

of these guidelines and relationships. This material may be helpful as a checklist in designing animal or human experiments in pharmacokinetics and in reviewing drug disposition reports; with greater elaboration, it has served as a basis for a graduate course in physiologic pharmacokinetics.

CONTEXT OF PHARMACOKINETICS

A pharmacokinetic analysis must be made in context of, be consistent with, and explain the array of basic data regarding the properties and disposition characteristics of the drug.

The tasks of model and equation selection and interpretation of data require a fundamental appreciation and integration of principles of physiology, pharmacology, biochemistry, physicochemical, analytical methodology, mathematics, and statistics. Pharmacokinetics has derived from these disciplines, and the relevant aspects of many of these areas must be considered in reaching any conclusions regarding a particular set of data. The physicochemical properties of a drug such as chemical form (salt, ester, complex), stability, partition coefficient, pKa, and molecular weight can affect drug absorption, distribution, and clearance. A drug disposition profile must be correlated with studies of structure-activity, disposition in alternative species, perfused organ experiments, tissue or microsomal metabolism, tissue drug residues, disease-state effects, and pharmacology and toxicology. For example, a much larger LD_{50} for oral doses of a drug compared with parenteral administration may be indicative of either poor gastrointestinal absorption (low aqueous solubility) or a substantial first-pass effect. Drug metabolism pathways may differ among species, but the biotransformation rate (V_{max} and K_m) of microsomes, homogenates, and perfused organs can often be applied directly to whole-body disposition rates and often correlate among species.¹⁻³

In general, the pharmacokinetic model and analysis should either conform to, or account for, the known properties and accumulated data related to the drug. One set of disposition data may misrepresent the characteristics of the drug because of any one or a combination of reasons. Experienced judgment is usually required in the final interpretation of any experimental findings and analysis.

ARRAY OF BASIC DATA

Pharmacokinetic studies often serve to answer specific questions about the properties of a drug. For example, a limited experimental protocol can easily resolve the question of how renal impairment affects the systemic clearance of an antibiotic. In the total design and implementation of

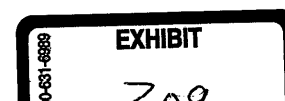
pharmacokinetic studies, an ideal and complete array of experimental data should include several considerations.

A. *The dosage form should be pre-analyzed.* All calculations stem from knowledge of the exact dose given (e.g., $CL = \text{Dose} / \text{AUC}$ [area under the plasma concentration-time curve]). Most commercial dosage forms are inexact, and content uniformity should be examined. Vials or ampules of injectables typically contain some overage and require analysis or aliquoting for administration of a precise dose. Solid dosage forms are required to yield an average of the stated quantity of drug with limited variability, but both injectable and solid forms may be inaccurate for pharmacokinetic purposes. Manninen and Korhonen⁴ provide an excellent example of both the variability and lack of stated quantity of digoxin in many commercial tablets. One product contained a range of 39 to 189% of the stated 0.25-mg dose of digoxin, whereas the most uniform product, Lanoxin, exhibited a range of about 95 to 106% for one batch of drug. To evaluate the potential uncertainty of the dose of drug used in disposition studies, it may be necessary to collect and analyze replicate doses of the product used. Poorly soluble and highly potent drugs are of most concern regarding erratic formulation.

B. *Accuracy in administration of the dose should be confirmed.* All doses should be timed exactly for starting time and duration of administrations. For ease in subsequent calculations, pharmacokinetic equations can be used to correct data from short-term infusion studies to the intercepts expected after bolus injection. The particular materials used in drug administration may cause loss of drug. In one of the most dramatic examples, Mackichan et al.⁵ found immediate loss of about 50% of a dose of intravenous diazepam by adsorption during passage through the plastic tubing of an infusion set. Inline filtration can also significantly reduce the potency of drugs administered intravenously.⁶

C. *Attention to methods and sites of blood collection is needed.* Ideally, blood samples should be collected by direct venipuncture in clean glass tubes without anticoagulant. Otherwise, the presence of possible artifacts should be tested. In the absence of any in vitro artifacts, serum and plasma concentrations are usually identical, and these terms are commonly used interchangeably. However, there are several reasons why they may not be identical. For example, the presence of heparin can result in increased free fatty acid concentrations, causing altered plasma protein binding.⁷ Also, the type of blood collection tube or anticoagulant may be a factor.⁸ If protein binding is temperature dependent, it may be necessary to centrifuge the blood sample at 37°C to avoid changes in red blood cell-plasma distribution of some compounds.⁹ These problems primarily pertain to weak bases, such as propranolol and imipramine for which binding to α_1 acid glycoprotein is appreciable and displacement alters plasma-red blood cell drug distribution.

