CHAPTER 7

Pre-clinical pharmacokinetics

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7.1 *Introduction*

When a patient is given a new medicine the doctor will instruct them how often the medicine should be taken, for example 'take a tablet three times a day' and any other conditions 'to be taken with food'. These are not arbitrary traditions but important instructions, which have a firm scientific basis. These dosing regimens are important in developing new medicines as potential advantages, which a new drug can provide over its competitors. How are the dosage regimens decided and what factors are important? These questions are examined in this chapter on pharmacokinetics.

Pharmacokinetics (PK) is a term applied to the quantitative study of the *in vivo* disposition of a xenobiotic, usually a drug. A good definition of PK is 'what the body does to the drug'. Typically pharmacokinetics describes how the concentrations of a drug in plasma change with time following a known dose being given by a specific route. The 'true' pharmacokinetic data on a drug molecule, obtained following intravenous dosing, provides an understanding of how readily the drug can distribute in the body, the ability of the body to eliminate the drug and how rapidly the drug leaves the body.

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Additionally dosing the drug by the oral route provides information on how rapidly and how much of this dose reaches the systemic circulation. Thus intravenous and oral pharmacokinetic parameters of a molecule provide a great deal of information about its disposition. The many routes of administration and elimination are illustrated in Figure 7.1. How pharmacokinetic information is obtained and interpreted is described in this chapter.

The study of PK is an important component of both the research and development phases in the discovery of new drugs. In research, chemical compounds will be screened in various biochemical and pharmacological tests to find compounds which show a positive effect. Typically the compounds will be categorised based on the biological or pharmacological potency with the compounds, which are most active at the lowest concentration, regarded as the most promising lead compound. However, *in vitro* potency is not the only factor required by a good drug because many compounds which are potent *in vitro* are inactive *in vivo*. It is critically important that pharmacokinetic properties are given just as much weight as

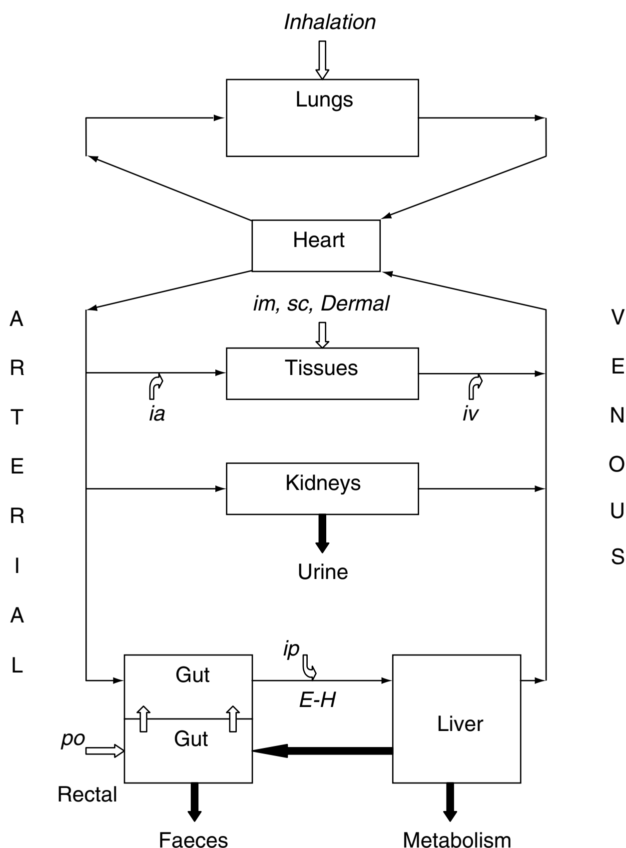

FIGURE 7.1 *Schematic representation of routes of administration and elimination.*

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efficacy. Good efficacy but poor PK is unlikely to lead to a successful medicine, and failure during the development phase due to inappropriate PK is both expensive and inefficient. The understanding of *in vivo* biological results is greatly enhanced if the disposition of the molecule is understood. A poor *in vivo* result may be due to inappropriate plasma concentrations rather than a poor model, or a molecule lacking in potency. Relating plasma concentrations to effect (pharmacokinetic/pharmacodynamic or PK/PD modelling) is covered in Chapter 8.

In pre-clinical development, PK has a key role to play in understanding the safety of the drug molecule. This manifests itself primarily in toxicokinetic studies in support of safety evaluation, which will be covered in Chapter 9. In addition during the development process detailed pharmacokinetic studies are performed to characterise the molecule to a level that satisfies the regulatory authorities.

Finally, through species comparisons and scaling, forecasts of the likely clinical parameters are made from the pre-clinical data. These forecasts are an invaluable contribution in designing efficient clinical trials and for evaluating the likely commercial and clinical success of the molecule.

