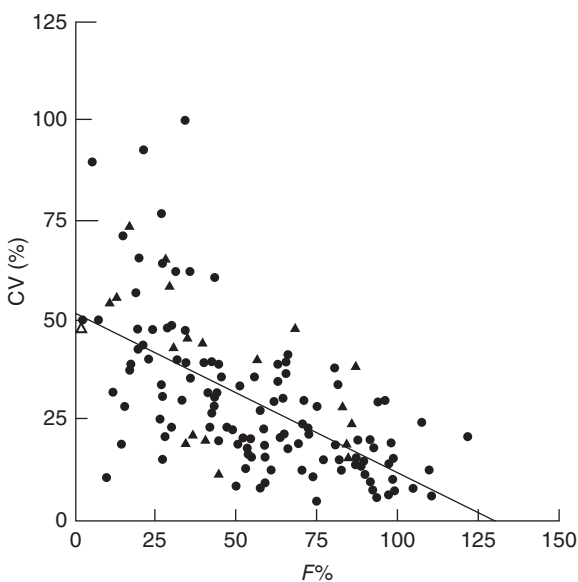


FIGURE 1.2 The relationship between drug oral bioavailability and interindividual variability reported as coefficient of variation (%). Hellriegel et al. 1996 [7]. Reproduced with permission of John Wiley & Sons.

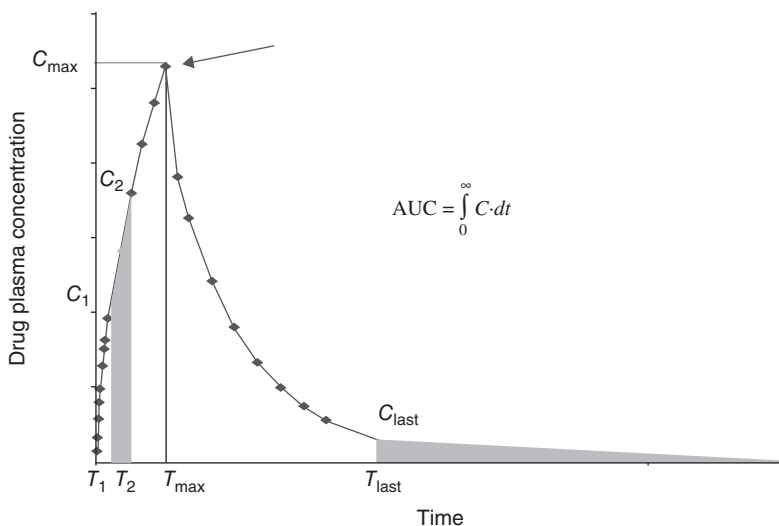


FIGURE 1.3 Estimation of the area under the plasma concentration–time curve (AUC).

A PK/TK study involves dosing animals or humans with NCE and collect blood samples at predefined time points. After sample preparation and quantification, a concentration–time profile is generated (Figure 1.3). In drug discovery, preliminary PK studies are usually conducted in rodents to evaluate the extent of drug exposure *in vivo*. These rodent studies are 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. Pharmacokinetic scaling, also known as allometry, is a discipline that was extensively used in the past to predict human PK profile using preclinical data and in predicting the drug human half-life, dose, and extent of absorption. 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, 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 (see Chapter 2) [9, 10]. Similarly, uptake and efflux transporters in the animal species may differ in substrate specificity or rate, as compared to humans, and thus may confound predictions of human PK [11]. Accurate prediction of human pharmacokinetic profile is imperative to minimize drug failure in development due to pharmacokinetic 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 [12–15]. An in-depth discussion of various PK concepts and their applications can be found in various references [16, 17].

1.2 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 based

upon 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 first in-human 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, PK/PD modeling, and set appropriate dosages for the subsequent good laboratory practice (GLP) 1-month general toxicology and safety pharmacology (i.e., cardiovascular testing in a nonrodent; CNS and respiratory function tests in a rodent) studies that proceeds the investigational new drug (IND) application before starting FIH clinical trials. Toxicokinetic assessment is based on the multiple samples obtained throughout the duration of the study along with the PK data. Such data are critical to define 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, then multiple dosing in normal healthy subjects. These studies are 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, carcinogenicity) of longer duration (3 months and up to 2 years) in both rodent and nonrodent species are conducted to support clinical trials of longer duration in patients.

The purpose of this chapter is to introduce the fundamentals of PK and TK, and their applications to 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, the implications of species differences, genomics, and exposure of the

metabolites in determining the safe dose in the first in human (FIH) clinical trials and further identification of clinical dosage regimen are discussed.

1.3 PARAMETERS THAT DEFINE PHARMACOKINETIC PROFILE

1.3.1 Area Under the Curve (AUC)

The first step in a pharmacokinetic experiment is to dose animals or humans with NCE and collect blood samples at predefined time points. Animals are generally dosed intravenously (IV) and/or orally (po). After sample preparation and quantification usually using LC/MS/MS, a plasma concentration–time profile is generated (Figure 1.3) [18].

Mathematically, area under the plasma (or blood) concentration–time curve (AUC) can be calculated from the obtained concentration–time profile by

$$AUC = \int_0^{\infty} C \cdot dt \quad (1.1)$$

AUC is a primary measure of the extent of drug availability to the systemic circulation (i.e., reflects the total amount of unchanged drug that reaches the systemic circulation following intravenous or extravascular administration). 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 1.1 or a linear trapezoidal method, which is the most widely used approach (Figure 1.3).

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

$$AUC_{t_1 \rightarrow t_2} = \frac{(C_2 + C_1)}{2} \times (t_2 - t_1) \quad (1.2)$$

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

$$AUC_{t_{last} \rightarrow \infty} = C_{last}/K_e \quad (1.3)$$

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

$$AUC_{0 \rightarrow \infty} = AUC_{0 \rightarrow t_{last}} + AUC_{t_{last} \rightarrow \infty} \quad (1.4)$$

AUC is used in the calculation of clearance, apparent volume of distribution, and bioavailability (see Sections 1.3.2, 1.3.3, and 1.3.5) and reflects the general extent of exposure over time.

1.3.2 Mean Residence Time (MRT)

Mean residence time (MRT) is the average time for all drug molecules to exist in the body. MRT is another measure of drug elimination and its unit is time (e.g., hour). Following intravenous dosing, MRT_{iv} is calculated as

$$MRT = \frac{AUMC}{AUC} = \frac{\int_0^{\infty} C \cdot t \cdot dt}{\int_0^{\infty} C \cdot dt} \quad (1.5)$$

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.

In some cases, MRT can be a better parameter to assess drug elimination compared to half-life ($t_{1/2}$). This assessment can be attributed to the greater analytical sensitivity shown with various analytical systems such as LC/MS/MS, the lower drug concentrations measured following drug administration appeared to yield longer terminal half-life, which are not related to the drug's pharmacologically relevant half-life. In a case like this, it would be recommended to measure MRT rather than half-life to assess drug elimination.

1.3.3 Clearance (CL)

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

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 to calculate its AUC (Figure 1.3). In this manner, the calculated CL (Equation 1.6) will not be confounded by complex absorption and distribution phenomena, which is commonly observed during oral dosing [7].

$$CL_{\text{tot}} = \text{Dose}_{\text{IV}} / \text{AUC}_{\text{IV}} \quad (1.6)$$

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. Therefore, total body clearance is an additive parameter and the sum of all clearances by various mechanisms. Mathematically, it is also expressed as shown in Equation 1.7 (Figure 1.4),

$$CL_{\text{tot}} = CL_{\text{hep}} + CL_{\text{ren}} + CL_{\text{bil}} \quad (1.7)$$

where CL_{tot} is the total body clearance from all different organs and mechanisms, CL_{hep} the hepatic blood clearance, CL_{ren} the renal clearance, and CL_{bil} the biliary clearance.

