9

Biliary Excretion

Biliary excretion can be an important hepatic elimination pathway for many drugs (Yamazaki *et al.,* 1996).

9.1. RELATIONSHIP BETWEEN HEPATIC AND BILIARY CLEARANCES

Hepatic clearance (Cl_h) of a drug is the sum of its hepatic metabolic clearance (Cl_{hm}) and its biliary clearance (Cl_{bl}):

(9.1) $$\boxed{Cl_h = Cl_{hm} + Cl_{bl}}$$

Cl_{bl} of a drug can be experimentally determined by measuring the amount of unchanged drug excreted in bile over an extended period of time:

(9.2) $$Cl_{bl} = \frac{\text{Amount of unchanged drug excreted in the bile from time 0 to t}}{AUC_{0-t,iv}}$$

$AUC_{0-t,iv}$ is the AUC in blood from time 0 to t and t is usually more than 24 hr for sufficient bile collection after intravenous administration of a drug.

9.2. SPECIES DIFFERENCES IN BILIARY EXCRETION

Rats and dogs are perhaps the most efficient biliary excretors, while guinea pigs and monkeys are very inefficient. The limited evidence available also suggests that humans are not efficient excretors for compounds with intermediate molecular weights.

Molecular-weight-threshold-theory: Important physicochemical properties of compounds governing the extent of biliary excretion include molecular weight, charge, lipophilicity (log P), and molar refractivity (MR). There have been attempts to establish a correlation between the molecular weight of a compound and the

Table 9.1. Molecular Weight Threshold for Biliary Excretion of Organic Anions and Cations in Different Species

	Species			
Compounds	Rat	Guinea pig	Rabbit	Human
Organic anions	325	400	475	500
Organic cations				
Monovalent	200 with little or no species variation			
Bivalent	500 with little or no species variation			

extent of its biliary excretion in animals (Hirom *et al.,* 1974). The findings from these studies have indicated that there might be a threshold for the molecular weight of a compound subject to relatively extensive biliary excretion. Rough guidelines for the molecular-weight threshold for biliary excretion of organic anions and cations among different species are summarized in Table 9.1. For a compound with a molecular weight well below the threshold, there will be more extensive renal elimination than biliary excretion. A compound of intermediate molecular weight (325–465) tends to be excreted in both the urine and the bile.

9.3. ACTIVE TRANSPORTERS FOR BILIARY EXCRETION

Several active transport systems for endogenous and exogenous substrates have been identified in the canalicular membrane of hepatocytes (Müller and Jansen, 1997; Smit *et al.,* 1995). These active transporters may have a significant effect on biliary excretion of certain organic compounds. In particular, recent studies with gene knock-out animals have revealed the physiological and toxicological functions of some of the transporter systems. A brief description of various transporters and their substrates is provided in Table 9.2.

Table 9.2. Various Active Transport Systems Located in Canalicular Membranes of Hepatocytes and Their Substrate Specificity

Transporters[a]	Substrate specificity	Known substrates
cBAT	Organic anions	Monovalent bile acids
MDR1	Organic cations	Daunomycin
MDR3	Organic cations (?)	Phosphatidyl choline
MRP1	Organic anions	Glutathione, glucuronide, and sulfate conjugates: dinitrophenol S-glutathione, leukotriene C_4 (CTC_4), oxidized glutathione, calcein
	Organic cations (?)	
MRP2 (cMOAT)	Organic anions	Glutathione, glucuronide, and sulfate conjugates
	Organic cations (?)	

[a]cBAT: canalicular bile acid transporter; cMOAT: canalicular multispecific organic anion transporter; MDR: multidrug resistance; MDR1, MDR3: *MDR1* and *MDR3* gene products, respectively (also known as P-glycoprotein); MRP: multidrug resistance-associated protein.

9.3.1. P-Glycoprotein

P-glycoprotein (P-gp or gp-170) is a multidrug resistance *(MDR)* gene product with a molecular weight of about 170 kDa, and functions as an ATP-dependent drug efflux pump in the membrane that lowers the intracellular concentrations of amphiphilic cytotoxic drugs (Silverman and Schrenk, 1997). P-gp is one of the causes of multidrug resistance of cancer cells to a wide range of chemotherapeutic agents. It is interesting that P-gp is also present in normal cells. Their locations are confined in the lumenal domains of cells in some (excretory) organs such as liver, intestine, kidney, and brain, and their physiological functions appear to be related to active transport of organic cations. There are two *MDR* gene products in normal human cells, i.e., MDR1 and MDR3, and three *mdr* gene products in rat and mouse, ie., mdr1a, mdr1b, and mdr2. MDR1, mdr1a, and mdr1b confer drug resistance on otherwise drug-sensitive cells, but MDR3 and mdr2 do not. Recent studies suggested that in normal hepatocytes MDR1 and MDR3 mediate active biliary excretion of hydrophobic organic (cationic) compounds and phosphatidylcholine across the canalicular membranes of the hepatocytes, respectively (Smit *et al.,* 1995).

9.3.1.1. Common Physicochemical and Structural Characteristics of P-Glycoprotein Substrates

Substrates for P-gp, especially the MDR1 gene product, appear to be bulky organic cations at physiological pH. Important general physicochemical and structural features are:

1. Molecular weight >400.
2. Log P >1.
3. The presence of at least one planar aromatic ring moiety, which can interact with hydrophobic P-gp drug binding domains.
4. Preferably, cationic at physiological pH, e.g., through amine moiety of the molecule that can be protonated at physiological pH.