7.2 *Pharmacokinetic parameters*

Pharmacokinetic parameters describe the exposure, distribution and time course of the drug in the body, and are generated from systemic drug concentration versus time data. Parameters of exposure are related to drug concentrations circulating in the blood, serum or plasma, and are usually expressed as an amount of drug per unit of volume. For simplicity the parameter descriptions in this chapter will relate to plasma, but they can be similarly applied to blood or serum.

When a drug is administered intravenously it enters the systemic circulation without an absorption phase, and drug is distributed from the blood to the tissues and organs. The blood levels obtained following intravenous administration provide the definitive PK of a compound reflecting the distribution and elimination phases.

7.3 *Bioavailability*

When a drug is administered by a route other than intravenous it has to pass absorption and metabolic barriers before it reaches the general circulation system. If a compound is poorly absorbed or extensively metabolised before it reaches the general circulation then only a fraction of the dose administered will reach the systemic circulation. Blood samples are taken for pharmacokinetic analysis from the systemic circulation; consequently they will only reflect the fraction of the drug reaching the systemic circulation. Bioavailability is the measure of the dose fraction

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available systemically after an extra-parenteral dose. For a compound to have good bioavailability, it must have good absorption and low clearance.

As a 'rule of thumb', low bioavailability results in high inter-subject variability and increases the difficulty in selecting the appropriate dosage level. As shown in Figure 7.1 when a drug is absorbed from the gastrointestinal tract the blood flow passes through the liver before reaching the general circulation. If a drug is cleared by metabolism the concentration of the drug in blood entering the liver is higher than the concentration in blood leaving the liver. This phenomenon is known as first pass metabolism, and removal of the drug by the liver is called hepatic clearance. Blood (plasma) levels taken for pharmacokinetic analysis inevitably reflect the level of drug in the general circulation consequently, when the plasma levels after oral administration are compared to those following intravenous administration (measure of bioavailability of the drug: see Section 7.5.10), the values are lower for oral administration because of the amount of drug removed in the 'first pass' through the liver. High hepatic clearance leads to low oral bioavailability, even if the drug is well absorbed.

7.4 *Calculation of pharmacokinetic parameters*

The main parameters of exposure are $C_{\max}$ (maximum plasma concentration – example: ng/mL) and *AUC* (area under the plasma concentration–time curve – example: ng.h/mL). Distribution parameters describe the extent of drug distribution and are related to body volumes (example: mL or litre), and time course parameters are related to time. For a comprehensive discussion about pharmacokinetic concepts see clinical pharmacokinetics: concepts and applications (Rowland and Tozer, 1989).

These pharmacokinetic parameters are calculated from mathematical formulae, and specific computer programs are usually used to do this (e.g. WinNonlin™). The parameters may be estimated by compartmental or non-compartmental approaches (or model-dependent and model-independent, respectively).

The plasma concentration and time point data may be fitted to a compartmental model using specially designed pharmacokinetic modelling programs (e.g. WinNonlin™). Modelling of data is based on an iterative process providing a solution to non-linear regression problems. The concept of 'compartments' is a hypothesis for the input, distribution and elimination of the drug from the body, and does not represent true bodily compartments. In general, non-compartmental PK is more than adequate to describe the time course of novel compounds in animals and is the method most often used in pre-clinical discovery. Schematic representations of the one- and two-compartment models are shown in Figures 7.2 and 7.3, respectively. Intravenous dosing provides the definitive PK of any compound. Parameters may also be derived after an oral dose, or a dose from any other route of administration,

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FIGURE 7.2 *Schematic representation of a one-compartment open model.*

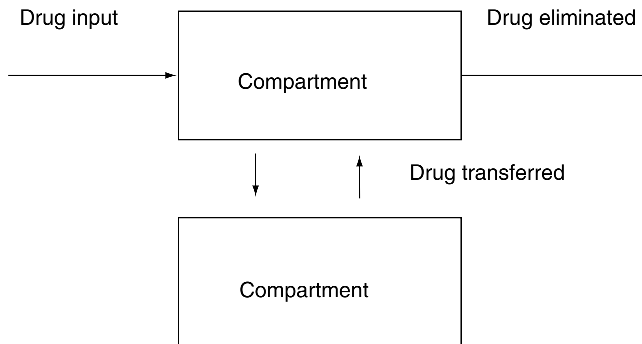

FIGURE 7.3 *Schematic representation of a two-compartment open model.*

but in these cases, parameters would need to be adjusted for the fraction of the dose absorbed because not all dose taken enters the systemic circulation.

This chapter will focus on describing the basic formulae for the parameters but will not endeavour to educate the reader in the mathematical derivations. For a comprehensive understanding of the derivation of the formulae, refer to PK (Perrier and Gibaldi, 1982).