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 [19]. The hepatic extraction ratio (E_h) is a pharmacokinetic parameter that is widely used to assess the liver's ability to extract

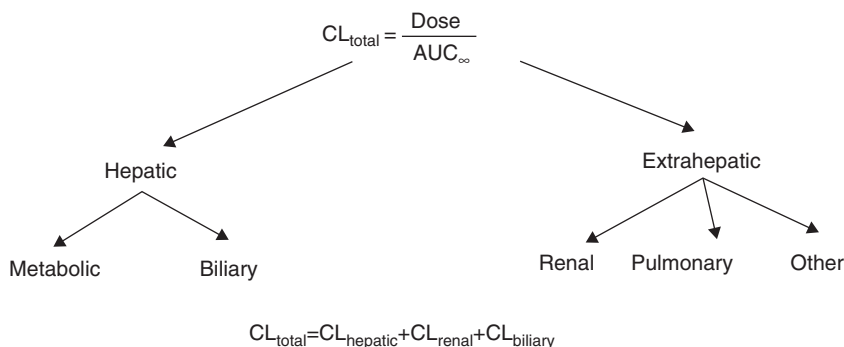


FIGURE 1.4 Various routes/mechanisms of eliminations that contribute to drug CL_{total} .

TABLE 1.1 Typical Body Weight and Hepatic Blood Flow for Various Preclinical Species and Human

Species	Body Weight (kg)	Liver Blood Flow (mL/min/kg)
Mouse	0.02–0.025	90
Rat	0.25	55
Rabbit	2.5–3	71
Cynomolgus monkey	4–5	44
Dog	10–12	31
Human	70	21

drug from the systemic circulation [17]. 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 CL_{hep} and the hepatic blood flow (Q) [17]:

$$E_h = \frac{CL_{hep}}{Q} \quad (1.8)$$

Typical values for the hepatic blood flow in various preclinical species and human are summarized in Table 1.1.

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

$$E_h = \frac{CL_{hep}}{Q} = \frac{CL_{total}}{Q} \quad (1.9)$$

Compounds that undergo hepatic metabolism can be classified 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.

1.3.4 Plasma versus Blood Clearance

Calculation of E_h from drug clearance in blood requires the determination of drug concentration in whole blood. Since determination of drug concentration is usually performed in plasma or serum, knowledge of the blood/plasma concentration ratio is necessary to estimate the blood clearance. Blood clearance is calculated using this equation:

TIP:

Various factors can lead to a total clearance of an investigated compound that is higher than hepatic blood flow (Table 1.1). For example, extrahepatic elimination pathways can play a key role in the elimination of xenobiotics, although hepatic clearance is commonly the main route of elimination [20]. Compounds with high blood to plasma ratio are preferentially distributed in red blood cells. Therefore, their plasma clearance would overestimate blood clearance. Furthermore, compounds with poor stability in blood/plasma tend to have high clearance. Overall, these factors should be considered and investigated when this trend is observed.

$$\frac{\text{Plasma clearance}}{\text{Blood clearance}} = \frac{\text{Blood concentration } (C_b)}{\text{Plasma concentration } (C_p)} \quad (1.10)$$

1.3.5 Apparent Volume of Distribution (V_d)

Volume of distribution is a proportionality factor that relates the amount of a drug in the body to its blood or plasma concentrations at a particular time,

$$\text{Amount of drug in the body at time } t = V_d \times C \text{ plasma at time } t \quad (1.11)$$

Following intravenous dosing and at $t = 0$ h, the amount of drug in the body is equal to the administered intravenous dose. V_d at $t = 0$ is termed volume of the central compartment (V_c).

TIP:

Need to always remember that volume of distribution has *no* physiological relevance. There are compounds that have a V_d that is significantly lower (e.g., acetyl salicylic acid $V_d = 0.15$ L/kg) than total body water (0.6 L/kg) and ones that are significantly higher (e.g., loratidine $V_d = 120$ L/kg). This question usually arises when V_d is smaller than total body water. The answer is simple: V_d is *not* physiologically relevant.

Similar to CL, V_d is a primary independent pharmacokinetic parameter and its unit is volume (e.g., L/kg). V_d is a mathematical constant that has no physiological relevance. V_d is used to assess the extent of drug distribution within or outside the total body water. In the literature, V_d ranges from 3 to more than 40,000 L per 70 kg human body weight. For example, if the drug has a V_d that is smaller than the total body water (human total body water = 42 L per 70 kg human body weight, which is equivalent to 0.6 L/kg), then the drug would be expected to have limited tissue distribution (e.g., acetyl salicylic acid has a $V_d = 10.5$ L per 70 kg human body weight, which is equivalent to 0.15 L/kg) [21]. On the other hand, if a drug has a V_d larger than the total body water, then the drug is likely able to distribute to body tissues (e.g., loratidine has a $V_d = 8400$ L per 70 kg human body weight, which is equivalent to 120 L/kg) (Figure 1.5) [22]. Therefore, the term apparent volume of distribution is usually used.

It should be emphasized that binding to both blood and tissue components such as lipids and proteins has a significant impact on the drug volume of distribution as outlined in the following equation:

$$V_d = V_{\text{blood}} + V_{\text{tissue}} \cdot \frac{f_{u,\text{blood}}}{f_{u,\text{tissue}}} \quad (1.12)$$

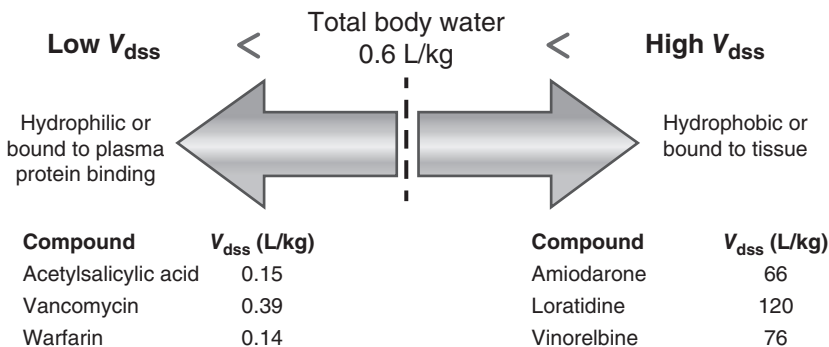


FIGURE 1.5 Volume of distribution and its relation with the extent of drug distribution in blood and tissues.

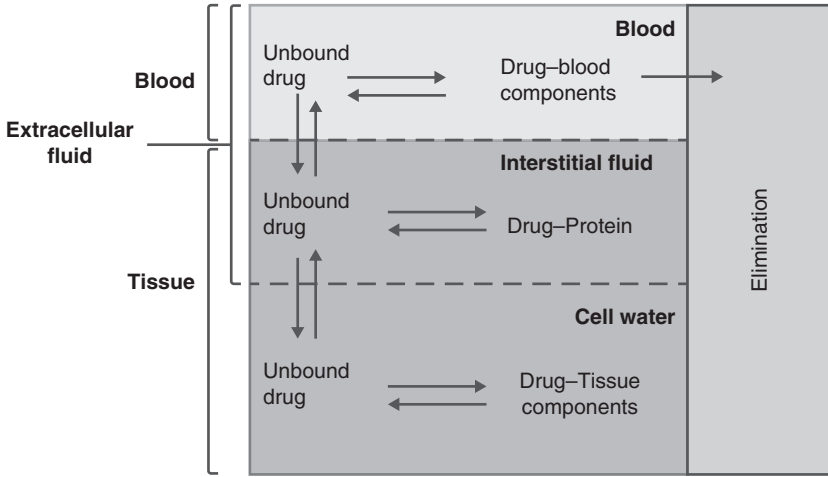


FIGURE 1.6 Tissue and blood binding and their impact on drug volume of distribution.

where $f_{u,blood}$ is the free fraction of the drug in blood, $f_{u,tissue}$ the free fraction of the drug in tissue, V_{blood} the volume of drug in blood, and V_{tissue} the volume of drug in tissue. As depicted in Figure 1.6, an increase in $f_{u,blood}$ is associated with an increase in drug V_d , whereas an increase in $f_{u,tissue}$ is associated with a decrease in drug V_d . Furthermore, increasing drug lipophilicity is associated with a decrease in $f_{u,tissue}$, which usually leads to an increase in the drug V_d .

