28

LINKAGE BETWEEN TOXICOLOGY OF DRUGS AND METABOLISM

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28.1 INTRODUCTION

Modern drug therapy relies on better understanding of molecular biology and genetics, the systems biology of diseases, and gene–environment and gene–drug interactions (Fig. 28.1a). In the last two decades, progress has been made in identifying, cloning, sequencing, and characterizing pathogenic genes (foreign and endogenous) important to disease onset and development, leading to novel approaches to drug target validation and to drug discovery and development (Fig. 28.1a). The fast-growing knowledge and technologies of genomics, proteomics, and systems biology are revolutionizing the process of drug discovery and development. For example, increasing numbers of molecular targets (DNA, RNA, and proteins), high-throughput screening technologies, and high-yield chemical syntheses have resulted in ever-increasing numbers of candidate compounds that are available for pharmacology and toxicology testing. In addition, genetic-based therapeutics, such as gene therapy and RNA/DNA-based therapeutics (aptamer, antisense, ribozyme, RNAi, miRNA, etc.), have been challenging the traditional paradigm of pharmacology and toxicology studies of new drugs [1–3].

Although our ability to identify candidate lead compounds with high potency and specificity has largely been improved, many candidate therapeutic agents are prevented from reaching the market due to inappropriate pharmacokinetic/ADME (absorption, distribution, metabolism, and excretion) properties and drug-induced toxicity; both are highly affected by many factors including pathogens, host genes, and drug–environment interactions (Fig. 28.1b). It is desirable that poorly behaved

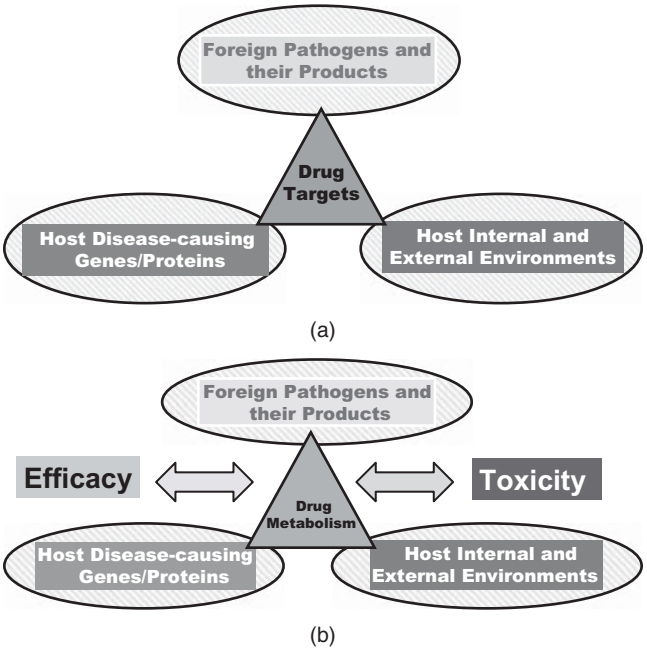


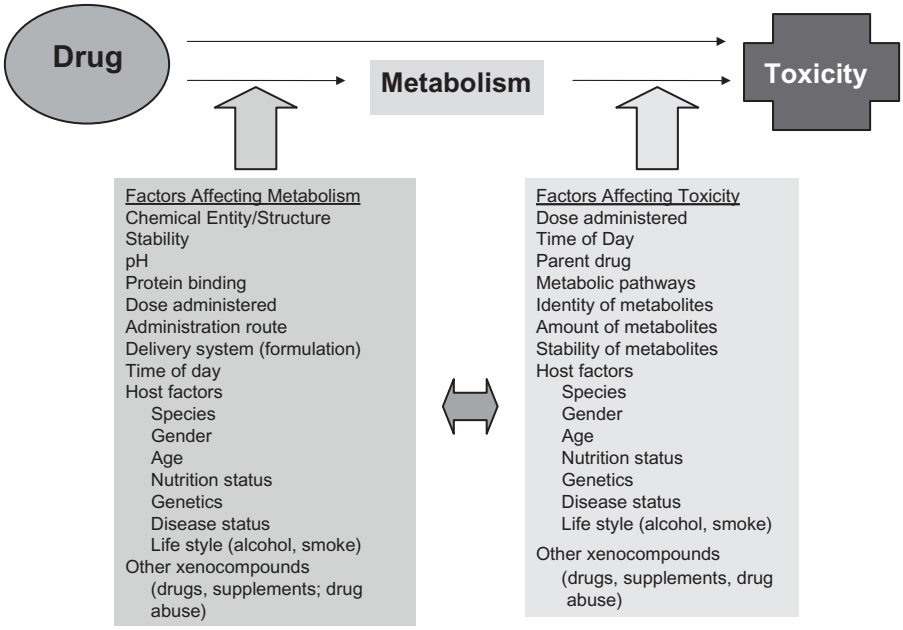
FIGURE 28.1 (a) Drug may target various subjects. (b) Drug metabolism may be affected by various factors.

compounds are removed early in the discovery and early development phases rather than during the more costly late development phases, especially during clinical development. This strategy has been termed "failing early and fast." As a consequence, over the past decades, ADME and toxicity (ADMET) screening studies have been incorporated earlier into the drug discovery phase. The intent of this chapter is to introduce the fundamental ADMET concepts, key tools, reagents, and experimental approaches for predicting human pharmacokinetics and assessing "drug-like" molecules, with an emphasis on the link between toxicology and metabolism (Figs. 28.1b and 28.2a).

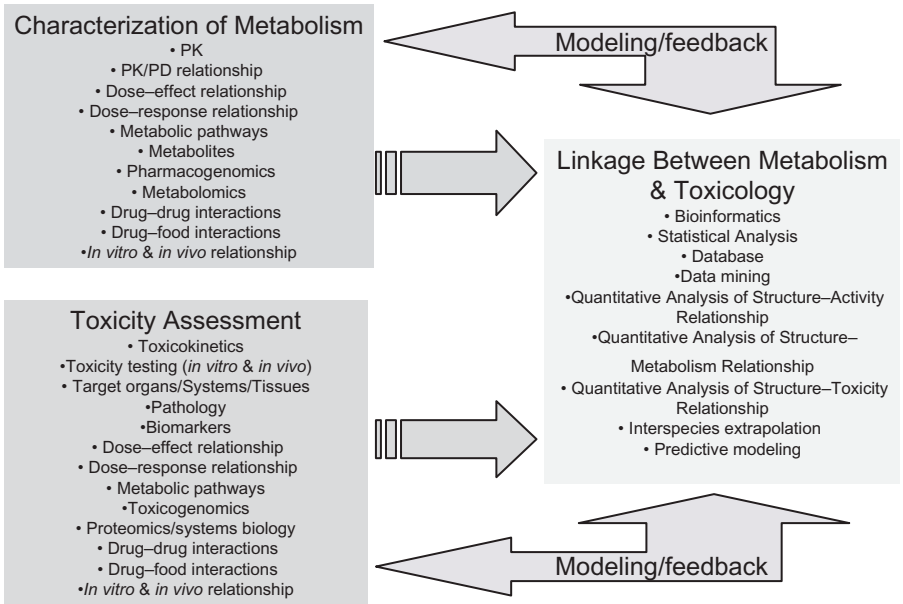
Pharmacokinetic (PK) studies, which evaluate the way in which a drug interacts with various barriers within a system, are an integral part of preclinical and clinical drug testing. A typical PK study may be divided into two sections, with respect to the fate of a given drug in the body: *absorption*, the movement of a drug into the bloodstream, and *disposition*, which involves the distribution, metabolism, and excretion of a drug. Preclinical PK studies often provide qualitative and/or quantitative measurements of these events, using various parameters and models to describe the speed, extent, and underlying mechanisms for a chemical entity to enter and remain in various systems/tissues of interest, generally including the plasma and blood cells, body fluids, various host normal tissues such as brain, heart, lungs, liver, kidneys, spleen, bone marrow, and skin, and drug-targeted tissues such as the tumor and infected tissues [4–6]. The related field of pharmacodynamics, which evaluates the effects of a drug and its metabolites on the test system under various physiological and pathological conditions, is often evaluated in tandem with pharmacokinetics. Pharmacodynamic (PD) studies are often carried out with a disease model that mimics the intended human diseases for the test drug and employ various biomarkers to link the biological effects of a drug with its use by various routes of administration under various conditions.

Also of equal importance for drug design are toxicokinetic studies. Toxicokinetics, as the name suggests, provides information on the behaviors and fate of a toxicant/drug in the test systems, with relation to the occurrence and time course of toxic events. While PK and PD studies provide information relative to drug distribution, metabolism, removal, and pharmacological activity, toxicokinetic studies are used to predict the behavior and safety of a compound/drug [7]. Knowing the distribution and especially the speed and extent of metabolism of a drug is helpful in determining its future efficacy and toxicity. Moreover, PK/PD and toxicity/safety studies are a routine part of human clinical trials, and the objectives of preclinical PK/PD and toxicology studies are to predict potential problems earlier in the drug development process. The results of these studies can be extrapolated to give at least a general idea of the likely effects of a particular compound in humans [8]. While still imperfect, numerous *in vitro*, *in vivo*, and *in silico* systems have been developed to evaluate the pharmacokinetics, pharmacodynamics, and toxicity of compounds prior to their use in humans. Of note, there is a trend to move toxicity testing to earlier phases of drug development.

Although typical pharmacology and toxicology studies can give an idea of the distribution and effects of a drug or compound with high predictive value for most cases, unexpected differences in the response to a given drug or compound among various patients have given rise to yet other fields of study—namely, pharmacogenetics, pharmacogenomics, and toxicogenomics—that investigate the effects of genes



(a)



(b)

FIGURE 28.2 (a) The linkage between drug metabolism and toxicity. Listed are factors affecting the metabolic and toxic responses to a drug. (b) The framework for studies aiming at the linkage between drug metabolism and toxicity.

of interest on the efficacy and toxicity of a drug or compound, utilizing various technologies in the fields of genetics, genomics, proteomics, and molecular biology [9, 10]. The differential genetic makeup of patients, including differences in the level of gene expression, polymorphisms, and gene deficiencies, can greatly alter the efficacy and toxicity of various therapies [9, 10]. Although many genes and their expression may be involved in various PK/PD and toxicology processes, the majority of pharamcogenomics/pharmacogenetics studies have been focused on genes related to drug metabolism. While differences in expression of the cytochrome P450 enzymes may be the most important factors that affect drug metabolism, there are ever-increasing numbers of various other genes that are shown to determine drug efficacy, metabolism, excretion, and toxicity (discussed in detail in Section 28.2). One of the well-documented cases is dihydropyrimidine dehydrogenase (DPD), a key metabolizing enzyme of 5-fluorouracil, one of the most clinically used anticancer agents. DPD deficiency has clearly been linked to severe 5-fluorouracil toxicity, including death. Pharmacogenetic and pharmacogenomic characteristics of DPD have been well defined by various experimental models, in the general population, and in cancer patients [11].

Another related area of study, metabolomics/metabonomics, has also been gaining recognition in recent years. This area investigates the time-dependent metabolic responses of organisms or physiological systems and the resulting metabolites of chemical entities, foods, and other compounds [12, 13]. The field is of particular interest for preclinical studies because it can be used to determine endpoints for further toxicity studies (clinical chemistry, histopathology, etc.) and to determine potential biomarkers [14–18]. It is also useful for screening purposes in order to eliminate agents with unfavorable profiles while still in early phases of development [19–21]. Because the metabolism of compounds differs based on a number of drug, patient/host, and environmental factors, the relatively new "-dynamics" and "-omics" areas are increasingly important for determining the efficacy and potential risks of new therapeutic agents. Figure 28.2a demonstrates the complexity of the interaction between these factors and their impact on drug metabolism, and thus toxicity.

Figures 28.1 and 28.2 provide a framework for concepts, scientific reasoning, and experimental design of studies aimed at linking drug metabolism and toxicity, the focal point of this chapter. As illustrated in Fig. 28.2b, three major components should be included in these studies: (1) the profile and mechanisms of drug metabolism; (2) the profile and mechanisms of drug toxicity; and (3) the methods and models for linking drug toxicity and metabolism. For drugs that are intended for human use, the major purpose of these preclinical studies should be to predict the potential risks to humans under both pathological and physiological conditions. While there have been significant improvements, there is still a need to develop more predictive models and better computational programs that can facilitate data analysis and extrapolation of the results from experimental models to human patients [22]. Recent progress made in bioinformatics and computational biology will certainly facilitate the fine-tuning of the modeling process and the development of models with better predictive values. The ability to predict the metabolism and toxicity of a compound and their relationship will both improve the results of human clinical trials and the likelihood of success of the new agents.

28.2 DRUG METABOLISM AND ITS ROLE IN TOXICOLOGY

28.2.1 General Principles of Drug Metabolism

As shown in Fig. 28.2a, the drug metabolism process relies on a number of factors. The human body encounters numerous foreign compounds (xenobiotics) during the course of an average day. While some compounds may be consciously taken in, such as food, vitamins, and pharmaceuticals, others, such as pollutants and bacteria with their toxins, are absorbed or consumed because of their ubiquitous presence in the environment.

All of these compounds need to be utilized or removed from the system, and this is accomplished by either metabolism or excretion. Here, we focus on metabolism, more specifically drug metabolism, which is the biotransformation of various drugs to (1) inactive compounds that can readily be excreted from the body or (2) more active compounds that can exert an effect (either positive or negative). Drug metabolism can result in four major outcomes:

1. A decrease in the biological activity of drugs and xenobiotics.
2. An increase in excretion due to the formation of a more water-soluble metabolite.
3. Conversion of a prodrug to an active drug, or conversion of an active drug to a different active compound.
4. Generation of toxic or carcinogenic metabolites.

Which of these processes occurs depends on a variety of factors. While the identity of the agent is the most important, its pharmacokinetics/pharmacodynamics within the human body is a close second. There are several factors that can affect the way that the body responds to xenobiotics.

- *Age.* Infants and children may not have a fully developed system to deal with xenobiotics and may then have different rates of drug metabolism and responses to the compound and toxicities. Older persons also may have differences in their ability to metabolize compounds due to changes that occur to the key metabolic systems, primarily the liver and kidneys.
- *Drug Interactions.* When several drugs are present in the body at the same time, they can cause adverse or unexpected side effects and can cause changes in metabolism. For example, combining two chemotherapeutic agents or a chemotherapeutic agent with an anti-inflammatory agent changes the pharmacokinetics of the chemotherapeutic agents [23, 24]. Common food items can also influence the metabolism of different compounds. It is important to note that there is an increased risk for drug–drug interactions in elderly persons and those who are hospitalized due to their higher frequency of polypharmacy. There is also growing concern over the increase in people who are now more actively self-medicating with over-the-counter agents.
- *Disease States.* Diseases that affect major excretory and metabolic organs (specifically those affecting the liver and kidneys) will change the rate of metabolism of a drug. Also, disease states that involve parenteral feeds can affect the

action and activity of a drug. Moreover, diseases that affect blood flow to various organs will also affect drug metabolism.

- *Occupation.* The occupation of a person in some cases can dictate metabolism. For example, pesticide applicators may have increased drug metabolism because liver enzymes can be induced by chronic exposure to pesticides. For example, exposure of male mice to piperonyl butoxide (PBO), a synergistic active ingredient in pesticides, leads to overexpression of cytochrome P450 genes, such as CYP1A1, CYP2A5, CYP2B9, and CYP2B10 [25].
- *Time of Day.* The expression and types of enzymes and cofactors expressed as well as the speed with which metabolism takes place varies with the host's circadian rhythm. For example, the expression of DPD and dThdPase varies with time in both human patients and experimental animals. This has implications for the metabolism of 5-fluorouracil and its toxicity [26, 27].
- *Genetics.* As mentioned in the Introduction, pharmacogenomics and toxicogenomics are now being studied to determine the metabolism and potential toxic reactions to drugs. Genetics and ethnicity are becoming increasingly recognized for their importance in drug metabolism. For example, one of the major drug metabolic pathways utilizes the cytochrome P450 system, which has several isoforms. One such isoform, CYP2D6, is responsible for metabolizing almost 20–30% [28] of all drugs, and there are about 51 [29] known allelic variants of this isoform.

  In Caucasian individuals with CYP2D6*4 or *5 alleles, 12–21% [29] have problems metabolizing drugs that are substrates for CYP2D6.

  Fifty-one percent [29] of the alleles in the Asian population are the CYP2D6*10 allele, which has a proline substitution for serine at position 34. This leads to the improper folding of the protein, which in turn affects its stability. Patients with this mutation have a reduced ability to metabolize some drugs, such as neuroleptics and antidepressants.

  The CYP2D6*17 allele is seen in about 20–35% [29] of black Africans.

  About 10–16% [29] of the Semitic population (people from Ethiopia, Saudi Arabia, and the Middle East) are ultrarapid metabolizers because they have 2–13 copies of the functional gene, resulting in a very high amount of the enzyme. Individuals with these mutations often do not respond well to neuroleptics and antidepressants because they metabolize the drugs too fast.

28.2.2 Metabolism and Metabolites in Toxicology

Drug metabolism involves various enzymatic pathways and transport systems that can convert xenobiotics to a form that can be more readily excreted. Although the enzymes needed for drug metabolism are present in almost every tissue, the rate at which compounds can be metabolized in a given tissue depends on the amounts and types of metabolic enzymes present in that tissue. Compared to other organs, the liver has very high concentrations of drug-metabolizing enzymes, which are also present in the GI tract, kidneys, lungs, and skin.