7.4.1 Half-life

Half-life is the time taken for the drug concentration in plasma to be reduced by half. This parameter is important because it helps define how often a drug should be administered. If a drug is dosed once every three half-lives, the ratio of peak to trough concentrations will be around 10-fold. In addition there will be no significant accumulation during the dosing regime. Thus, provided the safety profile of a molecule will allow it, an 8 hour half-life is ideal for once a day dosing. If the therapeutic window is small, a longer half-life or more frequent dosing will reduce the peak to trough ratio, but will lead to some accumulation during the initial doses. This in turn may necessitate an initial loading dose (e.g. two tablets at once and one tablet a day thereafter). If the half-life is too short the dosing frequency may have to be increased to provide an appropriate safety/efficacy profile, and this may

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be less attractive commercially. Conversely, if the half-life is too long, it will be difficult to reverse the drug action should an adverse event occur.

If the drug is administered by intravenous infusion or a slow release formulation a steady state can be achieved where the systemic drug concentration remains constant when the rate of absorption/infusion is equal to the rate of elimination. One example of this situation is the administration of anaesthesia.

The plasma time profile may be multiphasic with several different 'half-lives' over the elimination phase. It is difficult to define the terminal half-life without measuring the complete time course, and the measurements are often restricted by the sensitivity of the assay method used. Which half-life is important is dependent on the pharmacological activity of the drug. It is possible that the pharmacological duration of action may be long, despite a short half-life, in which case frequent dosing will not be required. This will be discussed further in the PK/PD chapter.

7.4.2 Clearance

Total plasma clearance is the volume of plasma cleared of drug per unit time. Clearance determines the overall exposure that the body receives from a drug. Furthermore, clearance determines the rate of dosing required to maintain a given average plasma concentration. Thus a high clearance may lead to low exposure and low plasma average concentrations during chronic dosing. A high dose will be required to compensate for this, which will be a burden on cost and patient acceptability.

There may be instances when high clearance is a very desirable property. For example, inhaled products for topical action in the lungs will have the best safety profile if a high clearance results in low systemic exposure. Clearance would be considered to be high if blood clearance approached hepatic blood flow indicating that the only factor limiting the rate of clearance was blood flow. A drug which is cleared by the liver passes from the blood into the liver cells where metabolism of the drug occurs. If the compound is only slowly metabolised then the rate of metabolism is the rate-limiting process and the clearance will not be significantly affected by blood flow.

The other major route of clearance is renal where the drug is eliminated by the kidneys and excreted in urine. This clearance can also be related to blood flow; however, it is also affected by the rates of glomerular filteration and active secretion or reabsorption. Renal clearance would be classed as low if significantly less than glomerular filtration rate.

It should be noted that whilst pharmacokinetic parameters are not physiological measurements these physiological values can be useful in interpreting the values obtained. Table 7.1 shows some typical physiological flow rates in various species (some data taken from Davies and Morris, 1993).

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TABLE 7.1 *Typical physiological flow rates*

	Body weight (kg)	Hepatic blood flow (mL/min/kg)	Glomerular filtration rate (mL/min/kg)
Mouse	0.02–0.025	90	14
Rat	0.25	55	9.0*
Marmoset	0.3		2.9*
Rabbit	2.5–3	71	3.1
Cynomolgus monkey	4–5	44	2.1
Dog	10–12	31	3.3*
Man	70	21	1.8

Data taken from *Pharm. Res.* 10, 1093–1095 (1993) except values marked with asterisk.
*in-house data.

7.4.3 Volume of distribution

The volume of distribution describes how well the drug distributes in the body. If the volume of distribution is too low, a molecule with an intracellular site of action, such as an antiviral agent, may not reach high intracellular concentrations even if the plasma concentrations are high. Conversely a molecule such as an antibiotic that acts in the extracellular space may suffer if wide distribution leads to low fluid concentrations. A volume of distribution of 0.07 L/kg or less generally suggests that the drug is confined to the systemic circulation. A volume of distribution of 0.25 L/kg may indicate that the drug reaches interstitial fluids, but does not penetrate cells. A large volume of distribution of 0.6 L/kg or more suggests that the drug is well distributed, but does not necessarily mean the drug enters all cells. The large volume may be due to uptake by a specific tissue or membrane, for example, highly lipophilic compounds are known to distribute into lipids in cell membranes and fat stores; these effectively form slow release depots of the drug and prolong the plasma levels. Table 7.2 shows typical physiological volumes, which may be applied as 'rules of thumb' to describe how well a particular drug is distributed.

TABLE 7.2 *Typical physiological volumes*

Plasma volume	Blood volume	Extracellular water	Total body water
4%	7%	26%	60%

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7.5 *Parameter derivations*

7.5.1 C_{MAX}, T_{MAX}

The maximum plasma drug concentration (C_{max}) and the time taken to reach C_{max} (T_{max}) are obtained directly from the observed concentration–time data. The plasma concentration at the intercept (C_0) is a hypothetical concentration relevant only for an intravenous dose. It is derived by extrapolation from the first measured plasma concentrations to time zero based on the slope derived from regression of the initial two or three time points. Figure 7.4 shows a representation of a plasma concentration–time curve showing C_{max} and T_{max} for an oral dose.

