EVALUATION OF DRUG CANDIDATES FOR PRECLINICAL DEVELOPMENT

Pharmacokinetics, Metabolism, Pharmaceutics, and Toxicology

Edited by

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PREFACE

In the past two decades, the pharmaceutical industry has experienced tremendous transformation. There have been significant scientific advances with the potential to revolutionize the treatment of human disease. Advanced technologies and automation have increased efficiency in the laboratory. Productivity of the industry as a whole, however, has not met the high expectations of society. As mature products lose patent protection pharmaceutical companies have struggled to fill gaps in their pipelines. Reorganization in the industry is commonplace; a wave of mega-mergers is under way as this book goes to press. Despite these challenges, small biotechnology companies and academic researchers continue to enter the fray, and competition in the industry remains fierce. Outsourcing of diverse discovery and development activities is increasingly common as the industry attempts to minimize infrastructure and maximize financial flexibility. These adaptations reflect the high attrition rates experienced during development, increasing costs, and the increased expectations of society that new medicines will be safe, effective, and affordable. It is in this complex and dynamic context that we edit this book on the preclinical evaluation of drug candidates.

We believe that selecting the "right" drug candidate for development is key to success. To lower attrition rates during early clinical development, pharmaceutical as well as pharmacological properties of the molecule should be optimized. This undertaking requires good science, perseverance, and often luck. There is precedence that the evaluation and optimization of pharmacokinetic properties early in drug discovery has a positive impact on the effort to lower attrition rates. We believe this example can be extended further and that a comprehensive evaluation of candidate developability at an early stage is an essential step.

This book presents three major scientific areas: pharmacokinetics and drug metabolism, pharmaceutical development, and safety assess-

ment. The various properties of a new chemical entity are typically evaluated by groups of scientists with diverse backgrounds and exquisite specialization, often working in isolation. Given the great potential for experimental findings in one discipline to profoundly influence outcomes in another, integration is essential. Our goal is not to emphasize the leading edge of science and technology but rather to stress the integration of activities and information essential for the advancement of new medicines during drug development. We expect this book will enhance the formulation of appropriate strategies for compound progression and improve decision-making. We hope this book will be valuable to readers from academia, industry, and service organizations, and thank the contributors for their dedication and patience.

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CHAPTER 1

INTRODUCTION

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The challenges faced by the pharmaceutical industry in the twenty-first century are potentially overwhelming. Nonetheless, there remains substantial demand for new medicines to address unmet medical needs. The global market for pharmaceuticals is growing. For cardiovascular, endocrine, metabolic, respiratory, neurological, infectious diseases, and oncology, the market is expected to exceed $500 billion by 2012.[1] The cost of drug development also is continuing to increase. The R&D expenditures for a single new chemical entity approach $1 billion.[2] Overall, attrition during drug discovery and development remains high. Thousands of compounds may be profiled before a development candidate emerges and only 1 or 2 in 10 that initiates testing in humans, is expected to reach the market.[3] The process overall may take 10–15 years. Despite R&D expenditures of $48 billion by Pharmaceutical Research and Manufacturers of America member companies in 2007, US drug approvals were the lowest in 24 years.[4]

Today, scientists in pharmaceutical R&D face unprecedented pressure from payers, regulators, ethicists, and the public, to bring to market safe and effective drugs while reducing costs. As recent events attest, even after having received regulatory approval, idiosyncratic drug reactions or infrequent adverse safety events may lead to "black-box" warning labels or potentially the removal of a drug from the market all together.[5,6] Serious adverse events may be extremely difficult to detect during the course of drug development given the numbers of patients

Evaluation of Drug Candidates for Preclinical Development: Pharmacokinetics, Metabolism, Pharmaceutics, and Toxicology, Edited by Chao Han, Charles B. Davis, and Binghe Wang

involved in pivotal clinical trials and the relative homogeneity of these patient populations. Despite numerous challenges, sponsors need to anticipate the most likely asset profile, as early as possible, to make intelligent investment and portfolio decisions. Resource must be minimized for compounds less likely to progress through development. Given the increased costs associated with late phase development terminations, "fail early and fail cheap" has become the mantra for many in drug discovery.