While the major function of drug metabolism is to inactivate and eliminate foreign compounds from the body, it can also generate reactive compounds that

TABLE 28.1 Classification of Phase I and Phase II Reactions

Phase I	Phase II
Oxidation	Glucuronidation
Reduction	Sulfation
Hydrolysis	Acetylation
Hydration	Methylation
Dethioacetylation	Glutathione conjugation
Isomerization	Amino acid conjugation
	Fatty acid conjugation
	Condensation

may be harmful. The generation of electrophiles is of particular concern as they can react with cellular components such as DNA and RNA and induce genetic mutations. It is essential to carefully evaluate the advantages and disadvantages of administration prior to the use of any drug. In addition to conventional testing methods (LD_{50} and MTD in animals, and safety evaluations in preliminary human clinical trials), the newer fields of pharmacogenomics, toxicogenomics, and metabolomics are being used to assess the spectrum of metabolites generated and their potential toxicity prior to administration of an agent to human patients.

Drug metabolism generally occurs through two categories of enzymatic reactions: classified as either phase I or phase II reactions. Phase I reactions structurally alter the original compound and are often regarded as ones that prepare a xenobiotic for conjugation in phase II. Phase I reactions include oxidation, reduction, hydrolysis, hydroxylation, dethioacetylation, and isomerization reactions. Phase II reactions, which are catalyzed by enzymes found in hepatocytes, are usually conjugation reactions and involve the addition of a large bulky side group to a compound. In many cases, phase II reactions produce water-soluble and charged metabolites that have reduced biological activity and can readily be excreted in urine or bile. It is worthwhile to note that phase II reactions can precede phase I reactions. For example, the drug isoniazid undergoes phase II metabolism first and results in the formation of an *N*-acetyl conjugate, which can then undergo a phase I hydrolysis reaction [30]. Table 28.1 gives a summary of the phase I and phase II reactions. Figure 28.3 is a scheme that displays the fate of administered drugs.

Drug Metabolism: Effects on Efficacy and Toxicity of the Test Drug

Phase I Reactions Phase I reactions are carried out by the mixed function oxidase (MFO) system. The most important MFO enzymes include the cytochrome P450s (CYP450). To date, the CYP450 MFO is regarded as the most powerful *in vivo* oxidizing system. This family of monooxygenases can be broken down into the liver microsomal MFO system and adrenal mitochondrial MFO system.

- The liver microsomal MFO system is made up of two components—NADPH cytochrome P450 reductase and cytochrome P450 (CYP450)—which are membrane-bound hemoproteins that use NADPH as a reducing agent and require molecular oxygen as well as FAD and FMN.

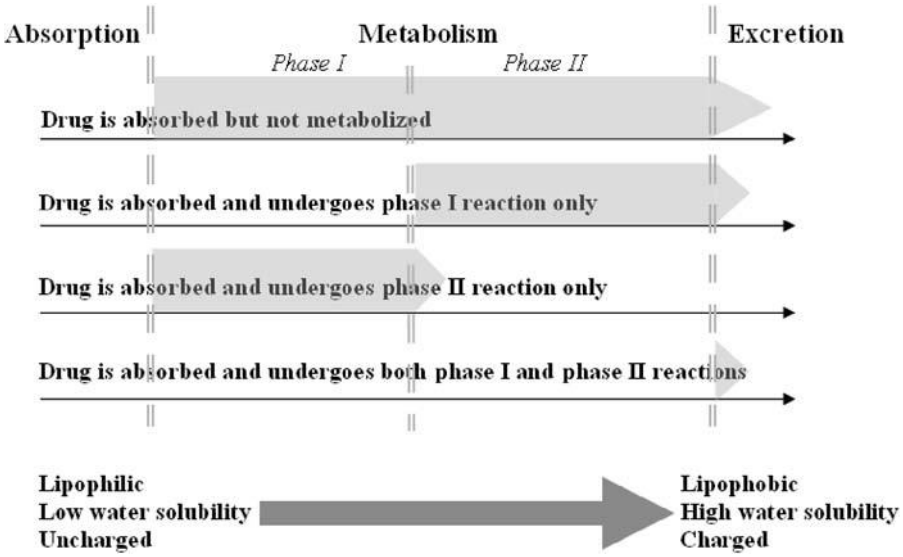


FIGURE 28.3 The fate of administered drugs. Drugs can be absorbed and undergo different fates but ultimately they are all excreted in urine, bile, or feces. As drugs are metabolized, they have decreased lipophilicity, increased water solubility, and increased charge.

- Instead of CYP450 reductase, the adrenal mitochondrial MFO system is made up of an iron–sulfur protein, adrenodoxin, and an adrenodoxin reductase. These two proteins are involved in transferring electrons from NADPH to CYP450 in steroid biosynthesis and bile metabolism. As such, the adrenal MFO system is not a major player in drug metabolism. However, it is important to note that, in the future, it may be necessary to make use of this system for specific agents, particularly to evaluate and optimize drugs that mimic endogenous steroids.

The liver microsomal MFO system catalyzes numerous reactions by transferring electrons from NADPH to CYP450. It does so by activating the iron component of CYP450, enabling the insertion of an oxygen atom into a substrate (drug). A scheme depicting this reaction appears in Fig. 28.4.

NADPH–CYP450 reductase catalyzes nonspecific reactions, and its main role is the transfer of electrons to CYP450 through the use of FAD and FMN. Thus, specificity depends on the different isoforms of the CYP450s. In humans, there are 57 CYP genes and 33 CYP pseudogenes, which can be divided into families and subfamilies. Table 28.2 [35] shows the relative abundance of some of the isoforms and their importance in drug metabolism. Substrate specificity is often hard to assess because one enzyme may be involved in more than one metabolic pathway and one pathway of drug metabolism can involve several different enzymes.

Although the CYP450 catalytic cycle is very important in drug metabolism, it can also create some problems, such as autooxidation. In this process, if the substrate cannot acquire the electrons properly, the system can generate superoxide anions, hydrogen peroxide, or water. This process is of major concern as the CYP450 system has a success rate ranging from 1% to 100%. The production of free radicals can

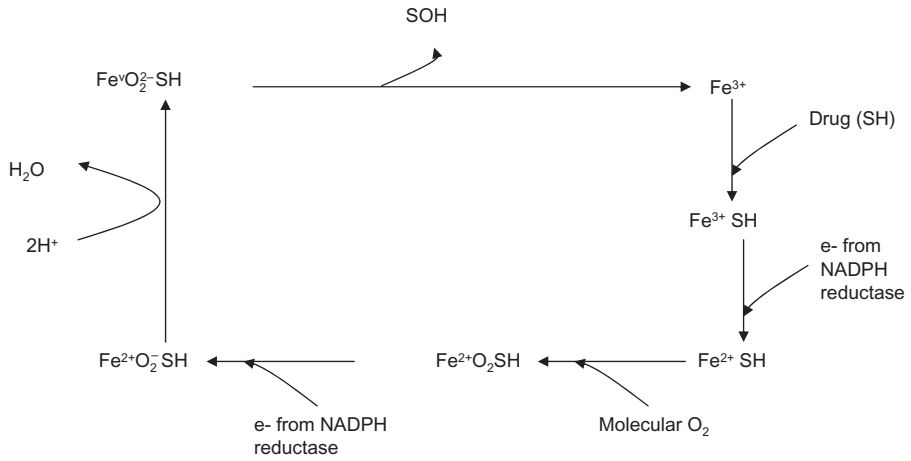


FIGURE 28.4 Drug (here represented by SH) binds to the oxidized form of CYP450, which accepts one electron from NADPH reductase. This reduced form can bind molecular oxygen. Another electron is added and the molecule rearranges to generate the oxidized form of the drug (here SOH), water, and the oxidized form of CYP450.

TABLE 28.2 Different Expression Levels of CYP450 and Their Contributions to Metabolism

Isoform	Percentage of Hepatic CYP450 Content	Isoforms	Percentage Contribution to Metabolism
3A	30	3A 4/5	36
2C	18	2D6	19
1A2	13	2C 8/9	16
2E1	7	1A2	11
2A6	4	2C19	8
2D6	1.5	2E1	4
2B6	0.2	2A6	4
Others	26.3	2B6	3

result in toxicity, and determination of possible reactive oxygen and nitrogen species that can be generated from the drug is important for predicting its toxicity.

Some phase I reactions do not involve the CYP450 MFO system, although these mostly represent reactions of endogenous compounds. However, they can be participants in drug metabolism. Following is a list of these types of reactions.

1. *Oxidations.*

(a) Alcohol oxidation is performed by alcohol dehydrogenase.

(b) Aldehyde oxidation is performed by aldehyde dehydrogenase and aldehyde oxidase.

(c) Xanthine oxidase is concerned with the metabolism of xanthine-containing drugs such as caffeine and allopurinol.

(d) Monoamine oxidase (A and B forms) will oxidatively deaminate primary, secondary, and tertiary amines.

(e) Flavin-containing monooxygenases (FMOs) can oxidize nitrogen-, sulfur-, and phosphorus-containing drugs, by using NADPH and molecular oxygen, in a similar manner as MFOs. However, FMOs will not catalyze the oxidation of negatively charged compounds such as organic acids.

(f) Peroxidase-dependent cooxidation is important in extrahepatic tissues, especially those with low CYP450 levels and activity. In this reaction, peroxidases couple the reduction of hydrogen peroxide and tissue hydroperoxide to the oxidation of other compounds.

2. *Reductions.* Other processes catalyze the reduction of azo compounds, nitro compounds, epoxides, heterocyclic ring compounds, and halogenated hydrocarbons. Examples of such reactions include the reduction of protonsil to triaminobenzene and sulfanilamide, and the successive reductions of nitrobenzene to nitrosobenzene to phenylhydroxylamine to aniline.

3. *Hydrolysis.* The addition of H_2O to compounds such as esters, amides, and carbamates by peptidases, amidases, and esterases results in the generation of new metabolites.

4. *Epoxide Hydrolase.* Epoxide hydrolases catalyze the hydration of epoxides. Since epoxides are known to be reactive species that can bind to DNA and RNA and thus induce mutations, epoxide hydrolase activity serves as a protective measure to decrease toxicity.

Phase II Reactions As explained previously, phase II reactions usually consist of the addition of a bulky charged group to phase I reaction products to facilitate excretion of drugs. Phase II reactions also involve several enzymes that are capable of catalyzing a wide variety of reactions. Table 28.3 lists phase II reactions, their enzymes, and the functional groups involved.

1. *Glucuronidation.* Glucuronidation involves the addition of UDP glucuronic acid to the substrate and is catalyzed by UDP-glucuronyltransferases (UGTs), which are microsomal membrane-dependent enzymes. It is the main phase II reaction, as UDP glucuronic acid can bind to O, N, C, or S atoms. While UGTs seem to have low specificity in drug metabolism, their specificity increases when dealing with

TABLE 28.3 Summary of Phase II Reactions

Reaction	Enzyme	Functional Group
Glucuronidation	UDP-glucuronyltransferase	—OH, —COOH, —NH_2, —SH
Glycosidation	UDP-glycosyltransferase	—OH, —COOH, —SH
Sulfation	Sulfotransferase	—NH_2, —SO_2NH_2, —OH
Methylation	Methyltransferase	—OH, —NH_2
Acetylation	Acetyltransferase	—NH_2, SO_2NH_2, —OH, —COOH
Amino acid, glutathione, and fatty acid conjugation and condensation	Glutathione-*S*-transferase	Epoxide, organic halide, —OH, various

endogenous substrates. There are three subfamilies of UGT: UGT1A, UGT2A, and UGT2B, which contain the 15 known isoforms of UGT. While the liver is the main site of glucuronidation, other organs such as the stomach and kidneys have high levels of some UGT isoforms [31]. Drugs that undergo glucuronidation include morphine and codeine.

2. *Sulfation.* Sulfation plays an important role in drug metabolism, especially when phenols and phenol-containing compounds, alcohols, amines, thiols, and some steroids are present. Sulfotransferases (SULTs) catalyze the transfer of a sulfate moiety from 3′-phosphoadenosine 5′-phosphosulfate (PAPS, a cofactor) to the substrate. Since this sulfate moiety has a very low pK_a (~2), sulfate conjugates will be charged at physiologic pH values, making them more water soluble and rapidly excreted. Similar to UGTs, SULTs are mostly found in the liver, as well as extrahepatic organs. It is important to note that the availability of PAPS is a rate-limiting condition of sulfation reactions, and that while there are both membrane-bound and membrane-free forms of SULTs, only the membrane-free forms are involved in drug metabolism. To date, 10 isoforms of cytosolic SULTs have been identified, and all of them have been reported to participate in the activation of procarcinogens. Unstable esters are frequently formed, which can spontaneously rearrange, producing reactive electrophiles capable of binding to DNA and RNA. Drugs that undergo sulfation include ethanol, acetaminophen, dopamine, and minoxidil (Rogaine™).

3. *Acetylation.* Aromatic amines and sulfonamides are the most common substrates for acetylation by *N*-acetyltransferase (NAT). During this process, the acetyl group from acetyl coenzyme A is transferred to the drug substrate, which contains a primary amine, a hydroxyl, or a sulfhydryl group. Unlike glucuronidation and sulfation, acetylation does not produce conjugates that have improved water solubility and excretion. Drugs that undergo acetylation include isoniazid, *p*-aminobenzoic acid, and hydralazine. NATs can be classified as NAT1 or NAT2 and each are regulated independently of the other. Point mutations in NAT2 have been associated with polymorphisms seen in the metabolism of isoniazid, which is often used to identify rapid and slow acetylators. For example, a point mutation at Ile114Thr is often seen in Caucasians but very rarely in Orientals. The latter population has a mutation at nucleotide 857 of NAT2, which results in lower level of active enzyme. Ninety percent of North Africans and 50% of Caucasians are slow acetylators, while 10–30% of the Korean and Japanese populations are slow acetylators [32]. Such genetic polymorphisms will affect the dose response to drugs and increase drug toxicities in slow acetylators.

4. *Methylation.* Drugs that contain O, N, and S atoms can be methylated by methyltransferases, which can be classified into four groups—catechol-*O*-methyltransferase (COMT), phenol-*O*-methyltransferase (POMT), thiopurine *S*-methyltransferase (TPMT), and thiol methyltransferase (TMT). Although the reactions have high substrate specificity, the methyl donor is *S*-adenosyl-methionine (SAM), irrespective of which enzyme is used. Methylation, like acetylation, does not necessarily result in increased water solubility or excretion. Substrates that undergo methylation include histamine, *L*-dopa, and 6-mercaptopurine.

5. *Amino Acid Conjugation.* Drugs that contain a carboxyl group can be conjugated with amino acids in a two-step reaction that requires acyl CoA ligase and amino acid acyltransferase. One of the most important amino acid conjugations is

in bile metabolism. Amino acids such as glycine, glutamine, or taurine (nonprotein amino acid) can be conjugated with their substrates to form products that can be readily excreted in urine [33]. One example of an amino acid conjugation substrate is salicylic acid.

6. *Glutathione Conjugation.* Glutathione *S*-transferase (GST) catalyzes the conjugation reaction of some drugs with glutathione, which is a tripeptide in which glutamate is bonded to cysteine through the γ-COOH group of glutamate. The other amino acid present in this molecule is glycine. As glutathione (GSH) is a nucleophile, it can bind reactive electrophiles present in the body and thus plays an important protective role. While GSH can spontaneously react with reactive electrophiles, GST can increase the rate of this reaction, as it converts GSH into its active anionic form. Once GSH conjugates have been formed, they undergo other reactions through peptidase and acetylase activities, to mainly produce cysteine and mercapturate conjugates, which can then be excreted in urine. More than 20 GSTs have been discovered and they can be classified as either cytosolic or microsomal [34].

The cytosolic forms are more important for drug metabolism and can participate in conjugation, reduction, and isomerization reactions, while the microsomal forms interact with endogenous substrates. Although concentrations of GSH and GST are relatively high in cells, depletion of GSH can occur, leading to toxicity. For example, high doses of acetaminophen are toxic because the drug covalently binds to GSH and effectively depletes GSH from the system. Acetaminophen bioprocessing usually involves glucuronidation and sulfation reactions, but in high doses, these systems are overloaded and CYP450 starts to process the drug. This produces the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) [35], which can attack cellular components. In most cases, NAPQI is eliminated by GSH, but in the case of overdose, there can be a decreased amount of GSH and thus hepatocellular necrosis and death can occur.

Similar to SULTs, NATs, and P450 enzymes, there are also GST polymorphisms. GST has five members (mu, alpha, pi, theta, and sigma). The mu (GSTM1*0) and theta (GSTT1*0) genotypes express a null phenotype, and in individuals with the GSTM1*0 allele (50% of the Caucasian population, ~60% in Chinese and Korean populations), it may indicate a genetic predisposition to lung, colon, and bladder cancers [36–38].

While GSH is usually protective, it has been shown that GST can lead to increased drug resistance to chemotherapeutic agents, possibly through the inhibition of MAP kinase activity and apoptosis [34], which are both mechanisms that are important for the action of many anticancer therapies. TLK199 is a GSH analogue that can produce a GSH inhibitor, TLK117, and thus decrease the effects of GSH on anticancer agents [34].

Drug Metabolism: Effects on Efficacy and Toxicity of Other Drugs When more than one medication is taken by a patient, there is always a risk that the patient may suffer side effects that are due to the interaction between the different drugs. Drugs may compete for binding sites on plasma proteins or transporters, or can alter the activity of the metabolic enzymes (most notably the CYPs). For example, ketoconazole, used as an antifungal drug, is a potent inhibitor of several CYPs including CYP3A4. When drugs that use CYP3A4, such as anti-HIV viral protease inhibitors, are coadministered with ketoconazole, they cannot be metabolized and their plasma

concentration increases, leading to toxicity [39, 40]. Some agents can also induce CYPs in a way to increase their own metabolism or to affect the metabolism of other drugs present in the body. For example, the herbal medicine St. John's wort will induce CYP3A4, leading to increased metabolism of other drugs such as oral contraceptives. The toxicity and efficacy of a drug can also be influenced by diet. For example, when the antihistamine terfenadine was taken with grapefruit juice, it led to arrhythmias. This was due to the fact that grapefruit juice contains furanocoumarins, which are inhibitors of CYP3A4, and terfenadine is a prodrug that requires this specific CYP for bioactivation. Terfenadine levels in the plasma would rise and cause arrhythmias [41].