7.5.2 Terminal phase rate constant (λ_z) and half-life ($t_{1/2}$)

The terminal phase rate constant (λ_z) is estimated by linear regression of logarithmic transformed concentration versus time data:

$$\lambda_z = \text{slope} \times -2.303$$

The terminal half-life ($t_{1/2}$) is calculated as follows:

$$t_{1/2} = \frac{\ln\ 2}{\lambda_z}$$

where ln 2 equals 0.693. The elimination may be monophasic (a single phase of elimination) or biphasic (comprising an initial or distribution phase and a terminal or elimination phase). These are illustrated in Figures 7.5 and 7.6.

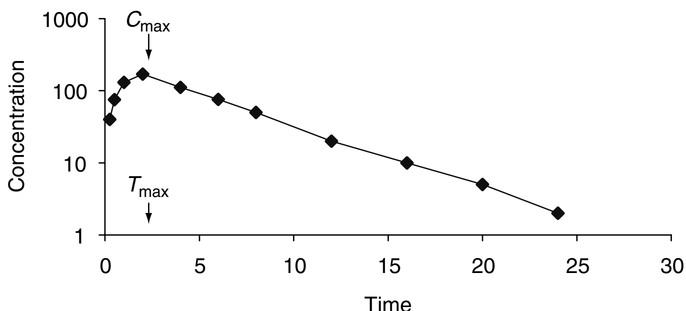

Figure 7.4 *Representation of a plasma concentration–time curve showing* C_{max} *and* T_{max} *for an oral dose.*

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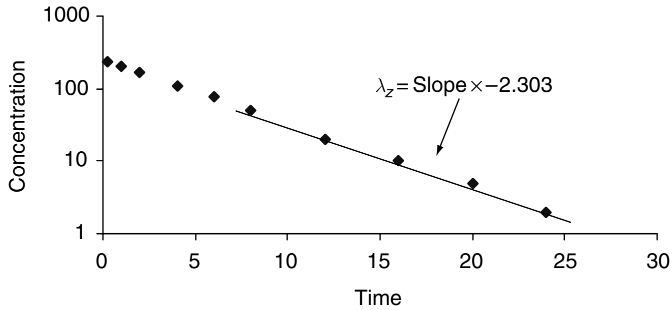

FIGURE 7.5 *Plasma concentration–time curve showing a monophasic elimination.*

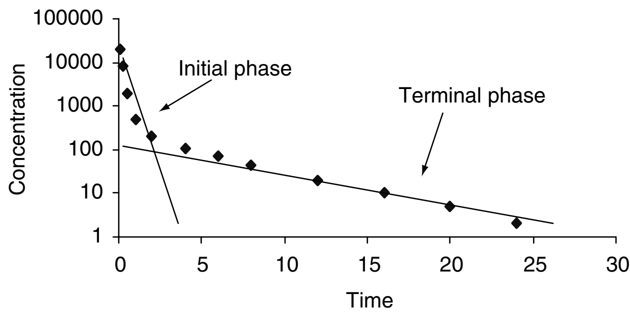

FIGURE 7.6 *Plasma concentration–time curve showing a biphasic elimination.*

7.5.3 Area under the plasma concentration–time curve (AUC_{last} and AUC_{∞})

The AUC from zero time to the time of the last quantifiable concentration (AUC_{last}) is calculated by the linear trapezoidal rule, or a combination of linear and logarithmic trapezoidal methods. These are illustrated graphically in Figure 7.7.

The linear trapezoidal method alone may be used if the time between terminal sampling times is less than the terminal half-life of the drug. However, this is rarely the case with pre-clinical data, as in the interest of reducing animal usage and stress, relatively few time points may be collected from animals.

(a) Linear trapezoidal area method

The trapezoidal area between the two data points (t_1, C_1) and (t_2, C_2), where $t_2 > t_1$ and $C_2 \geq C_1$, is given by:

$$AUC_{t_1-t_2} = 0.5 \times (t_2 - t_1) \times (C_1 + C_2)$$

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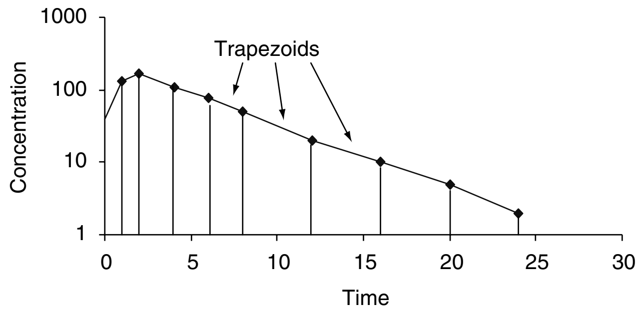

FIGURE 7.7 *Area under the plasma concentration–time curve showing the constituent trapezoids.*

where concentrations have been sampled at intervals greater than the elimination half-life, a combination of the linear and logarithmic trapezoidal rules can minimise overestimation of the AUC, which is a factor of exponential elimination. The linear trapezoidal method is employed when the plasma concentrations are rising and the logarithmic trapezoidal method when the concentrations are declining.