9.3.1.2. Overlapping Substrate Specificity of Cytochrome P450 and P-Glycoprotein

It has been found that there is a significant overlap of the substrates for cytochrome P450 3A (CYP3A) and P-gp, including a wide range of hydrophobic (chemo)therapeutic agents, and their tissue distribution patterns. These findings suggest that CYP3A and P-gp may play complementary roles in drug disposition via metabolism and active secretion of drugs, especially in the villi of the small intestine, where CYP3A and P-gp can act synergistically as a barrier against the oral absorption of drugs (Wacher *et al.,* 1995; Zhang *et al.,* 1998).

9.3.2. Multidrug Resistance-Associated Protein

Multidrug resistance-associated protein (MRP) was first detected in some multidrug-resistant cell lines, in which no overexpression of either P-gp nor mRNA

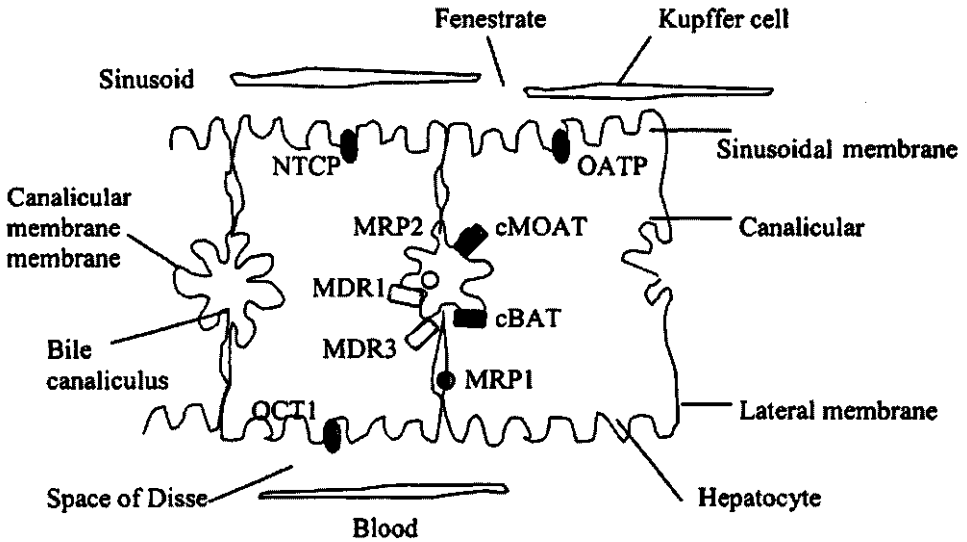

Figure 9.1. Current concept of carrier-mediated transport systems for organic cations and anions at the sinusoidal, the lateral, and the canalicular membrane domains of hepatocytes. cBAT: canalicular bile acid transporter; cMOAT: canalicular multispecific organic anion transporter; MDR1, MDR2: multidrug resistance gene products (in human hepatocytes), MRP1, MRP2: multidrug resistance-associated proteins; NTCP: Na^+-taurocholate co-transporting polypeptide; OATP: organic anion transportingtt polypeptide; OCT1: organic cation transporter.

from the encoding MDR1 gene could be detected. Later, it was found that MRP is a member of the ATP binding cassette (ABC) family of transporters along with P-gp, and the MRP gene can confer drug resistance in tumor cells. The molecular weight of MRP is about 190 kDa and it has a broad spectrum of substrates (Kusuhara *et al.,* 1998; Meijer *et al.,* 1997). MRP is also found in normal cells and its tissue distribution patterns are similar to those of P-gp, including the liver (canaliculus), erythrocyte membranes, heart, kidneys, intestinal brush border membranes, and lungs.

At least two MRP isoforms, MRP1 and MRP2, have been found in human and rodent hepatocytes. MRP1 is present in lateral membrane domains of normal hepatocytes at a very low level, whereas MRP2 is localized exclusively in canalicular membranes of hepatocytes (Fig. 9.1). MRP1 is known as an ATP-dependent glutathione S-conjugate transporter and functions as an ATP-dependent transporter for amphiphilic organic anions such as leukotriene C_4 (LTC_4), dinitrophenol S-glutathione, and calcein. It has been suggested that MRP1 functions as a transporter not only for glutathione S-conjugates but also for glucuronide and sulfate conjugates of various compounds. MRP2 is also known as the canalicular multispecific organic anion transporter (cMOAT), and it has been suggested that it functions as an ATP-dependent transporter for various amphipathic organic anions as well as organic cations in a glutathione-dependent manner.

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10

Nonlinear Pharmacokinetics

In general, pharmacokinetic profiles of most drugs over therapeutic concentration ranges are concentration-independent. This concentration-independent pharmacokinetics is often referred as linear or first-order kinetics, implying that the rate of change in drug concentration is *proportional to the drug concentration* without saturation of any kinetic processes. At high concentrations, however, drug pharmacokinetics tends to change in a concentration-dependent way owing to saturation of certain processes (Jusko, 1989; Ludden, 1991). In this chapter, nonlinear pharmacokinetics will be defined and explained and the ways in which it can be elucidated from experimental data will be discussed.