Routine use of absorption, distribution, metabolism, and elimination (ADME) screening in drug discovery has successfully reduced attrition due to poor human pharmacokinetics from about half of all development failures in 1990,[7] to approximately 10% presently.[3] Experimental ADME screening remains a cost effective and robust way to assure a thorough understanding of the desired and undesired biological effects of a new chemical entity in animals and humans. For this, sufficient free drug concentrations must be maintained at the site of action, for an appropriate period of time, to enable a thorough evaluation of biological effects. This finding is as critical for comprehensive animal toxicology studies as it is for successful, decision-making clinical investigation.

This book describes powerful experimental approaches employed today by modern laboratories within pharmaceutical R&D, biotechnology companies, and academia to characterize ADME properties of drugs with a focus on small molecules. The primary *in vivo* and *in vitro* tools used to characterize a drug candidate are discussed. Included are theoretical and practical aspects of preclinical pharmacokinetics (in Chapter 2), the important role of transporters (Chapter 3) and the cytochromes P450 (Chapter 4), the role of metabolism and metabolite identification in drug discovery (Chapter 5), plasma protein binding (in Chapter 6), and the prediction of human pharmacokinetics (Chapter 7). Effort has been made to integrate the subject matter to account for important interdependencies. The concepts should be applied in a cross-functional manner and with due consideration of the context including potential clinical implications.

One of the most important sources of development termination today is animal safety. Our ability to predict toxicological effects of new drugs, particularly those that develop over time, continues to be limited due to the enormous complexity and dynamic nature of biological systems. Therefore, in conjunction with ADME, successful drug discovery depends on experimental toxicology. Chapters 9 and 10 of this book discuss general, genetic, and cardiovascular toxicology as it is applied in the drug discovery setting. Central to the field of

safety assessment is the consideration of the therapeutic window of a drug: the difference between exposure associated with the desired therapeutic benefit and exposure associated with adverse effects. Preferably, there is substantial separation between these drug exposures (a large therapeutic window) to permit safe and effective treatment for a heterogeneous patient population. The therapeutic window may decrease as the duration of dosing increases. Acute effects (desired and undesired) may differ from those observed with intermittent or chronic drug administration. The therapeutic window may or may not be conserved between preclinical species and humans (one reason to study multiple preclinical species). Different species may have different sensitivity to drug treatment (same effect at different exposures) or the biological effects themselves may differ from one species to another. The many challenges of early safety assessment include the provision of cost-effective *in vitro* and *in vivo* technologies that can be integrated into the drug discovery process and are predictive of clinical outcomes.

Additionally we included a chapter (Chapter 8) on pharmaceutics, encompassing theoretical and practical aspects of the physical characterization of drug substance, the importance of selecting an appropriate version (parent or salt) of the chemical for development and formulation considerations for definitive animal safety studies, and initial clinical trials. When fully integrated within a drug discovery program, drug metabolism and pharmacokinetics, safety assessment, and pharmaceutical development will play a crucial role. Together, they will assure the best chance of success by building the appropriate properties into the drug molecule as early as possible in the process. They will help to identify potential liabilities as the asset progresses, as well as areas for further specialized study. This is the nature of the developability assessment.

It is important not to underestimate the interrelatedness of these developability activities in drug discovery. Understanding and addressing issues at the interfaces can have a significant impact on the development plan, the time and resource involved in the activities, as well as the success of the program overall. For example, as previously indicated, animal safety studies will need to be performed to evaluate the full range of biologic effects including exaggerated pharmacology and off-target effects, acute and chronic, to appropriately manage potential liabilities. In many cases, prerequisites for this will include low to moderate *in vivo* clearance and acceptable oral bioavailability from a solid dosage form. This in turn will require well-characterized drug substance, a suitable formulation, and an understanding of

factors influencing the rate and extent of dissolution of drug at the absorption site.