(b) Logarithmic trapezoidal area method

The trapezoidal area between the two data points (t_1, C_1) and (t_2, C_2), where $t_2 > t_1$ and $C_2 < C_1 (C_2 > 0)$, is given by:

$$AUC_{t_1-t_2} = \frac{(t_2 - t_1) \times (C_1 - C_2)}{\ln(C_1/C_2)}$$

Extrapolation of AUC_{last} from zero time to infinity (AUC_{∞}) is calculated as follows:

$$AUC_{\infty} = AUC_{\text{last}} + \frac{C_{\text{last}}}{\lambda_z}$$

where C_{last} is the last observed quantifiable concentration.

The percentage of AUC_{∞} obtained by extrapolation ($\%AUC_{\text{ex}}$) should be less than 20 per cent for reasonable confidence in the extrapolated parameter. The $\%AUC_{\text{ex}}$ can be calculated as follows:

$$\%AUC_{\text{ex}} = \frac{(AUC_{\infty} - AUC_{\text{last}})}{AUC_{\infty}} \times 100$$

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7.5.4 Clearance (CL)

The total plasma clearance (CL) is calculated as follows:

$$CL = \frac{Dose}{AUC_\infty}$$

or for continuous infusion

$$CL = \frac{R_o}{C_{ss}}$$

where R_o is the infusion rate and C_{ss} the plasma concentration at steady state.

7.5.5 Volume of distribution (V)

Volume of distribution, in non-compartmental PK, may be estimated as follows. The volume of distribution immediately after an intravenous bolus dose is:

$$V = \frac{Dose}{C_o}$$

The volume of distribution associated with the elimination phase can be estimated from the clearance and elimination rate constant:

$$V_z = \frac{CL}{\lambda_z}$$

7.5.6 Statistical moment theory ($AUMC_{last}$ and $AUMC_\infty$)

The plasma concentration–time curve can also be regarded as a statistical distribution curve, and statistical moment theory may be applied to the derivation of parameters. Although not generally quoted in pharmacokinetic interpretation, the area under the first moment curve can be used to estimate the volume of distribution at steady state.

The area under the first moment of the plasma (serum or blood) concentration–time curve ($AUMC$) from zero time (pre-dose) to the time of last quantifiable concentration ($AUMC_{last}$) may be calculated as shown below.

The linear trapezoidal method alone may be used if an appropriate sampling scheme (i.e. sampling time intervals $\leq$ to the elimination half-life) has been

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employed. The trapezoidal area between the two data points $(t_1, t_1 \times C_1)$ and $(t_2, t_2 \times C_2)$, where $t_2 > t_1$ and $C_2 \geq C_1$, is given by:

$$AUMC_{t_1-t_2} = 0.5 \times (t_2 - t_1) \times (t_1 \times C_1 + t_2 \times C_2)$$

If sampling intervals are longer than the half-life of elimination of the drug, a combination of the linear trapezoidal method (employed when the plasma concentrations are rising) and the logarithmic trapezoidal method (employed when the concentrations are declining) should be used.

The trapezoidal area between the two data points $(t_1, t_1 \times C_1)$ and $(t_2, t_2 \times C_2)$ where $t_2 > t_1$ and $C_2 < C_1 (C_2 > 0)$, is given by:

$$AUMC_{t_1-t_2} = \frac{(t_2 - t_1) \times (t_1 \times C_1 - t_2 \times C_2)}{\ln(C_1/C_2)} - \frac{(t_2 - t_1)^2 \times (C_1 - C_2)}{(\ln(C_1/C_2))^2}$$

AUMC from zero time to infinite time ($AUMC_\infty$) may be calculated as follows:

$$AUMC_\infty = AUMC_{\text{last}} + \frac{(t_{\text{last}} \times C_{\text{last}})}{\lambda_z} + \frac{C_{\text{last}}}{\lambda_z^2}$$

where C_{last} is the last observed quantifiable concentration.

The percentage of $AUMC_\infty$ obtained by extrapolation ($\%AUMC_{\text{ex}}$) may be calculated as follows:

$$\%AUMC_{\text{ex}} = \frac{(AUMC - AUMC_{\text{last}})}{AUMC_\infty} \times 100$$

If the percentage of $AUMC_\infty$ obtained by extrapolation constitutes more than 50 per cent of the total, caution must be exercised if subsequent parameters are estimated using these values (e.g. *MRT*, V_{ss}).