10.1. DEFINITIONS

Any pharmacokinetic process of a drug, i.e., absorption, distribution, metabolism, and excretion, that cannot be adequately described with first-order (linear) kinetics of drug concentrations can be considered nonlinear kinetics. In other words, nonlinear pharmacokinetics implies deviations in the rate of change in the amount (or concentration) of a drug in any physiological or experimental system (whole body, organs, or compartments) from first-order kinetics in a dose (or concentration)- and/or a time-dependent manner.

10.1.1. Dose Dependency

Dose dependency in exposure generally indicates that the dose-normalized plasma drug concentrations or the dose-normalized AUC is not constant and depends on the dose levels. This can be due to transient saturation of any enzyme or carrier-mediated process such as metabolism or active transport at high doses (or concentrations). For instance, if clearance processes of a drug can be saturated at high concentrations, the dose-normalized AUC values after intravenous administration at high dose levels are higher than those at lower dose levels. Dose dependency can be viewed as transient and reversible at high drug doses (or concentrations), which reverse at low doses (or concentrations). Dose (or concentration)-dependent nonlinear kinetics is also called “capacity-limited” kinetics, and can be often described with the Michaelis–Menten equation.

10.1.2. Time Dependency

Time dependency in exposure implies that dose-normalized individual concentrations (or AUC) of a drug after multiple dosing or at a particular (reference) time after single dosing differ from those after single dosing or at a different time after single dosing at the same dose, respectively. A major distinctive feature of time dependency as opposed to dose (or concentration) dependency is that it results from actual physiological (or biochemical) changes or differences with time in the organ(s) associated with the corresponding disposition parameters of the drug. For instance, when a drug induces its own metabolism (autoinduction), its exposure levels decrease after multiple dosing as compared to those after single dosing owing to an actual increase in the amount of metabolizing enzymes with time. Another type of time-dependent pharmacokinetics is the so-called "chronopharmacokinetics," which describes the effects of rhythms in physiological or biochemical functions of the body on the pharmacokinetic behavior of a drug in a time-dependent manner (Levy, 1982).

10.2. MICHAELIS–MENTEN KINETICS

Generally, the initial rate of an enzyme reaction such as biosynthesis of an endogenous compound or metabolism of a xenobiotic is directly proportional to the concentration of the substrate at low concentrations. However, as the substrate concentration increases, the reaction reaches a certain maximum rate. In the simplest case, the overall processes of an initial enzymatic reaction can be described by followingscheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

In brief, the substrate (S) binds to free enzyme molecule (E) and forms an enzyme–substrate complex (ES), which then can either dissociate back to E and S, or further break down to E and product (P); k_1 and k_{-1} are the association and dissociation rate constants, respectively; and k_2 is the rate constant for the production of P. During the early stages of the reaction, the reverse process, i.e., E + P→ ES, is assumed to be negligible, because the concentration of P is essentially zero. The equation describing the *initial* rate of reaction as a function of the substrate concentration can be derived from this scheme, and is known as the Michaelis–Menten equation [Eq. (10.1)], first proposed by Henri in 1903 and originally formulated from the equation describing a simple in vitro enzymatic reaction. This rather simple equation has been found to be extremely useful for describing apparent nonlinear plasma concentration–time profiles of many drugs in both *in vivo* and *in vitro* situations.

MICHAELIS–MENTEN EQUATION:

(10.1)
$$v = \frac{V_{max} \cdot C}{K_m + C}$$

C is the original concentration of the substrate ([S]) at the beginning of the reaction; *v* is the *initial* rate of reaction [substrate depletion (–dC/dt), or product formation (dP/dt)]; K_m is the Michaelis–Menten constant ($[E] \cdot C/[EC]$); and V_{max} is the maximum rate of the reaction ($k_2 \cdot [E]_t$), with $[E]_t$ being the total concentration of the enzyme.

In practice, consumption of no more than 5% of the original amount of substrate is considered acceptable for estimating the initial reaction rate with the original substrate concentration at the beginning of a reaction to satisfy the Michaelis–Menten equation. K_m is inversely related to the affinity between the substrate and the enzyme, i.e., the smaller the K_m value, the stronger the affinity between the substrate and the enzyme, and vice versa. K_m is also equal to the substrate concentration at which the rate of the process is half of $V_{max} \cdot V_{max}$ would be attained at infinite substrate concentration where all the enzymes are saturated with the substrate and present as ES, and is directly proportional to the total enzyme concentration. The important assumptions required for the Michaelis–Menten equation include:

1. Only a single substrate and a single enzyme–substrate complex with 1-to-1 stoichiometry between substrate and enzyme are involved, and the enzyme–substrate complex breaks down directly to form free the enzyme and the product.
2. The reaction rate is measured during the very early stages of the process so that the reverse reaction from the enzyme and the product to the enzyme–substrate complex ($E + P \rightarrow ES$) is negligible.
3. The substrate concentration is significantly higher than the enzyme concentration so that the formation of an enzyme–substrate complex does not alter the substrate concentration to any significant extent.