Although some aspects of the process and strategy will be very similar from program to program, others will not. Development hurdles will differ depending on the therapeutic area, the availability of existing treatments, and ultimately the level of risk that may be acceptable given the potential benefit to the patient (the risk/benefit ratio). Therefore, the lead optimization strategy, including the staging of assays and the acceptance criteria will adjust accordingly. An analgesic or antibiotic may require relatively higher free drug concentrations thus rapid dissolution, high intestinal permeability, and low protein binding may be required. Some drugs will need to effectively penetrate the blood–brain barrier (e.g., an anticonvulsant). For other drugs, it may be desirable to have limited brain penetration. On this basis, assays to assess central nervous system (CNS) penetration may be included in the screening cascade.

Drugs administered intravenously will require relatively higher solubility and will need to have limited hemolytic potential. An asthma drug may be inhaled directly into the lungs and therefore relatively higher metabolic clearance may be desirable to minimize potential systemic effects. Others drugs will be used to treat a chronic condition (e.g., osteoporosis) and may be taken for many years on a regular basis. In this case, a longer biological half-life may be desirable. Some drugs will be taken in combination with others [e.g., antiretrovirals for human immunodeficiency virus (HIV) infection]. For these, it may be particularly important to study cytochrome P450 enzymology, to minimize the potential for drug–drug interactions. For diseases where there are limited or no therapeutic alternatives, convenience of administration will be less important. For life-threatening illnesses, there may be less of a concern regarding manageable side-effects, long-term or reproductive toxicities. Therefore, drug discovery strategy should be customized following thoughtful consideration of the desired product profile.

How does this complex process begin? In the earliest phase of drug discovery, a biological target (receptor, enzyme) is identified and its relationship to the disease process is elucidated. As confidence builds that inhibition of the target represents a valid approach for therapeutic intervention, assays are developed and a high-throughput screen is conducted. Libraries containing potentially millions of chemicals are tested for their ability to inhibit the target and hits are identified. When hits are deemed an appropriate starting point, lead optimization begins. During lead optimization, the structure of chemical leads is modified

to optimize potency, selectivity, cell-based activity, pharmaceutical, and ADME properties while assuring structural novelty that will form the basis of successful patent applications.

Patents provide market exclusivity for the innovator for a defined time period after which generic drug companies can manufacture and sell the same active ingredient. They must establish bioequivalence with the innovator's product (a statistical analysis of the rate and extent of absorption in humans). In so doing, they avoid conducting extensive clinical trials to evaluate safety and efficacy, which have been demonstrated previously by the innovator. The situation is more complicated for biologics since these products tend to be heterogeneous, and it is generally not possible to demonstrate chemical identity to the innovator's product. Regulatory agencies around the world are developing strategies for approval and marketing of well-characterized biologics given the potential for substantial savings and increased benefit to patients and society.

During lead optimization, a team of scientists including chemists, biologist, and drug metabolism and PK experts will work closely together to develop an appropriate screening cascade. This is a series of assays of various priority and throughput that are performed sequentially to optimize compound properties. Higher throughput assays designed to measure and incorporate the most critical attributes of the molecule are typically performed earlier in the screening cascade and require relatively smaller amounts of compound for testing. More detailed and resource intensive studies take place subsequently on a more limited number of promising compounds. These studies often require a larger quantity of drug for testing. It always requires some work to be performed in parallel, at risk, to avoid unnecessary delay. Turn-around time becomes critical in such a cascade because test results influence the subsequent round of chemical synthesis and biological testing, the order that compounds may be studied subsequently, and their priority for scale-up and further evaluation.

Assays with insufficient capacity to accommodate leads that have passed previous tests have the potential to become a bottleneck. Although assays may be redeveloped or resources redeployed to improve the situation (or acceptance criteria changed), bottlenecks often persist or may move to other areas within the screening cascade. Scientists involved in profiling compounds during lead optimization will require perseverance and creativity to adjust their experimental approaches to meet the needs of the program. Appropriate distinctions will be made between assays used for more definitive assessments and predictions, compared to those used primarily for rank ordering or

screening compounds. Thus, drug discovery assays will be fit for this purpose.

