Progress in Drug Metabolism

Volume 4

Edited by
J. W. Bridges

L. F. Chasseaud

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J. W. Bridges

Institute of Industrial and Environmental Health and Safety, and Department of Biochemistry. University of Surrey

L. F. Chasseaud

Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre

A Wiley—Interscience Publication

Preface

Drug absorption and drug measurement are the two main themes of this volume of *Progress in Drug Metabolism*.

The many different aspects and implications of the former are discussed in the first three chapters. There is no doubt that despite the information available on this subject, insufficient attention is still accorded to the role and impact of drug absorption in some important areas such as drug safety evaluation and therapeutics.

As amplified in the preceding volume of this series and emphasized in this volume, liquid chromatography, in less than a decade, has become established as an invaluable technique for the measurement of drug concentrations in biological materials. It is appropriate also to include a companion review of the important contribution of gas–liquid chromatography to drug measurement.

J. W. BRIDGES
L. F. CHASSEAUD

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CHAPTER 1

Pharmacokinetics as a tool in drug development

S. A. Kaplan and M. L. Jack

Department of Pharmacokinetics and Biopharmaceutics, Hoffmann–La Roche Inc., Nutley, New Jersey 07110, U.S.A.

INTRODUCTION

Pharmacokinetics is an applied scientific discipline that achieves its greatest potential when considered during the early stages of drug development. It encompasses the relationships between the physicochemical properties of a drug and both its physiological disposition by the organism and its pharmacological response.

Since oral administration is the route of choice for the administration of most drugs, properties such as low aqueous solubility, slow dissolution, permeability limitations, gastrointestinal degradation, and biotransformation may contribute to the bioavailability characteristics observed following oral administration. These parameters can be studied individually and many expected problems can be avoided or overcome prior to clinical testing of the drug.

The value of pharmacokinetic studies during the early stages of drug development is to enable critical decisions to be made as to which form of the active compound should be recommended for the time-consuming and costly

animal tolerance, formulation design, and clinical studies. This is an important consideration early in the drug development programme since subsequent modification of the drug, even if it is as apparently simple as micronization or salt formation, may require repetition of many studies.

At the onset of drug development, it is important to have the limits of absorbability of a drug defined, that is, the maximal or optimal rate and extent of absorption obtainable with the drug in its most readily available form. Delivery systems must be used to standardize drug absorption, especially if the drug is not optimally absorbed. This is essential since a slow absorption rate may result in an erratic and variable drug level profile that may not be reproducible from administration to administration. Finally, in the design of pharmacokinetic studies, each drug must be considered individually since the various physicochemical properties, intended therapeutic uses, and physiological disposition characteristics of each drug will influence the study design. Therefore, drug development programmes must consider not only the pharmacological and toxicological characteristics of the potential drug but also the pharmacokinetic properties.

Such drug development programmes are by their nature multi-disciplinary in scope, and systematic coordination of the various disciplines is required. This text will focus on such a multi-disciplinary programme in which the scientific investigators responsible for defining and evaluating the pharmacokinetic and bioavailability characteristics of a drug are intermediary in the programme.

PHARMACOKINETICS

Pharmacokinetics is the study of the rate processes associated with the absorption, distribution, metabolism, and excretion of a drug, i.e. quantitating drug and/or metabolite(s) levels in body fluids, tissues, and excreta at any point in time from the moment of administration until elimination from the body is complete.

The primary standard of a pharmacokinetic study is based upon the data obtained following intravenous administration. Since the entire dose administered is placed directly into the bloodstream following intravenous administration, the pharmacokinetic parameters obtained are unaffected by all the potential rate-limiting and metabolic factors associated with drug absorption following other routes. Subsequent to obtaining the primary pharmacokinetic parameters, the drug absorption parameters following other routes of administration may be evaluated and defined in relation to the intravenous standard.

Bioavailability is defined as a measure of the rate and extent of absorption of an administered dose into the systemic circulation. This can be reported in absolute terms based on an intravenous standard or in relative terms by comparison to a standard which may or may not have a known absolute bioavailability. A relative or comparative bioavailability study is also referred to as a bioequivalence study.