10.3. PHARMACOKINETIC IMPLICATIONS OF MICHAELIS–MENTEN KINETICS

For most drugs, the rate of change in the amount of drug in the body after dosing is governed primarily by enzymatic reactions such as metabolism or carrier-mediated transport including, e.g., absorption, biliary excretion, and renal secretion. Therefore, most pharmacokinetic processes can in principle be subject to nonlinear kinetics. Despite the fact that the Michaelis–Menten equation is most applicable to *in vitro* reactions, it can also be used to describe apparent nonlinear plasma concentration *vs.* time profiles of a drug *in vivo,* if the body behaves like a single compartment [Eq. (10.2)]. It is important to note that K_m and V_{max} estimates based on *in vivo* data are *apparent* values. This is because unlike *in vitro* experiments, numerous factors other than enzymatic reactions cause nonlinear kinetics in *in vivo* situations (Cheng and Jusko, 1988):

$$-\frac{dC_p(t)}{dt} = \frac{V_{max,app} \cdot C_p(t)}{K_{m,app} + C_p(t)} \tag{10.2}$$

$C_p(t)$ is the concentration of the drug in the plasma at time t; $-dC_p(t)/dt$ is the rate of drug disappearance in the plasma at time t over a short period of time, dt; $K_{m,app}$ is the apparent Michaelis–Menten constant; and $V_{max,app}$ is the apparent maximum rate of elimination of the drug. Under two different extreme conditions, Eq. (10.2) can be simplified into either first- or zero-order kinetics as shown below.

10.3.1. First-Order Kinetics

When drug concentrations are substantially lower than $K_{m,app}$ (usually $C_p(t) < 0.1 \times K_{m,app}$), the rate of change in the plasma drug concentration becomes a first-order function of the concentration, i.e.,

(10.3)
$$-\frac{dC_p(t)}{dt} = \left(\frac{V_{max,app}}{K_{m,app}}\right) \cdot C_p(t) = k \cdot C_p(t)$$

In this case, the rate of drug disappearance is directly proportional to its concentration, and, thus, the plasma concentration–time profile of the drug follows first-order kinetics with a rate constant of V_{max}/K_m.

10.3.2. Zero-Order Kinetics

When plasma drug concentrations are substantially higher than $K_{m,app}$ (usually $C_p(t) > 10 \times K_{m,app}$), the value at which the enzymatic process can be saturated, the rate of change of the drug concentration becomes independent of the concentration itself

(10.4)
$$-\frac{dC_p(t)}{dt} = V_{max,app}$$

Under these conditions, the rate of drug disappearance is constant, i.e., zero-order kinetics.

10.3.3. Characteristics of the Plasma Concentration–Time Profile of a Drug Subject to Michaelis–Menten Kinetics

Figure 10.1 illustrates plasma drug concentration–time profiles on a semilogarithmic scale after intravenous injection in a one-compartment body system, for which the slopes are shallower at initial high concentrations than at low concentrations. These apparent concentration-dependent changes in the slopes indicate that at high concentrations during the initial phase, drug elimination is saturated and governed by zero-order kinetics, but as the concentration decreases during the later phases, the drug elimination processes follow first-order kinetics.

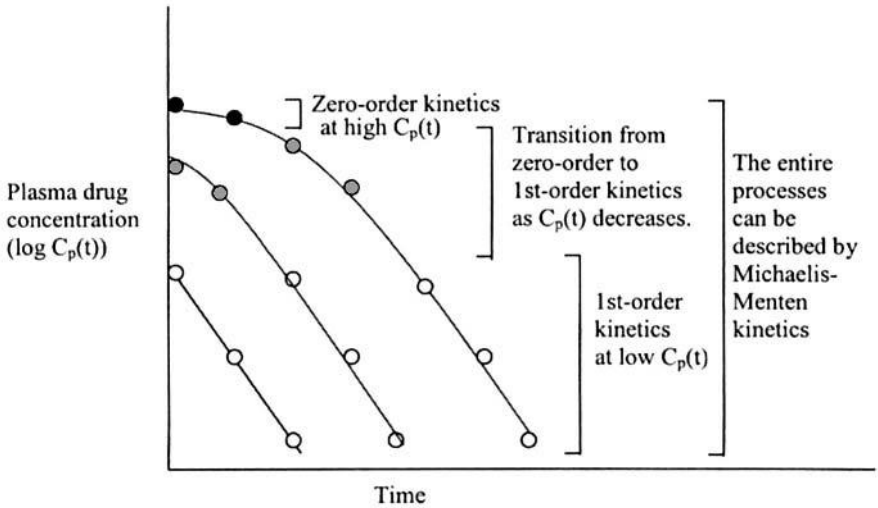

Figure10.1. Semilogarithmic plasma concentration–time profiles of a hypothetical drug after intravenous injection at three different doses. Elimination of the drug is assumed to be mediated by an apparent single Michaelis–Menten process. A one-compartment system is assumed for drug disposition. At drug concentrations much higher than the apparent Michaelis–Menten constant ($K_{m,app}$) (●) the slopes of the plasma concentration–time plot are shallower and more variable than those at concentrations much lower than $K_{m,app}$ (O), which become steeper and constant. At the intermediate concentrations (◍), the transition of shallow slopes at high concentrations toward the steeper slope is noticeable as the concentration decreases.