1.3.5.1 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.

$$V_{dss} = \frac{\text{Amount of drug in the body at equilibrium conditions}}{\text{Steady state plasma concentrations } (C_{ss})} \quad (1.13)$$

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

$$V_{dss} = CL \times MRT \quad (1.14)$$

Furthermore, V_{dss} is used in the calculation of a loading dose as

$$\text{Loading dose} = \frac{V_{dss} \times C_{ss}}{F} \quad (1.15)$$

Use of loading dose is important especially for those drugs in which it is desirable to immediately or rapidly reach the steady-state plasma concentration (C_{ss}) (e.g., anticoagulant, antiepileptic, antiarrhythmic, and antimicrobial therapy).

1.3.6 Half-life ($t_{1/2}$)

$t_{1/2}$ is the time that is required for the amount (or plasma concentration) of a drug to decrease by one half. It is calculated by the following equation:

$$t_{1/2} = \frac{0.693 \times V_d}{CL} \quad (1.16)$$

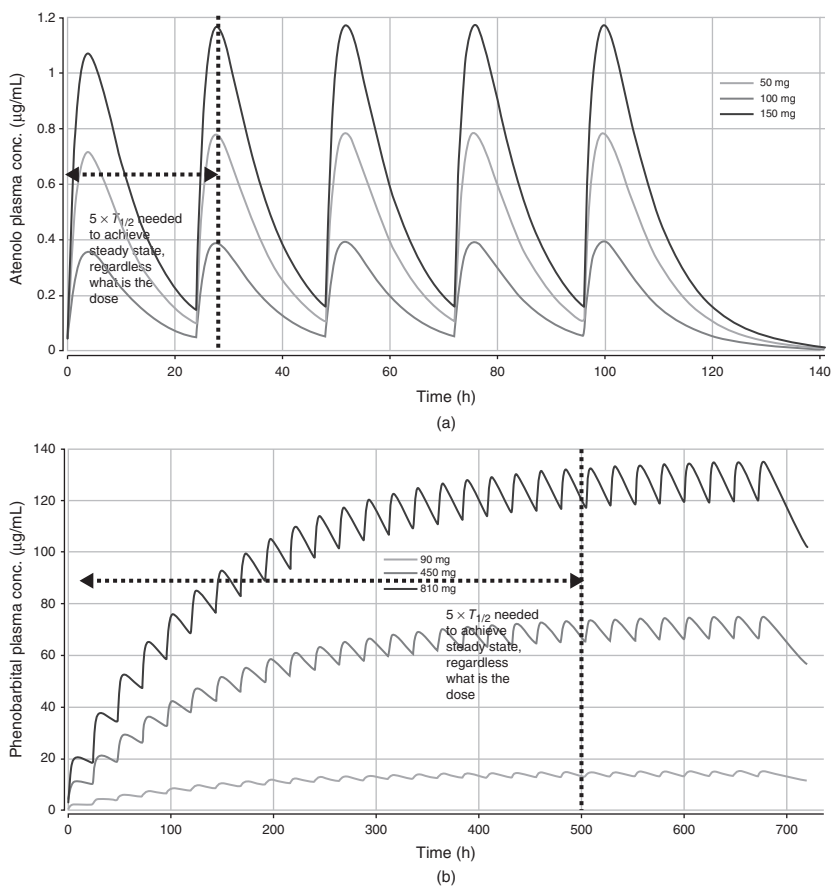


FIGURE 1.7 Plasma concentration–time profiles for drugs with half-lives of 6, 36, or 99 h administered once daily. Simulations were performed using Berkeley Madonna Software®. (a) Half-life is 6 h (e.g., atenolol); (b) half-life is 99 h (e.g., phenobarbital).

$t_{1/2}$ is a dependent pharmacokinetic parameter that is determined by both CL and V_d , which are independent primary pharmacokinetic parameters. Therefore, $t_{1/2}$ is increased by a decrease in CL or increase in V_d and vice versa. $t_{1/2}$ is the most widely reported pharmacokinetic parameter since it may constitute a major determinant of the duration of action after single and multiple dosing. The unit for $t_{1/2}$ is time (e.g., h). In addition, $t_{1/2}$ 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. In general, for a drug that follows one compartment kinetics, it takes five half lives for it to reach steady-state concentrations after multiple dosing and under linear conditions. For example, for a drug with a half-life of 6 h (e.g., atenolol), steady-state concentrations are reached in about 30 h regardless of its dose or dosage regimen. Similarly, a drug such as phenobarbital with a $t_{1/2}$ of 99 h, would take 495 h to reach its steady-state concentrations (Figure 1.7).

If a drug follows one compartment model following intravenous dosing, then its $t_{1/2}$ is calculated as follows:

$$t_{1/2} = 0.693 \cdot \text{MRT}_{\text{iv}} \quad (1.17)$$

where MRT_{iv} is the mean residence time following intravenous dosing. This calculation assumes that $t_{1/2}$ is proportional to MRT_{iv} .

The elimination rate constant k_e is a first-order rate constant that is used to describe drug elimination from the body. The k_e can be calculated directly from the slope of the straight line or from biologic $t_{1/2}$ using Equation 1.18.

$$k_e = \frac{0.693}{t_{1/2}} \quad (1.18)$$

It is interesting to note that in light of the major advancements realized in the field of drug analysis and as greater analytical sensitivity has been achieved, lower concentrations are being detected, therefore, using the $t_{1/2}$ calculated from the terminal elimination phase resulted in significantly longer terminal $t_{1/2}$. For example, a $t_{1/2}$ of 120 h was calculated with indomethacin, whereas 2.4 h pharmacologically relevant $t_{1/2}$ is reported. Therefore, scientists are recommended to determine the most biologically relevant $t_{1/2}$ by using Equation 1.16 where $t_{1/2}$ is defined by the drug clearance and volume of distribution.

TIP:

Develop a habit of double-checking the $t_{1/2}$ calculated from the terminal elimination phase following intravenous dosing by comparing it with that calculated using Equation 1.16. If the two numbers are similar, then this is the pharmacologically relevant $t_{1/2}$. Otherwise, report the value determined using Equation 1.16.

1.3.7 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 (Figure 1.3). For some drugs, the biological 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 the minimum inhibitory concentration (MIC) to obtain a clinical response $\geq 90\%$ [23, 24]. 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 and its unit is time (e.g., h) (Figure 1.3). As depicted in Equation 1.18, t_{max} is independent of drug dose, bioavailability, or volume of distribution and is only determined by the rate constants of absorption (k_a) and elimination (k_e).

1.3.8 Absorption Rate Constant (k_a)

The k_a for a drug administered by a route other than intravenous is the rate of absorption of a drug absorbed from its site of administration. The rate of absorption usually follows first-order kinetics. Many approaches are used to calculate this parameter. For example, rate of absorption can be calculated from the following equation:

$$t_{max} = \frac{2.303}{k_a - k_e} \log \frac{k_a}{k_e} \quad (1.19)$$

The k_a can also be calculated using the method of residuals also known as feathering. The calculation is made with the assumption that the pharmacokinetics of the compound tested

follows one compartment model with first-order input and output and is described using Bateman equation (Equation 1.19). The shape of the compound plasma profile is described by k_a and k_e . In general, k_a is larger than k_e and suggests that the compound absorption is faster than its overall elimination rate:

$$C_p = \frac{F \cdot \text{Dose} \cdot k_a}{V \cdot (k_a - k_e)} \cdot [e^{-k_e \cdot t} - e^{-k_a \cdot t}] \quad (1.20)$$

The following steps can be used to calculate k_a :

1. Graph measured plasma concentration in semilog scale plot.
2. If $k_a > k_e$, then $e^{-k_a \cdot t}$ achieves zero faster than $e^{-k_e \cdot t}$. As a result, the plasma concentration (C') is described by

$$C'_p = \frac{F \cdot \text{Dose} \cdot k_a}{V \cdot (k_a - k_e)} \cdot e^{-k_e \cdot t} \quad (1.21)$$

3. Determine the intercept (Equation 1.22) and k_e (slope) of the terminal linear portion of the graph using either linear regression or graphically (Figure 1.8).

$$\text{Intercept} = \frac{F \cdot \text{Dose} \cdot k_a}{V \cdot (k_a - k_e)} \quad (1.22)$$

4. Calculate the difference between C' that depicts the terminal phase of the oral plasma profile and C (Bateman equation).
5. Plot ($C' - C$) values in the same semilog scale plot.
6. Calculate the k_a from the slope using either linear regression or graphically (Figure 1.9).