During lead optimization there will be occasions when a particular challenge presents itself and the team will need to pull together to address the challenge. Changes may need to be made in the screening cascade temporarily to solve a particular problem. Or, a parallel screening cascade may need to be put in place temporarily. Identifying and addressing these challenges will be critical for the success of the team, which requires strong leadership, excellent working relationships among team members, and thoughtful integration of data and information.

Various organizational models have proven successful in promoting collaboration and efficient decision making. In one model, the line functions [e.g., chemistry, biology, drug metabolism and pharmacokinetics, pharmaceutical development, and safety assessment] are separately managed. In this case, individuals are appointed to represent their discipline on a matrix program team and senior line management assures resources are aligned in a manner that is consistent with the overall strategic intent of the organization. In another model, smaller drug discovery units are dedicated to a therapeutic area or therapeutic approach and have, more or less, ring-fenced resource and potentially considerable autonomy. Typically, these drug discovery units include the minimal essential complement of scientists required considering the phase and maturity of the program (for lead optimization, often chemistry, biology, and DMPK). Ideally, these scientists are colocated to facilitate frequent discussion, interaction, and collaboration.

The former model may be more bureaucratic, accountability may be less clear, and loyalty may be split between the line function and the team. On the other hand, the larger line functions will likely have more specialized expertise and may be better able to respond to peaks and troughs in activity by reassigning staff to the most active and/or highest priority projects. In the latter model, the entrepreneurial model, there may be a greater sense of ownership, empowerment, and engagement. Of course, another model that has developed recently matches various aspects of the above with an aggressive outsourcing strategy. In this case, much of the laboratory work is performed by contract research organizations (CRO). More often than not, the CRO is located in a market where the cost of labor may be substantially lower than in the United States or western Europe.

In any case, it is inevitable that as teams advance compounds further into development, substantially more resource will be required and more discussion and debate will take place to assure organizational

consensus, as well as continued commitment to the project and the underlying development plans. Most teams will eventually require expertise and resource outside of their direct control and thus the importance of skilled matrix management and team work should not be underestimated. The most successful teams will take full advantage of expertise on and off the team, tapping into know-how and experience where ever it may exist. Transparency and communication will be critical as issues often arise within one area that have the potential to impact strategy and planning in another.

One of the major challenges discovery and development teams will face is to assure that there is an appropriate balance between what needs to be done now and what can be done later. The critical path must be well defined and there must be consensus around what activities are most essential in advancing the program to the next major decision point. What activities need to be completed when and at what cost? What activities can be postponed without affecting the critical path? What kinds of enabling activities need to be considered? What are the issues and risks associated with delaying a resource intensive study? What is the asset profile and how does it compare to the desired product profile? In a world of limited time and resource, these types of questions need to be considered proactively and on an on-going basis as new data and information become available.

On behalf of my co-editors, Dr. Chao Han and serial editor, Dr. Binghe Wang, I would like to take this opportunity to thank the contributing authors for sharing their considerable scholarly expertise, for their tireless effort preparing their contributions, and for their patience as this monograph was compiled. We hope our readers find this book to be relevant if not insightful and we wish you the best of fortune in your journey to bring important new medicines to patients.

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PART I

PHARMACOKINETICS AND DRUG METABOLISM IN DRUG DISCOVERY

CHAPTER 2

PHARMACOKINETICS IN PRECLINICAL DRUG DEVELOPMENT: AN OVERVIEW

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2.1 INTRODUCTION

At its most basic level, the interaction of a drug with its target receptor for activity is almost always associated with a definable concentration

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versus response relationship. Usually, these target receptors take the form of macromolecular entities, usually proteins. Other entities including messenger ribonucleic acid (mRNA), or other forms of nucleic acid [e.g., deoxyribonucleic acid (DNA) as part of genes and chromosomes], may also be the foci of a pharmacodynamic change in response to presence of a drug. In most cases these drug–receptor interactions occur within cells of the body, which with the exception of the blood cells, are usually fixed as part of tissue structures. For this reason, a precise tissue drug concentration versus effect relationship may not be readily discernable due to the practical issues involved in obtaining tissue samples after dosing. Such study designs are by nature destructive and are not ideal for *routine* characterization of a drug–receptor interaction and response.