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Plasma or serum protein binding and red blood cell partitioning should be measured at 37°C over the expected range of plasma drug concentrations. Both rate and degree of binding and uptake are theoretically important. This information may be especially needed for interpretation or normalization of nonlinear disposition patterns. Sometimes the site of blood collection and the presence of a tourniquet can alter the composition of the blood sample: serum proteins, calcium, and magnesium concentrations rise by 5 to 13% during venous stasis.¹¹

One of the major assumptions used in most pharmacokinetic studies is that venous blood collected from one site adequately reflects circulating arterial blood concentrations. For practical purposes, venous blood samples are usually collected. The pharmacokinetic analysis may need to be somewhat qualified, because arterial and capillary blood concentrations may differ markedly from venous blood concentrations of many drugs.¹¹ The AUC of arterial versus venous blood is expected to be identical for a nonclearing organ, and thus the principal difference expected is in distribution volumes. Physiologically, organ uptake of drugs occurs from the arterial blood, and clearance organ models are based on arterial-venous extraction principles.

D. *Serum (or blood) concentration data after intravenous injection (bolus or infusion) provides partial characterization of drug disposition properties.* Accurate assessment of volumes of distribution, distribution clearance (CL_D), and systemic clearance (CL) can best be attained with intravenous washout data.

E. *Serum (or blood) concentration data after oral doses of the drug in solution and common dosage forms provides additional pharmacokinetic parameters related to absorption and intrinsic clearance.* The doses (or resultant serum or blood concentrations of drug) should be comparable to those from the intravenous dose. These data permit assessment of either oral clearance (CL_{oral}) or bioavailability (F), and of the mean absorption time (MAT). If relevant, other routes of administration should be studied. For these, the U.S. Food and Drug Administration (FDA) guidelines for bioavailability studies should be consulted.¹²

F. *Three dosage levels (both oral and intravenous) should be administered to span the usual therapeutic range of the drug to permit assessment of possible dose-dependence (nonlinearity) in absorption, distribution, and elimination.*

G. *Urinary excretion rates of drug (as a function of time, dose, and route of administration) should be measured to accompany the above studies.* Urinary excretion is often a major route of drug elimination, and analyses permit quantitation of renal clearance (CL_R). Collection of other excreta or body fluids (feces, bile, milk, saliva) may permit determination of other relevant elimination or distributional pathways.

H. *Many drug metabolites are either pharmacologically active or otherwise of pharmacokinetic interest.* Phase I products such as hydroxylated or demethylated metabolites are most commonly either active or toxic.¹³ Their measurement will allow evaluation of AUC and mean residence time (MRT) and perhaps permit quantitation of metabolite formation and disposition clearances.

I. *Multiple-dose and steady-state experiments are necessary if therapeutic use of the drug relies on chronic dosing or steady-state concentrations.* The duration of multiple-dosing in relation to the terminal half-life is crucial for ascertaining applicability to steady-state conditions. Comparative single-dose and multiple-dose studies permit further assessment of linearity or allow determination of chronic or time-dependent drug effects (nonstationarity), such as enzyme induction,¹⁴ unusual accumulation,¹⁵ or drug-induced alterations in disposition. For example, aminoglycoside uptake into tissues is extremely slow and difficult to assess from single-dose studies. Multiple-dose washout measurement (Fig. 2-1) led to observation of a slow disposition phase for gentamicin that was the result of tissue accumulation and release.¹³

J. *Tissue analyses add reality and specificity to drug distribution characteristics.* Comprehensive studies in animals permit detection of unusual tissue affinities while generating partition coefficients (K_p) for individual tissues ($V_{t,i}$). This can lead to complete physiologic models for the drug in each species studied.^{1,2} Autopsy or biopsy studies in man may extend or complement pharmacokinetic expectations. This approach was found to be extremely helpful in confirming the strong tissue binding of gentamicin in man that was anticipated on the basis of serum concentration profiles (see inset of Fig. 2-1).¹⁵

K. *Suitable drug disposition studies in patients with various diseases and ages or given secondary drugs form the basis of clinical pharmacokinetics.* Perturbations in organ function, blood flow, or response will often alter drug disposition in a way that may warrant quantitative characterization. General principles may not always apply, and each drug needs individualized study. For example, although hepatic dysfunction may diminish the rate of oxidation of many drugs, some compounds, such as oxazepam and lorazepam, are predominantly metabolized by glucuronide conjugation, a process largely unaffected by liver diseases such as cirrhosis.¹⁶ Each disease state may require evaluation of direct effects on pharmacokinetic processes such as changes in renal clearance caused by kidney disease. However, indirect changes also require attention, such as the effects on both distribution and clearance caused by altered plasma protein binding.¹⁷ Commonly encountered patient factors such as smoking habit¹⁸ and obesity may cause unusual changes in drug disposition and require specific study and notation in patient surveys.