Finally, k_a can also be calculated using the moment method:

$$k_a = \frac{1}{\text{MRT}_{\text{po}} - \text{MRT}_{\text{iv}}} \quad (1.23)$$

where MRT_{po} is the mean residence time after oral dosing and MRT_{iv} the mean residence time after intravenous dosing.

1.3.9 Flip-Flop Kinetics

TIP:

To determine if a drug undergoes flip-flop kinetics following oral administration, both intravenous and oral plasma profiles for the drug should be characterized. If observed, the cause, usually associated with poor solubility, dissolution, and/or permeability of the tested article, may need to be investigated [25].

Flip-flop kinetics is a phenomenon where the terminal phase of the plasma profile of a drug following its oral administration is determined by the drug absorption. Here, the drug k_a is much slower than its k_e . This condition is usually associated with sustained absorption characterized by a decrease in C_{max} and increase in t_{max} (Figure 1.10). It should be emphasized that the obtained AUC stays the same with similar oral dose.

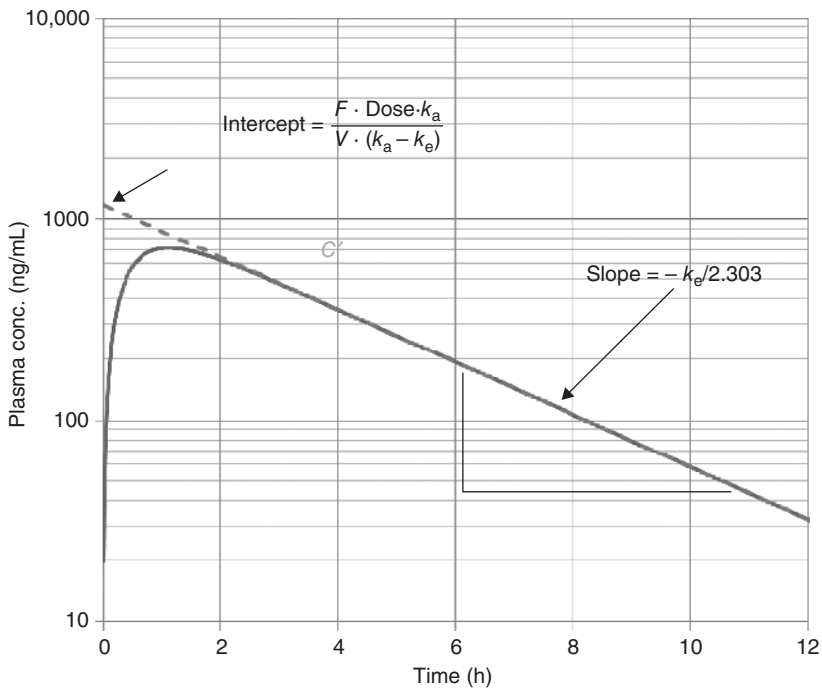


FIGURE 1.8 The semilog plot of plasma profile versus time of a compound that follows one compartment model with first-order input and first-order output.

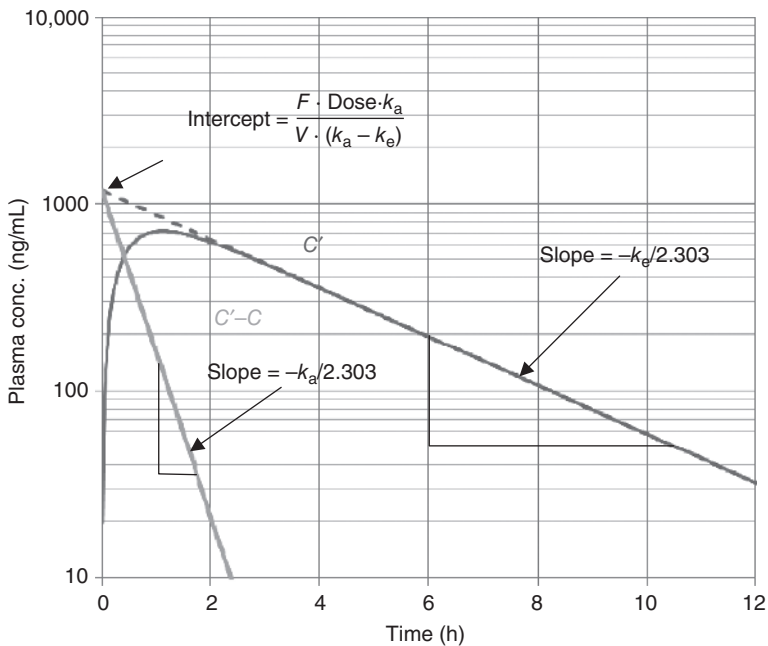


FIGURE 1.9 The semilog plot of residual versus time.

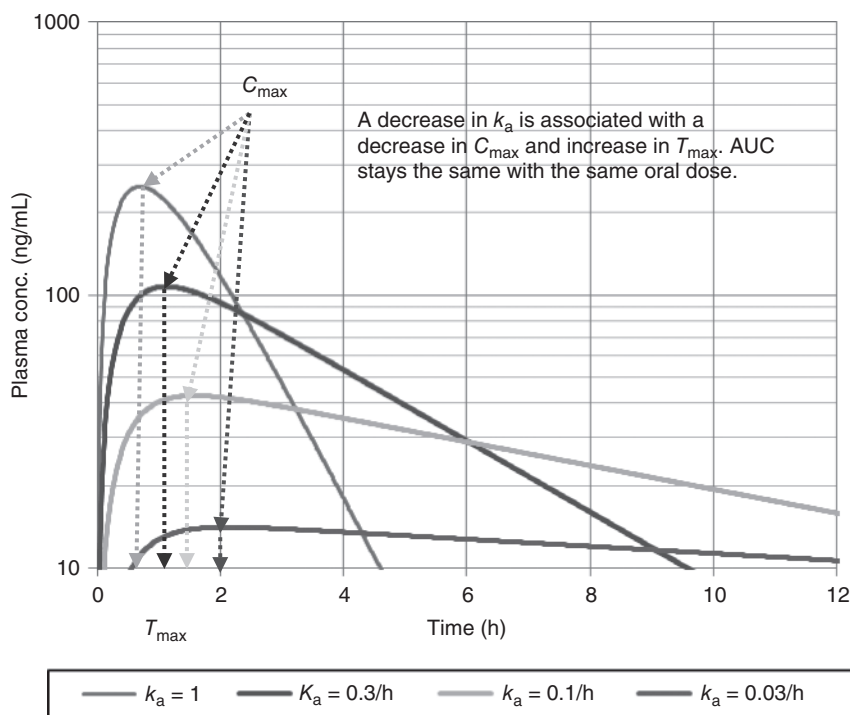


FIGURE 1.10 The impact of changes in k_a values on the oral plasma profile of a compound.

1.3.10 Mean Absorption Residence Time (MAT)

Mean absorption residence time (MAT) is the average time for a molecule to cross the intestinal membrane and arrive at the systemic circulation [26]. It is calculated using the following equation:

$$\text{MAT} = \text{MRT}_{\text{po}} - \text{MRT}_{\text{iv}} \quad (1.24)$$

where MRT_{po} and MRT_{iv} are the mean residence time of a drug after PO and IV dosing, respectively. Takahashi et al. used MAT to determine gastric emptying rate (GER) in monkey and compared it to that in human. The team used acetaminophen as a probe substrate since it has high passive permeability. Interestingly, acetaminophen MAT of 1.02 h in cynomolgus monkeys was only slightly longer than that in humans suggesting that monkey GER is comparable to that in human [27].