In tandem with this reality, there is also a relationship between the concentrations of the drug in the blood and the concentrations of the drug in the tissues in which the target pharmacologic receptors might reside. This relationship is possible because in order for a drug to be considered to possess systemic availability, it must first find its way into the posthepatic blood. Blood is an important compartment in the body because it is the primary fluid that connects all tissues of the body as a circuit. It transports nutrients (including oxygen) to the cells, and removes byproducts of cellular metabolism. It also helps to maintain homeostasis by performing its essential buffering functions. Another role is to act as a transport pathway for hormones, which allows specific endocrine tissues to influence the biochemical processes of anatomically far removed tissues. In a manner akin to hormone transport, the blood also serves as a conduit by which drugs can be introduced directly, as in the case of intravenous administration, or absorbed from the intestinal tissues (oral route), skin (transdermal route), or depots (intramuscular or subcutaneous injection) into the blood, where it can be transported to the tissue possessing receptors. This cascade is illustrated in Figure 2.1.

The processes that dictate the magnitude of plasma concentrations in response to a given dosage of a drug fall into the general realm of pharmacokinetics (PK). Pharmacokinetics encompasses the processes that are related to what the body does to the drug when the two come into contact with one another. The four basic PK processes are absorption (input) of drug into the body, distribution of drug through the body, metabolism of drug by the body, and excretion of the drug from the body. The moniker usually used to denote the processes is "ADME"; namely, the absorption, distribution, metabolism, and excretion of drugs. In recent times, another subset of processes has been

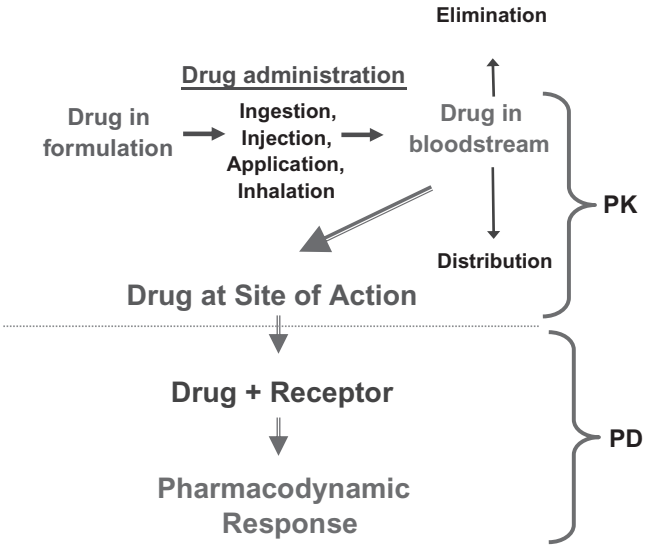

Figure 2.1. The link between pharmacokinetics (PK) and pharmacodynamics (PD).

introduced into this scenario and the moniker ADMET has been coined, wherein the "T" represents the transport of drug across cell membranes, facilitated by specialized protein. Conceptually, however, transport processes might be considered to be part of the subprocesses involved under the wider umbrellas of absorption, distribution, and excretion of drugs. Hence, the use of the term ADMET could be viewed as being superfluous.

Pharmacokinetics incorporates a wide body of knowledge, and borrows extensively from many disciplines including biochemistry, physiology, mathematics, physical pharmacy, and chemistry. The underlying foundation for the need for PK information during the development of new drug candidates is the concentration in blood fluids versus effect relationship. Pharmacokinetic information may aid in the decision-making processes pertinent to selection of a lead compound for further development.

The purpose of this chapter is to provide an introduction to PK in a general sense, including a discussion of the different processes involved in the PK of a drug, with special focus on the use of pharmacokinetics in preclinical studies. The chapter will begin with some basic PK concepts and follows with some discussion of the place of PK data in lead selection decision making.