10.3.4. Estimating $V_{max,app}$ and $K_{m,app}$ from the Plasma Concentration–Time Profile *In Vivo*

The values of $V_{max,app}$ and $K_{m,app}$ can be estimated directly from log $C_p(t)$ *vs* time profiles following the intravenous administration of a drug, when its disposition profiles can be adequately described with a one-compartment model and a single Michaelis–Menten equation, according to Eqs. (10.5) and (10.6):

(10.5)
$$\frac{V_{max,app}}{(2.303)\cdot K_{m,app}} = \frac{\log[C_0^*/C_p(t)]}{t}$$

and

(10.6)
$$K_{m,app} = \frac{C_0}{(2.303)\cdot \log(C_0^*/C_0)}$$

where C_0 is an estimated concentration at time zero obtained by backextrapolating the log $C_p(t)$ *vs.* time plot to time zero. C_0^* is a zero-time intercept of a plot extrapolated from the terminal log–linear portion of the log $C_p(t)$ *vs* time plot (Fig.10.2).

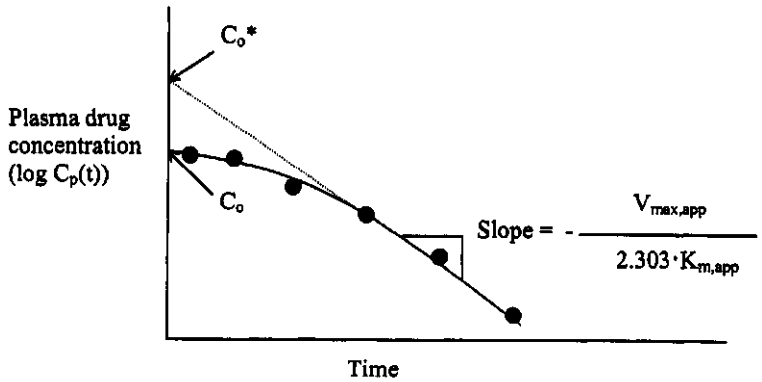

Figure 10.2. Estimate of $V_{max,app}$ and $K_{m,app}$ from a log $C_p(t)$ *vs.* time plot of a drug exhibiting a Michaelis–Mententype elimination after intravenous administration. The terminal slope of the plot equals $-V_{max,app}/(2.303 \cdot K_{m,app})$, and the estimates of C_0^* and C_0 by curve-fitting can be used to calculate $K_{m,app}$.

10.3.5. Systemic Clearance and Nonlinearity

When the elimination process of a drug can be described by simple Michaelis–Menten kinetics with a one-compartment model, based on Eq. (10.2), its systemic clearance (Cl_s) can be expressed as

(10.7)
$$-\frac{dA(t)}{dt} = \underbrace{\frac{V_{max,app} \cdot V}{K_{m,app} + C_p(t)}}_{= Cl_s} \cdot C_p(t)$$

(10.8)
$$\boxed{Cl_s = \frac{V_{max,app} \cdot V}{K_{m,app} + C_p(t)}}$$

where A(t) is the total amount of the drug present in the body at time t [$A(t) = C_p(t) \cdot V$] and V is the volume of drug distribution. Depending on the relative magnitudes of $C_p(t)$ and $K_{m,app}$, Cls can be concentration-dependent (non-linear) or concentration-independent (linear),

10.3.5.1. First-Order Kinetics

Under the first-order kinetic conditions, i.e., at $C_p(t) << K_{m,app}$, Cl_s of the drug becomes concentration-independent and constant:

(10.9)
$$Cl_s = \frac{V_{max,app} \cdot V}{K_{m,app}} (= \text{constant})$$

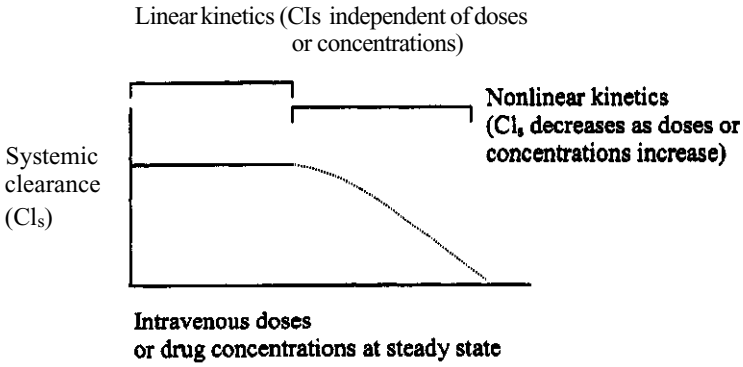

Figure 10.3. Potential changes in the systemic clearance of a drug as a function of an intravenous dose or plasma (or blood) drug concentration at steady state after continuous infusion, when elimination of the drug follows simple Michaelis–Menten kinetics, assuming a one-compartment body system.

10.3.5.2. Zero-Order Kinetics

Under the zero-order kinetic conditions, i.e., at $C_p(t) \gg K_{m,app}$, Cl_s of the drug is inversely related to Cp(t) and becomes a function of drug dose or concentration:

(10.10) $$Cl_s = \frac{V_{max,app} \cdot V}{C_p(t)}$$

Potential changes in Cl_s of a drug as a function of the intravenous dose levels (or concentrations at steady state after continuous infusion) are illustrated in Fig. 10.3.