7.5.7 The mean residence time (*MRT*)

The *MRT* similarly is rarely used for interpretative purposes in pre-clinical evaluation. *MRT* for an intravenous bolus dose can be calculated as follows:

$$MRT_{\text{iv}} = \frac{AUMC_\infty}{AUC_\infty} - \frac{T}{2}$$

The *MRT* following an intravenous infusion may be calculated as follows:

$$MRT = \frac{AUMC_\infty}{AUC_\infty} - \frac{T}{2}$$

where *T* is the infusion time.

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7.5.8 Volume of distribution at steady state (V_{SS})

The volume of distribution at steady state (V_{ss}) provides us with a more homogeneous measure of volume, as it is not dominated by the distribution in the initial or terminal phases. It is calculated as follows:

$$V_{ss} = CL \times MRT$$

7.5.9 Renal clearance (CL_R)

If sufficient urinary excretion data are available, the renal clearance (CL_r) may be calculated as follows:

$$CL_r = \frac{Ae_\infty}{AUC_\infty}$$

where Ae_∞ is the total amount of unchanged drug recovered in the urine.

7.5.10 Bioavailabiliy (F)

The fraction of an oral dose that reaches the systemic circulation unchanged is calculated as follows:

$$F = \frac{AUC_{\infty po}}{AUC_{\infty iv}} \times \frac{Dose_{iv}}{Dose_{po}}$$

multiplied by 100, this is also referred to as 'bioavailability'.

7.6 *Study design and data handling in pre-clinical drug development*

7.6.1 Study design

The objective of the pre-clinical pharmacokinetic study is to evaluate the PK of the developmental drug in the animal species used in safety studies. The dose level is often selected as the lowest dose used in toxicology studies, another toxicity test dose or a pharmacologically therapeutic dose. Single dose pharmacokinetic studies provide the definitive pharmacokinetic profile in the pre-clinical species and systemic exposure to metabolites with the use of a radiolabel.

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It is important to perform the pharmacokinetic assessment at a dose level that is within the range of linear kinetics (i.e. not where elimination is saturable) as many drugs will exhibit non-linear kinetics over a wide dose range. This is because the mechanisms for drug clearance can be saturated by, for example, metabolic clearance. Metabolism is a biological process mediated by enzymes, and the rate of metabolism is related to the concentration of the drug substrate and amount of enzyme available. It is also dependent on blood flow and the intrinsic rate of metabolism of the compound. Some compounds may act as inducers or inhibitors of their own metabolism. At higher doses the metabolism may be rate limiting, and greater levels of unchanged drug may reach the systemic circulation. Likewise mechanisms involved in renal clearance notably glomerular filteration and active secretion can be saturated at higher doses resulting in decreased clearance of unchanged drug. These situations are further complicated if the pharmacological action of the drug results in a decrease in blood flow.

Absorption of drug may also be concentration dependent and in some cases the drug formulation, dissolution and solubility factors lead to a decrease in the percentage of the dose which is absorbed.

Generally non-linear kinetics is only observed in the dose ranges used in pre-clinical toxicity studies which are much greater than those used in human studies.

Intravenous data is required to obtain the definitive clearance and volume of distribution data. Oral and intravenous data together provide a measure of the bioavailability of the drug.

During a single dose pharmacokinetic study, blood should be collected over a period of at least four half-lives of the drug if the terminal half-life is to be accurately determined.

'Mean profiles' in which the plasma time profile is constructed from blood samples taken from different animals are typically used for small animals and may be conducted using a destructive (single time point from each animal) or composite design (several samples taken from different animals at different times). The reason for this is the difficulty in obtaining sufficient sample volume for analyses if all samples were taken from an individual animal (see, the toxicokinetics, Chapter 9).

The nominal times when plasma samples are taken are used to calculate the mean and standard deviation (or median and range) drug concentrations at each time point for each period and dose.

Individual profiles are more typical for larger animals which may be serially bled. Blood sampling times should be designed to minimise large gaps between time points (i.e. where concentrations are not detectable at 24 hours, consideration should be made to include a 12 or 16 hour time point).

Before a pharmacokineticist can begin to estimate the pharmacokinetic parameters, it is important that the data is inspected to ensure no gross deviations from the protocol have occurred. For example, on occasion, the actual sampling times during a pharmacokinetic study may deviate from the target sampling times.

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Deviations of greater than 5 per cent between the target sampling time and the actual sampling time should be taken into account especially for drugs with rapid absorption/distribution rates. If the rate of change in the plasma time profile is rapid then a significant difference between the nominal time and the actual time can give errors in the calculated pharmacokinetic parameters. In these cases, the actual sampling times should be used in the individual concentration–time profiles. Otherwise, it is sufficient to use the target sampling times for the analysis.