2.2 BASIC KINETIC PROCESSES INVOLVED IN MOVEMENT OF DRUG

Drug movement into, through, and from the body can be separated into zero- and first-order types of processes. The nature of the differences between these sorts of kinetic processes are readily seen when dealing with PK data, which typically takes the form of concentrations measured in blood, plasma, or serum at different time points after administration of a dose.

Zero-order processes are those that proceed at a constant rate and are independent of concentration. When the concentration versus time data are plotted on linear scaled graphs, a straight line can be drawn through the concentration or amount versus time data points (Fig. 2.2). If the same data is plotted on semilog graph paper (i.e., paper where the *x*-axis plot representing time is linear, and the *y*-axis representing

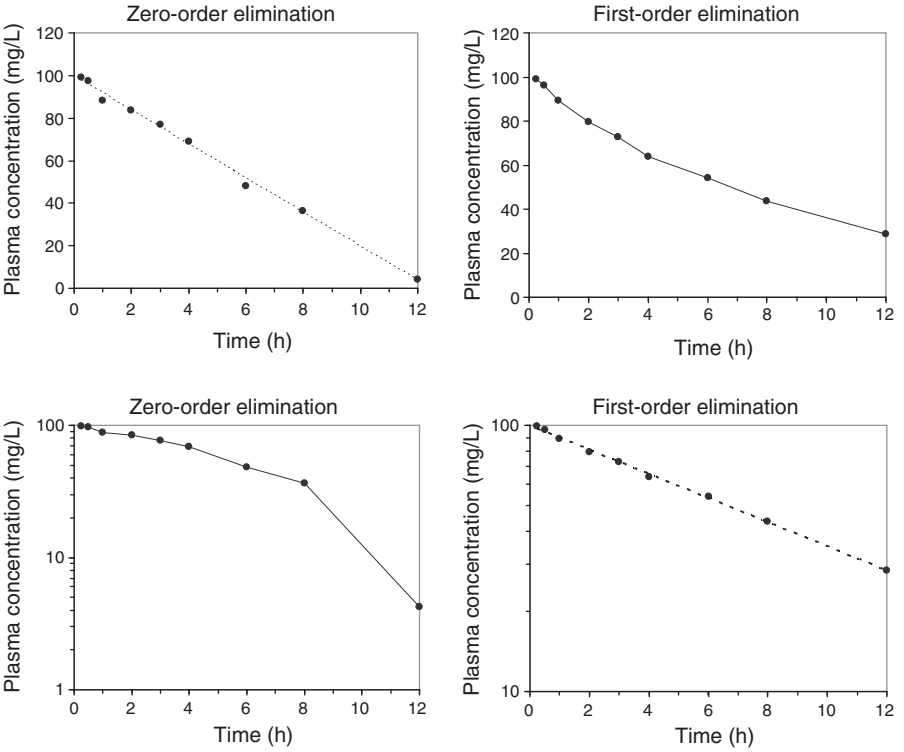

Figure 2.2. Differences between zero (constant rate) and first order (concentration-dependent rate) elimination kinetics are readily apparent when concentration versus time data are plotted on linear (top panels) or semilog graph paper (lower panels). Dotted lines represent best-fit lines extrapolated using regression analysis.

concentration is log-transformed), then curvature is observed (Fig. 2.2).

In contrast to zero-order processes, first-order processes proceed at a rate that is fractional in nature (Fig. 2.2). As an example of a first-order process, let us assume that we have 100 mg/L of drug in the body, and over each hour, 10% of the drug present in the body at the beginning of the hour is removed. The net result is a curved line through the data points when plotted on a linear plot, but a linear line through the data points when plotted on semilog graph paper.

In PK, first-order decline in blood fluid concentration versus time is most frequently observed. In first-order kinetics, the mechanism is either one of passive movement of drug, or one that involves a facilitative protein/enzyme for transport or metabolism, but where the concentrations are so low that the majority of the protein-binding sites are unoccupied with drug. In essence, the concentrations of drug are far below the concentration where the process occurs at maximal rate (i.e., far below the Michaelis–Menten (k_m) affinity constant of the process).