Toxicokinetics, Toxicodynamics, and Toxicogenomics In order to predict these types of toxicities, the past few decades have witnessed the exponential expansion of the boundaries of scientific knowledge. Core discoveries, such as DNA and its structure, as well as technical milestones including genetic engineering and initial quantification of cellular processes and their pathways, have been made. Such findings have triggered the evolution of drug evaluation and toxicology from its traditional aspects to one that now includes genetics. These discoveries have led to the development of toxicogenomics, pharmacogenomics, and better ways to study pharmacodynamics and toxicodynamics [42, 43].

In light of the discovery of the multiple enzyme isoforms that are present in metabolic pathways, such as the CYP450s, it is important to consider how genetic diversity plays a role in the metabolism and side effects of xenobiotics. The development of new techniques in the field of toxicogenomics is proving very useful in the elucidation of the genetic components of diseases. For example, by using nucleic acid microarrays, protein chips, and denaturing HPLC (DHPLC), one can identify pathways and mechanisms that are affected by administration of different agents. These methods allow the comparison between multiple nucleic acid sequences and their variants concurrently [42, 43]. This is particularly useful as toxicity is now being recognized as the result of multiple gene interactions, rather than a largely uncharacterized response within the entire body or within certain organ systems [42].

This new trend necessitates the investigation of genetic variability and its relation to phenotypic observations, as well as its importance in drug metabolism and subsequent toxicity. It is clear that toxicogenetics and pharmacogenetics will now play an integral part in drug development and are likely to be increasingly important in the future. Moreover, the use of these strategies by various methods can predict the patient response to treatment, thus helping physicians to select the most appropriate treatment options for individual patients that will improve the efficacy and minimize toxicity of the therapy [42–44].

28.2.3 Examples for Pharmacogenetics and Pharmacogenomics of Therapeutic Drugs

Example 1: 5-Fluorouracil 5-Fluorouracil (5-FU), an antimetabolite of pyrimidine, is commonly used in the treatment of head, neck, breast, ovarian, and gastrointestinal tract cancers [45–47]. However, it is associated with toxicities ranging from stomatitis, diarrhea, skin changes, myelosuppression, and neurological com-

plications [45]. While these symptoms may be observed at standard doses of 5-FU, they have also been shown to be dependent on the dose and schedule of drug administration. A genetic basis to these toxic effects has also been discovered and is thought to be the main cause of the difference in toxicities between patients. The enzyme dihydropyrimidine dehydrogenase is responsible for the catabolism of 5-FU, converting it (about 85% of administered drug) [48] to its inactive and less toxic metabolite, dihydrofluorouracil, and increasing its excretion. 5-FU can also undergo an anabolic pathway, which accounts for its host and tumor toxicity. In patients, the aim is to achieve a balance between the two pathways to limit the amount of toxicity to normal tissues through the catabolic pathway, while not reducing the effects of its anabolic metabolites in tumor cells. However, this balance can be affected in patients who have a partial or complete deficiency of DPD [47]. The dihydropyrimidine dehydrogenase gene (*DPYD*) is known to have 39 alleles [49], and a point mutation at the DPYD*2A allele has been linked to deficiency in DPD [47, 50–52]. It is clear that the alteration in 5-FU metabolism can lead to toxicity to the host. The main factor involved in its toxicity is genetics, emphasizing the need for more accurate risk and safety assessments based on individual patient profiles.

Example 2: Thiopurine Methyltransferase Azathioprine and 6-mercaptopurine (6-MP) are immunosuppressant drugs that are used to treat and prevent reoccurrence of diseases such as acute lymphoblastic leukemia (ALL), inflammatory bowel disease (IBD), ulcerative colitis, rheumatoid arthritis, and organ rejection in transplant patients [53, 54]. The metabolism of these two drugs requires thiopurine methyltransferase (TPMT), which is a cytosolic enzyme that can catalytically methylate these drugs. TPMT has been found to have several alleles that can be used to classify the general population as normal/high, intermediate, and deficient methylators. In Caucasians, this accounts for 90%, 10%, and 0.3–0.6% of the population, respectively [47, 54]. Three main alleles have been linked to deficient methylators, namely, TPMP*20, TPMP*21, and TPMP*22 [54], and these result in a reduction in enzyme activity. Other alleles are also known to be involved in the deficiency (TPMT*2, *3A, *3B, *3C) [47]. This can lead to toxic accumulation of metabolites such as azathioprine and 6-MP along the thiopurine metabolic pathway. Hence, similar to Example 1, patient genetics greatly influence the toxicity of administered drugs.

28.2.4 Examples of the Link Between Toxicology of Drugs and Metabolism

Drug metabolism is vital to the bioactivation, inactivation, and eventual clearance of drugs, but it also represents a major source of toxicity. There are many naturally toxic parent compounds; however, a large portion of therapeutic drugs exert acute or chronic toxic effects only following metabolism. During the drug discovery process, it is important to carefully perform risk and safety assessments of drugs, and to keep in mind issues such as dose-dependent toxicity, species differences with respect to drug action, metabolism and toxicity, and long- and short-term toxicity, as well as reproductive toxicity. In addition to these factors, there are numerous others that affect the potential toxicity of a compound following metabolism. Some of these are listed in Table 28.4.

TABLE 28.4 Examples of Factors that Affect Metabolism and the Subsequent Toxicity of Drugs

Factor	Subclassification	Example
Identity of the compound	Innate toxicity of the parent compound	Vinca alkaloid compounds bind to and disrupt microtubules, resulting in cell cycle arrest [55].
	Presence of toxic constituent atoms or structures	Methoxyflurane releases free inorganic fluorine in the blood, leading to nephrotoxicity [56].
	Generation of free radicals	Many compounds lead to the generation of free radicals following metabolism (e.g., hydroquinones in cigarette tar lead to the production of free radicals and subsequent DNA damage) [57].
	Metabolism/ activation	Prodrugs (e.g., capecitabine) result in different toxicity compared to their parent compound (5-FU) [56].
Polypharmacy	Drug–drug interactions	Warfarin binds to albumin. When phenylbutazone is administered to the same patient, phenylbutazone competes for warfarin's binding site, displacing warfarin and causing the level of free warfarin in the plasma to increase [26].
	Activation or repression of enzyme activity	Drugs can influence the activity of metabolic enzymes, including cytochrome P450s. For example, ketoconazole inhibits CYP3A4. Anti-HIV protease inhibitors are also metabolized by CYP3A4. When the enzyme's activity is repressed, they cannot be metabolized, and their plasma concentration increases [31].
Host status	Genetics and ethnicity	There are 51 known allelic variants of CYP2D6 • 12–21% of Caucasians with CYP2D6*4 or CYP2D6*5 alleles have problems metabolizing 2D6 substrates. • Within the Asian population, the CYP2D6*10 allele leads to improper 2D6 protein folding, reducing the ability to metabolize neuroleptics and antidepressants. • About 10–16% of the Semitic population are ultra-rapid metabolizers because they have 2–13 copies of the functional gene [34].
	Age	Elderly patients have different expression and activity of metabolic enzymes compared to adults, and typically metabolize drugs more slowly [58].
	Concommittant disease	Various disease states can influence metabolism (e.g., cirrhosis, gastrointestinal disorders, hypertension) [59].
Epidemiological factors	Diet	Consumption of grapefruit juice by patients taking the antihistamine terfenadine led to arrhythmias because of high levels of terfenadine in the plasma due to CYP3A4 inhibition by furanocoumarins in the juice [31].
	Occupation	Many occupations can lead to exposure to activators/ repressors of metabolic enzymes (e.g., farmers are likely to experience increased exposure to pesticides) [27].

28.3 METHODS USED IN LINKING DRUG METABOLISM AND TOXICOLOGY

28.3.1 General Principles

As part of the regulatory process, toxicologists face the need for both qualitative and quantitative studies to assess hazardous risks versus benefits of substances that are potentially toxic. Qualitative studies provide the toxicologist with information about the possible hazardous effects of the toxicant via various exposure routes and at various doses. They also provide information about the mode of action, progression of toxic effects, and susceptibility of subjects to the agent [60–63]. To understand the biological factors that affect the dose–response curve, a quantitative relationship among the varying factors must be understood.

Modern toxicology has three key assumptions: (1) toxicity in humans can be predictively modeled in other organisms; (2) selection of an appropriate model is key to accurate prediction in humans; and (3) to understand the relevance to humans, you must understand the strengths and weaknesses of the model selected [60–64]. *In vivo*, *in vitro*, mathematical, and computer models (*in silico*) are all used to study the effects of metabolism on the toxicity of drugs and xenobiotic compounds. Table 28.5 shows the different methods used to assess the metabolism and toxicity of target drugs and their advantages and disadvantages.

TABLE 28.5 A Summary of the Systems and Methods Used to Evaluate Metabolism and Toxicity, and Their Advantages and Disadvantages

System	Examples	Acquisition	Maintenance	Expense	Human Correlation
In Vivo	Nonhuman primates	Difficult	Difficult	High	High
	Rodents	Easy	Easy	Low	Moderate–high
	Other mammals	Moderate	Moderate	Moderate	Moderate–high
	Nonmammalian models	Easy–moderate	Easy–moderate	Low–moderate	Low–moderate
	Lower animals	Easy	Easy	Low	Low–moderate
In Vitro	Primary cells	Moderate	Moderate–difficult	Low	Moderate (tissue dependent)
	Cancer cells	Easy	Easy–moderate	Low	Moderate (cell and study dependent)
	Isolated organs	Moderate–difficult	Difficult	Moderate	Moderate (cell and study dependent)
	Microsomes	Easy–moderate	Easy–moderate	Low–moderate	Low–moderate
	P450 kits	Easy	Easy	Low–moderate	Low–moderate
In Silico	Scaling	Easy–moderate	Easy–moderate	Low	Moderate (depends on model)
	QSAR	Moderate–difficult	Moderate–difficult	Moderate–high	Moderate, but improving
	PBBK	Moderate–difficult	Moderate–difficult	Moderate–high	Low–moderate, but improving
	BMD	Easy–moderate	Easy–moderate	Moderate–high	Moderate

28.3.2 *In Vivo* Models

In vivo modeling has long been the gold standard in toxicity testing, and extensive *in vivo* studies are still required prior to use of new agents in human clinical trials. *In vivo* models provide the ability to assess the actions or effects of particular drugs and their metabolites in an intact animal before human trials [60–64]. While other research models may provide useful information, only *in vivo* models provide the full picture of the pharmacokinetics, metabolism, and effects of the agent and its metabolites. In order to most effectively use animals for predicting human efficacy and toxicity, it is vitally important that the proper model is chosen.

Nonhuman Primates Although they are the most closely related (genetically) to humans, nonhuman primates (most often chimpanzees, gibbons, macaques, and baboons) make up only a small percentage of the animals used for biomedical research. However, nonhuman primates are susceptible to many of the same diseases as humans, so primates are used for studies of surgery and diagnostics, as well as for studies of drug efficacy and toxicity of agents that cannot be accurately evaluated in other animals. The most common use for primates is to study new agents for the prevention and treatment of HIV/AIDS.

Nonrodent Mammalian Models Many models are available for use in biomedical research, each serving to closely model different human systems. While not the most popular models, cats and dogs will continue to play a vital role in biomedical research and for toxicity and PK studies. Because of their similarity with human anatomy and physiology, dogs and cats make excellent models for some diseases and for evaluating toxicity to certain systems. Dogs are especially well suited to studies of compounds with potential cardiotoxicity. They are also frequently used to study renal toxicity of compounds due to their high incidence of kidney disease, and dogs have been a valuable source of preclinical data used to develop novel therapeutic agents [65]. Due to the similarity in anatomy and biochemistry of the cat brain to that of humans, cats have served as important models in the field of neurophysiology and synaptic response. Research using this model has also focused on leukemia, a disease shared by cats and humans, providing information important in understanding immunodeficiency and cancer [66]. Numerous other animals, including mini-pigs and ferrets, are used to study specific types of agents.

Rodent Models Rodents are the most frequently used model for toxicology testing. Rodents have many advantages that make them valuable as an *in vivo* model system, including their small size, short gestation period, and ease of housing and care. Popular examples include hamsters, rats, and mice. Hamsters provide a model that is relatively free of spontaneous disease but is susceptible to several introduced pathogens. They also have a characteristic cheek pouch that provides an excellent site for the evaluation of proposed carcinogenic agents. Short life spans, rapid reproduction, and a virtual absence of spontaneous disease make the hamster a good model for reproductive toxicology. With gestation time of 15–18 days and a very regular estrus cycle of 4 days, the hamster has the shortest gestation time of all laboratory animals, giving it an advantage over other rodent models [67].

Rats are in the same subfamily as mice, Muridae. The majority of laboratory rats are domesticated varieties of the Norway rat. With their well-defined genetic makeup,

this extensively used model has been purpose-bred to be susceptible to specific human diseases, such as hypertension. A wide variety of transgenic and chemically induced rat disease models are also available for laboratory use. Additionally, rats have a ridge that covers the opening of the esophagus, making them unable to vomit. This enables rats to be used for extensive oral/ingestion toxicity studies. Rats are the most commonly used animal to test median lethal doses (LD_{50}).

Disadvantages to the use of rats compared to mice include their size (requiring larger amounts of test compounds compared to mice) and longer life span. Additionally, while the rat genome has been studied extensively, the mouse genome is more well defined. In addition, the rat's blood supply to the heart makes it a poor model for heart-related studies.

The mouse model is the most frequently used model in *in vivo* research. Mouse subspecies origins can be traced back to the beginning of human civilization when mice became commensal with human settlements [68]. Coat color mutations of mice have been noted and recorded for millennia, with widespread domestication of the mouse by the 1700s [68]. Haldane's report in 1915 began the genetic mapping of the mouse. Haldane described the linkage between pink-eye color and albino loci that were later defined as part of mouse chromosome 7. Knowledge of the mouse genome grew slowly over the next 50 years until the discovery of recombinant DNA technology. By 2002, 96% of the mouse genome had been sequenced, leading researchers to even further specialize the mouse model.

There are now a wide variety of mouse models available for evaluating the efficacy and toxicity of compounds for various diseases including cancer, diabetes, lupus, and infectious diseases. Even for just the study of cancer, there are thousands of possible models, including xenograft models (where human tumor cells are injected subcutaneously into immunocompromised animals), syngeneic models (where mouse cancer cells are implanted subcutaneously into normal or immunocompromised mice), transgenic models (with overexpression or targeted knockout of specific gene expression), and chemically induced models (where agents with proven carcinogenic activity are administered to animals in order to determine whether the formation of tumors can be slowed or prevented).

While rabbits are not technically rodents, they are often grouped within the same category. Rabbits have been used at length in biomedical testing as well as in consumer product safety testing. Rabbits are easy to handle, and their large ear veins make blood collection easily accessible. The rabbit, like the rat, does not have the ability to vomit. Extensive evaluations of atherosclerosis and potential therapies for atherosclerosis have been done in rabbits because studies have demonstrated that they develop lesions similar to human fatty streaks when fed a high fat diet [69]. The rabbit model is also often used in irritation testing that is required by law for certain consumer products. Rabbits are also used to evaluate pyrogenicity and muscle irritation by medical devices that are administered parenterally [64].

Guinea pigs were once a very popular subject for scientific research; however, they are used with much less frequency than other rodent models today. While in the past they were used for a variety of studies, including the development of a vaccines and antiviral agents, guinea pigs are now most commonly used to study sensitivity and hypersensitivity to agents for toxicity testing and for studying potential preventive and therapeutic approaches for juvenile diabetes and preeclampsia

[70, 71]. Mice and rats are now used for most of the studies that were previously done in guinea pigs.

Nonmammalian Models While they are not currently used as a primary model for evaluating new therapeutic agents, fish (especially zebrafish, *Danio rerio*) represent an excellent model for studying embryonic development and exposure to environmental toxicants and are gaining in favor for evaluating pharmaceutical agents [72]. The zebrafish genome is well characterized, and many transgenic and genetically modified fish have been generated [73]. Moreover, it is possible to inject antisense oligonucleotides or siRNA into embryonic fish to generate fish with knocked down genes. Like rodents, the small fish are inexpensive, easy to breed, and relatively easy to maintain. However, given that they are not mammals, they do not represent an accurate translational model for all types of toxicity testing.

Other animals, including avian models (most frequently chickens and embryonic chicks) and frogs (*Xenopus laevis* and other *Xenopus* species) are also frequently used to study embryonic development and gene function [74, 75]. The genetics of these other animals are still being determined (although the *Xenopus* genome has been fairly well characterized), although animals with gene mutations or polymorphisms have been obtained for many species. However, because their genome is not as well characterized as that of other research animals, and because they do not possess some of the anatomical features of mammals, these models are less frequently used for drug development and evaluation.

Finally, lower organisms, including fruit flies (*Drosophila melanogaster*) and roundworms (*Caenorhabitis elegans*) are also being used to study gene function and could provide novel models for toxicity testing. The advantage of these models is that they have been extensively characterized, with the *C. elegans* genome being the first to be completely sequenced, and with all of its cells having been studied and mapped [76, 77]. Drosophila are also frequently subjected to gene manipulation to study gene function and development. Both of these lower organisms are easily bred and maintained in labs and, given their relative simplicity, can facilitate studies of reproductive and developmental toxicity. However, while they represent excellent models for some studies, the use of these models for drug design and evaluation studies is limited.