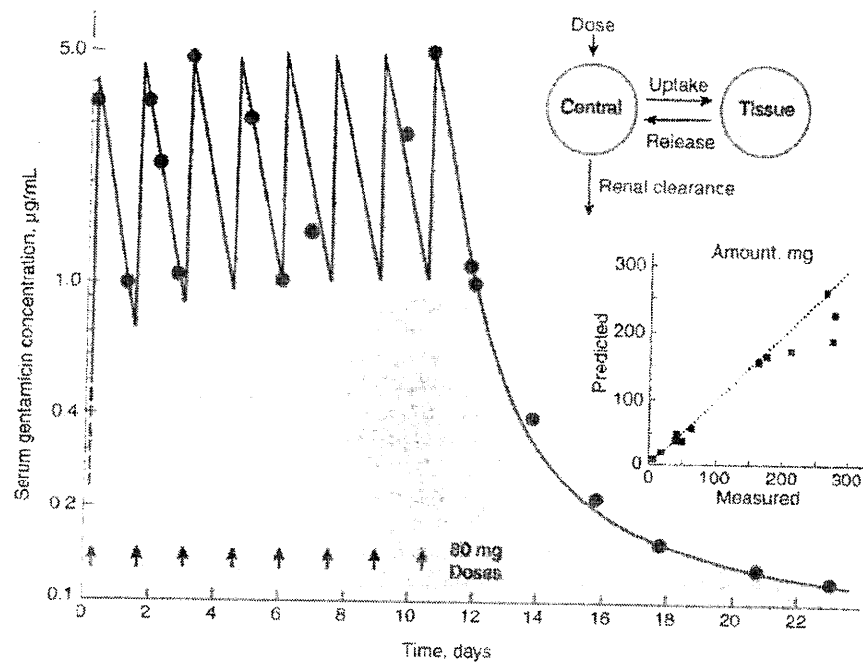


Figure 2-1 Plasma concentration-time profile for gentamicin disposition during multiple-dosing in a patient showing the prolonged terminal phase caused by strong tissue binding. These data were characterized with a two-compartment model (inset) that included prediction of drug remaining in the body on autopsy of some patients (inset). Data from Schentag et al.¹⁵

L. Many questions of drug disposition can be resolved from selected, carefully designed studies, and alternative types of information may be sufficient to validate various assumptions and reduce experimental procedures. The investigator's obligation is to adequately assess the literature, to avoid unwarranted assumptions, and to seek experimental strategies that would resolve a proposed hypothesis.

A comprehensive overview of pharmacokinetic needs in drug development has been constructed by Balant et al.¹⁶

DRUG ASSAYS

Certainty of specificity, sensitivity, and accuracy in measurement of drugs and their metabolites is a *sine qua non* in pharmacokinetics and deserves considerable attention. Guidelines for quality assurance in laboratory analyses have been concisely summarized by the American Chemical Society.²⁰ It is now commonplace to report the linearity, the coefficient of variation of the assay at low and high drug concentrations, the minimum level of detection, and the procedures used to assure specificity and stability, especially in the presence of metabolites, secondary drugs, and in specimens from diseased patients. Microbiologic assays are notoriously unreliable with problems caused by other antibiotics and active metabolites.

An extreme case of metabolite inclusion is in use of radioisotopic tracers: total radioisotope counts generally

yield total drug and metabolite activity and possibly the products of radiolysis. Separation of parent drug and individual metabolites is required for specificity. Microbiologic, enzymatic, and immunoassays are often of uncertain specificity, and matrix effects may require preparation of standards in each patient's pretreatment plasma. Most drug companies provide analytical grade samples of their drugs (and sometimes metabolites) to qualified investigators on written request.

Sample Handling

Coupled with assay reliability is concern for the stability of drug in biologic specimens, even in the frozen state. Ampicillin is unusual in that it is less stable when frozen than when refrigerated.²¹ Some drug esters, such as hetacillin (a pro-drug of ampicillin), continue hydrolyzing in blood and during the bioassay. Penicillamine is unstable in the presence of plasma proteins, and immediate deproteination after blood collection avoids loss of reduced penicillamine before analysis.²² Cyclosporine is best assayed in EDTA rather than heparinized blood as the latter yields red blood cell aggregates that increase assay variability.²³ Measurement of drug stability in blood will reveal whether hydrolysis can occur in blood or whether exposure to other body organs is required. Additional concerns in handling samples from a pharmacokinetic study include labeling and record-keeping procedures and documentation of storage conditions.