Mechanistically, zero-order processes always require an energy-consuming facilitative protein/enzyme to proceed, which are capable of transporting drug against a concentration gradient. Further, they are observed only when the concentrations are at a high enough level whereby essentially all of the binding sites on the protein are occupied by the drug. In contrast to first-order processes, there are few drugs that behave according to true zero-order concentrations after therapeutic doses of a drug. A good example of a compound that displays zero-order elimination with ingestion of normal dose levels in humans is ethanol.[1]

2.3 PHARMACOKINETIC METHODOLOGY

2.3.1 Compartmental Models

In order to allow for an understanding of the processes involved in the constitution of the pharmacokinetics of a drug, or to allow for predictions of blood fluid concentrations in the presence of altered conditions or changes in dosage, compartmental models can be used to quantitatively describe drug disposition (Fig. 2.3). The rationale for classical compartmental modeling is based on differences in rates of tissue uptake of drug, which is related to permeability and physicochemical properties of the drug, and perhaps even more importantly, differences in blood perfusion through organs. If a drug has good permeability

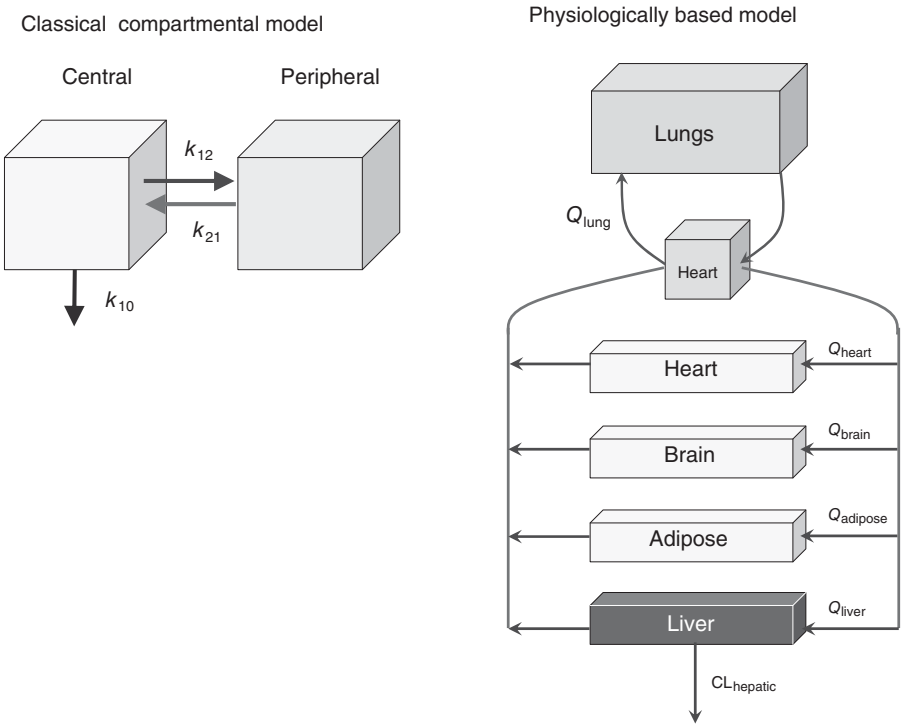

Figure 2.3. Examples of two basic types of PK models. Classical compartmental models "lump" tissues that behave similarly from a distribution perspective into nonspecific compartments. Intercompartmental transfer events are described by micro-rate constants. Physiologically based models typically represent specific tissues as discreet compartments with varying volume terms. Rather than rate constants, these models include blood flows into and out of the organs. Although both have their advantages and disadvantages, both can be used to predict the relationship between dose and plasma concentrations.

characteristics into most of the tissues into which it will be taken up, and if the blood flow going through those tissues is high, then a rapid uptake of drug will ensue. In this case, uptake is almost instantaneous, and as a consequence, if the drug follows first-order kinetics, a single straight line can best describe the decline in concentrations when a semilog concentration versus time plot is used. This hallmark presentation of a drug follows a one-compartment open model. On the other hand, many drugs penetrate significantly not only into well-perfused tissues, but also medium or poorly perfused tissues. In these cases, curvature will be present in the log concentration versus time plot. These sorts of models are multicompartmental. The number of com-

partments involved (i.e., the number of different tissue types based on blood flow) can be identified using, most reliably, nonlinear curve-fitting programs, or by manual graphical manipulation (method of residuals). The judge of model fit can be made using visual assessment of predicted to actual data, and objective statistical criteria, such as Akaike Information Criterion, Schwartz Criteria, and sum of least squares, or a combination of all of these.[2]