Despite their many advantages, the use of animals is generally costly, can be controversial, and is not necessarily correlative with human results. Other *in vitro* and computer modeling systems are being used to evaluate the toxicity of compounds and are especially useful for preliminary studies so that fewer animals will be required.

28.3.3 *In Vitro* Models

In addition to the above disadvantages, there are increasing numbers of compounds that need to be screened. Therefore, faster and less expensive models of toxicity evaluation have been established. The use of *in vitro* models for the screening of cytotoxic drugs began in the 1940s with the development of nitrogen mustard derivatives. With the development of chemically defined media in the 1950s, qualitative evaluations in cells grown as monolayers in Petri dishes became easier, and by using protein dyes, dose–response relationships could be determined [78].

Compounds affect cells directly in *in vitro* systems until medium containing compound is removed, allowing observation of direct toxic effects [79]. The advances in *in vitro* methods have allowed for elucidation of many of the mechanisms underlying clinical toxicity. Moreover, in many cases there is a high degree of correlation between the results obtained from animal studies and cell culture studies, providing an alternative to animal models. This allows for both high throughput screening and decreased use of laboratory animals.

The use of *in vitro* models is now generally well accepted as a means of prescreening for toxicity. In early stages, *in vitro* models can assist drug design via permeability (barrier penetration) assays and structure–activity relationships. The preliminary *in vitro* models often are used to decide the fate of compounds and whether they will be selected to continue in the development pipeline.

In vitro toxicity testing can involve everything from bacteria or yeast, to isolated organ preparations or embryos, to cultured human or animal cells. Isolated organ preparations were among the first systems used for *in vitro* toxicity studies. While the data from isolated organ studies may be the most similar to the *in vivo* situation, the nature of these types of studies (require animals, often laborious surgical procedures, and there is often large variation between samples) largely negates their usefulness. Similarly, the use of embryos is labor intensive and not practical for studying anything other than developmental toxicity. Rather, cultured cells (immortalized or isolated and cultured from primary tissue sources) now are the most commonly used model for *in vitro* studies. For example, hepatocytes can be used to evaluate hepatotoxicity, and other cell types (such as endothelial cells or colon cells) can be used to determine whether a drug is likely to enter the bloodstream, to be absorbed by oral administration, or to cross the blood–brain barrier.

28.3.4 Human Cells *In Vitro*

Human Tissues In Vitro Special consideration must be given to the use of human tissue or primary human cells in *in vitro* modeling. *In vitro* modeling using human tissue could offer valuable preclinical research data and possibly increased safety, but there are still numerous legal and ethical issues involved in the use of human tissues [80, 81]. Improvements in cell culture techniques, along with increasing availability of human tissues, have led to increased use of human primary and immortalized cells in toxicology and pharmacodynamic studies [82]. Human *in vitro* models are being used with physiological models to predict quantitative metabolism, transport, clearance, and pharmacodynamic outcome [82]. The use of human cells may be able to tease apart differences between human responses and animal responses, including differential expression and activity of genes involved in detoxification or in converting drugs to toxic metabolites.

Various methods exist for evaluating the effects of compounds on cells, including methods to evaluate viability (trypan blue staining), proliferation (MTT or MTS assays, BrdUrd incorporation), apoptosis (Annexin-V staining/flow cytometry), and cell cycle progression (propidium iodide staining/flow cytometry) [5, 83]. Due to the ease of isolation of proteins, DNA, and RNA from cells, cell culture also lends itself to evaluation of effects on individual molecular targets. Of particular importance are gene arrays, which allow changes in numerous genes to be observed and quantitated together from a small sample. While the resulting array data are incredibly

complex, better statistical methods and data mining software are allowing for interpretation of the effects of compounds and metabolites on gene expression, and vice versa.

Primary Cell Cultures Primary cell cultures are prepared directly from tissue removed from an organism during biopsy or necropsy. As a result, primary cultures have high variability in the types of cells that can grow from a given sample. The well-differentiated heterogeneous nature of primary cell culture allows for it to retain many complex biochemical properties of the tissue of origin [64]. However, cell viability is variable and depends on factors such as damage to the cell membrane, cell isolation (anoikis), and the nutrients, growth factors, and hormones available in the media. While heterogeneous cultures are sometimes desirable, more often, single cell types (e.g., B lymphocytes) are required. Many cells can be extracted or purified from heterogeneous samples using gating procedures that eliminate or allow for the passage of cells with expression of certain surface proteins [64]. Other cells can be selected for by the type of container and medium in which they are grown. As a result of individual genetics, the growth kinetics differ between cultures, making results obtained from primary cultures difficult to reproduce. Other complications encountered with primary cell lines are their finite life span in culture and the loss of specific cell functions and metabolic capabilities [64].

Permanent and Immortalized Cell Lines Most *in vitro* systems are established with permanent cell lines, which will grow consistently in culture. Often derived from primary cell line subcultures, permanent cell lines will form continuous diploid or clonal lines that will grow consistently for extended periods of time. Of the population of diploid cells, at least 75% of the culture will contain the same complement genetic material as the host organism [64]. These cells can typically undergo approximately 40–50 divisions in culture.

Continuous cell lines often originate from tumor cells. Spontaneous or induced transformation (e.g., via infection with Epstein–Barr virus) of a primary cell line results in a cell line that is immortalized. Immortalized cells retain many, but not all, of the characteristics of the primary cells from which they are derived. Transformation can often result in genetic differences from the tissue of origin, including a heteroploid or aneuploid karotype [64]. However, most of the immortalized and tumor-derived cell lines are actually clonal. Clonal cell lines are formed from a genetically homogeneous subline through single cell isolation. Through reisolation and cloning, genetic drift is minimized and the homogeneity of the cell line can be maintained. Via selection media, desirable phenotypes or markers can be selected for. Transformed cells and established tumor cell lines can grow indefinitely and often can grow in suspension since the cells have lost their need for attachment [64].

Cell-Free Systems Still other *in vitro* systems exist (in addition to cell cultures, and isolated organ or embryo cultures) for evaluating drug metabolism and the presence of potentially toxic metabolites. These typically involve incubation of the target drug with metabolic enzymes or tissues expressing the enzymes. Incubation of a drug with microsomes (isolated vesicles containing metabolic enzymes) can indicate the likely metabolites of the drug [84]. Microsome studies can also be used to assess

differences in metabolism of compounds by mutant enzymes or different enzyme isoforms or to assess whether certain compounds activate or repress the activity of the metabolic enzymes [85]. While whole hepatocytes can also be used, isolated microsomes allow for easier examination of the metabolism of a compound by specific enzymes and complexes. However, microsomes do not contain phase II enzymes [86], so results obtained from microsome studies should be considered carefully. In some cases, it may be preferable to use whole hepatocyte preparations, which contain both phase I and II enzymes [86].

To evaluate the effects on particular cytochrome P450 enzymes, or to determine which enzymes metabolize a drug, there are also kits available containing preparations of specific P450 enzymes (available from Human Biologics International, BD Biosciences, Sigma-Aldrich, and others). While not necessarily reflective of what occurs *in vivo*, these kits and microsome preparations are an excellent way to collect preliminary data on likely metabolites of new drugs.

28.3.5 *In Silico* Models

With the advances in technology in the last few decades, the development of *in silico* (computer-based) testing offers a vast array of toxicity testing requiring small sample sizes [87]. While traditional predictive scaling models utilize equations to determine starting and likely maximum doses for more advanced studies, newer *in silico* studies use computer simulations to model the interaction of various compounds with important receptors and to predict efficacy and toxicity. Computer-aided models focus on replacing animal models in toxicity testing through structure-based techniques, quantitative structure–activity relationship (QSAR) methodologies, and PBBK (physiologically based biokinetic) modeling [88]. Preliminary testing has been very promising. QSAR can be used for a wide range of toxicity endpoints including lethality [89–91]. The results of QSAR analysis continue to improve as more compounds are evaluated and can be compared. PBBK can be used to predict toxicity by simulating the kinetic behavior of the compound of interest using available data. Systems biology attempts to use the interaction of the complete system, that is, metabolic, regulatory, signaling, and transport processes. By understanding the interactions between small molecules and their targets, models should be able to better predict toxic effects of test compounds. Besides reducing the number of animals needed in toxicity testing, reliable *in silico* testing could also predict and prevent failure of a compound in later phases of development [90, 91].

Mathematical modeling of toxicity often involves the use of a dose–response model to define the benchmark dose (BMD) that will give a standard response. The user-friendly BMD software is readily available and is a good tool for establishing a reference point/point of departure within an observable dose range [92]. BMD can be applied with as few as two dose groups and one control group, and it makes use of all data points on a dose–response curve.

The major limitation of these alternative models is data reliability. The toxicity predicted through the use of computer simulations is only as accurate as the data used. However, as the state of the science improves through the resolution of dynamic protein structures, more defined metabolic pathways, and characterized genetic variations within the human population, it is likely that *in silico* technology will also improve.

Traditional toxicity testing involved large numbers of animals and it was often impossible to predict the type of toxicity that would result. Current toxicity testing in animals has been greatly streamlined, partly by the use of *in silico* and *in vitro* models to predict possible target organs and useful endpoints. It is likely that as our knowledge of genetics and pharmacogenetics improves, our use of animal models will be further streamlined, and results from the few animal studies that are done will allow more accurate prediction of human toxicity. While it is likely that the number of animals used can be further decreased, it is unlikely that it will ever be possible to completely avoid their use. No other research model can provide investigators with so much information or can reproduce the complex environment found within a living animal.

Fortunately, however, as more data is generated using these various *in vitro*, *in vivo,* and *in silico* models, it will become easier to develop an understanding of the SARs that exist among different drugs and classes of molecules. This will then facilitate the *in silico* studies, further eliminating the need for using a large number of animals. Combined, all of these technologies will help to ensure that unsafe and ineffective agents do not enter into human clinical trials, thus saving human lives, preserving human quality of life, and guarding the bottom line for the companies developing novel agents.

28.3.6 Predictive Models

Recently, significant progress has been made in predictive toxicology that, from a chemistry perspective, uses a structure–activity relationship (SAR) built on available test data (chemical characteristics, pharmacokinetics, pharmacodynamics, metabolism, toxicity, toxicokinetics, pharmacogenetics, pharmacogenomics, gene profiling, metabolomics, and bioassays) to predict the potential biological activity (e.g., toxicity) of a chemical /drug based solely on its molecular structure and computed properties. Of note, the predictive value of any predictive toxicology approaches that are based on SAR modeling are entirely dependent on and limited by the availability, quality, and quantity of the reference data. In the field of linking metabolism and toxicology, the basic data for SAR modeling include, but are not limited to, the following:

- *In vitro* and *in vivo* toxicology studies
- Characteristics of the chemicals/drugs and their analogues
- *In vitro* and *in vivo* metabolic studies
- *In vivo* and *in vitro* pharmacology screening
- *In vitro* and *in vivo* pharmacology profiling
- Biomarkers/experimental endpoints
- Human accidental exposure studies

Among these data, the following characteristics are particularly important:

- Dose–effect relationship
- Dose–response relationship
- *In vitro*/*in vivo* relationship

- Therapeutic index/window
- Interspecies scaling

To predict biological activities from chemical structure, there are many computational approaches and programs available; interested readers are directed to several excellent recent reviews [93–96]. Such approaches have proven capabilities when applied to well-defined toxicity endpoints or well-documented metabolic pathways. However, these approaches are less suited to the challenges of global toxicity prediction, that is, for predicting the potential toxicity of structurally diverse chemicals across a wide range of endpoints of regulatory and pharmaceutical concern. In this field, there are several major trends improving the predictive values of the models:

- Large-scale data generation involving more fundamental, interdisciplinary technologies such as systems biology, genomics, proteomics, and metabolomics.
- Increasing public information resources to support these efforts.
- In-depth, well-focused mechanistic studies on chemical biology and toxicology using advanced molecular and genetic approaches (e.g., gene transfer, gene knockout, transgenic animal models) and analytical methods (e.g., real-time quantitative PCR, gene arrays, LC/MS, MS/MS).

Figure 28.5 illustrates a systems approach for SAR modeling to predict the linkage between metabolism and toxicology. The role of bioinformatics (Fig. 28.5)

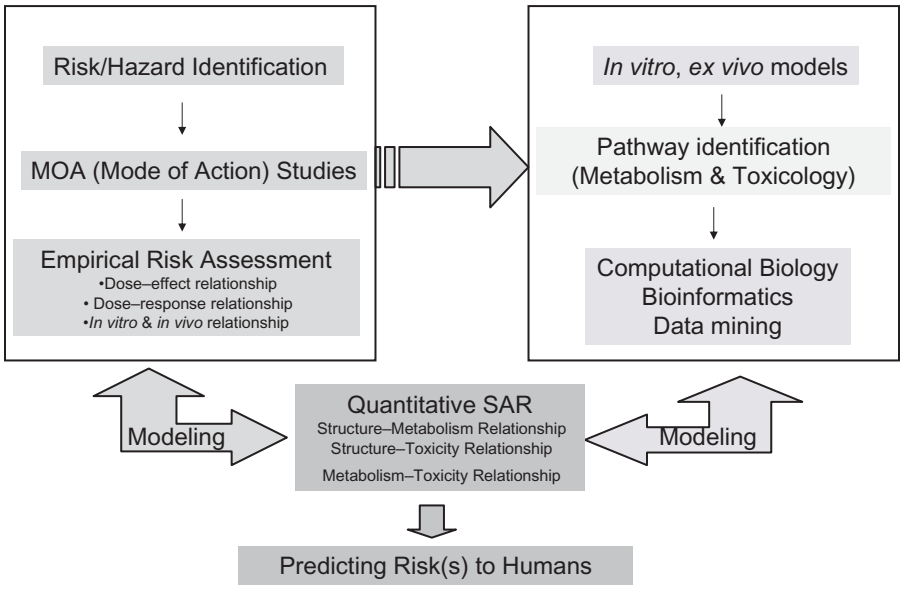


FIGURE 28.5 Systems approaches to SAR modeling of the linkage between drug metabolism and toxicity.

in toxicology data analysis and data mining is critical to SAR modeling, including the following: (1) increasing standardization and digitization of available toxicity data and migration of these data into the public domain; (2) establishing database standards and models that facilitate data integration and mining for both historical and new findings; and (3) linking these toxicology data with chemical structures, leading to hypothesis-driven SAR modeling. Of note, the predictive value of a SAR model should ultimately be confirmed by experimental or clinical data.

28.4 TOXICITY/METABOLISM IN DRUG DEVELOPMENT

28.4.1 Toxicity, Toxicogenomics, and Metabolism

One of the most important steps in preclinical drug development is the evaluation of the metabolism and toxicity of the compound. Many effective drugs fail during toxicity testing. As recent events in the news have shown, the toxicity of compounds is often difficult to predict using traditional toxicity testing. Even extensive animal studies do not give the full picture of toxicity. Moreover, while clinical trials give the most accurate prediction of the response of patients, there are still frequently problems when the new agent is used in a larger population. Depending on the risk–benefit ratio, some toxic agents are still useable, but major toxicity not only costs the pharmaceutical industry time and money that would have been better used for other drugs in the pipeline, but can also result in life-threatening toxicity for patients.

Even when the majority of a population does not experience any major toxicity from a compound, there are genetic variations to important metabolic enzymes (i.e., the cytochrome P450s) that can result in increased or decreased metabolism of a drug, leading to decreased efficacy or increased toxicity. Preclinical testing, including computer modeling, and especially cell culture will be important as more becomes known about the human genome. Pharmacogenomics and toxicogenomics (which study the effect of genes on the efficacy, metabolism, and toxicity of compounds) will likely be used to dictate which agents will continue in development, and whether there are subpopulations of patients who should be cautioned against the use of certain agents.

Although the P450 enzymes may be the most important and well-known metabolic enzymes, there are many others that can affect drug pharmacokinetics and dynamics and can result in otherwise unforeseen toxicity, including examples mentioned in Section 28.2 [50, 54].

While genetic polymorphisms may preclude the use of drugs for certain patients, prior knowledge of potential toxicity caused by these differences can save human lives and ensure that a therapeutic agent can remain in the clinic for other patients. As mentioned, certain mutations within the DPYD gene (which codes for dihydropyrimidine dehydrogenase) result in life-threatening toxicity for patients administered a routine dose of 5-FU. Demonstrating the advances in genetics, molecular biology, and instrumentation, a simple breath test using ^{13}C-labeled uracil was developed that can detect patients who are likely to have DPYD mutations. These patients can be administered alternative therapies while their genotype is confirmed using other methods, such as DHPLC (denaturing HPLC) [97].

28.4.2 "Omics," Toxicology, and Risk Assessment

Being able to predict major toxicity, either for all patients or for certain subsets of patients, could result in saved lives and can lead to a more efficient drug development program. Pharmacogenomics and toxicogenomics can help predict the overall toxicity of compounds and can be used for screening certain subpopulations who may have metabolic differences that could lead to toxicity or inefficacy. In this way, clinical trials and subsequent large-scale use of new agents could be tailored to decrease toxicity and increase efficacy for patients.

Evaluating toxicogenomics and pharmacogenomics is becoming a trend among pharmaceutical companies. For example, Bristol-Myers Squibb started a Department of Applied Genomics in 2001 to evaluate pharmacogenomics and toxicogenomics. Most other major pharmaceutical companies are following suit. Although still in its infancy, pharmacogenomics and toxicogenomics programs have the potential to improve drug development programs by eliminating compounds earlier in the program, allowing more money to be invested in the development of other compounds.

The toxicogenomics "revolution" was sparked by the advent of microarray technology. These arrays utilize small samples of cDNA from cells or tissues to determine the effect of compounds on gene expression [98, 99]. By examining the types of genes that are up- or downregulated by a given compound, it is possible to predict whether a compound might be toxic, and to gain insight into the type of toxicity it causes (e.g., oxidative stress) [99].