10.3.6. Effects of Nonlinearity on Pharmacokinetic Parameters

Table 10.1 summarizes the potential effects of changes in various saturable pharmacokinetic processes at high doses or concentrations on several pharmacokinetic parameters, including clearance, volume of distribution, dose-normalized AUC, and terminal half-life of a drug.

10.3.7. Terminal Half-Life and Nonlinear Kinetics

Nonlinearity may or may not alter the terminal half-life ($t_{1/2}$) of a drug. If a drug's disposition profile can be readily described with simple Michaelis–Menten kinetics and a one-compartment system, its $t_{1/2}$ over the same concentration range should be the same regardless of the dose level. Thus, the differences in $t_{1/2}$ over the same concentration range at different dose levels can be indicative of the presence of nonlinearity in the system, which cannot be readily described with simple Michaelis–Menten kinetics, or a multicompartment system for the body. Assay sensitivity and product inhibition, which are two of the important factors in the apparent changes in $t_{1/2}$ at different dose levels, are discussed below.

Table 10. 1. Potential Effects of Changes in Saturable Pharmacokinetic Processes on Systemic Clearance (Cl_s), Volume of Distribution (V_{ss}), Dose-Normalized AUC (AUC/dose), and Terminal Half-Life ($t_{1/2}$) of a Drug, as Doses or Concentrations Increase.[a]

Pharmacokinetic parameter	Change at high doses	Potential effect on			
		Cl_s	V_{ss}	AUC/dose	$t_{1/2}$
Ratio of unbound and total drug concentrations in plasma	↑	↑	↑	↓	?
Intrinsic hepatic clearance	↓	↓	↔	↑ (likely)	↑
Dose-normalized rate of carrier-mediated intestinal absorption	↓	↔	↔	↓	↔
Concentration-normalized rate of carrier-mediated distribution into organs or tissues	↓	↔, ↓[a]	↔	↔, ↑	↔, ↑
Biliary or renal clearance	↓	↓	↔	↑ (likely)	↑

[a]Organ clearance can be affected by the rate of drug transport from the blood into the organ, when membrane transport of the drug is mediated by active carrier system.

10.3.7.1. Product Inhibition

If a metabolite(s) inhibits the biotransformation of its parent drug (product inhibition) *in vivo,* $t_{1/2}$ of the drug can increase with the dose. In order to verify product inhibition, metabolite in question can be identified and the initial drug elimination rates in the absence and presence of the metabolite can be compared in *in vitro* metabolism studies (Lin *et al.,* 1984; Perrier *et al.,* 1973).

10.3.7.2. Assay Sensitivity

In some cases, an apparent increase in a drug's $t_{1/2}$ with increasing doses may be due simply to limited assay sensitivity, which precludes detection of drug levels during the terminal phase at low doses in a multicompartmental body system. This is a rather common issue with drugs exhibiting multiexponential concentration–time profiles (Fig. 10.4). Important differences in definitions and terminology of linear and nonlinear pharmacokinetic processes are summarized in Table 10.2.

10.4. FACTORS CAUSING NONLINEAR PHARMACOKINETICS

Various factors for nonlinear kinetic behaviors in ADME processes and corresponding examples are summarized below.

Absorption

- Poor aqueous solubility and/or slow dissolution (griseofulvin).
- Site-specific absorption along the GI tract (phenytoin).

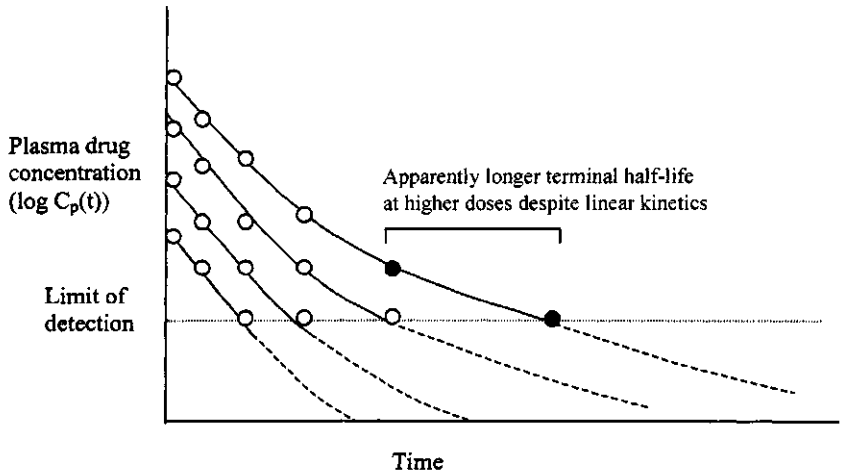

Figure 10.4. Limited assay sensitivity resulting in an apparent increase in the terminal half-life of a drug with increasing doses after intravenous administration in a multicompartment system. The dotted lines represent exposure levels of the drug below the limit of detection.

- Carrier-mediated absorption (riboflavin).
- P-glycoprotein efflux in intestinal epithelial cells (cyclosporin A).
- Saturable first-pass effect by the intestine and/or the liver (propranolol).
- Dose/time-dependent changes in gastrointestinal physiology including: (a) gastric emptying, (b) gastrointestinal motility, (c) gastrointestinal blood flow rate.