1.3.11 Bioavailability ($F\%$)

According to the European Medicines Evaluation Agency (EMA), 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.” As a parameter, there are two types of bioavailability:

1. Absolute bioavailability, which refers to the fraction of the extravascular (e.g., oral) dose that reaches the systemic circulation unchanged in reference to an intravenous dose.

It is usually determined by calculating the respective AUC after oral and intravenous administration as depicted in Equation 1.24. This calculation assumes that the drug complies with linear kinetics following dosing by both routes. Therefore, to avoid the effect of nonlinearity, the plasma concentrations following both intravenous and oral dosing should be similar.

$$\text{Absolute bioavailability} = \frac{\text{AUC}_{\text{PO}}}{\text{AUC}_{\text{IV}}} \times \frac{\text{Dose}_{\text{IV}}}{\text{Dose}_{\text{PO}}} \quad (1.25)$$

2. Relative bioavailability, which refers to the fraction of a dose of drug reaching the systemic circulation relative to a reference product, is usually calculated as

$$\text{Relative bioavailability} = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{ref}}} \times \frac{\text{Dose}_{\text{ref}}}{\text{Dose}_{\text{test}}} \quad (1.26)$$

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) (Figure 1.11). Oral bioavailability is mathematically expressed by the following equation:

$$F = f_a \cdot f_g \cdot f_h \quad (1.27)$$

Furthermore, oral bioavailability is a multiplicity parameter due to the anatomical sequential nature of the overall process (Figure 1.11).

f_h is calculated using the following equation:

$$f_h = 1 - E_h = 1 - \frac{\text{CL}_h}{Q} \quad (1.28)$$

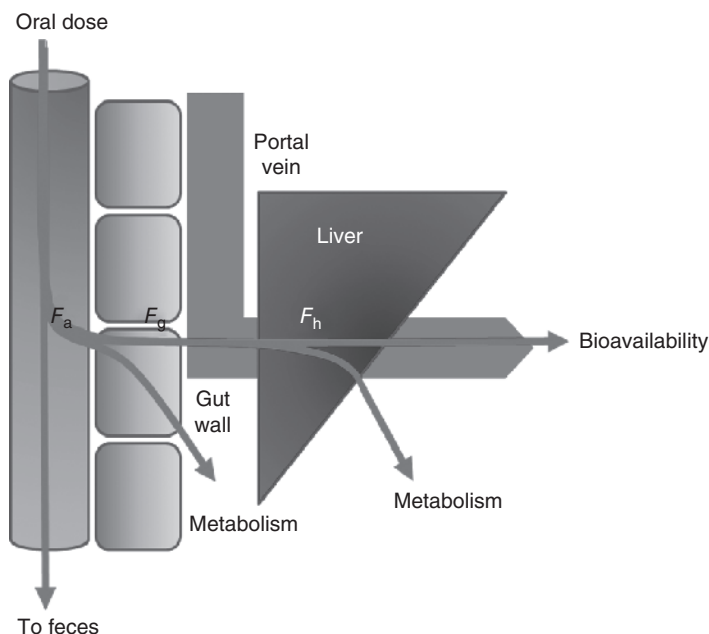


FIGURE 1.11 Oral bioavailability is a multiplicity parameter and a product of f_a , f_g , and f_h . (See color plate section for the color representation of this figure.)

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

TIP:

Always keep the values of the hepatic blood flow in preclinical species and human in mind. Develop a habit of calculating extraction ratio and f_h from CL using Equation 1.28. Based on which, you would be able to determine if hepatic first pass is a major contributing factor for your compound poor bioavailability, if observed following oral dosing.

1.3.12 Linear and Nonlinear Pharmacokinetics

Drug metabolism, renal tubular secretion, biliary secretion, and other active processes are usually mediated by metabolizing enzymes or transporter proteins. These systems usually have good substrate selectivity and are capacity limited. They are usually described by Michaelis–Menten kinetics:

$$v = -\frac{dc}{dt} = \frac{V_{\max} \cdot C}{K_m + C} \quad (1.29)$$

where v is equal to $-dc/dt$, which is the differential rate of change in free drug concentration with time, C the free drug concentration that can undergo the change, $V_{\max}$ the maximum elimination or transport rate, and K_m the Michaelis constant, which is equal to free drug concentration that results in $V = V_{\max}/2$. The values of $V_{\max}$ and K_m are dependent on the nature of the drug and enzymatic/transporter process involved. This equation implies that when the free drug concentration is lower than K_m , no saturation of the enzymes or transporters protein occurs (Case I) (Figure 1.12). However, when the free drug concentration is larger than K_m , saturation of the enzymes or transporter proteins occurs and the rate of elimination or transport is maximized and approaches that of $V_{\max}$ (Case II) (Figure 1.12). Here, pharmacokinetic parameters such as CL, V_{dss} , and $t_{1/2}$ become time, concentration, and dose dependent.

In drug discovery and development, dealing with drug molecules that follow linear pharmacokinetics principles is a desirable property. Since, an increase in dose is associated with a proportional increase in AUC and all relevant pharmacokinetics parameters such as CL, V_{dss} , MRT, and $t_{1/2}$ are constant and dose, time, and concentration independent (Figure 1.13). Therefore, prediction of the plasma exposure following various dosing regimens and over multiple dosing can be more easily achieved. However, there are situations where drug molecules exhibit nonlinearity, where increasing doses is associated with more than or less than proportional increase in AUC. As a result, extrapolation and projection of drug pharmacokinetic profile at different doses or for different dosage regimen cannot be easily accomplished using modeling techniques such as noncompartmental or compartmental modeling, which are based on the assumption that various biological processes comply with first-order kinetics. Moreover, these drugs will likely require more careful monitoring when dosage adjustment is made in order to achieve the desired therapeutic effects and minimize the potential for adverse effects.

1.3.12.1 Causes for Drug Nonlinearity Saturation of active processes mediated by enzymes or transporters is usually associated with nonlinearity that affects drug overall absorption, distribution, metabolism, or elimination (ADME). The causes of nonlinear kinetics are depicted in Table 1.2.

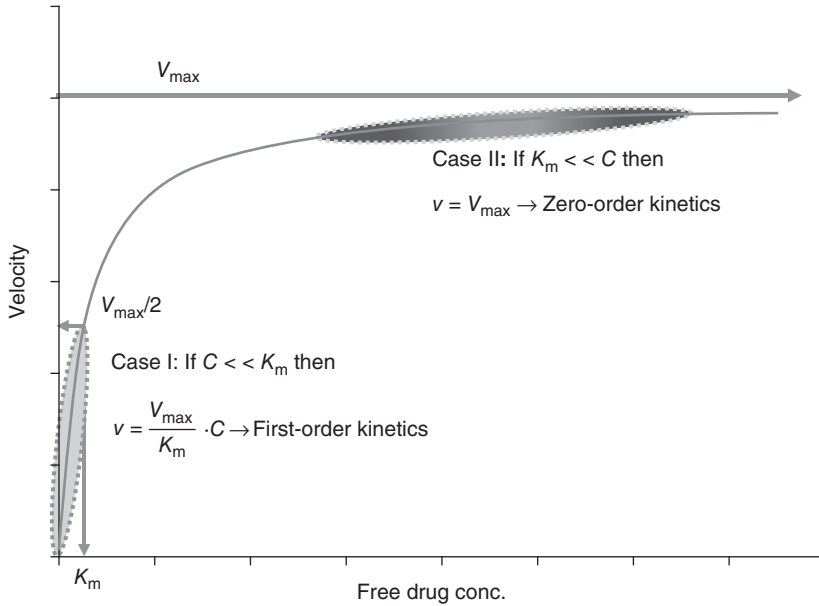


FIGURE 1.12 The relationship between drug elimination/transport rate and free drug concentration for a Michaelis–Menten kinetics complying biological process.