A common question for the novice pharmacokineticist is 'how should values below the quantitation (BQL) limit be handled?'. Non-quantifiable values are treated differently by various scientists; either being considered to reflect zero, or the actual limit of quantification, or even some value half way between. One recommendation for handling of BQL data is as follows:

1 BQL values at early time points, when appreciable absorption will not have occurred, should be set to zero.
2 When two consecutive BQL values are encountered, all subsequent non-BQL values should be excluded from the pharmacokinetic analysis.
3 When a BQL value occurs between two adjacent non-BQL values, it may be more appropriate to exclude it from the pharmacokinetic analysis.

Descriptive statistics (median, range or mean $\pm SD$) are often used to summarise the data. To calculate the mean plasma concentration in the presence of BQL values, the BQL values should be set to zero. If the mean value calculates to a value below the quantitation limit the mean should be reported as BQL.

The bioanalytical method is important as it underlines the integrity of the pharmacokinetic evaluation. While the method must be demonstrated to be valid, a formal validation report is not required for pre-clinical studies. The method of validation includes analyte stability, specificity, precision, accuracy and sensitivity. A calibration line with appropriate limits of quantification is defined. It may be necessary to quantify metabolite as well as parent compound, and this should optimally be done with the same assay.

7.7 *Application of PK in drug discovery*

7.7.1 Pharmacokinetic screening methods during lead optimisation

When designing a new drug, the desirable pharmacokinetic profile of the compound can be predicted from deficiencies in existing medicines or based

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on the required pharmacological target. On discovery a number of compounds will be screened to select those whose pharmacokinetic characteristics are closest to those desired. This process is called lead optimisation. The pharmacokinetic parameters investigated are half-life, clearance, volume and bioavailability. In the past, significant numbers of molecules have failed in the early stages of clinical development due to inappropriate pharmacokinetic properties, and the aim of lead optimisation should be to reduce this attrition rate.

Until recently the time taken to conduct a pre-clinical pharmacokinetic study was too slow to allow a pharmacokinetic screen to be used on the critical path of lead optimisation. Much of the bottleneck was with bioanalytical methods. Advances in the use of LC–MS–MS in bioanalysis have changed the situation radically. Rapid development and use of sensitive methods for many compounds per week is now a reality. Decisions now have to be made on how best to use this new technology. Conventional pharmacokinetic studies may not be appropriate in a lead optimisation programme. There must be a balance between compound throughput and the depth of information gained. There are a number of study designs now in general use.

Conventional studies will provide detailed characterisation of the pharmacokinetic parameters of molecule, but are relatively time-consuming. They are most appropriate for characterising lead molecules, or differentiating within a limited group of molecules in which small differences could be significant.

For the selection of the best molecules during a lead optimisation programme, it may only be necessary to rank molecules according to specific pharmacokinetic parameters. For example, if high oral bioavailability is required for a series of compounds that is known to be well absorbed, it is not necessary to dose by both oral and intravenous routes. A simple intravenous screen for low clearance will identify the best and worst compounds. Furthermore, to rank compounds according to clearance an estimate of plasma concentrations at two, or at most, three time points may be all that is needed. Whilst an *AUC* based on these limited points would not give an accurate measure of clearance, it will, with the right study design, correlate with clearance and hence be a reliable ranking tool. Both of these approaches reduce analytical and *in vivo* workload, and if terminal bleeds are involved animal numbers are also reduced.

Another approach currently being explored by a number of pharmaceutical companies is N-in-One dosing. Individual animals receive a cocktail of up to around ten compounds in a single dose formulation. The specificity of the LC–MS–MS bioanalysis then enables a plasma concentration–time profile and associated pharmacokinetic parameters to be obtained for each individual compound. To minimise drug–drug interactions, the doses are kept low, and to monitor such interactions a standard compound is included in each cocktail. Whilst this system is not foolproof it can dramatically increase throughput, reduce animal numbers and produce valid and useful PK screening information. Clearly the risk of interactions

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is ever present, and the method should be validated for any compound class prior to use in a decision-making role.

A further increase in throughput, mirrored in a further reduction in the detail of the PK output, can be obtained by incorporating the limited time point approach into N-in-One studies. The clear disadvantage of N-in-One studies is the potential for drug–drug interactions. If this precludes their use in a particular chemical series, some gains in throughput can still be obtained by N-in-One assays. In this study design, animals only receive one compound in a dose, but plasma samples from animals that have received different compounds are pooled prior to LC–MS–MS assay. Whilst the *in vivo* workload is not reduced, the sample processing and Mass Spec time are markedly reduced, leading to greater efficiency in the lab. Of course there is still a chance of interactions during LC–MS–MS analysis but these are usually both predictable and avoidable. In a lead optimisation environment, N-in-One bioanalysis should be the norm for all PK work so as to make best use of the resource available.

In summary, there are a number of ways to increase PK throughput by using novel study designs of N-in-One dosing, N-in-One analysis and limited sample time PK. The decision on whether to use any of these, or to use conventional study design, must be made on the balance between throughput on the one hand and accuracy and confidence in the PK parameters on the other.