Nevertheless, changes at the gene expression level are not necessarily sufficient for assessing the potential risks of new compounds. Often, while there are no changes in gene transcription, there are alterations in protein expression, stability, or activity level. Moreover, different pathways may be activated even while expression levels remain the same. Hence, other "omics" technologies are necessary to more effectively evaluate the effects of a given compound. Proteomics, typically evaluated using two-dimensional (2D) electrophoresis gels (where proteins are separated by charge and then by molecular weight) and mass spectroscopy, can be used to evaluate the expression level and, to a degree, the posttranslational modifications made to numerous proteins. Enzyme assays are usually required to determine the activity of proteins. The newer field of metabolomics (or metabonomics) evaluates the relationship between the genetic profile of an organism/cell and the complement of small molecules present in the plasma/serum or tissues/cells following administration of the agent. Combining all of these "omics" strategies can lead to a more global picture of the response of cells, tissues, or animals to new compounds, can define which genes are important for processing and reacting to the compound, and can be used to predict potential problems with efficacy, resistance, and toxicity.

28.4.3 Future Directions

One of the most exciting outcomes of toxicogenomics is the potential to predict the type of toxicity that may result from administration of a particular agent. Along with this is the ability to determine potential biomarkers that can be used to assess the risk for individual patients. In addition to the already known polymorphisms

(i.e., to the cytochrome P450 enzymes), gene and protein expression levels at baseline and following preliminary dosing (using a lower dose of the agent) can be used to determine whether a particular patient is likely to respond to the agent or is especially sensitive to the compound, potentially leading to toxicity. Biomarkers can also be used to detect whether a patient has been exposed to an environmental toxicant [100].

The "omics" studies also present several ethical issues. In addition to perhaps leading to a mandate for more individualized therapy, or at least a screening for polymorphisms in the most common and important metabolic enzymes, there are many other potential applications for "omics" data. For example, toxicogenomics data could be used to predict whether an individual would be more/less sensitive to toxic chemicals. These data could be used to determine which individuals would be allowed to engage in work where exposure could occur [101]. While this type of practice could prevent toxicity due to exposure to common chemicals, it would also constitute a new (and still legal in some states) type of employment discrimination. It may become necessary to pass federal legislation banning discrimination on the basis of genetic information.

On a more basic level, "omics" studies can provide insight into the complex reactions within living organisms. While none of the *in silico*, *in vitro*, or *in vivo* models are currently sufficient for determining the potential metabolites and toxicity of a compound, the addition of pharmacogenomics, toxicogenomics, and metabolomics may result in a more accurate picture of the toxicity of the compound in humans.

In conclusion, while advances have been made in the fields of toxicology, pharmacology, and molecular biology, there is still a lot of work yet to be done in order to make the drug development process faster, easier, and more effective. Study of the toxicity of both the parent compound and its metabolites can be greatly facilitated by examining the interplay between the genome of the experimental model or patient and the agent being administered. Moreover, when the full spectrum of metabolites is known, a more accurate picture of the toxicity and pharmacokinetics of the agent can be derived. With advances in our knowledge about disease processes, new technology, data processing, and statistical analysis, the evaluation of the toxicity of new agents will become faster and more accurate.

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29

ALLOMETRIC SCALING

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29.1 INTRODUCTION

29.1.1 Role of Allometric Scaling in Preclinical Development

Allometric scaling is useful in the preclinical drug development phase to project pharmacokinetic parameter values determined experimentally in a few animal species to other species and ultimately to humans. Such projection permits early decisions about the developmental prospects for candidate drugs and forms a basis for selection of drug dosage to provide a desired level of systemic exposure. Projection of human pharmacokinetic parameter values from preclinical animal studies permits calculation of a first dose in humans that achieves a desired systemic exposure. Allometric scaling also plays a quality control function in that failure to observe an expected scaling relationship raises a concern about the validity of pharmacokinetic parameter values on hand or about possibly unusual interspecies variability. The purpose of this chapter is to present allometric scaling techniques in the context of the preclinical drug development process.

29.1.2 Historical Perspectives

Allometry in Biology The term *allometry* was first coined by Huxley and Teissier [1] in 1936. The *allometric growth* can be described by an algebraic formula, $Y = aM^b$, in which the sizes (Y) of the parts of an organism follow a power law relationship with the overall body mass (M) [1]. While the original concept of allometry was limited to the size-dependent morphological or structural changes within or between species, it has been applied to other size-related attributes, such as biochemical and physiological processes. To date, allometry (or allometric scaling) is often used to denote any size-dependent power law relationship found in biology.

A topic pursued for more than a century is the allometric scaling of metabolic rate across species. Over 160 years ago, Sarrus and Rameaux first recognized the importance of size as a determinant of metabolic rate (MR) (cited by White and Seymour [2]). In 1883 the German scientist Max Rubner [3] described a proportional relationship between MR and body surface area (BSA) in dogs. He then suggested that metabolic rate is proportional to BSA and therefore to body mass (M) to the power of two-thirds, that is, $MR \propto M^{2/3}$, which has been considered as the *surface law*. In 1932 Kleiber [4] concluded that basal metabolic rate was not proportional to surface area, but scaled with an exponent close to three-fourths. Kleiber's finding was later supported by Brody's [5] famous mouse-to-elephant curve. *Kleiber's law* has been generalized as the quarter-power law, which has been considered as ubiquitous and "universal" in biology [6].

Allometry in Pharmacokinetics While some ponder theories of allometry, biomedical and pharmaceutical scientists face the practical question of how to extrapolate physiological, pharmacological, and toxicological data from animals to humans. In 1949 Adolph [7] listed 34 allometric equations that correlated morphological, physiological, and biochemical parameters with interspecies body mass. For example, creatinine clearance followed the allometric equation with a b value of 0.69 [7]. His quantitative power law relationships suggested overwhelming similarities across

species, which formed a biological basis for extrapolation of experimental data from one animal species to another [8]. Adolph's work also had a significant impact on modern development of physiologically based pharmacokinetics (*PBPK*).

Animal scale-up of pharmacokinetics was actively pursued as early as the 1950s. Pinkel [9] proposed the use of body surface area (*BSA*) as a criterion of drug dosage in cancer chemotherapy, based on the observation of species invariant dose when expressed on a mg/m^2 basis for five antineoplastic agents. Freireich et al. [10] confirmed Pinkel's observation by evaluating the predictive relationship between toxic doses in five adult mammals with adult human data for 18 anticancer drugs. Furthermore, Mellett's [11] analysis of kinetic data for amethopterin and cyclophosphamide in six species, including humans, suggested that an allometric relationship applied for AUC and half-life ($t_{1/2}$).

The concept of scaling pharmacokinetics gained momentum in the early 1970s. Dedrick et al. [12, 13] assumed that pharmacokinetic processes correlated with internal physiological processes and demonstrated that drug concentration profiles in several lab animals were superimposable when a normalization procedure based on the concept of *equivalent time* was adopted. The *equivalent time* was chosen to correlate with body mass to the one-quarter power, based on the similarity principle [14] and the allometric relationship between half-life of methotrexate and body mass of several mammals [13]. This was the first attempt where the similarity principle in biology was applied in animal scale-up of pharmacokinetics [12]. Klotz et al. [15] showed that diazepam plasma clearance was proportional to species body mass to the two-thirds power. Soon thereafter, Weiss et al. [16] detailed the dependence of pharmacokinetic parameters on body mass. They drew the link between pharmacokinetic parameters and body mass by first introducing equations that related pharmacokinetic parameters (hepatic clearance, renal clearance, etc.) to physiological parameters (liver blood flow, *GFR*, etc.), followed by application of the allometric equations for the physiological parameters. Thus, for a drug eliminated by glomerular filtration, the renal clearance would be $CL_{renal} = GFR = aM^b$, where b was cited as 0.82 [16].

In 1980 Boxenbaum [17] characterized interspecies scaling of liver weight, hepatic blood flow, and antipyrine clearance. A log–log plot of antipyrine intrinsic clearance versus body mass yielded a straight line that well described the data from 10 out of 11 mammalian species, but the human value was a pronounced outlier [17]. The seven fold deviation between estimated and observed human antipyrine intrinsic clearance suggested that extrapolation of human pharmacokinetic parameters from animal data using simple allometry may not generally apply. Later, Boxenbaum [18] introduced maximum life span potential (*MLP*) into the allometric equation, producing an allometric relationship between intrinsic clearance of antipyrine per *MLP* and body mass. Boxenbaum's papers drew significant attention and the concept of allometric scaling in pharmacokinetics was well discussed in the 1980s [8, 19–25]. Meanwhile, the suitability of the allometric equation for predicting human pharmacokinetic parameters was further examined [26–29]. Sawada et al. [28] predicted total body clearance, renal clearance, hepatic clearance, volume of distribution, and half-life for six β-lactam antibiotics in humans. Extrapolations were performed using data from mice, rats, rabbits, dogs, and monkeys. For the first time, differences in the plasma protein binding among species was considered an important determinant for the prediction of volume of distribution [28]. Sawada et al. [29] further showed

that similarities among animals and humans in tissue distribution of 10 basic drugs failed to produce a successful prediction of the human value. To predict the volume of distribution it was suggested that unbound fraction in plasma (f_{up}) should be included for analysis. In the same period, prediction of human pharmacokinetic parameters using the allometric equation was applied to other antibiotics, such as cephalosporins, monobactams, and macrolides [26, 30].

In the early 1990s an insightful review by Ings [31] heralded a new era for allometry in pharmacokinetics. As this new era began the concept and technique of allometric scaling had become familiar to drug development scientists. But it had also become apparent that the allometric scaling technique was not universally reliable. While for humans the technique sometimes gave acceptable predictions for the pharmacokinetics of those drugs whose elimination was either renal or liver-blood-flow dependent, poor predictions often occurred for low clearance drugs whose elimination was primarily via metabolism. Moreover, the application of the technique was limited to only a few drug classes. These shortcomings contributed to increased research activity in the 1990s. The features of interspecies pharmacokinetic studies in this period include (1) a publication boom focused on interspecies pharmacokinetic scaling for old and new agents and small versus large molecules (proteins); (2) the use of single animal species to extrapolate human pharmacokinetics; (3) the integration of *in vitro* metabolic data into the allometric prediction; (4) the use of the *rule of exponents* along with additional studies of correction factors such as *MLP* and brain weight; and (5) more successful examples of human dose estimation for Phase I studies.

A commentary by Bonate and Howard [32] highlighted an inherent problem faced by those interested in prediction—the uncertainty associated with the prediction. The authors argued that a large bias was associated with publication of predominantly successful allometric scaling attempts, which could lead to a false sense of security with regard to use of allometric scaling to prospectively select dose for a first-time-in-human study. One major problem pointed out by the authors was that prediction intervals are often too wide to be of practical use [32]. By using linear regression and log–log coordinates for prediction, the authors cautioned that an excellent point estimate might not prescribe a good prediction. Moreover, they questioned whether the use of "correction factors" in allometric scaling was appropriate [32, 33].

Hu and Hayton [34] tackled the uncertainty issue in a different way. They carefully analyzed clearance values for 115 xenobiotics from published studies in which at least three species were used for the purpose of interspecies comparison of pharmacokinetics. The allometric exponent for each xenobiotic was calculated along with its confidence interval (*CI*). While it was of interest to note that for each individual drug either 0.67 or 0.75 appeared to be the central tendency of the allometric exponent, the confidence interval of the exponent was often large due to considerable random variability in the clearance values from each species [34].

After so many years of endeavor, it is somewhat discouraging that no matter which allometric method is used for predicting human pharmacokinetic parameters, some drugs will be very poorly predicted. The incorporation of *in vitro* metabolic data improves the prediction performance. Perhaps the most urgent task for the future is to understand what makes certain drugs so unique and how to improve the predictability of these drugs.

29.2 ALLOMETRIC EQUATION

29.2.1 Mathematical Description, $Y = aM^b$

As used here, *scaling* refers to relationships between objects and their representation (e.g., a scale model where 1 cm represents 1 m) or to relationships between elements of a series of similar objects that vary in size only. For example, when geometric bodies such as squares, rectangles, and cubes vary in size, their corresponding linear dimensions differ by a constant ratio. Two geometrically similar rectangles that differ in width by a factor of 2 will differ similarly in length. Length is then said to scale in direct proportion to width, while area scales in direct proportion to the square of width. Geometrically similar bodies are also referred to as *isometric* bodies (*iso* means *equal*).

When bodies are not geometrically similar they are referred to as *allometric* (*allo* means *different*). Characterization of scaling relationships for a series of geometrically dissimilar bodies, for example, mammalian species, is therefore termed *allometric scaling*. For organisms, many aspects of their morphology and physiology change systematically as body mass (M) changes, according to the power function:

$$Y = aM^b \tag{29.1}$$

where a and b are constants, and Y is the particular variable of interest. Because Eq. 29.1 relates so many biological variables to body mass, it has become known as the *allometric equation*. In its logarithmic form, it is

$$\log Y = \log a + b \log M \tag{29.2}$$

Use of the allometric equation for scaling in biology was popularized by Huxley [35]. During the ensuing decades, use of the equation became widespread even though no satisfactory underlying theoretical basis supported its use [7, 36]. The allometric equation was often invoked whenever a linear relationship between log Y and log M was observed. Critics of the nondiscriminatory, routine use of the allometric equation have pleaded for more thoughtful application of the equation, noting that use of the "cookbook approach to study the comparative biology of size has falsely simplified a complex problem" and that "use of the method has been considered equivalent to study of the problem" [37].

The foundations for a theoretical underpinning of the allometric equation were laid by the landmark paper of West, Brown, and Enquist [6], which were strengthened in following years [38, 39]. The paper proposed that organisms utilize a similarly structured material supply network, which provides the common mechanism that underlies the allometric equation and in addition explains the often observed quarter-power (and its multiples) value for the body mass exponent, b. The theory is based on three assumptions: (1) the supply network has "a space-filling fractal-like branching pattern"; (2) "the final branch of the network . . . is a size-invariant unit"; and (3) "the energy required to distribute resources is minimized." An extensive variety of quantifiable biological characteristics conform to the theory, which removes much of the empirical nature of the allometric equation [40].

29.2.2 Interpretation of *a* and *b*

Empirically the values of the body mass coefficient a and exponent b are simply those that best fit the allometric equation to the particular dataset. The value of a is the value of Y when the value of M equals unity. The b value shows the sensitivity of the Y value to a change in the M value. As is often the case, $b < 1$ and a change in M results in a less-than-proportional change in Y. In the special case of $b = 1$, Y varies in direct proportion to M. While Heusner [41] argued that the a value is not a constant but rather increases with the size of M, applications in scaling pharmacokinetics have generally ignored this possibility.

The b value is often interpreted in comparison with the values obtained from simple geometrical relationships; for example, a value of two-thirds that occurs when the variable Y is proportional to body surface area rather than body mass [42]. In this regard, the allometric scaling of basal metabolic rate in mammals is of interest as a potential surrogate for the body mass sensitivity of drug metabolism rate. Kleiber [43] proposed for basal metabolism a b value of three-fourths, a value that was disputed for several decades, with an alternative value of two-thirds proposed [41, 44–46]. The two-thirds value garnered support from a heat-dissipation mechanism, where it was argued that heat loss is proportional to body surface area and that heat loss is an important constraint that forced its production per unit body mass to decline as body mass increased, in proportion to body surface area. The three-quarters value lacked a compelling mechanistic explanation until the model of West, Brown, and Enquist [6], which convincingly supports the three-fourths value [40].

29.2.3 Using the Allometric Equation

Allometry has been broadly defined as "the study of size and its consequences" [47], and more narrowly described as the specific use of the allometric equation to analyze the body mass influence on quantifiable biological phenomena [37]. In preclinical drug development, the narrow description more aptly applies as the usual problem involves characterization of the association between body mass and quantitative pharmacokinetic and biopharmaceutic parameters—for example, apparent volume of distribution, clearance, half-life, and bioavailability—and using the quantitative association to make predictions. Both inter- and intraspecies applications are possible.

Choice of Species Preclinical drug development uses laboratory animals to gain insights about the pharmacology and toxicology of candidate drugs. Because animals and human beings show marked congruence in their genome, physiology, and biochemistry, it is hoped that insights obtained from animal studies will be applicable to humans. Dose–response relationships, metabolite profiles, bioavailability properties, pharmacokinetic behaviors, and so forth observed in animal studies are hoped to be predictive for humans. Decades of drug development research have validated the utility of animal studies in preclinical development, and informed the uncertainty involved in extrapolation of animal results to humans, but have also borne witness to marked divergences, which sometimes have been unpredictable and difficult to explain.

Thus, an important and hard-to-answer question is: Which species should be used in preclinical studies? While it is tempting to believe that those species with the closest phylogenetic relationship to humans will be the most reliable for prediction of drug behavior in humans, it has become apparent that substantial differences in metabolic clearance occur between closely related species. Environmental and dietary adaptations are also important determinants that shape quantitative differences (and similarities) in drug behavior among species. In addition, nonscientific considerations such as cost and public acceptance of experimentation with particular species cannot be divorced from the selection of species to be studied. It appears uncontroversial to suggest that test species should be from the Class *Mammalia*, and from the 4000+ members of this class the species most commonly used are rat, dog, rabbit, mouse, and monkey [31] (Table 29.1). While popularity of usage does not necessarily point to the species that provide the best prediction of behavior in humans, there is scant evidence to suggest that alternative species would be better in this regard. A recent review concluded that no animal species consistently shows absorption, distribution, metabolism, and excretion behaviors similar to those of humans [48]. Animals "humanized" by transgenesis may improve this situation [48, 49].