Sample Timing

Appropriate pharmacokinetic evaluation requires properly timed specimens. The simplest and least ambiguous experiment is the determination of systemic plasma clearance during continuous infusion at steady-state:

$$CL = k_0 / C_{ss} \quad (\text{Eq. 2-1})$$

where k_0 is the infusion rate and C_{ss} is the steady-state plasma concentration. For this equation to apply, the infusion period must be sufficiently long (about five terminal disposition half-lives) to allow steady-state to be attained. Alternatively, a loading dose or short-term infusion may be administered to more rapidly achieve equilibrium.²⁴

Practical methods are available²⁵ for designing optimal sampling strategies for kinetic experiments in which the number of specimens is limited, such as in the clinic. Optimal designs largely depend on the likely "true" model parameter values, the structure of the model, and measurement error. A sequential approach has been advocated with pilot studies and a sampling schedule that distributes time points over the major phases of drug disposition as the first step. Subsequent experiments can then resolve a specific hypothesis.

A common and severe problem in applied pharmacokinetics is the inadequate or incomplete measurement of drug washout from the system, either because of premature termination of sample collection or because of analytical limitations. The "true" terminal disposition phase must be examined for most aspects of data treatment and interpretation to be accurate. For example, the early distributive phase of aminoglycoside disposition measured by bioassay had long been accepted as the only phase, yet more sensitive radioimmunoassays, lengthier sample collection, and evaluation of multiple-dose washout revealed the slower phase of prolonged drug release from tissues (Fig. 2-1).

The two primary physiologic parameters in pharmacokinetics, namely systemic clearance and steady-state volume of distribution, can be most easily calculated by use of the area under the plasma concentration-time curve (AUC) and the area under the moment curve (AUMC). Both area values require extrapolation of plasma concentrations to time infinity, and the AUMC is, in particular, prone to exaggerated error from an inaccurate terminal slope.²⁶ If analytical or ethical constraints limit blood sample availability, extended saliva or urine collection may aid in defining the terminal disposition slope while adding one or two other pharmacokinetic parameters to the analysis. Urine may be particularly useful in this regard (if renal clearance is linear), as sample volumes are large and urine concentrations often greatly exceed plasma values.

The "midpoint" (C_{mid}) is generally the most desirable time to collect blood samples to match an excretion interval to assess a time-dependent clearance process.²⁷

$$\text{Clearance} = \frac{\text{Excretion Rate}}{C_{av}} = \frac{\text{Amount Excreted}}{AUC} \quad (\text{Eq. 2-2})$$

The arithmetic mean time is acceptable for slow processes, but errors will be incurred if the kinetic process produces rapid changes in plasma concentrations; it is common to miss an early exponential phase of drug disposition because of infrequent blood sampling. For a polyexponential curve with intercepts C_1 and slopes of λ_1 , the total AUC is

$$AUC = \sum (C_i / \lambda_i) \quad (\text{Eq. 2-3})$$

If the initial distributive phase is missing (area = C_1 / λ_1), then the error incurred in calculation of a clearance parameter ($CL = \text{Dose} / AUC$) is

$$\% CL \text{ error} = \frac{100(C_i / \lambda_i)}{AUC} \quad (\text{Eq. 2-4})$$

BASIC PHYSIOLOGIC PARAMETERS

The evolution of complete physiologic models¹ and clearance concepts applied to perfused organ systems,^{28, 29} with the restrictions incurred by the limited in vivo visibility offered by most blood or plasma drug disposition profiles, has led to the use of partial physiologic models for description of pharmacokinetic data. One such model is shown in Figure 2-2. Its construction and use should be viewed with some conceptual flexibility, and this material will apply to linear processes unless stated otherwise.