Distribution

- Nonlinear plasma protein binding (valproic acid).
- Carrier-mediated membrane transport (thiamine).
- Nonlinear tissue binding (prednisolone).

Metabolism

- Saturable metabolism (ethanol).
- Product inhibition (dicoumarol).
- Cosubstrate depletion (acetaminophen).
- Nonlinear plasma protein binding (prednisolone).
- Autoinduction.

NOTE: Capacity-limited metabolism is the most common and fundamentally important nonlinear mechanism in pharmacokinetics.

Excretion

- Nonlinear protein binding and/or glomerular filtration (naproxen).
- Carrier-mediated tubular secretion (cimetidine)/reabsorption(riboflavin).
- Carrier-mediated biliary excretion (iodipamide).

Table 10.2. A Summary of Definitions and Characteristics of Linear and Nonlinear Kinetics in a One-Compartment System

Kinetics	Definition	Elimination rate[a] $[-dC_p(t)/dt]$	Systemic clearance	Slope of plasma concentration–time profile after intravenous injection on a semilog scale	Synonyms
Linear	First-order kinetics	$k \cdot C_p(t)$ [proportional to $C_p(t)$]	Constant	$-k/2.303$	Dose (concentration- or time-) independent kinetics
Nonlinear[b]	Zero-order kinetics	k_0 (= constant)	Inversely related with $C_p(t)$	No definitive description available[c]	Dose (concentration- or time-) dependent, or capacity-limited kinetics
	Michaelis–Menten kinetics	$\frac{V_{max,app} \cdot C_p(t)}{K_{m,app} + C_p(t)}$	Constant at low $C_p(t)$ and inversely related to $C_p(t)$ at high $C_p(t)$	Changing from shallower slopes at high concentrations during the initial phase to steeper slopes reaching $-V_{max,app}/(2.303 \cdot K_{m,app})$ at low concentrations during the terminal phase	

[a] $C_p(t)$: concentration of drug in plasma at time t after intravenous injection; k, k_0: first-order or zero-order rate constants, respectively; $K_{m,app}$: apparent Michaelis–Menten constant; and $V_{max,app}$: apparent maximum rate of the process.
[b] Any process other than linear kinetics, e.g., zero-order or Michaelis–Menten kinetics.
[c] $C_p(t) = C_p(0) - k_0 \cdot C_p(0)$ is drug concentration at time 0 after intravenous injection.

Other

- Pharmacological effects of a drug that affect animal physiology, such as a decrease in blood flow rate after administration of antihypertensive agents.
- Chronopharmacokinetics: circadian rhythm or seasonal variability in kinetics
- Pathophysiological changes influencing pharmacokinetics of a drug, such as an increase in hepatic CYP2E1 content in diabetes or an increase in α_1-acid glycoprotein after surgery.

10.5. RECOGNIZING NONLINEAR PHARMACOKINETICS

When the system is linear, all the pharmacokinetic processes become concentration- and time-independent, and the pharmacokinetic parameters such as dose-normalized exposure levels (AUC/dose, AUMC/dose, and C_{max}/dose), t_{max}, $t_{1/2}$, Cl_s, V_{ss}, and MRT should remain constant regardless of the dose or concentration level. The most noticeable characteristic of nonlinearity of drug disposition is the lack of superimposibility of dose-normalized concentration *vs.* time profiles at different dose or concentration levels.

Principle of superposition (or dose proportionality): Superposition (also called "dose-proportionality") of drug disposition under linear conditions implies that dose-normalized drug concentrations or $AUC_{0-\infty}$ at different dose levels are the same. In other words, when the system follows linear kinetics, $C_p(t)$ or $AUC_{0-\infty}$ values at different doses become superimposible when they are dose-normalized:

$$\frac{C_p(t) \text{ or } AUC_{0-\infty}}{\text{Dose}} = \text{Constant} \tag{10.11}$$

On the other hand, nonlinearity can be viewed as "lack of superposition" of exposure levels at different dose levels. Virtually, all pharmacokinetic systems can be nonlinear at high dose (or concentration) levels owing to the saturation of various enzymatic or carrier-mediated processes. Figure 10.5 illustrates the relationship between dose normalized $AUC_{0-\infty}$ and dose levels under linear and nonlinear conditions.

In general, there is a more than dose-proportional increase in $AUC_{0-\infty}$ as a dose increases. Occasionally, however, an increase in $AUC_{0-\infty}$ with dose that is less than dose-proportional can be observed, which may be due to an increased systemic clearance associated with an increase in the unbound drug fraction at higher drug concentrations. In this case, a further increase in dose will eventually result in an overproportional increase in $AUC_{0-\infty}$ owing to saturation of the clearance mechanisms. Another example of an increase in $AUC_{0-\infty}$ after oral administration that is less than dose-proportional is impairment of absorption due to limited solubility of a drug or saturation of active transporter(s) in the intestinal membranes at high doses.