Ideally, the number of test species to use is at least four, particularly if the test animal body mass range does not encompass 70 kg. This recommendation is based on a review of clearance (CL) values for 115 xenobiotics subjected to allometric analysis, where failure to find a statistically significant correlation between log CL and log M occurred for 24 compounds [34]. The correlation failure rate declined as the number of species studied increased: a total of 20 studies used only three species and 16 of those failed to find correlation. The correlation failure rate was dramatically lower for $n \geq 4$: for $n = 4$, five failures to find correlation out of 43 studies; for $n = 5$, 2/31; for $n = 6$, 1/15; and for $n = 7$, 0/6.

TABLE 29.1 Incidence of Use of Animal Species in Scaling Clearance Values for Xenobiotics

Species	Usage (% of 115 Xenobiotics)
Rat	97
Dog	73
Rabbit	62
Mouse	56
Monkey	53
Pig	7.8
Guinea pig	6.1
Sheep	5.2
Hamster	5.2
Baboon	3.5
Chimpanzee	2.6
Cat	2.6
Cow	2.6
Goat	0.9

Source: From Ref. 34, with permission.

While at least four species is optimum, data from fewer species is not unusual and effort has been expended toward development of scaling techniques for this case [50–55]. Allometry using three and even two species was investigated using a 103 nonpeptide xenobiotic training set, along with nonallometric single species approaches to *CL* estimation based on the product of hepatic extraction ratio estimated in animal and human hepatic blood flow [50, 51]. For the 103 drug candidates, volume of distribution (*V*), total body clearance, and mean residence time (*MRT*) were determined from the plasma (serum) concentration–time profile after intravenous (IV) administration in rat, dog, monkey, and human, and it was possible to compare the various prediction techniques using animal data with measured human values. Allometry based on all three animal species, and pairs of species (rat–monkey, rat–dog, and dog–monkey), predicted human *V* and *CL* values within an error of –50% to +100% (exception: dog–monkey pair for *CL*), for about two-thirds of the test compounds, and MRT was predicted within –50% to +100% for about two-thirds of compounds using allometry based on three species and on the rat–dog pair only.

Ideal Body Mass Range Clearly, the selected species should span a "reasonable range" of body mass values, and if the objective is to scale the animal values to estimate the corresponding human value, it is desirable to include species with *M* values below and above 70 kg. However, resource constraints often make it difficult to study animals larger than 70 kg and very few published studies of pharmacokinetic parameter scaling include species larger than humans (Table 29.1). While pharmacokinetic parameter values frequently conform to the allometric equation, use of a small number of values from experimental animals to prospectively predict human values by extrapolation beyond the range of *M* values is "outright perilous" [36].

It is challenging to state precisely what an appropriate *M* range would be; two orders of magnitude seems reasonable, although not based on theoretical principles. For example, if rat were the smallest species (0.2 kg), then the largest species should be on the order of 20 kg. Also, while it is desirable to avoid extrapolating beyond the *M* value range, it is often necessary to do so, in which case one should minimize the extrapolation. A given error in the extrapolation line would be expected to produce a larger error in the extrapolated value the further the extrapolation. For example, if mouse (0.02 kg) were the smallest species and the largest species were 2 kg, the two-orders-of-magnitude suggestion would be met, but the extrapolated human value would be less certain due to the extrapolation from 2 kg to 70 kg. In this case, the *M* range should be increased to minimize extrapolation.

Optimal Species for Scale-up to Human Drug development studies mostly utilize species from the mouse–rat–rabbit–monkey–dog group (the standard five) and data are insufficient to fully address this question beyond these five species. Furthermore, only these species have well established background pathology and other species would not be used for toxicology testing even though they might be advantageous for extrapolation of pharmacokinetic behavior to human [31]. As preclinical pharmacokinetic data are usually obtained as part of the toxicology studies, it is pertinent to consider whether some of the five most commonly used species would serve better than the others for prediction of human pharmacokinetic parameter values.

Rat is nearly universally used in preclinical pharmacokinetic studies and its pharmacokinetic parameter values generally relate well to corresponding human values in allometric scaling analyses [51]. A review of the 61 drugs for which *CL* values were available in human and at least three species showed that qualitatively most of the standard five species were close to the fitted allometric equation line and that the human *CL* was reasonably predicted most of the time (data used in Ref. 34). Significant deviations were relatively few although more prevalent for rabbit and monkey than for mouse, rat, and dog. On the other hand, in their analysis of a 103 compound dataset, Ward and Smith [50, 51] concluded that monkey was somewhat superior to other species for prediction of human pharmacokinetic parameter values when single species extrapolation was conducted. It therefore appears that none of the standard five species stands out as particularly superior or inferior to the others.

Fitting the Allometric Equation to Data Approaches that can be used to fit the allometric equation to *Y,M* data include linear regression of the logarithms of *Y* and *M*, and nonlinear least squares or maximum likelihood fitting of the allometric equation to the *Y,M* data. Both approaches assume that the *M* values are independent variables and that the error associated with their determination is zero. It is therefore essential to accurately determine the mass (weight) of each animal used for determination of the corresponding *Y* value, with the associated experimental error being negligible compared with that of the *Y* value.

While individual *Y,M* values for each animal could be fitted, it is customary practice to average the values from each species and to fit the allometric equation to the means. On log–log coordinates with a two order of magnitude range for *M*, individual values for each species cluster close together, and as a practical matter, there is little difference between analysis of individual or lumped data, with regard to the appearance of the plot and the values of *a* and *b*.

Avoid application of allometry to *M*-normalized *Y* values as this can create spurious correlation [56]. Weighting the *Y* values is not usually done, probably because the linearized form of the equation is usually fit to log *Y*, log *M* points and the magnitude of the range is thereby much reduced. If the equation is fitted directly to *Y*,*M* data, a weighting function should be used to prevent the excessive influence of the larger *Y* values on the *a* and *b* values; for example, Y^{-1} or Y^{-2}, or by the reciprocal of the variance of the means. Pertinent statistical characterizations of the goodness of fit should also be calculated and reported; namely, correlation coefficient and its statistical significance, and variance for *a* and *b*. Prothero [56] developed by simulation an empirical equation to calculate the standard error (*se*) for *b* from the mean percent deviation (*PD*) of the points about the line, the number (*n*) of points, and the log of the body mass range (*pWR*), where *WR* is M_{max}/M_{min}:

$$se = \frac{0.019(PD)}{pWR \times n^{1/2}} \tag{29.3}$$

This alternative to the value supplied by standard statistical packages alerts the user to suspect unreliability in the latter when it is smaller than predicted by Eq. 29.3.

29.3 SCALING CLEARANCE

29.3.1 Physiological Basis and Theoretical Considerations

Clearance is generally defined as the proportionality constant that relates the steady-state rate of drug elimination to its concentration in a reference fluid, which is commonly plasma or serum and sometimes whole blood [57]. For expediency plasma concentration (C_p) is used in the following sections with the understanding that it represents the other reference fluids as well. The total body (systemic, plasma) clearance (CL) is a primary pharmacokinetic parameter, often determined experimentally as the ratio of dose to area under the plasma concentration–time profile after IV administration of the dose:

$$CL = \text{Dose} \Big/ \int C_p \, dt$$

The total body clearance is the sum of the clearance values contributed by the various organs involved in the elimination of the particular substance. Hepatic clearance (CL_H) is the major contributor to CL of drugs; it contributes more than three-fourths the CL for about 60% of the drugs used in therapeutics [58]. For most hepatically cleared drugs, metabolism is the major mechanism, with biliary excretion of importance for a relatively small number of drugs, usually those that are amphipathic and have molecular weight threshold that differs from species to species in the range 400 ± 100. Renal clearance (CL_R) is a distant second to CL_H in overall importance, being the principal contributor to CL for only about 15% of drugs in current use. For interspecies scaling of CL values it is helpful to know the relative contributions of the liver, kidneys, and other organs to the total body clearance.

Physiologically based models of clearance provide a useful basis for understanding interspecies scaling relationships [16]. These models incorporate the plasma flow (Q) to the clearing organ, the free fraction of drug in the plasma (f_{up}), and the intrinsic activity of the clearance process within the organ (CL_{int}). Each of these components varies among species, and for each there is the possibility that its allometric scaling relationship differs from the others. In the following three sections, expectations for allometric scaling relationships are developed based on relevant physiologically based clearance models.

Hepatic Clearance Of the several available models of CL_H [57], the well stirred or venous equilibrium model will be used, as other models lead to similar expectations with regard to allometric scaling relationships. The standard representation of the well stirred liver model of CL_H is [59]

$$CL_H = \frac{Q_H f_{up} CL_{int}}{Q_H + f_{up} CL_{int}} \tag{29.4}$$

CL_{int} here represents the activity of metabolism enzymes or the activity of biliary transport mechanisms.[1] There are two limiting cases, one where $Q_H >> f_{up} CL_{int}$ (low

[1]It may be drug concentration dependent if the metabolism enzyme and/or the transport mechanism are significantly occupied by the drug. This greatly complicates characterization of the pharmacokinetics and thereby the scaling of pharmcokinetic parameter values across species. This situation is not addressed in the present chapter.

extraction case) and the other where $Q_H << f_{up}CL_{int}$ (high extraction case). Most drugs fall into the low extraction category and for these $CL_H \cong f_{up}CL_{int}$. The extraction class is readily identified by comparison of the values of CL_H and Q_H; Q_H values are available in the literature [60]. For low extraction drugs the ratio CL_H/Q_H is below 0.25 and for high extraction drugs it is above 0.75. In the low extraction case, interspecies differences in both f_{up} and CL_{int} contribute to interspecies differences in CL_H. To remove the influence of f_{up}, it is recommended that CL_{int} be the hepatic clearance parameter to scale; it is calculated as the ratio CL_H/f_{up}. The human CL_H value is then calculated as the product of the measured human f_{up} and the human CL_{int} predicted by allometric scaling. Interspecies scaling of CL_{int} is problematic; there are examples of high interspecies variability and of a tendency for the predicted human CL_{int} value to substantially exceed the measured value. Predicted human CL_{int} values by allometry applied to animal data should therefore be regarded with appropriate skepticism. Approaches to dealing with this uncertainty are described in Section 29.3.2.

For the high extraction class of compounds, $CL_H \cong Q_H$, and the expectation in allometric scaling is that CL_H would scale as does Q_H; f_{up} is not an issue in this case. Liver blood flow for the standard laboratory animals and humans is well fitted by the allometric equation with the b value being 0.894 [17]. For a high extraction drug therefore, the expectation is that the b value for CL_H would be about 0.9, similar to that for Q_H.

For intermediate extraction drugs (CL_H/Q_H between 0.25 and 0.75), CL_H shows sensitivity to f_{up}, Q_H, and CL_{int}. The influence of f_{up} cannot be removed by scaling CL_H/f_{up} as an f_{up} term remains on the right-hand side of Eq. 29.4. This case is perhaps the most difficult to handle as all three independent determinants can vary in different ways across species, causing CL_H values to vary erratically, the result being no systematic way to scale them.

Renal Clearance Drug elimination by the kidneys is quantitatively less important than that by the liver; it contributes more than 75% to total clearance for ~15% of drugs used in therapeutics [58]. Renal clearance (CL_R) may be more complex than hepatic clearance as it may involve a combination of glomerular filtration, tubular secretion, tubular reabsorption, and metabolism. Physiological models are available for each process, but their combination is difficult since the processes act in series and are therefore not simply additive. For the filtration component, $f_{up} \times GFR$ is its contribution to CL_R, which may then be reduced due to subsequent tubular reabsorption. The tubular secretion contribution may be modeled along the lines of the well stirred liver model, where Q_R replaces Q_H and represents the postglomerular plasma flow. Tubular reabsorption may also reduce the secretion clearance. Finally, tubular reabsorption of drug from the preurine of the tubule may be passive or involve transporter, and it may be dependent on preurine flow. Clearly, several physiological processes may contribute to the CL_R value; those operative for a particular test compound and their relative contributions are generally only poorly understood at the preclinical stage of development. Nevertheless, CL_R values typically scale relatively well across test animal species and the human CL_R value is typically well predicted by allometric scaling of animal CL_R values. Appropriate adjustments for interspecies differences in f_{up} should be considered; see Role of Protein Binding in Section 29.3.2. For example, both filtered and secreted test sub-

TABLE 29.2 Body Mass Exponent (*b*) Values for Renal Clearance of Renal Function Test Substances [7]

Test Substance	Renal Clearance by	*b*
Urea	Filtration	0.72
Inulin	Filtration	0.77
Creatinine	Filtration	0.69
Diodrast	Filtration + secretion	0.89
Hippurate	Filtration + secretion	0.80

stances followed the allometric equation reasonably well, and *b* values based on measured renal clearance values in rat, rabbit, dog, and human were in the range 0.69–0.89 (Table 29.2) [7]. These substances are little bound (urea, inulin, creatinine) to plasma proteins or highly extracted (diodrast, hippurate) so f_{up} was not a confounding variable.

Total Body Clearance Total body clearance may be dominated by a single organ and in that case scaling of *CL* would conform to the expectation for the major pathway. In the event that multiple organs were involved (e.g., liver and kidneys contribute significantly), *CL* in principle could be a sum of allometric expressions:

$$CL = a_L M^{b_L} + a_R M^{b_R} \tag{29.5}$$

If the *b* values differed significantly, the relative contribution of each pathway would vary across species and a simple log *CL*–log *M* graph would be curved. In practice, *b* values for the hepatic and renal pathways are not sufficiently different that this behavior is observed, and other pathways generally do not contribute significantly.

29.3.2 Empirical Observations

The following subsections present approaches to the allometric scaling of drug clearance and the performance of each approach as experienced in preclinical drug development. The unembellished application of the allometric equation is considered first and this is followed by a series of modifications aimed at improvement of its performance.

Simple Allometry A study of the application of Eq. 29.1 to interspecies scaling of drug clearance for 115 xenobiotics in three or more species showed that *CL* generally followed the allometric equation (Fig. 29.1) (Fig. 3 in Ref. 34). While the equation failed to fit *CL* values for 24 of the compounds, as indicated by the failure to show a statistically significant correlation, most of the failures could be attributed to use of only three species (see Section 29.2.3, Choice of Species). For 91 compounds, the *CL* values followed the allometric equation; in most of these cases four or more species were used. From the set of 91 compounds, 68 included humans as

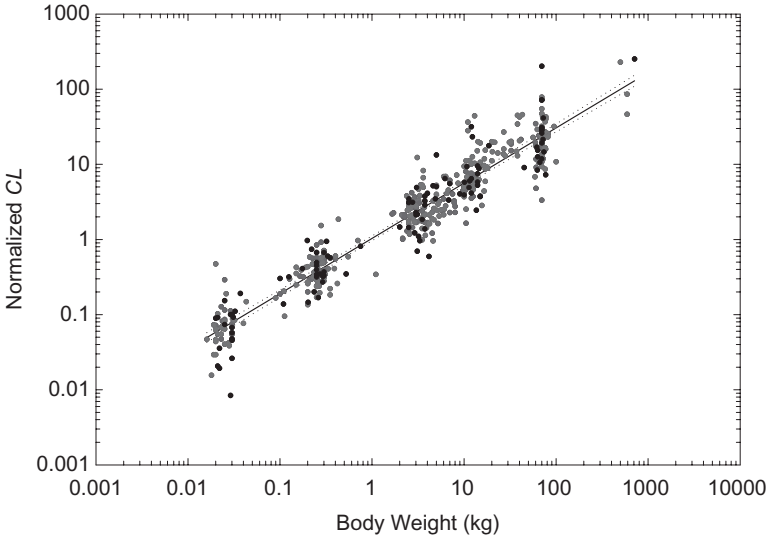


FIGURE 29.1 *CL* values for 91 substances determined in three or more species. Values were normalized by adjustment of all values of *a* to 1 [34].

one of the species. For all but 7 of the 68 compounds, the observed human *CL* value deviated from that predicted by the fitted allometric equation by less than 50%; that is, the measured value was within a range of one-half to twice the predicted value, which is a reasonable marker of precision [52, 55].

This relatively rosy record of success (~90%) has to be tempered by two realities. First, because the human *CL* was included in the fitted datasets, the human *CL* would be expected to lie closer to the predicted value than if it had been omitted from the values used to fit the equation. Second, the data for the various compounds were from the literature, mostly from papers that reported on the allometric scaling of the compound. As negative results are rarely reported, it is not known how often the allometric equation fails to scale *CL* values across species.

Ward and Smith [51] compiled from the literature *CL* values for 103 nonpeptide xenobiotics in rat, dog, monkey, and human after IV administration. Allometric scaling of the three animal species' *CL* values to predict the human *CL* value had a 66% success rate, based on whether the human *CL* was correctly predicted to be in the low, moderate, or high *CL* category, defined as a percentage of hepatic blood flow (<30%, 30–70%, and >70%). The success rate differed across categories, being 80–90% for low *CL* substances and only 30–50% for high *CL* substances.

It might be concluded that allometric scaling of human drug *CL* using three or more animal species is a viable approach. For renally eliminated drugs, the success rate is quite high, particularly when renal elimination is primarily by glomerular filtration, plasma protein binding is low, and tubular reabsorption is minimal. For more complex renal clearance mechanisms, the success rate would tend to decline. For drugs eliminated hepatically, the success rate is not as high as when the kidney is the dominant pathway. There are dozens of hepatic drug metabolism enzymes that across species vary in their activity and substrate selectivity. That along with

interspecies variability in the extent of plasma protein binding make the success rates reported above surprisingly high.

To improve the predictive capability of allometry, several embellishments have been proposed. The first to consider is the use of brain weight or maximum life span potential along with M.