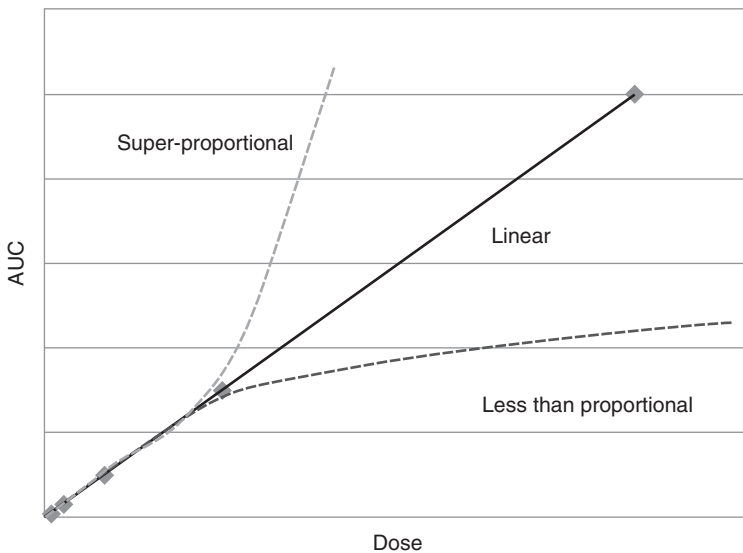


FIGURE 1.13 The relationship between dose and AUC is indicator of presence or absence of drug linearity.

TABLE 1.2 The Factors that may Contribute to Drug Nonlinear Kinetics

Process	Factor	Example
Absorption	Poor solubility and/or dissolution with high oral dose	Amphotericin, griseofulvin, danazol, and chorothiazide [25, 28, 29]
	Intestinal active uptake-mediated absorption saturation e.g., system L and OATP2B1	Gabapentin and celirolol [30, 31]
	Intestinal P-glycoprotein-mediated efflux saturation	Talinolol and UK343,664 [32, 33]
Distribution	Plasma protein binding saturation	Ceftriaxone and warfarin [34–36]
	Transport into and out of tissues saturation e.g., OATP1B1	Methotrexate and bosentan [37, 38]
Metabolism	Intestinal and/or hepatic-mediated first-pass effect saturation	Salicylic acid, lorcaidine, theophylline, and simepravir [39–41]
	Cofactor and/or metabolizing enzyme depletion	Acetaminophen [42]
	Enzyme induction	Avasimibe, carbamazepine, phenobarbital, phenytoin, ritonavir, efavirenz, and bosentan [38, 43–48]
	Metabolite-dependent inhibition	Paroxetine, erythromycin, clarithromycin, and diltiazem [49–51]
Renal excretion	Active renal secretion	Clofarabine, adefovir, cidofovir, and tenofovir [52, 53]
Active hepatic uptake	Active biliary secretion	Rosuvastatin and pravastatin [54–56]
Biliary excretion	Active biliary secretion	Rosuvastatin and pravastatin [54–56]

1.3.13 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 and drug ADME. Generally, the extent and duration of exposure is 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.

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. Due 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 to 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 lesser, 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).

A range of modeling approaches is used to simulate and project plasma exposure in preclinical species and human. Below is a summary of these approaches with emphasis on their advantages and limitations.

1.3.14 Noncompartmental Pharmacokinetics

Various pharmacokinetic parameters such as CL, V_d , $t_{1/2}$, MRT, and $F\%$, can be determined using noncompartmental methods. These methods are based on the empirical determination of AUC and AUMC described above. Unlike compartmental models (see below), these calculation methods can be applied to any other models, provided that the drug follows linear pharmacokinetics. However, the main limitations of the noncompartmental method are it is not robust and lacks any mechanistic interpretation of the data since derived pharmacokinetic parameters have no meaningful physiological relevance. Furthermore, the method does not provide insight into the mechanism of drug–drug interaction. The method cannot be used for the simulation of different plasma concentration–time profiles when there are alterations in dosing regimen or multiple dosing regimens are used.

1.3.15 Compartmental Pharmacokinetics

Compartmental models of pharmacokinetic 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 are virtual and 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. Compartmental models are usually arranged in a mammillary format, such that there is a one or more compartment that feeds from a central compartment.

1.3.15.1 One-Compartment Open Model In the one-compartment model, the body is assumed to be a homogenous unit where the drug is rapidly distributing throughout the body and once eliminated it follows a monoexponential decline (Figure 1.14). Following intravenous dosing, the plasma drug concentration can be calculated as

$$C = C^{\circ} \cdot e^{-K_e t} \quad (1.30)$$

where C° is the plasma drug concentration immediately after intravenous dosing. C° is also calculated as

$$C^{\circ} = D/V_c \quad (1.31)$$

Unlike other compartmental models, there is only one V_d , where $V_c = V_{dss}$.

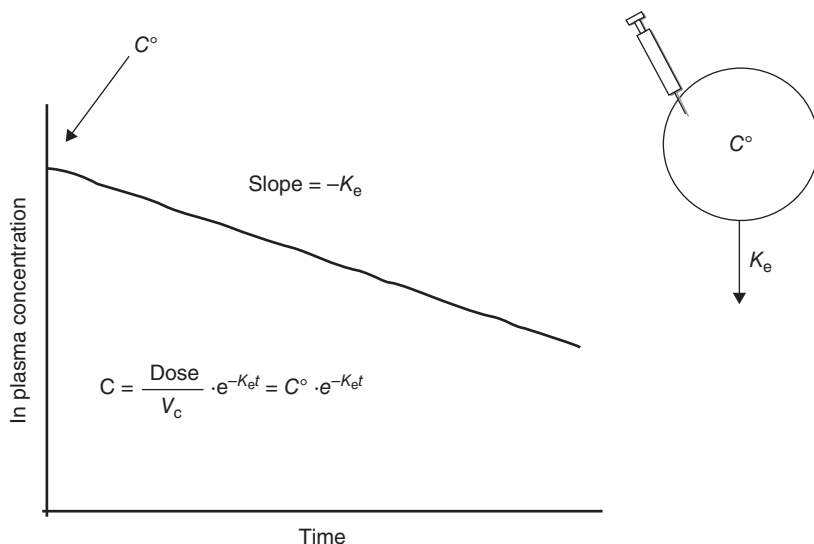


FIGURE 1.14 One-compartment model.

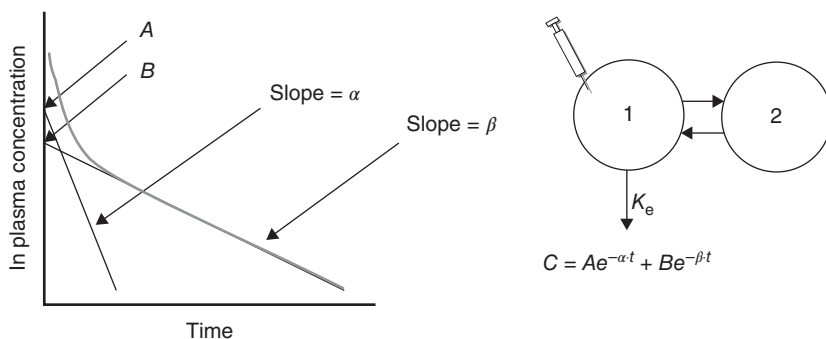


FIGURE 1.15 Two-compartment model.

1.3.15.2 Two-Compartment Open Model 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 (Figure 1.15). A drug that follows the pharmacokinetics of a two-compartment model does not rapidly distribute throughout the body as evident in the one-compartment model. In the two-compartment model, the drug is assumed to distribute into two compartments, the central and tissue compartments. The central compartment represents the highly perfused body organs where the drug distributes rapidly and uniformly. On the other hand, in the tissue compartment, the drug distributes more slowly.

For a drug that follows the two-compartment model, the rate of drug plasma concentration change following intravenous dose can be determined as

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1.32)$$

where A and B are functions of the administered dose and α and β the first-order constants for the distribution and elimination phase, 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 are described with additional exponential terms. However, these models are more complex.