In pharmacokinetics, attempts are made to describe a biological event(s) in mathematical terms, usually by developing mathematical models. In the early stages of drug development, the simplest model or curve-fitting procedures that are compatible with the observed data should be used. Extensive pharmacokinetic analyses are generally not necessary until clinical protocols are designed. The assessment of drug absorption is of prime importance during the initial drug development studies. In this respect, preclinical pharmacokinetic parameters obtained following intravenous administration can be utilized in the evaluation of data obtained following oral administration so that the distribution, metabolism, and excretion parameters can be separated from those of absorption. Such *in vivo* absorption parameters defined in animal studies will be shown to allow for meaningful predictions of the absorption characteristics of a drug in man, even when the preclinical and clinical physiological disposition profiles are different. In addition, preclinical pharmacokinetic parameters such as distribution volume, elimination rate, and extent of recovery of intact drug in the urine are useful in evaluating *in vivo* pharmacological and toxicological studies, and also in planning for the first clinical studies. However, until clinical and preclinical parameters can be obtained and compared, such additional pharmacokinetic evaluations should be kept simple.

In the evaluation of intravenous blood, serum, or plasma level data the first assumption to be made and tested is that the drug is eliminated exponentially, i.e. via first-order kinetics. This assumption, which is valid in most instances, is easily verified. This section will deal with drugs eliminated via first-order kinetics. Other elimination profiles will be discussed later.

First-order kinetics can be demonstrated by plotting the logarithm of blood, serum, or plasma levels *versus* time using semi-logarithmic paper and observing visually or by curve-fitting computer techniques that the curve is linear. The pharmacokinetic profile is determined as a function of the number of exponential phases observed in the drug level–time curve. In most instances, the data will be found to be mono-, bi-, or triexponential. The mathematical considerations associated with the curve-fitting procedures are beyond the scope of this discussion and are presented in detail by Gibaldi and Perrier (1975) and Wagner (1971).

The most useful pharmacokinetic parameters obtained following intravenous administration are half-life, clearance, area under the drug level–time curve, and volume of distribution. The half-life of a drug is simply the time required to reduce the postabsorption concentration of drug in the bloodstream by 50% (Wagner, 1971, Gibaldi and Perrier, 1975). This parameter is calculated by dividing 0.693 by the calculated slope of each exponential phase of a multiphasic blood level curve, i.e.

$$t_{\frac{1}{2}} = \frac{0.693}{\text{slope}}$$

In figure 1, the half-life for the monoexponential curve was calculated to be 10.0 hours.

Whereas the half-life of a drug tends to be relatively constant in any given animal or human subject, the half-life for many drugs can vary between subjects and species as a function of sex, age, disease state, and environment. Variation of half-life as a function of dose suggests non-linear (zero-order) pharmacokinetics and/or prolonged absorption or formation of the compound under study (Wagner, 1971; Gibaldi and Perrier, 1975).

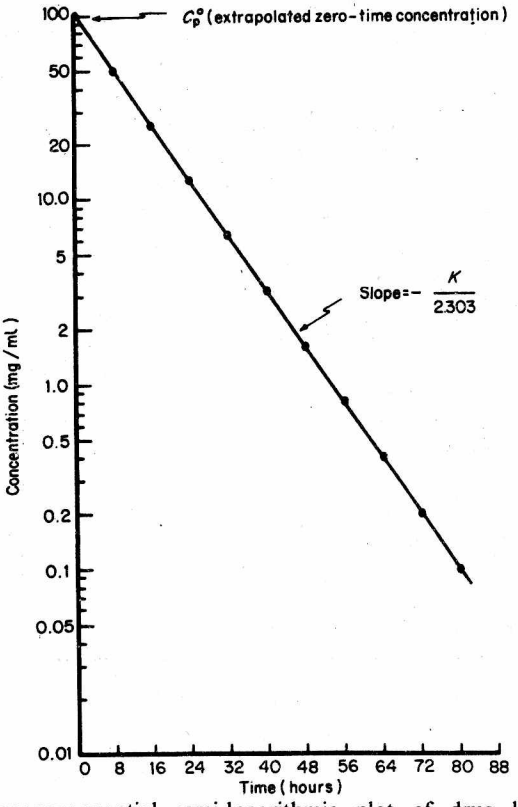

Figure 1 A monoexponential semi-logarithmic plot of drug level *versus* time (K = elimination rate constant)