7.8 *Interspecies scaling*

The PK of a novel compound in humans may be forecast by allometric scaling of pre-clinical data. This is a method of interpolation and extrapolation based on the anatomical, physiological and biochemical similarities in mammals. Mathematical analysis of pre-clinical pharmacokinetic data may permit the extrapolation of animal data to human data, and thus predict pharmacokinetic parameters and therapeutic dose levels in man. The two approaches described below are particularly successful if the major route of elimination of the test compound is as unchanged drug in the urine or if hepatic clearance is high.

In conjunction with non-clinical PK–PD data (see PK–PD chapter), the scaled parameters can be used to forecast effective doses for humans. This is important both in early discovery when the suitability of lead compounds for clinical effectiveness needs to be judged, and during drug development when the dose levels for early clinical studies need to be estimated.

Once the pharmacokinetic parameters and dose levels for early human studies have been predicted, data may be simulated to show the exposures at the selected dose levels. The exposure cover generated from the toxicology studies should be compared to the predicted exposures in man to aid in assessing risk. The exposures in the toxicology species should be higher than the predicted exposure in humans at

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the recommended dose levels for early human studies and ultimately for therapeutic use (see toxicokinetics, Chapter 9).

Two methods of interspecies scaling will be described here, simple allometry and flow-based scaling.

(a) *Simple allometry*
All available pharmacokinetic data from research or ADME (absorption, distribution, metabolism and excretion) studies should be used for the scaling; the more species the better. Experience has shown that it may be a disadvantage to use toxicokinetic data for scaling. This is because there is usually a smaller number of time points available from these types of studies, and more complex kinetics (like biphasic elimination) may not be evident from the profile. A simple allometric approach may be used to scale to humans using the formula $y = aW^b$, where y the pharmacokinetic parameter in question, a the value at the intercept at 1 kilogram, W body weight, and b the slope of the regression line. This is illustrated in Figure 7.8.

Based on the above example, parameter estimates for humans were $t_{1/2} = 2.6\,\text{h}$, $\lambda_z = 0.27\,\text{h}$, $CL = 0.21\,\text{L/h/kg}$ and $V = 0.77\,\text{L/kg}$.

(b) *Flow-based scaling*
Flow-based scaling is based on the premise that in all species the volume of distribution (L/kg) of a given molecule is the same, and that clearance is the same fraction of either glomerular filtration rate (renally cleared) or liver blood flow (hepatic clearance). This fact allows us to forecast the human pharmacokinetics from pre-clinical studies in only one or two species by comparing physiological flows in different species.

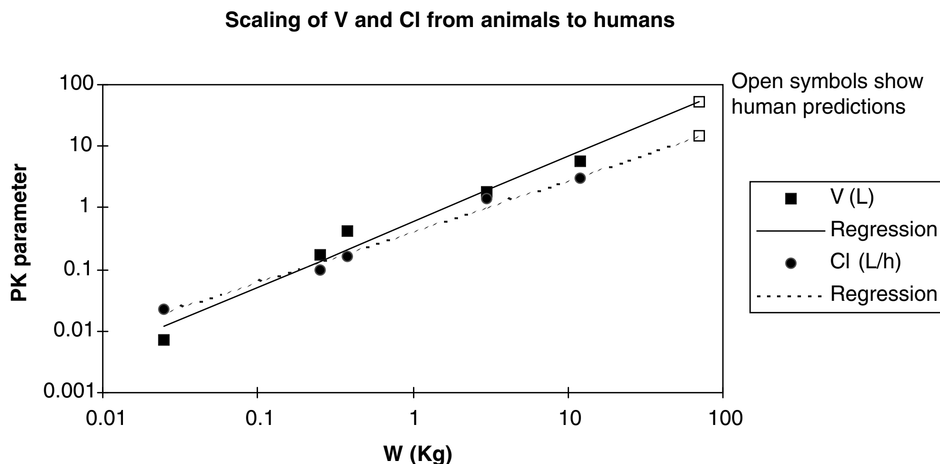

FIGURE 7.8 *Simple allometric scaling of clearance and volume.*

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Using this technique for a renally cleared compound:

$$half/life_{\text{man}} = \frac{half/life_{\text{animal}} \times GFR_{\text{animal}}}{GFR_{\text{man}}}$$

where *GFR* is glomerular filtration rate (mL/min/kg). The calculation is the same for hepatically cleared compounds using liver blood flow. The inverse equation is used for clearance.

The outcome of this is that the half-life in man is most likely to be around three-fold higher than that in rat and dog. Clearly significant differences in intrinsic clearance of moderately cleared molecules can render this model (as with graphical models) erroneous. Nonetheless, experience has shown that the forecast is more often right than not. The advantage of being able to scale with data from only one or two animal species is evident as human forecasts can be made from the very first pre-clinical pharmacokinetic study on a compound. In addition, if the human forecasts vary widely from different species they would be viewed with more uncertainty than if they were all similar.

7.9 *References*

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