1.3.16 Physiology-Based Pharmacokinetic (PBPK) Modeling

PBPK modeling is quantitative description of pharmacokinetics using both drug-specific *in vitro* and physiochemical properties as well as the species-specific anatomical and physiological information. Mathematically, the model constitutes multiple compartments, each corresponding to different body organs or tissues and linked together based on their anatomical placement with respect to blood flow (Figure 1.16). The vital physiological information in building these models are tissue volumes, blood flow to the tissue and tissue composition, which are considered system parameters that are drug independent (Figure 1.17). The compound-specific physicochemical and *in vitro* information (drug parameters) such as

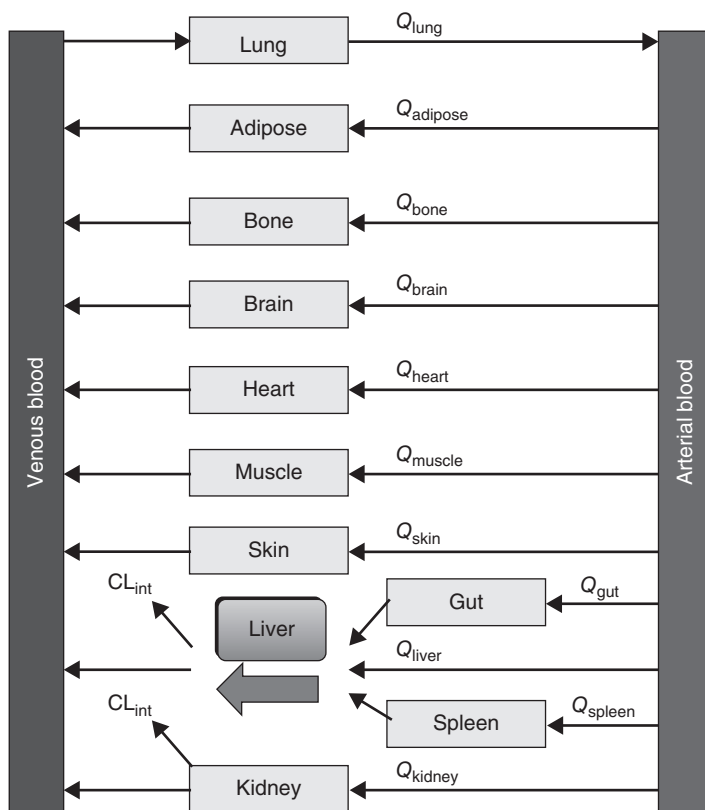


FIGURE 1.16 Physiologically based pharmacokinetic (PBPK) model incorporating physiological compartments depending on the drug's distribution.

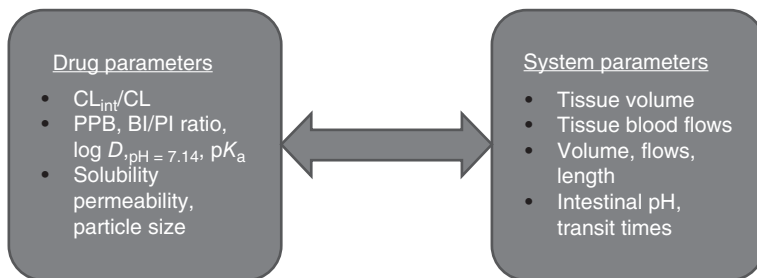


FIGURE 1.17 Known drug and system parameters used to build PBPK models.

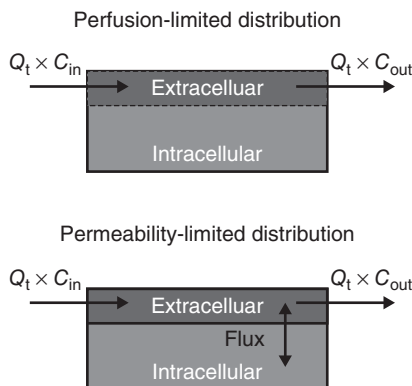


FIGURE 1.18 Perfusion- and permeability-limited distribution.

tissue to plasma partition (K_p) and the intrinsic clearance are provided as inputs (Figure 1.17). The distribution of the investigated molecule in various body organs is governed by whether it is either perfusion- or permeability-limited distribution. For perfusion-limited distribution, this is considered the most common type of compartment and mainly attributed to the leaky nature of the blood capillaries and rapid passive diffusion of drug molecules that is associated with high extent of mixing upon entrance into the tissue compartment. The rate of entry of drug molecules into the interstitial and intracellular spaces is faster than the rate of blood flow into the compartment. This rate is associated with instantaneous partition into the tissue and $C_{out} = C_f$, where C_f the free concentration of the chemical in the tissue and C_{out} the free concentration in the systemic circulation (Figure 1.18). As for permeability-limited distribution, the rate of drug molecules entry into the interstitial and intracellular spaces is slower than organ blood flow and usually governed by transporters and tight junctions such as those reported in the blood–brain barrier. Therefore, the free drug tissue concentration is typically lower than the free blood concentration in systemic circulation.

The major advantages of such modeling are twofold. First, unlike the empirical models (e.g., allometry), the obtained pharmacokinetic parameters are physiologically relevant and second, concentration–time profiles of each tissue can be obtained simultaneously. With the exploratory relationships between tissue concentration profiles and the pharmacological or toxicological effects, PBPK modeling provides framework for mechanistic pharmacokinetic/pharmacodynamic (PK/PD) modeling. PBPK models are also most reliable in dose–response and tissue exposure assessments under various physiological conditions (e.g., age, disease condition) [57].

1.3.17 Modeling to Predict Single and Multiple Dose Pharmacokinetic Profile

As previously discussed, compartmental models can be effectively used to project plasma concentrations that would be achieved following different dosage regimen and/or multiple dosing. However, for these projections to be accurate, the drug pharmacokinetic profile should follow first-order kinetics where various pharmacokinetic parameters such as CL , V_d , $t_{1/2}$, and $F\%$ do not change with dose.

1.4 TOXICOGENOMICS AND BIOMARKERS

Toxicology safety biomarkers are functional or structural measurements that correlate with a morphologic histopathologic or clinical pathology change in an organ system such as the liver or heart. 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 an 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. The issues with toxicogenomics experiments are variability in experimental design, strain, gender, age of experimental animals, husbandry, and nutrition; interanimal variation and clinical health effects of test compounds; and organs heterogeneity. For example, although the liver appears heterogeneous, it is composed of approximately 58% hepatocytes, 19% endothelial cells, 14% Kupffer cells, 4% 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 for 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 [58]. Multidisciplinary collaboration among toxicologic pathologists, toxicologists, biologists, toxicokinetics, and statisticians is needed for successful toxicogenomic efforts.

1.5 SPECIES DIFFERENCE 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 [59]. There are significant species differences in bile flow rate and hepatic

blood flow (Table 1.1), 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 pharmacokinetics disposition of various drugs [60].

Nelson et al. 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 [61]. Similar findings were also reported with uridine diphosphoglucose transferases and carboxylesterases [19, 62]. Overall, these small differences in the amino acid sequences can lead to significant differences in substrate affinity and specificity, which translates 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 [63]. 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% [64], but was 74% in dog [64]. 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 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 [64]. 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 [64]. 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 has been observed in preclinical species, ranging from 72% in dogs to 19% in monkeys, and was 24% in rats [65]. 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 [65], which is consistent with the observation that CYP3A4 is the main isoform responsible for the oxidative metabolism of indinavir in human liver microsomes [66]. The *in vitro* profile of indinavir metabolism was qualitatively similar across species [65]. In addition, an *in vitro*–*in vivo* correlation was established in rats and dogs using the *in vivo* hepatic clearance 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 clearance 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 [65] [67]. 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 clearance 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. Thus, protein binding may be another contributing factor to the differences in both the binding affinity and sites of drugs in protein molecules among different species [68]. For more information on the impact of species differences of metabolizing enzymes and transporters on drug disposition following oral dosing, see Chapter 11.

1.6 MIST (METABOLITES IN SAFETY TESTING)

Guidance for Industry on Safety Testing of Drug Metabolites, MIST, published in 2008 from the Center for Drug Evaluation and Research (CDER) defining the threshold for human metabolites that raises safety concerns as 10% of total drug exposure. Additional guidelines issued from the International Conference on Harmonization Guideline (ICH) on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, ICH M3[R2], defines 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, which account for dose is a more relevant threshold [69, 70]. 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; area-under-the-curve) in any one of the preclinical safety species is critical for addressing MIST implications. While rare, this early assessment is exceedingly important if the metabolite is formed in human 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 pharmacokinetic and toxicokinetic 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 1.3). 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 clearance pathways and potential target organ toxicity to evaluate if measuring metabolites in preclinical pharmacokinetic/toxicokinetic 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.