Another pharmacokinetic parameter in frequent use is the area under the curve of blood level *versus* time. The methods of calculating area are discussed by various authors (Wagner, 1971; Gibaldi and Perrier, 1975). The principle of area analysis has been used as a means of assessing the bioavailability of a drug. The area under a blood level–time curve reflects the amount of drug reaching the systemic circulation. Many authors have used area analysis in order to evaluate

the extent of absorption (Wagner and Nelson, 1963, 1964; Kaplan *et al*, 1973b). Kaplan *et al* (1973b) used the principle of area analysis to develop a pharmacokinetic pathway and to obtain the rate constants associated with the proposed pharmacokinetic model.

Total body clearance is the sum of the individual clearances of the drug by the various organs and tissues. Total body clearance (TBC) is calculated by

$$\text{TBC} = \frac{\text{Dose or amount absorbed}}{\text{Area under curve } (0 \rightarrow \infty)} = \text{Volume of distribution} \times \frac{0.693}{t_{\frac{1}{2}}}$$

When a drug is eliminated from the body via hepatic metabolism and urinary excretion, then TBC is defined as the sum of renal and hepatic clearances. If a drug is eliminated via hepatic metabolism only, then hepatic clearance is equal to the TBC. Similarly, when drugs are eliminated exclusively via the kidney, the renal clearance is equal to the TBC.

The volume of distribution (V_d) is inversely proportional to blood level, i.e. the larger the volume of distribution the more extensively the drug distributes from the bloodstream into the various tissues, organs, and binding sites of the body. There are various ways of calculating V_d depending on the pharmacokinetic profile of the drug (Wagner, 1971, 1975; Gibaldi and Perrier, 1975).

The pharmacokinetic parameters obtained following single-dose administrations may be used to predict the steady-state blood levels following chronic

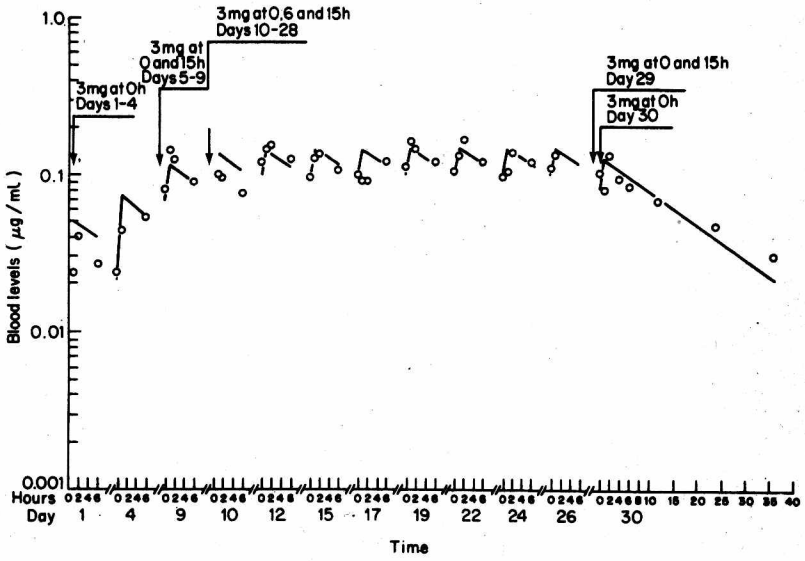

Figure 2 Oral blood level data for bromazepam in a human subject receiving a chronic dosing regimen as indicated for 30 days. The solid lines represent the simulated blood level curve. From Kaplan *et al* (1976)

administration (Boxer *et al*, 1948). The calculated and experimental steady-state blood level profiles of bromazepam, a drug exhibiting linear first-order disposition characteristics, are presented in figure 2. Such calculations may be used to determine dosing regimens which will maintain a desired blood level range, and permit the determination of dosing regimens which may be safely used in a diseased state in which the disposition profiles of a drug may be altered.

