Second Edition

APPLIED PHARMACOKINETICS

Principles of Therapeutic Drug Monitoring

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Guidelines for Collection and Analysis of Pharmacokinetic Data

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Efforts in both theoretical and applied pharmacokinetics over the past decade have emphasized the utilization of the principles of **physiological pharmacokinetics** and the use of **noncompartmental** approaches to analysis of drug disposition data. Physiological pharmacokinetics involves the deployment of pharmacokinetic models and equations based on anatomical constructions and functions such as tissue masses, blood flow, organ metabolism and clearance, specific drug input rates and sites, and processes of partitioning, binding, and transport. While the complete applications of physiologic systems analysis may require extensive models,[1] even the simplest of pharmacokinetic treatments should have a physiologic basis for interpretation. Noncompartmental techniques in pharmacokinetics can serve in this regard. This term applies to curve analysis methods of data treatment which do not require a specific model and which yield the prime pharmacokinetic parameters such as systemic clearance (CL) and steady-state volume of distribution (V_{ss}) which summarize the major elimination and distribution properties.

This chapter is intended to provide an overview of major components of experimentally-applied pharmacokinetics. A summary is provided of the most relevant concepts, models, equations, and caveats which may be useful in the design, analysis, and interpretation of pharmacokinetic studies. References are provided for more complete details of the assumptions, derivations, and applications of these guidelines and relationships. This material may be helpful as a checklist in designing animal and (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 physiological 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, physicochemistry, 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 between species, but the biotransformation rate (V_{max} and K_m) of microsomes, homogenates, or perfused organs can often be applied directly to whole-body disposition rates and often correlate between 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 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 a number of considerations:

A. *The dosage form should be pre-analyzed.* All calculations stem from knowledge of the exact dose given [e.g., CL = dose / 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 Koriionen[4] 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, while 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 drugs are susceptible to erratic formulation.

B. *Accuracy in administration of the dose should be confirmed.* All doses should be timed exactly for starting time and duration of administration. 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 in small doses.[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 that 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 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 cell drug distribution.

Plasma or serum protein binding and red 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.[10]

One of the major assumptions employed 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 non-clearing organ, and thus the principal difference expected is in distribution volumes. Physiologically, organ uptake of drugs occur from the arterial blood, and clearance organ models are based on arterial-venous extraction principles.

D. Serum (or blood) concentration data following **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 following **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 transit time for absorption ($\bar{t}_a$). If relevant, other routes of administration should be studied. For these, the 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 transit time 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 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- and multiple-dose studies permit further assessment of linearity and (or) allow determination of chronic or time-dependent drug effects 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 measures (Figure 2-1) led to observation of a slow disposition phase which was the result of tissue accumulation and release.[16]

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_{pi}) for individual tissues (V_{ti}). This can lead to complete physiologic models for the drug in each species studied.[1,2] Autopsy or biopsy stud-

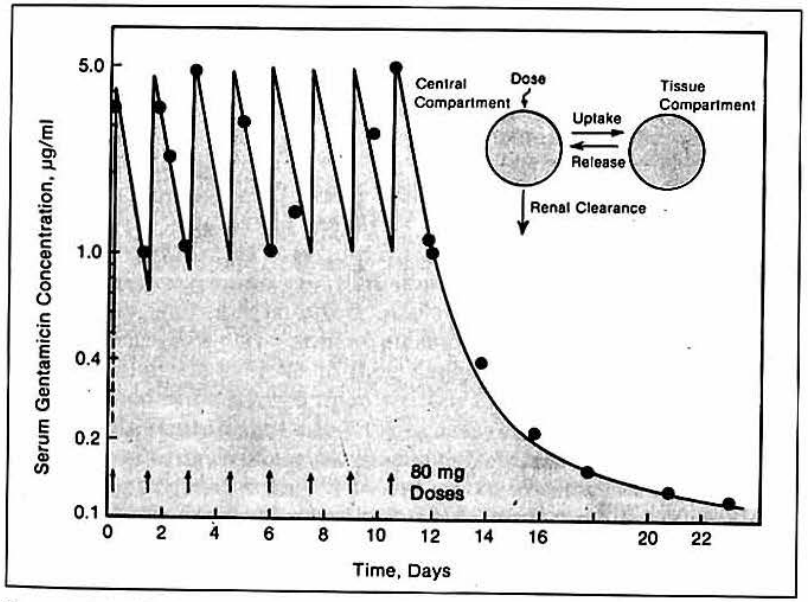

FIGURE 2-1. Plasma concentration-time profile for gentamicin disposition during multiple dosing in a patient showing the prolonged terminal phase caused by strong tissue binding. These data were characterized with a two-compartment model (inset) which included prediction of drug remaining in the body at the time of death of the patients. Data from reference 15.

ies in man may extend or complement pharmacokinetic expectations. This approach was found to be extremely helpful (Figure 2-2) in confirming the strong tissue binding of aminoglycosides in man which was anticipated on the basis of serum concentration profiles (Figure 2-1).[16]

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, while 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.[17] 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.[18] Finally, commonly encountered patient factors such as smoking habit[19] and