Pharmacokinetic profiles at a minimum of three different dose levels are required to adequately determine nonlinearity of a system. The dose-dependent changes of the following parameters are indicative of the presence of a nonlinear

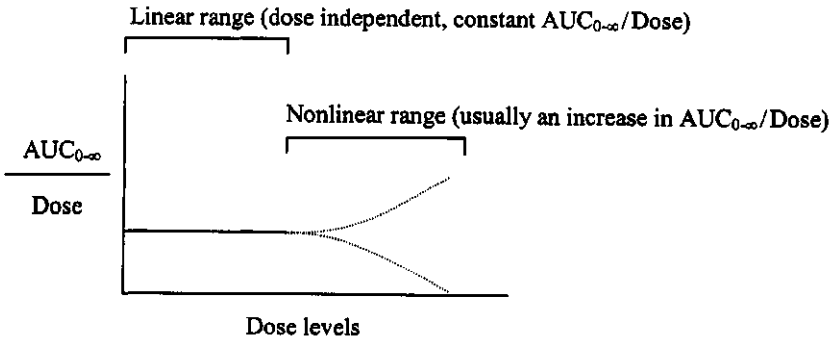

Figure 10.5. Schematic description of the relationship between dose-normalized $AUC_{0-\infty}$ and dose levels of a drug. At high dose levels, the dose-normalized $AUC_{0-\infty}$ can increase or decrease depending on the disposition properties of the drug.

pharmacokinetic system or process. Potential causes for the lack of superposition of those parameters are also discussed.

1. $C_p(t)$/dose: Some type of dose-dependence exists, but further study is needed to determine the causes for nonlinearity.
2. $AUC_{0-\infty}$/dose: Bioavailability or systemic clearance is nonlinear.
3. $AUMC_{0-\infty}$/dose: Absorption rate, Cl_S, or V_{ss} is nonlinear.

10.6. CHRONOPHARMACOKINETICS

Chronopharmacokinetics is defined as the circadian variations in pharmacokinetics of a drug, which are due to parallel changes in the physiological functions involved in drug absorption, distribution, metabolism, and excretion. Chronopharmacokinetic behaviors can result in apparent nonlinear kinetic profiles of a drug in a time-dependent manner (Bruguerolle, 1998; Lemmer and Bruguerolle, 1994). Circadian variations in different pharmacokinetic processes are summarized below (Labrecque and Belanger, 1991; Reinberg and Smolensky, 1982).

10.6.1. Absorption

It is known that there are circadian variations in gastric emptying time, gastric motility, and gastrointestinal blood flow, which in general are faster during the activity period than the sleeping period in both animals and humans. These circadian changes in physiological functions can alter the exposure profiles of orally dosed drugs.

10.6.2. Distribution

It has been reported that there are circadian variations in the blood albumin concentrations in both laboratory animals and humans, which in general are higher

during the period of activity than during the sleep. The free drug concentration can be affected by these rhythmic changes in albumin levels resulting in time-dependent variations in drug distribution in addition to the circadian changes in blood flow.

10.6.3. Metabolism

Circadian variations in the activity of different metabolizing enzymes have been noted in some laboratory animals. In rats, the metabolizing enzyme activity usually increases at night and decreases while the animals are asleep during the daytime. In many cases, the circadian changes in the activities of both phase I and phase II enzymes are found to be positively correlated with the enzyme concentrations.

10.6.4. Excretion

Studies in both animals and humans have indicated that the glomerular filtration rate is highest in the middle of the activity period and lowest during sleep. This seems to be due to the circadian rhythms in the systemic blood pressure and the circulating vasoactive hormones producing time-dependent variations in renal hemodynamics. Circadian changes have also been found in urinary pH, which is more acidic at night than during the day. In rats, it is reported that the bile flow is higher in the middle of the activity period.

10.7. TOXICOKINETICS

Toxicokinetics (TK) is pharmacokinetic principles and techniques applied to concentration *vs.* time data generated at the (high) dose levels that are usually used in toxicity studies, in order to determine the rate, extent, and duration of exposure of the test compound in the animal species examined (Chasseaud, 1992; Clark and Smith, 1982; Smith *et al.*, 1990; Smith, 1993; Welling, 1995). The main objectives of TK for regulatory purposes are:

1. To establish a kinetic relationship between doses and exposure levels.
2. To evaluate the results of toxicity observed in the test animal species based on the rate, extent, and duration of exposure levels of the test compound at different dose levels.
3. To provide information on the relationship between exposure levels of the test compound and the extent of toxicity found in the test animal species for direct comparison with potential human exposure to the test compound.
4. To support clinical study designs and data interpretation by assessing the safety margin, i.e., no observed adverse effect level (NOAEL) in the test animal species divided by a target therapeutic exposure level in a clinical setting.

Owing to the high dose levels used in TK studies, it is not uncommon to observe nonlinearity in drug disposition. Usually three dose levels are examined in short- or long-term TK studies, i.e., low (close to target clinical dose levels), medium, and high

(producing toxicity in animals). Although a thorough understanding of the causes of nonlinearity in exposure can be important for the evaluation of toxicity of the compound, the main purposes of TK studies as stated above are to determine NOAEL and the absolute exposure levels of the test compound associated with toxicity in the animals. The purposes of toxicity studies (drug safety evaluation) in animals [usually one rodent (rat) and one nonrodent species (dog); sometimes mice and monkeys can be used] in addition to those of TK studies are:

1. To ascertain toxicity in the test animal species.
2. To identify the organ(s) or tissue(s) associated with the toxicity.
3. To characterize types of toxicity in conjunction with exposure levels of the compound.

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