Volumes

The drug concentration in blood or plasma (C_p) is considered to be part of the central compartment (V_c). The mini-

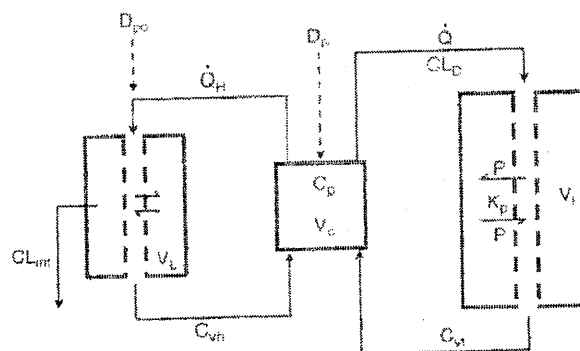


Figure 2-2 Basic semiphysiologic pharmacokinetic model for drug distribution and elimination (symbols are defined in the text). The clearance organ is pharmacokinetically perceived as separate from other compartments for drugs with high intrinsic clearances (CL_m), allowing characterization of the first-pass input.

TABLE 2-1 ■ PHYSIOLOGIC DETERMINANTS OF DRUG PARTITION OR DISTRIBUTION RATIOS BETWEEN TISSUES AND PLASMA

Active transport	Plasma protein binding
Donnan ion effect	Tissue binding
pH differences	Lipid partitioning

num value of V_d is plasma volume (V_p), but, either because drug diffuses rapidly out of plasma or the number of early time data are limited, the V_d value often exceeds V_p .

Drug located outside of V_p or V_c is, of course, present in tissues. The apparent volume of the tissue compartment (V_t) has two basic determinants: physiologic weight or volume of each tissue (V_{ti}) and partition or distribution factors (K_{pi}). In analysis of plasma concentration-time profiles, tissues must commonly be clustered together (including the clearing organs), thus:

$$V_t = \sum K_{pi} \times V_{ti} \quad (\text{Eq. 2-5})$$

This equation leads to the definition of one of the primary pharmacokinetic parameters with a physiologic basis, volume of distribution at steady-state (V_{ss}):

$$V_{ss} = V_c + V_t \quad (\text{Eq. 2-6})$$

If plasma and tissue binding are the sole determinants of nonhomogeneous distribution of drug in the body, then one definition of V_{ss} is

$$V_{ss} = V_p + \frac{f_u}{f_{ut}} \cdot V_t \quad (\text{Eq. 2-7})$$

where f_{up} and f_{ut} are the fractions of unbound drug in plasma and tissue.³⁰ Other factors may also contribute to the apparent partition coefficient of drugs between tissues and plasma (Table 2-1). Since, by definition, V_p and $\sum V_{ti}$ constitute total body weight (TBW),

$$TBW = V_p + \sum V_{ti} \quad (\text{Eq. 2-8})$$

then the quotient of

$$K_D = V_{ss} / TBW \quad (\text{Eq. 2-9})$$

defines the distribution coefficient (K_D) a physicochemical and physiologic measure of the average tissue to plasma ratio of the drug throughout the body. Approximate values of K_D and the primary rationalization of the size of K_D are provided in Table 2-2 for several common drugs. Normalization of V_{ss} for TBW is thus of value for generating the K_D and for making inter-individual and interspecies comparisons of this parameter.

One qualification of V_{ss} is needed. Drug equilibration between plasma and tissue of a clearing organ is affected by blood flow (Q_{it}) and intrinsic clearance (CL_{int}).³¹ For hepatic tissue, this yields the following relationship between the true partition coefficient (K_{ph}) and the lower, apparent value K_{ph}^{app} that would be experimentally measured at steady-state.

$$K_{ph}^{app} = K_{ph} \left(1 + \frac{CL_{int}}{Q_{it}} \right) \quad (\text{Eq. 2-10})$$

Distribution Clearance

The least appreciated element of the basic pharmacokinetic properties of drugs is the distribution clearance (CL_D) or intercompartmental clearance. This term reflects the flow

TABLE 2-2 ■ DISTRIBUTION COEFFICIENTS (K_D) FOR VARIOUS DRUGS AND PROBABLE PHYSIOLOGIC (PHYSICOCHEMICAL) CAUSE

DRUG	$K_D = \frac{V_{ss}}{TBW}$	EXPLANATION/INDICATION
Indocyanine green	0.06	Strong binding to plasma proteins and limited extravascular permeability
Inulin	0.25	Distribution limited to plasma and interstitial fluid owing to large molecular weight (5,500) and lipid insolubility
Theophylline	0.5	Moderate plasma binding and distribution primarily into total body water
Antipyrine	0.6	Slight plasma binding and fairly uniform distribution into total body water
Gentamicin	1.1	Strong tissue binding (common to aminoglycosides)
Tetracycline	1.6	Strong tissue binding to calcium in bone
Diazepam	1.7	Appreciable lipid partitioning
Digoxin	8.0	Strong binding to Na/K transport ATPase in cell membranes
Imipramine	10.0	Strong tissue binding (common to weak bases)

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