Maximum Life Span Potential and Brain Weight In scaling the CL of extensively metabolized drugs, investigations found early on that the human CL value often fell considerably below the value predicted in animals. Examples include the intrinsic hepatic clearances of antipyrine and phenytoin, which were below the values predicted by application of the allometric equation to animal data by factors of 7 and 4.4, respectively [17]. Compared with other mammals, humans were also noted to have relatively long life spans, and life span was observed to relate to brain weight. As enhanced longevity may necessitate slowed energy consumption, it was thought that drug metabolism rate may also correlate inversely with life span. A quite successful test of this proposition with antipyrine, phenytoin, and clonazepam intrinsic clearances involved allometric scaling of the product of CL_{int} and MLP, the maximum life span potential [18]. This adjustment brought the human CL_{int} values into alignment with the animal values (e.g., antipyrine) (Fig. 29.2).

A variation of this approach is to use brain weight (BW, literature values) as a second independent variable in the simple allometric equation. For example, antipyrine CL_{int} (mL/min) in 15 mammalian species including humans [21] is given by

$$CL_{int} = 0.3865M^{1.316}BW^{-0.6188} \tag{29.6}$$

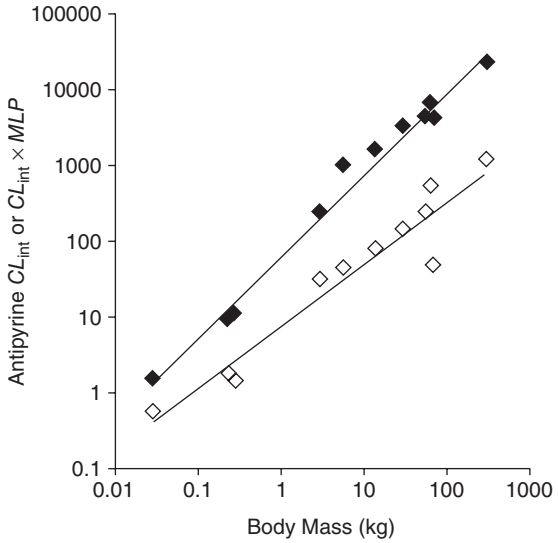


FIGURE 29.2 Allometric scaling of antipyrine intrinsic clearance (CL_{int}) alone (◇) and after multiplication by MLP (◆). CL_{int} from Ref. 21 and MLP from Ref. 18. CL_{int} for human and pig moved from below and above the allometric line (lower plot) to be near the allometric line for $MLP \times CL_{int}$ (upper plot).

where M and BW are in kilograms. The coefficient and exponents were determined by fitting the equation to the data using a computer-based algorithm. All 15 calculated CL_{int} values were within –67.4% to +57.6% of the observed values.

However, not all drugs eliminated by metabolism show overprediction of human clearance upon allometric scaling of animal values; for example, Boxenbaum [18] noted that while some do (antipyrine, benzodiazepines, propranolol), others such as tolbutamide and cyclophosphamide do not. More recent work indicates that human CL values may fall above the allometric line as often as they are below it [34]. Use of MLP or BW generally moves the human CL_{int} value more that it does the values from animals. Thus, the MLP or BW technique would tend to move a human value that was on the simple allometric line to well above the line. As a *prospective* technique to predict human CL_{int}, use of MLP or BW is problematic as the result would be to increase the number of overpredictions as the number of underpredictions was decreased. From the standpoint of first-dose-in-human estimation, this may be undesirable as overprediction of CL would lead to a less conservative first human dose.

The "Rule of Exponents" For prediction of human CL from animal values, Mahmood [61] and Mahmood and Balian [62, 63] examined the predictive capacity of simple allometry, the MLP and BW techniques described in the previous section, and a variant that involved allometric scaling of the $CL \times BW$ product. These studies pointed to the empirical use of the b value obtained from simple allometry to select one of the following methods to predict human CL:

1. For $0.55 < b < 0.75$, use the simple allometric equation.[2]
2. For $0.75 < b < 1.0$, use the MLP technique:

$$CL_{human} = \frac{a70^b}{8.18 \times 10^5} \tag{29.7}$$

   where a and b are obtained from fitting the allometric equation to the product $CL_{animal} \times MLP$ (h), and 8.18×10^5 h is the human MLP.
3. For $b > 1.0$, use

$$CL_{human} = \frac{a70^b}{1.53} \tag{29.8}$$

   where a and b are obtained from fitting the allometric equation to the product $CL_{animal} \times BW$ (kg), and 1.53 kg is the human BW.

This empirical approach based on the b value after simple allometry is known as the *rule of exponents*. For the set of 46 test substances, CL values were available in three or more of mouse, rat, rabbit, guinea pig, goat, monkey, and dog. The predicted human CL was within the range 0.5–2.0 times the observed CL for 33

[2]The upper limit (0.75) of the b range was given as 0.70 in Refs. 62 and 63 and as 0.75 in Ref. 61. The 0.75 value was used in this analysis.

substances (72%), and an additional 6 (13%) substances were included in the range 0.2–5.0.

A comprehensive evaluation of the approaches described above for prediction of human *CL* utilized a 103 compound dataset with *CL* values determined in rat, dog and monkey [52, 55]. Using the limits 0.5–2.0 for the ratio of predicted to observed *CL* in humans to define acceptable predictive performance, none of the allometric approaches was particularly successful. Simple allometry with no correction factor performed better (53% of acceptable predicted human *CL*) than did incorporation of *MLP* (38%) and *BW* (45%), and use of the rule of exponents to select among the three approaches (49%). In addition, subsets of test compounds grouped according to physicochemical property (*c* log *P*, polar surface area, number of H-bond acceptors, number of rotatable bonds) and route of elimination failed to improve predictive performance. This discouraging analysis suggests that allometry applied to *CL* values determined in animals will not reliably predict the human *CL* value. As noted earlier (Section 29.2.3, Choice of Species) use of only three animal species does not provide a robust dataset for projection to other species and the relatively poor performance reported by Nagilla and Ward [52] may reflect in part this constraint.

The search for more reliable techniques to improve the allometric-based approach to prediction of human *CL* has more recently turned to incorporation of *in vitro* measurements such as activity of hepatic enzymes and plasma protein binding. These efforts are summarized in the following three subsections.

In Vitro *Data for* In Vivo *Prediction* Liver weight follows the allometric equation in mammals with a *b* value of 0.849 [17], which is similar to that for hepatic blood flow. Thus, in the common mammalian species hepatic blood flow per unit mass of liver is invariant, at about $1.5\,\mathrm{L\,min^{-1}}$ (kg liver)$^{-1}$ and failure to scale *CL* among species is generally not attributable to liver mass or blood flow issues. It has been known for some time that *in vitro* enzyme kinetic parameters can be quantitatively related to *in vivo* hepatic clearance in a single species. This *in vitro*/*in vivo* correspondence was first demonstrated in rat by Rane et al. [64]. For seven compounds having a wide range of hepatic extraction ratio, they successfully predicted *in vivo* hepatic extraction ratio from K_M and V_{max} determined in $9000g$ supernatant or microsomal preparations along with liver mass and blood flow, and blood free fraction. Others reported similar success [57]. The feasibility of *in vivo* hepatic CL_{int} prediction ($CL_{int} = V_{max}/K_M$) pointed to the potential to use K_M and V_{max} values in animals to predict the human CL_{int}.

A significant problem remained, however. Among the dozens of hepatic enzymes available for xenobiotic metabolism, relatively few are primarily involved in the metabolism of any particular compound. As there are interspecies differences in the relative proportions of the various enzymes and structural differences that can lead to interspecies differences in intrinsic activity and metabolic pathways, it is not so simple a matter to use *in vitro* metabolism parameters determined in laboratory animals to predict CL_{int} in humans [48, 65]. Possible reasons for failure to predict human CL_{int} include nonhepatic metabolism, slow equilibrium between blood and hepatocytes, and involvement of transporters [66]. Recent effort has therefore focused on integration of *in vitro* measures of metabolic activity with *in vivo* CL_{int} values in animals to improve the accuracy of human CL_{int} prediction.

Incorporation of In Vitro Data in Allometry The first successful combination of *in vitro* and *in vivo* data to predict the human CL_{int} took the approach of adjusting each *in vivo* animal clearance (CL_{an}) value by multiplying by the human/animal ratio of *in vitro* V_{max}/K_M [67, 68]. This effectively adjusted each animal CL to the value it would have had if the overall intrinsic activity of drug metabolism enzymes were the same in the test animal and humans on a unit mass basis; that is, CL_{an} could be "humanized" (CL_{an}^{h}) by the following adjustment:

$$CL_{an}^{h} = CL_{an} \frac{(V_{max}/K_M)_{human}}{(V_{max}/K_M)_{an}} \tag{29.9}$$

This adjustment to CL_{an} reduces the problem associated with interspecies differences in metabolism pathways and enzyme activities so that CL_{an}^{h} values will more likely follow the allometric equation and will also more likely predict the *in vivo* human CL. It is only appropriate for low or intermediate hepatic extraction drugs since for high extraction drugs hepatic blood flow rather than CL_{int} is the principal determinant of CL_H. Because liver size and blood flow scale according to the allometric equation, adjustments for their interspecies differences are unnecessary. At least three animal species should be used.

The *in vitro* V_{max} and K_M values were determined using a hepatic microsome preparation. The impact of inefficiency in the microsomal isolation procedure is minimized if it is similar for all determinations, as assured by simultaneous determination of *in vitro* parameters using the same protocol. Lavé et al. [67] also used an *in vitro* hepatocyte preparation to adjust the *in vivo* CL_{an} with equally good results. For hepatocytes, *in vitro* CL_{int} (volume/10^6 cells) = Dose/$AUC(0-t)$, where $AUC(0-t)$ is the area under the drug concentration–time profile in the hepatocyte suspension over the duration of the incubation. The hepatocyte method for assessment of *in vitro* CL_{int} is the method of choice as it provides the widest array of enzyme activities and generally shows the same metabolite pattern as is seen *in vivo* [69].

Role of Protein Binding Both renal and hepatic clearance may be affected by the degree of plasma binding; namely, when clearance is predominantly composed of low extraction hepatic or active renal tubular secretion clearance, or glomerular filtration. Xenobiotic binding affinity can vary considerably among species, due to differences in the concentration of the particular protein and to differences in the affinity of the xenobiotic for the binding protein [70, 71]. The influence of interspecies variability on the allometric scaling of CL can be reduced by calculation of the "unbound" clearance [72] before the allometric analysis. This adjustment can be added to that shown in Eq. 29.9:

$$CL_{an}^{h} = CL_{an} \frac{(V_{max}/K_M)_{human}}{(V_{max}/K_M)_{an}} \frac{f_{up,human}}{f_{up,an}} \tag{29.10}$$

Oral Clearance Clearance determined as Dose/$\int C_p\, dt$ after oral administration of test substance is uncertain due to lack of knowledge about the bioavailability, characterized as the fraction of dose that reaches the systemic circulation, F:

$$CL_{\text{oral}} = \frac{CL}{F} = \frac{Dose}{\int C_p dt} \tag{29.11}$$

Interspecies variability in F due to species-to-species differences in bioavailability (incomplete absorption, presystemic degradation) will be reflected in the apparent oral clearance ($CL_{\text{oral}} = CL/F$) values and will contribute to their scatter around the fitted allometric line. A special case is when $F < 1$ due solely to presystemic hepatic first-pass elimination of the test compound. Here, CL/F is in fact $f_{up}CL_{int}$, assuming that $C_p << K_M$. For this special case, the adjustments described in Eqs. 29.9 and 29.10 may be carried out and the human CL estimated using the allometric equation. When $F < 1$ for reasons other than hepatic first-pass extraction, there is no adjustment procedure available.

Projections Based on One or Two Species Lin [48] noted that the per kg rat/human GFR ratio was 4.8, which is generally found for the rat/human CL_R ratio of renally cleared drugs. He suggested that the human CL_R ($\text{mL min}^{-1}\text{kg}^{-1}$) could therefore be estimated as the rat CL_R ($\text{mL min}^{-1}\text{kg}^{-1}$) divided by 4.8. This seems appropriate since renal clearance is scaled relatively well by the allometric equation.

Ward and Smith [51] examined two-species allometry for estimation of human CL, using 103 test compounds and their CL values determined in rat, dog, monkey, and human. Rat–dog and rat–monkey, but not dog–monkey, pairs provided correct qualitative estimates of human CL category (low, intermediate, or high fraction of hepatic blood flow) with about 60% success. They also investigated calculation of the human CL according to the animal CL expressed as a fraction of liver blood flow multiplied by human liver blood flow:

$$CL_{\text{man}} = \frac{CL_{\text{an}}}{Q_{\text{H,an}}} \times Q_{\text{H,human}} \tag{29.12}$$

While CL was assumed to be primarily hepatic, a check of the 103 test compound list against a database of fraction of dose eliminated in urine (*Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 9th and 10th editions [58]) showed that about one-fourth of the 55 test compounds in the database were predominantly (>50%) eliminated in the urine of humans. Use of Eq. 29.12 makes the tacit assumption that the ratio CL/Q_H is a constant across species, which seems unfounded even when hepatic clearance is the dominant contributor.

29.3.3 Recommended Approach

1. Determine the organ(s) primarily involved in clearance of the test compound. Generally, this will be liver or kidneys with the likelihood of liver being greater.
2. Determine CL after IV administration if possible in a minimum of four lab animal species. Fewer than four will raise the uncertainty considerably in the extrapolated human CL value. CL value for each species should be an average from several animals with interfacility differences minimized. Body mass

should span a range of 100, and the mass of the largest animal should be ~10 kg.

3. If renal clearance is the primary elimination pathway, simple allometry will generally predict the human *CL*. If f_{up} varies among species, use the unbound clearance in the allometric analysis.
4. If hepatic clearance is the primary elimination pathway, assess whether the clearance is low or high extraction. If the latter, simple allometry will generally predict the human *CL*.
5. If the primary elimination pathway is hepatic and low extraction, prediction of the human *CL* by simple allometry has a relatively low probability of success. Use f_{up} and *in vitro* intrinsic clearance measurements in the animals and humans to adjust the *in vivo* animal clearance values prior to allometric analysis.
6. Recognize that the extrapolated human *CL* value may be far from the true value. Extrapolation is considered successful if it is within a range of one-half to twice the true value.

29.4 SCALING VOLUME OF DISTRIBUTION

29.4.1 Physiological Basis and Theoretical Considerations

Drug distribution is often expressed in terms of the volume of distribution (V_d), which is usually defined as the ratio of drug amount in the body to the plasma (or blood) concentration. It is generally believed that only the unbound drug can freely diffuse across membranes that separate vascular and tissue compartments. Changes in drug protein binding in plasma and tissue can therefore affect drug distribution in the body, which can be described by the following relationship [59]:

$$V_d = V_p + \left(\frac{f_{up}}{f_{ut}}\right) V_t \tag{29.13}$$

V_p and V_t are the volume of plasma and tissue, respectively, and f_{up} and f_{ut} are the unbound fraction of drug in plasma and tissue, respectively. Considering allometric scaling of V_d, three scenarios are apparent: (1) $V_d \approx V_p$, (2) $V_d >> V_p$, and (3) $V_d = V_p + V_t$.

When a drug is extensively bound to plasma protein (i.e., $f_{up} \rightarrow 0$), the drug is confined in the vascular space; therefore, $V_d \rightarrow V_p$, which may satisfy condition 1. For a drug that fulfills the first condition, the interspecies scaling of V_d would be expected to follow that of blood volume. Generally, the total blood volume tends to scale across species with the first power of body mass ($M^{0.99}$ [7] and $M^{1.02}$ [73]). Therefore, for drugs with extremely high protein binding in plasma,

$$V_d \approx aM^{1.0} \tag{29.14}$$

On the other hand, for a drug that is extensively bound to tissue protein (i.e., f_{ut} very small), V_d may be significantly larger than V_p and the second condition

may be fulfilled. Usually if $V_d \geq 30\,L$, V_p has only a minor effect on V_d [59]; in this case,

$$V_d \approx \left(\frac{f_{up}}{f_{ut}}\right) V_t \tag{29.15}$$

As indicated in Eq. 29.15, both f_{up} and f_{ut} would affect the magnitude of V_d; therefore, if interspecies variation of protein binding were substantial, it would be difficult to find an allometric relationship for V_d.

When a drug has very low plasma and tissue binding (i.e., f_{up} and $f_{ut} \rightarrow 1$), the V_d of the drug will reflect the volume of total body water (condition 3). Accordingly, the scaling of V_d would correlate with the scaling of the total body water (TBW). The reported b value is 0.89 for the TBW [74]; therefore, for a drug that satisfies condition 3, the allometric relationship could be

$$V_d = aM^{0.89} \tag{29.16}$$

The scaling relationship of the mass (or volume) of the major drug distribution organs could also be indicative for the scaling of V_d. The scaling exponent of major organs ranged from 0.70 to 0.99 [7].

The V_d defined in the compartmental models usually does not represent a real physiological volume; however, it is an important pharmacokinetic parameter for the estimation of clinical loading dose. The rationale behind the scaling of V_d in drug development is at least twofold: (1) the volume of distribution of the central compartment (V_c) can be used to estimate initial plasma concentration, which may serve as an additional indicator for safety/toxicity for first-time dosing in humans; (2) combined with a known CL value, the value of V_c can be used to estimate the half-life, since $t_{1/2} = 0.693(V_d/CL)$. For compounds exhibiting multicompartmental kinetics, other volume terms (V_β, V_{ss}) have also been considered. V_{ss} would be expected to scale according to the principles embodied in Eqs. 29.14–29.16. V_β includes influences of distribution and elimination kinetic parameters and its scaling according to the allometric equation is less certain.