TABLE 1.3 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	<i>N</i> -Acetyl- <i>p</i> -benzoquinone imine (NAPQI)	Toxicity
Troglitazone	GSH conjugation/ring scission	Idiosyncratic toxicity
Losartan	5-Carboxylic acid (10–40× potency)	Pharmacologically active
Dexfenfluramine	<i>D</i> -Norfenfluramine	Pharmacologically active

1.7 PHARMACOLOGICALLY ACTIVE METABOLITES

Metabolism of drug candidates can occur at sites of the molecule that do not alter the active pharmacophore, and sometimes, result in metabolites that have greater pharmacological activity or longer pharmacodynamic duration. This result is usually first suspected when existing PK/PD 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.

1.8 REACTIVE METABOLITES

The MIST guidance addresses a majority of direct or indirect conjugates; the latter class of metabolites considered harmless but toxicity continues to be a major challenge 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 confirm the formation of these species *in vivo* [71]. Not only is the confirmation of suspected reactive metabolites informative, but the design of enzymology studies to understand pathways that lead to their formation allow for an understanding of the potential for species-specific reactions.

1.9 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 [72].

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 pursue metabolite assessment are 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.

1.10 CONCLUSION

PK and TK are important surrogates of drug exposure, efficacy, and safety in early and late drug discovery and development. Determinants of a drug PK/TK include absorption, distribution, metabolism, and elimination. 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, 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 clearance pathways. Ultimately, a well-designed PK/TK evaluation would provide the discovery team with knowledge to design and advance a best-in-class compound.

1.11 CHAPTER 1 TUTORIAL

- Which of the following statements are true (T) or false (F) with regard to first-order or zero-order processes?
 - A constant $t_{1/2}$ is characteristic for a first-order process.
 - The rate constant for a zero-order process has the unit [concentration per time].
 - A drug that is eliminated by a first-order process with the rate constant 0.1 h^{-1} has a longer half-life than a drug eliminated by a first-order process with the rate constant 0.3 h^{-1} .
 - If a drug follows linear pharmacokinetics, then the transfer of drug between different compartments in a compartmental pharmacokinetic model always follows zero-order kinetics.
- Define the following concepts:
 - Area under the curve
 - Clearance
- What is the impact of increasing clearance on volume of distribution and half-life according to Equation 1.16?
- A patient received an intravenous bolus injection of the Amikacin. Amikacin elimination follows first-order kinetics. The plasma concentration 2 h after the dose is $31 \mu\text{g/mL}$. Six hours after the dose was given, it is $2.3 \mu\text{g/mL}$. Calculate the elimination rate constant, the half-life of Amikacin, the initial concentration of the drug, and the area under the curve using integration method?

5. The data summarized in the following table depict the average plasma exposure of a new medication obtained from 10 healthy volunteers (average weight 70 kg).

Time (h)	Mean Plasma Conc. (ng/mL)		
	IV Dose 1 mg/kg	Oral Solution 10 mg/kg	Oral Tablet 10 mg/kg
0	10,000	0	0
0.25	7,650	495	100
0.5	6,260	1,330	250
1	4,630	3,540	600
2	3,710	4,650	800
3	2,110	3,500	1,100
4	1,940	2,730	1,200
6	1,020	1,450	800
8	390	807	500
12	138	288	150
24	9	68	100

- From the above data, calculate the following:
 - Total body clearance (CL)
 - Volume of distribution (V_d)
 - Half-life ($t_{1/2}$)
 - First-order elimination rate constant (K).
- What is the relative oral bioavailability of the oral tablet to oral solution dosing? Why it is not 100%?
- Which of the oral formulations tested, demonstrated flip–flop kinetics? What is the basis for your observation?

1.12 CHAPTER 1 TUTORIAL ANSWERS KEY

- Which of the following statements are true (T) or false (F) with regard to first-order or zero-order processes?
 - A constant $t_{1/2}$ is characteristic for a first-order process. (T)
 - The rate constant for a zero-order process has the unit [concentration per time]. (F)
 - A drug that is eliminated by a first-order process with the rate constant 0.1 h^{-1} has a longer half-life than a drug eliminated by a first-order process with the rate constant 0.3 h^{-1} . (T)
 - If a drug follows linear pharmacokinetics, then the transfer of drug between different compartments in a compartmental pharmacokinetic model always follows zero-order kinetics. (F)
- Define the following concepts:
 - Area under the curve:

It is the total amount of unchanged drug that reaches the systemic circulation following intravenous or extravascular administration. The unit for AUC is concentration per unit time (e.g., $\text{ng}\cdot\text{h/mL}$)

- Clearance:

It is a primary pharmacokinetic 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.

3. What is the impact of increasing clearance on volume of distribution and half-life according to Equation 1.16?

Clearance and volume of distribution are independent primary parameters where changes in either won't affect the other. Any change in either volume of distribution or clearance will only affect the half-life.

4. A patient received an intravenous bolus injection of the Amikacin. Amikacin elimination follows first-order kinetics. The plasma concentration 2 h after the dose is 31 µg/mL. Six hours after the dose was given, it is 2.3 µg/mL. Calculate the elimination rate constant, the half-life of Amikacin, the initial concentration of the drug, and the area under the curve using integration method?

$$-K/2.3 = [\log 2.3 - \log 31]/6 = -0.188, \quad K = 0.434 \text{ h}^{-1}$$

$$t_{1/2} = 0.693/K = 0.693/0.434 = 1.6 \text{ h}$$

$$\text{AUC}(0 \rightarrow 6 \text{ h}) = (C_2 + C_1)/2 * (t_2 - t_1) = (31 + 2.3)/2 * 6 = 99.9 \text{ µg h/mL}$$

5. The data summarized in the following table depict the average plasma exposure of a new medication obtained from 10 healthy volunteers (average weight 70 kg).

Time (h)	Mean Plasma Conc. (ng/mL)		
	IV Dose 1 mg/kg	Oral Solution 10 mg/kg	Oral Tablet 10 mg/kg
0	10,000	0	0
0.25	7,650	495	100
0.5	6,260	1,330	250
1	4,630	3,540	600
2	3,710	4,650	800
3	2,110	3,500	1,100
4	1,940	2,730	1,200
6	1,020	1,450	800
8	390	807	500
12	138	288	150
24	9	68	100

- a. From the above data, calculate the following:

- Total body clearance (CL)

$$= \text{Dose}/\text{AUC} = 1,000,000 \text{ ng/kg} / 22,120 \text{ ng h/mL} / 60 \text{ min/h} = 0.75 \text{ mL/min/kg}$$

- Volume of distribution (V_d)

$$V_{\text{dss}} = \text{CL} * \text{MRT} = \text{CL} * \text{AUMC}/\text{AUC}$$

$$= 0.75 \text{ mL/min/kg} * 60 \text{ min/h} * 3.493 \text{ h} * /1000 \text{ mL} * 1 \text{ L}$$

$$= 157 \text{ mL/kg}$$

- Half-life ($t_{1/2}$)

$$= 0.693 * V_{\text{dss}} / \text{CL} = 0.693 * 157 \text{ mL/kg} / 0.75 \text{ mL/min/kg} / 60 \text{ min} * \text{h} = 2.42 \text{ h}$$

- First-order elimination rate constant (K)

$$K = 0.693 / t_{1/2} = 0.693 / 2.42 = 0.286$$

- b.** What is the relative oral bioavailability of the oral tablet to oral solution dosing? Why is not 100%?

The relative oral bioavailability is 50% and the cause of this is the potential poor solubility and dissolution of the tablet formulation in comparison to oral solution.

- c.** Which of the oral formulations tested, demonstrated flip–flop kinetics? What is the basis for your observation?

The oral tablet appears to have a flip–flop kinetics since its half-life following PO dosing is longer than that observed in the IV formulation.

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