A number of digital computer programs are available for the nonlinear least-squares estimation of pharmacokinetic parameters and for simulation of the time-course of drug levels as a function of input variables. Some programs, such as Continuous System Modeling Program (CSMP) and MIMIC do not have least-square regression analysis capabilities but do permit simulation of data, while others such as NLIN, SAAM, and NONLIN have both capabilities (see references for details).

NONLIN is a versatile and widely used program for pharmacokinetic evaluations. Once the nature of the elimination kinetics is determined, NONLIN fits the terminal data by the nonlinear least-squares methods to the appropriate equation, e.g. first-order or Michaelis–Menten. Least-squares estimates of the

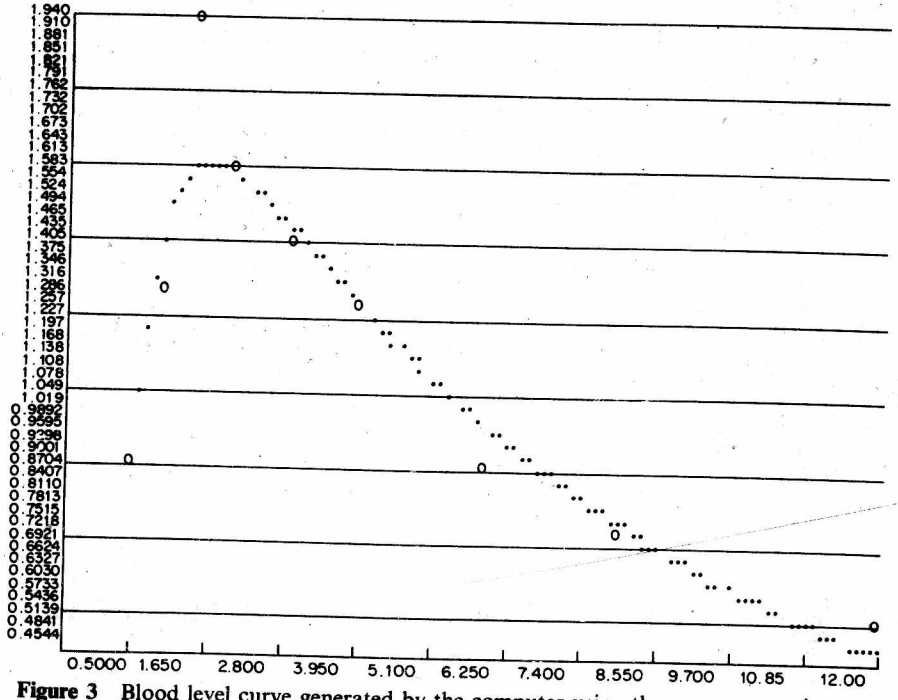

Figure 3 Blood level curve generated by the computer using the parameter estimates obtained from NONLIN. The O's are the experimental data points (ordinate, drug concentrations in blood; abscissa, time)

NONLIN PROGRAM
SUMMARY OF NON-LINEAR ESTIMATION

X	OBS. Y	CALC. Y	FUNCTION 1 OBS-CALC	% DEVIATION	WEIGHT	WEIGHTED RESIDUAL
0.50000	0.88000	0.85598	0.24016E-01	2.73	1.1446	0.25693E-01
1.0000	1.2900	1.4585	−0.16852	−13.06	0.78080	−0.14891
1.5000	1.9400	1.6013	0.33867	17.46	0.51919	0.24403
2.0000	1.5900	1.5881	0.18692E-02	0.12	0.63348	0.14877E-02
3.0000	1.4300	1.4406	−0.10617E-01	−0.74	0.70435	−0.89101E-02
4.0000	1.2700	1.2736	−0.36379E-02	−0.29	0.79309	−0.32397E-02
6.0000	0.88000	0.98539	−0.10539	−11.98	1.1446	−0.11275
8.0000	0.75000	0.76134	−0.11342E-01	−1.51	1.3430	−0.13144E-01
12.000	0.52000	0.45443	0.65569E-01	12.61	1.9370	0.91256E-01