Once an appropriate model is selected, the compartmental estimates of PK parameters are based on the estimated data points from the model fitting, rather than the actual measured data as reported by the drug analysis laboratory. There are a number of compartmental equations that are used for estimation of volume of central compartment, area under the concentration versus time curve, area under the concentration versus time curve (AUC), clearance, and so on. Compartmental modeling is a very useful tool for obtaining data that can be used to predict plasma concentrations in response to a change in a rate constant, or for predicting plasma concentrations obtained with repeated dosing of a drug.

A unique type of modeling used in PK, which is arguably more rational than classical compartmental modeling, is physiologically based modeling (Fig. 2.3). This approach still makes use of compartments in the model structure. However, rather than lumping tissues in a compartment in an empirical way based on similarities in rate of tissue penetration, physiological-based PK modeling uses compartments to represent specific organs.[3] Actual organ volumes may be incorporated into the model, with unknowns being the unbound fraction in the tissues. Another difference from classical compartmental modeling is that the physiologically based model links compartments by blood flows into and from the organ. In contrast, classical compartmental modeling typically links tissues in a mammillary design with arrows representing movement into and out of compartments, with the arrows representing a rate or rate constant. Conceptually, physiologically based models are more true to the actual situation, although there level of complexity raises some issues with respect to validation of the model.

2.3.2 Noncompartmental Methods

Because compartmental methods require a derived model that may or may not be valid, in most applications of PK, especially for drug discover in pharmaceutical R&D, it is most common to see the use of noncompartmental methods to estimate parameters. This approach is

truly descriptive, and its major advantage is that the actual data is used, with no need to worry about model choice. Noncompartmental approaches to PK require AUC to be calculated by the trapezoidal rule, which in turn is used to calculate clearance (CL) and volume of distribution of drug at steady state (V_{dss}) using an approach that does not rely on any specific predefined model. This finding is a major advantage, in that validation of a model is not necessary; one simply uses the data as is to gain the important parameters that best describe the PK properties of the drug (CL and V_{dss}). It must be recognized that noncompartmental methods are not useful for the purpose of predicting a plasma concentration versus time curve. This result is best achieved by use of an appropriate PK model and compartmental fitting.

2.4 PHYSIOLOGICAL PROCESSES AND RELATED CONSIDERATIONS INVOLVED IN PHARMACOKINETICS

2.4.1 Absorption of Drug

With the exception of the intravenous (iv) and intraarterial (ia) routes, all other routes of drug administration are associated with an absorption step. These include parenteral injection via the subcutaneous, intramuscular and intraperitoneal routes, inhalation, transdermal, and most importantly due to its ease, safety and frequency of use, the oral route.

The half-life ($t_{1/2}$) of a drug after iv or ia administration is a reflection of the distribution and elimination properties of a drug. A theoretical terminal half-life is determined when the distribution phase is complete. However, after dosing by a route with an absorption step it is possible for the terminal phase $t_{1/2}$ to represent the absorption rate constant, rather than elimination rate constant of the drug. This finding is often referred to as the "flip–flop" phenomenon.

2.4.1.1 Absorption and Nonoral Routes of Administration. In the intramuscular and subcutaneous routes, the drug is directly injected into the muscle or under the layers of the skin, respectively, from where it is absorbed into either the adjoining capillaries or the lymphatic drainage.[4] Highly lipophilic or large molecules tend to gravitate toward lymphatic absorption. When a drug is injected into the peritoneal cavity, it is mostly absorbed by the mesenteric blood system lining the serosal side of the intestinal tract. Although the normal absorption steps and enteric metabolism or efflux is largely avoided, the drug is