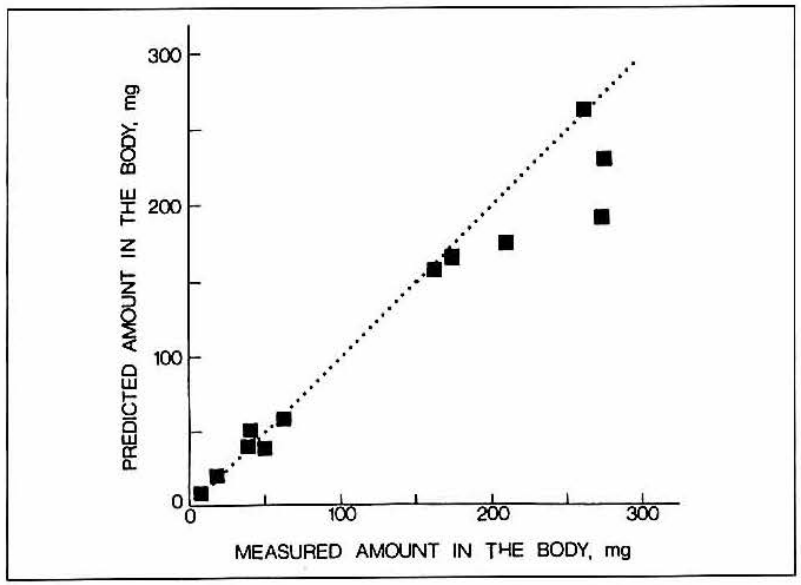

FIGURE 2-2. Correlation of gentamicin accumulation in the body determined by pharmacokinetic analysis of serum concentration data (see Figure 2-1) and by direct analysis of body tissues obtained at autopsy from the same patients who were evaluated pharmacokinetically prior to death. Dotted line indicates perfect correlation. Data from references 15 and 16.

obesity may cause unusual changes in drug disposition and require specific study and notation in patient surveys.

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 permit some experimental procedures to be omitted. However, it is the investigator's obligation to adequately assess the literature, to avoid unwarranted assumptions, and to satisfy the demands of experimental strategies that would resolve a proposed hypothesis.

DRUG ASSAYS

Certainty 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. Microbiological assays are notoriously unreliable. Other antibiotics often interfere in detection of the main drug. Active metabolites such as the desacetyl form of some cephalosporins[21] may be included in the measurements unless prior separation is made of the two active compounds. An extreme case of metabolite inclusion is in the 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 pharmacokinetic specificity. Microbiologic, enzymatic, and radioimmunoassays 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 upon written request.

Sample Handling. Coupled with assay reliability is concern for the stability of drug in biological specimens, even in the frozen state. Ampicillin is unusual in that it is less stable frozen than when refrigerated.[22] Some drug esters such as hetacillin (a prodrug of ampicillin) continue hydrolyzing in blood and during the bioassay, and imprudent handling of the blood specimens can confound the true disposition profiles of both prodrug and drug. Penicillamine is unstable in the presence of plasma proteins, and immediate deproteination after blood sample collection is necessary to avoid loss of reduced penicillamine prior to analysis.[23] Measurement of drug stability in blood will complement the pharmacokinetic characterization of drug by revealing

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 specimen 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:

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

where k_o is the infusion rate and C_{ss} is the steady-state plasma or serum 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 and cost-effective methods are available for designing optimal sampling strategies for kinetic experiments where, such as in the clinic, the number of specimens is limited.[25] Optimal designs largely depend on the likely "true" model parameter values, the structure of the model, and the measurement error. A sequential approach has been advocated with pilot studies and a sampling schedule which distributes time points over the major phases of drug disposition as the first step. Subsequent experiments can then be designed to 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 in order 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 (Figure 2-1).

The two summary 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 the sample volume is large and urine concentrations often exceed plasma values by one or more orders of magnitude.

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

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

The arithmetic mean time is acceptable for slow processes, but errors will be incurred if the kinetic process produces rapid changes in plasma concentrations.[27] It is common to miss an early exponential phase of drug disposition because of infrequent blood sampling. For a polynomial curve with intercepts C_i and slopes λ_i, the total AUC is:

$$AUC = \Sigma\, C_i / \lambda_i \qquad \text{(Eq. 2-3)}$$

If the initial distributive phase is missing (area = C_1 / λ_1), then the error incurred in calculation of a clearance parameter (CL = dose / AUC) is

$$\%\ \text{of CL error} = 100 \times (C_1 / \lambda_1) / AUC \qquad \text{(Eq. 2-4)}$$

BASIC PHYSIOLOGIC PARAMETERS

The evolution of complete physiologic models[1] 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 employment of partial physiologic models for description of pharmacokinetic data. One such model is shown in Figure 2-3. 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 in blood or plasma (C_p) is considered to be part of the central compartment (V_c). The minimum value of V_c 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_c value often exceeds V_p.

Drug which is 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:

$$V_T = \Sigma\, K_{pi} \cdot V_{ti} \qquad \text{(Eq. 2-5)}$$

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