CORRECTED SUM OF SQUARED OBSERVATIONS = 1.628356
SUM OF SQUARED DEVIATIONS = 0.1593374
SUM OF WEIGHTED SQUARE DEVIATIONS = 0.1036891
R−SQUARED = 0.936 COR = 0.951
S = 0.1440063 WITH 5 D.F.

Scheme I Output generated from the NONLIN program. Columns one and two are the input data, blood/plasma levels (OBS. Y), and their corresponding times (X)

parameters, standard deviation, and confidence intervals are part of the computer printout. The observed X and Y values, the calculated Y values and the percentage difference between the observed and calculated Y values are also included in the printout. A typical example of the input and output factors for NONLIN is presented in Scheme I.

Columns 'X' and 'Obs. Y' are input data which were fitted to a biexponential

AFTER 6 ITERATIONS THE ESTIMATES AND THEIR VARIABILITY ARE:

NO.	ESTIMATE	STD. DEV.	95% CONFIDENCE LIMITS	
1	1.64635	0.717039	−0.196834	3.48953
			−1.67790	4.97059
2	0.213566	0.137117	−0.138898	0.566031
			−0.422116	0.849248
3	0.164810	0.318131E-01	0.830332E-01	0.246587
			0.173224E-01	0.312298
4	1.20000	0.135760	0.851022	1.54898
			0.570606	1.82939

NO.		% CV
1	UNIVAR	43.5533
	S PLANE	
2	UNIVAR	64.2032
	S PLANE	
3	UNIVAR	19.3029
	S PLANE	
4	UNIVAR	11.3133
	S PLANE	

ALPHA = 0.24528003E 01
ALPHA HALF-LIFE = 0.28259415E 00
BETA = 0.12901000E 00
BETA HALF-LIFE = 0.53728159E 01
K21 = 0.19200000E 01
K12 = 0.49700000E 00
KEL = 0.16481030E 00
F = 0.11999988E 01
VP = 0.12888000E 05
DOSE = 0.26700000E 05
KA = 0.16463460E 01
KA HALF-LIFE = 0.42102146E 00
BETA/KEL = 0.78277877E 00
AUC = 0.15084198E 02
CP-ZERO = −0.43368087E-18
TBC = 0.21240752E 04
VD-EXTRAP = 0.16722042E 05
VD-SS = 0.16224112E 05
VD-AREA = 0.16464422E 05
KEL*AUC = 0.24860312E 01

Scheme II Computer output containing a summary of pharmacokinetic parameters generated by the NONLIN program

equation with a weighting factor of 1/Y. The calculated drug levels are reported in Scheme I, 'Calc. Y'

The slopes and intercepts, parameters 1, 2, 3, and 4 reported in Scheme II, result from the nonlinear least-squares fit of the data. The remaining pharmacokinetic parameters in Scheme II are calculated based on the NONLIN estimates of the slopes and intercepts. Parameters 1, 2, 3, and 4 are used to generate the theoretical drug level curve as shown in figure 3.

NONLIN, therefore, permits the calculation and simulation of pharmacokinetic profiles based on the drug level–time profile obtained following the administration of the drug under investigation.

BIOPHARMACEUTICS

Physiological and Pharmacokinetic Variables

The primary pharmacokinetic parameter of concern in a drug development programme is the absorption profile or bioavailability of a drug. The study of the relationships of the physicochemical properties of drug substances and their delivery systems, to absorption and hence bioavailability into the systemic circulation, following all routes of administration, except for the intravenous route, is the subject of the discipline of biopharmaceutics.

Since oral administration is the route of choice for the administration of most drugs, *in vitro* screening procedures that are predictive of individual *in vivo* processes are useful in identifying potential bioavailability problems during the initial stages of drug development. Following oral administration, the *in vivo* rate and extent of absorption of a drug depend upon a number of physicochemical and physiological factors. Evaluation of these parameters can provide information as to the nature of a bioavailability problem. Once the physicochemical properties associated with impaired absorption are identified, appropriate attempts to overcome such problems pharmaceutically and/or chemically can commence.

Physicochemical properties of a drug that directly affect its rate and extent of absorption include aqueous solubility (Gibaldi, 1977), stability (Kaplan, 1973), particle size (Dittert *et al*, 1968), crystal size (Paul *et al*, 1967), polymorphism (Aguiar *et al*, 1967), pK_a (Schanker, 1964), and partition coefficient (Hogben *et al*, 1959). Bioavailability problems associated with the physicochemical factors and/or chemical degradation of the drug can usually be overcome by proper control of the drug substance and the formulation of the drug.

A compound that is being screened as a drug must be chemically stable in both the solid state and in solution. The stability of drug in the pH range of the gastrointestinal tract as well as in the delivery system is of concern. This degradation can vary depending on the rate and extent of drug absorption. Drugs which are unstable in the physiological pH range may degrade to inactive products prior to absorption, resulting in incomplete bioavailability of the

administered drug, e.g. erythromycin derivative (Nelson, 1962). Conversely, prodrugs such as clorazepate (Abruzzo *et al*, 1977) and some of the penicillins (Tuano *et al*, 1966) require degradation in the gastrointestinal tract to render the active moiety available for absorption.

An orally administered solid drug must dissolve in the gastrointestinal tract prior to absorption across the gastrointestinal mucosa. Although it may not always be feasible to ascertain the potential effects of a low aqueous solubility and slow dissolution rate on the absorption of a drug candidate at an early stage of drug development, certain biopharmaceutical and bioavailability judgements can be made.

It has been suggested that a minimum aqueous solubility of 1% be considered a guideline relative to predicting the potential of solubility-limited absorption problems (Kaplan, 1974). The 1% solubility 'rule-of-thumb' limit is an arbitrary guideline which might alert the investigator to the potential of dissolution rate-limited absorption. However, other factors such as solubility and intrinsic dissolution rate as a function of pH within the physiological pH range of 1 through 8 should also be considered. If the solubility decreases with increasing pH, the potential for incomplete absorption is greater, especially if dissolution is not attained rapidly in the more acidic environment of the stomach. The solubility and dissolution rate must also be considered as a function of the intended size of the therapeutic dose. The smaller the projected therapeutic dose the less reliable the 1% factor becomes, since very small doses of even highly insoluble drugs may be well absorbed. Therefore, despite the complexity in interpretation, studies on the intrinsic dissolution rate of moderately and poorly soluble drugs over the physiological pH range are useful in assessing the potential for problems pertaining to dissolution rate-limited absorption. Various suitable dissolution techniques are reported in the literature (Swarbrick, 1970; Kaplan, 1974). Digoxin is probably the most widely studied drug *vis-à-vis in vitro* dissolution–*in vivo* absorption correlations (Mattok *et al*, 1977; Reissell *et al*, 1977), and it is believed to be a drug for which an *in vivo* prediction of bioequivalence can be made from *in vitro* dissolution data. Figures 4 and 5 exemplify the correlation between the percentage of drug dissolved in 30 and 60 minutes and the 24-hour urinary excretion of digoxin.

Although a rank order correlation exists, with the exception of product B, an asymptote to the line, i.e. a cut-off point, is missing. A simple rank order correlation merely confirms that dissolution is an absorption rate-limiting factor. The steepness of the slope of most of these correlation curves indicates that we are dealing with a moving target. Therefore, a correlation in which the line does not asymptote so that a minimum *in vitro* dissolution rate can be established is inappropriate as an *in vitro* predictor of the *in vivo* bioavailability of a new formulation. However, such a correlation may be valid as a quality control tool to assess reproducibility of an established product.

More often, *in vitro* dissolution data do not correlate with *in vivo* data. For