29.4.2 Empirical Observations

Generally, good correlation between body mass and V_c across species is expected. Most reported allometric exponents for V_d scattered about 1.0. Some drugs have b values that deviate significantly from 1.0 [75]. Mahmood [76] showed that V_c can be more accurately predicted than V_β and V_{ss}. It has been suggested that extrapolating V_β or V_{ss} from animal data is of limited significance for the first-time dosing in humans, because both parameters can be estimated later from human data [75]. Obach et al. [77] compared four different methods to predict human V_{ss} and demonstrated that when the allometric method was used, a better prediction was obtained when protein binding was taken into account. The main purpose of predicting V_d was to use the predicted V_d value in combination with a predicted CL value for predicting human $t_{1/2}$, "which is a more meaningful parameter with decision-making impact on the drug discovery and development processes" [77].

Interspecies scaling of V_d has also been applied to protein drugs and the reported allometric exponents were around 1.0. Mordenti [78] analyzed the volume of distribution of five human proteins in humans and laboratory animals as a function of body mass using allometric scaling techniques. The analyses showed that the volume data for each protein were satisfactorily described by an allometric equation. The allometric exponent for V_c ranged from 0.83 to 1.05, and the allometric exponent for V_{ss} ranged from 0.84 to 1.02. In another study, Mordenti [79] reported that the allometric exponents for recombinant human factor VIII were 1.04 and 0.84 for V_c and V_{ss}, respectively.

29.4.3 Limitations and Challenges

Prediction involves uncertainty. To establish a meaningful allometric relationship, it is always desirable to include as many animal species as possible. However, in the real world of drug development, pharmacokinetic data are often obtained from a limited number of preclinical species. It becomes a challenging task to predict any pharmacokinetic parameters when data are only available from two or three species. Mahmood and Balian [80] showed that allometric scaling of V_d from only two species is as predictive as that observed from three or more species. Ward and Smith [50], however, showed a contradictory result, indicating that three-species allometry provided improved quantitative prediction compared with two-species allometry. Moreover, single-species extrapolation has been proposed for making early go/no-go human PK decisions for drug discovery candidates [50, 81]. When the interspecies variation in pharmacokinetics, for a given drug candidate, cannot be understood prospectively, the claim of superiority of any projection based on limited data (i.e., from one to three species) may be futile. In other words, the overall prediction may be equally good or equally bad, depending on how one determines the prediction performance. For estimating volume of distribution, it may imply knowing in advance whether the protein binding (plasma and tissue) of a given drug is likely to be so variable across species. In this regard, the use of *in vitro* binding data may be helpful. Further studies may aim at characterizing the molecular and physicochemical properties of drugs that show high interspecies variability.

29.5 SCALING HALF-LIFE

29.5.1 Rationale and Theoretical Considerations

Half-life is an important pharmacokinetic parameter in that (1) it provides the basis for the selection of dosage regimen and (2) it offers prediction of the time to reach steady-state concentration and of the degree of accumulation. In drug development, the utility of scaling half-life across species lies mainly in predicting the dosing frequency of a lead compound. This is true because a frequently asked question in the compound selection process is: Will compound X be a once daily drug? [77].

Theoretically, half-life can be written

$$t_{1/2} = 0.693\left(\frac{V_d}{CL}\right) \tag{29.17}$$

If one assumes that the V_d scales with $M^{1.0}$ and CL scales as $M^{0.75}$, then the expected scaling relationship would be

$$t_{1/2} = aM^{0.25} \tag{29.18}$$

Practically, there are three ways to predict the half-life in humans: (1) direct extrapolation from animal data using the allometric relationship for half-life, if there is any; (2) indirect prediction using Eq. 29.17, where V_d and CL in humans are predicted *a priori* [63, 77, 82]; and (3) indirect estimation from predicted mean residence time (MRT), using the following relationship [76]:

$$t_{1/2} = 0.693MRT \tag{29.19}$$

29.5.2 Empirical Observations

Predicting the half-life in humans from animal data is challenging. One study reported that the exponents of the half-life for drugs varied from –0.066 to 0.547, with the average of 0.19 [76]. Riviere et al. [83] conducted an interspecies allometric analysis of the $t_{1/2}$ of 44 drugs across veterinary and laboratory animal species. A total of 11 drugs (25%) showed statistically significant correlations between log $t_{1/2}$ and log M. The exponent b value for this group of drugs ranged from 0.1 to 0.415, with an average of 0.24 ± 0.09. The remaining 33 drugs showed lack of correlation; many of these drugs were low hepatic extraction drugs. Lack of quality data (e.g., narrow body mass range, multiple data sources) may account for the lack of correlation observed with some drugs [83].

29.5.3 Limitations and Challenges

Difficulty in obtaining consistent correlation between $t_{1/2}$ and body mass across species might be due to the fact that $t_{1/2}$ is a hybrid parameter of V_d and CL; that is, both drug distribution and elimination processes determine the value of $t_{1/2}$. Successful prediction of $t_{1/2}$ tended to occur for drugs that are mainly renally excreted and have low protein binding [26]. It would be more difficult to obtain an allometric relationship for drugs that follow complex disposition mechanisms, such as extensive metabolism involving multiple enzymatic systems and high plasma and/or tissue binding. Use of the above-mentioned indirect methods may improve the predictability of $t_{1/2}$. Moreover, the reliability of prediction may be increased by the incorporation of *in vitro* metabolic data [77, 84] and of calculated molecular properties [53]. The main focus of predicting $t_{1/2}$ should be placed on assigning compounds to appropriate dosing regimen categories (e.g., once daily, twice daily), rather than on a precise estimate of $t_{1/2}$ value.

29.6 CONCLUSION

Allometric scaling can be useful in the projection of preclinical pharmacokinetic parameter values to humans. Apparent volume of distribution will generally scale successfully, more so when interspecies differences in plasma protein binding are

accounted for. Also, renal clearance generally will scale successfully to humans and accounting for interspecies differences in plasma protein binding will improve the success rate. Scaling metabolic clearance is problematic but progress on incorporation of *in vitro* metabolism parameters and plasma protein binding to adjust animal clearance values for interspecies differences in intrinsic metabolism activity has improved the prospects for successful prediction of human *CL* for compounds cleared primarily by metabolism. In future, transgenic animals with human intrinsic enzyme activities may further improve predictive success.

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30

INTERRELATIONSHIP BETWEEN PHARMACOKINETICS AND METABOLISM

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30.1 INTRODUCTION

For most drugs, the magnitude and duration of their pharmacological effect depend on their concentration–time profile within the body. This profile is largely the result of two opposing processes, the rate of drug input into the body and the rate of drug elimination by the body. The study of the time course of drug in the body is termed pharmacokinetics and incorporates the processes of absorption, distribution, metabolism, and excretion (ADME). For most drugs, absorption and distribution determine the speed of onset of drug effect, while metabolism and excretion terminate the action of a drug by removing the active form (with the exception of prodrugs, which undergo biotransformation to the therapeutically active metabolite). The major pharmacokinetic parameters that describe the ADME processes are clearance (CL), volume of distribution (V), elimination half-life ($T_{1/2}$), and bioavailability (F). These pharmacokinetic parameters are key determinants in the design of a suitable dosage regimen, providing information about the magnitude and frequency of dosing (Fig. 30.1).

In drug development, it is estimated that approximately 40% of all new chemical entities (NCEs) fail to become clinically successful drugs due to undesirable pharmacokinetic properties [1–4]. The single greatest factor contributing to inappropriate pharmacokinetics is the process of metabolism. Most drugs are fat/lipid soluble, and metabolism plays a crucial role in their deactivation and the determination of their pharmacokinetic profile. For such drugs, the *in vivo* capacity for metabolism is measured by clearance, which has a major impact on the secondary pharmacokinetic parameters, such as half-life and oral bioavailability. A knowledge of the capacity for the metabolism of an NCE and an understanding of the human metabolism pathways and the enzymes involved have now become key components in the selection of compounds for clinical development. This knowledge is important not only for the optimization of the metabolic stability of the NCE but also to avoid

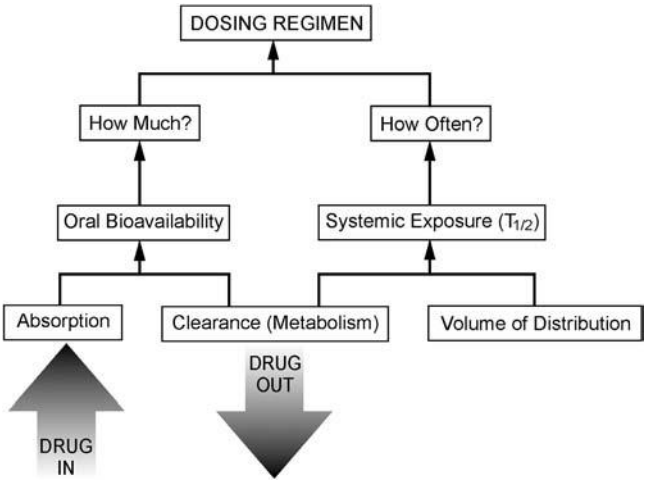


FIGURE 30.1 Relationship between metabolism, pharmacokinetic parameters, and dosing regimen. (Adapted from Ref. 4.)

the likelihood that it is subjected to clinically significant genetic polymorphic metabolism, drug–drug interactions (either by induction or inhibition), or the formation of biologically active or reactive metabolites. This chapter appraises the relationship between metabolism and the resulting pharmacokinetic profile of a drug, and reviews the major factors that may have an impact on a drug's metabolism and may have a detrimental effect on its pharmacokinetics and clinical use.

30.2 IMPORTANT PHARMACOKINETIC PARAMETERS

A basic tenet of pharmacokinetics is that the magnitude of both the desired therapeutic response and/or toxicity of a drug is a function of its concentration in the body [5]. If concentrations are too low at the site of action, therapeutic failure may occur, and conversely, if concentrations are too high, adverse effects and toxicity may result. Thus, successful pharmacotherapy is very often dependent on maintaining the drug concentration within a defined range, known as the *therapeutic range* or *window* [5]. The best place to measure drug concentration presumably would be at the site of action, but most often this is not accessible, being in a tissue such as the brain or the heart, and a blood sample (usually plasma or serum) is used instead. At steady-state conditions when the rate of drug administration is balanced by the rate of drug elimination, the plasma concentration for many drugs is a relatively acceptable index of the concentration at the site of action.

Pharmacokinetics is the study of the drug/metabolite concentration–time profiles resulting from ADME, and the factors that may influence these processes. A knowledge of pharmacokinetics is crucial to determine a rational dosing regimen; that is, how much and how often a drug should be given to achieve the desired therapeutic effect. Rational dosing regimens can be established from several simple pharmacokinetic parameters mainly derived from drug plasma concentration–time curves. The key pharmacokinetic parameters to provide information on dose size and dose frequency are clearance, elimination half-life, and bioavailability. The volume of distribution may also be useful to calculate a loading dose at commencement of treatment in order to more rapidly achieve a particular target concentration. Obviously, the greater the therapeutic index (i.e., the separation of the concentration at which adverse effects appear and the minimum concentration required to produce an effective pharmacological response), the less important is the consideration of a drug's pharmacokinetics and the individualization of the dosage regimen.

30.2.1 Systemic Clearance (CL_P)

Systemic or total plasma clearance (CL_P) is arguably the most important of all pharmacokinetic parameters. Clearance is the most useful parameter for the evaluation of the elimination processes and has the most potential for clinical application. Clearance may be defined as the proportionality constant relating the rate of drug elimination to the plasma concentration (C): thus,

$$CL_P = \text{Elimination rate}/C \tag{30.1}$$

This equation can be re arranged to give

$$\text{Rate of drug elimination} = CL_P \times C \tag{30.2}$$

Alternatively, the clearance can be defined as the volume of plasma irreversibly cleared of drug per unit time, and usually it has units of L/h or mL/min [5]. Clearance is an index of the capacity for the elimination of an active drug from the body and may involve both metabolism and excretion processes, depending on the physicochemical properties of the drug. For example, a water-soluble drug, such as gentamicin, is eliminated almost entirely unchanged by the kidney, and its clearance will be determined entirely by the ability of the kidney to remove it from the blood flowing through the kidney. However, for most drugs, the total plasma clearance is the sum of clearances by the kidney (CL_R) and liver (CL_H), plus any other route (CL_O), and thus

$$CL_P = CL_R + CL_H + CL_O \tag{30.3}$$

The balance between these processes depends on the relative efficiencies of each process for a particular drug and the drug's physicochemical properties. However, most drugs are relatively lipid soluble (necessary for acceptable oral absorption and tissue distribution), and consequently, metabolism is the major determinant of elimination, usually converting them into inactive and more excretable forms. Thus, for most drugs, hepatic metabolism is the major component of the total plasma clearance.

For any particular organ, the physiological determinants of drug clearance are the organ blood flow, the inherent ability of the organ to extract the drug from the blood, and the extent of binding to plasma proteins and cells. Thus, an equation for the clearance of an eliminating organ, such as the liver, can be derived using the simplest (and most widely employed) model of hepatic clearance (known as the *well-stirred* or *venous equilibrium model*):

$$CL_H = Q_H \times \left[\frac{fu \times CL_{int}}{Q_H + (fu \times CL_{int})} \right] \tag{30.4}$$

where Q_H is the hepatic blood flow, CL_{int} is the intrinsic clearance, and fu is the fraction unbound in blood. This model assumes that the distribution of the drug is so fast in this highly vascular organ that the concentration of the unbound drug in the blood leaving the organ is equal to that within the organ. The full derivation of Eq. 30.4 can be found in Rowland and Tozer [5].

The CL_{int} represents the intrinsic ability of the liver to remove (metabolize) the drug in the absence of any restrictions imposed by protein binding or blood flow to the liver. The term contained in the square brackets of Eq. 30.4 is referred to as the hepatic extraction ratio (ER_H) and is a measure of the efficiency of hepatic removal of drug and may range from 0 (when no drug is extracted) to 1, when the drug is completely removed during a single pass through the liver. Thus, Eq. 30.4 can be simplified to

$$CL_H = Q_H \times ER_H \tag{30.5}$$

Thus, if a drug has a very high intrinsic clearance compared to blood flow, the extraction ratio will tend toward 1, and the drug's hepatic clearance will approach the value of Q_H, the hepatic blood flow (but it can never be greater than this blood flow). Such drugs will exhibit blood-flow-dependent elimination kinetics. In this situation, the liver extraction processes or metabolizing enzymes are so active that the liver removes all, or nearly all, of the drug presented to it. Thus, the actual hepatic clearance is determined by the rate of supply of the drug to the liver, that is, the hepatic blood flow. As nearly all of the drug is already being removed, changes in enzyme activity make little or no difference to clearance. Similarly, protein binding is not important for such drugs as the liver has such high affinity/capacity for the drug that even bound drug can be stripped off the plasma proteins in a single pass through the liver. Thus, the hepatic clearance of a drug such as propranolol, lidocaine, and verapamil, for which the liver has a high metabolic capacity, is limited by hepatic blood flow and is uninfluenced by plasma protein binding [5]. The hepatic clearance of such drugs is relatively insensitive to changes in metabolizing activity brought about by induction or inhibition of hepatic metabolizing enzymes. However, a reduction in the rate of drug transport to the liver, such as a decrease in blood flow in heart failure, for example, would result in a decrease in hepatic clearance [5].

At the other extreme, when a drug's intrinsic clearance is very low compared to blood flow, then the hepatic extraction is low, and Eq. 30.4 for hepatic clearance reduces to

$$CL_H = fu \times CL_{int} \tag{30.6}$$

The hepatic clearance of such drugs is dependent only on enzyme activity and the unbound fraction in the blood. Thus, for a drug such as theophylline, for which the liver has a low metabolic capacity resulting in a low extraction ratio, its hepatic clearance will be affected by a change in enzyme activity but will not be influenced by changes in hepatic blood flow [5]. Thus, the hepatic extraction ratio may be used to roughly divide drugs into three categories: high ($ER_H > 0.7$), intermediate (ER_H 0.3–0.7), and low ($ER_H < 0.3$) extraction ratio drugs. This classification of a NCE is useful in drug development to predict the influence of blood flow, enzyme induction and inhibition, and protein binding on its hepatic clearance and oral bioavailability (see below).

The extraction ratio across any organ may also be defined as the fraction of the drug removed from blood in one passage across that organ and may be determined by measuring the drug concentration in the plasma entering and exiting the organ. For example, ER_H can be calculated using the following equation:

$$ER_H = (C_{in} - C_{out})/C_{in} \tag{30.7}$$

where C_{in} is the drug concentration in the plasma entering the liver, and C_{out} is drug concentration in the plasma exiting the liver.

In biochemical terms, the hepatic intrinsic clearance of a particular drug is a measure of how active the liver drug-metabolizing enzymes are with this drug as a substrate. Using the principles of enzyme kinetics and various *in vitro* enzyme systems (e.g., isolated perfused liver, hepatocytes, liver slices, human microsomes, S9

fraction, and various recombinant enzyme expression systems), it is possible to calculate a value for the *in vitro* intrinsic clearance. For example, using a one-site enzyme model and the Michaelis–Menten equation:

$$\text{Rate of metabolism (elimination)} = (V_{max} \times C)/(K_m + C) \tag{30.8}$$

where V_{max} is the maximal velocity of the reaction, K_m is the substrate concentration at which half maximal velocity is attained, and C is the substrate concentration. When substrate concentration is much less than K_m, Eq. 30.8 reduces to

$$\text{Rate of elimination} = (V_{max}/K_m) \times C \tag{30.9}$$

This ratio of V_{max}/K_m estimated from *in vitro* data can be used as an approximation of the expected *in vitro* intrinsic clearance of a drug and usually has units of μL/min/mg of microsomal protein (if using a microsomal preparation). More recently in drug development, it has become popular to use the "depletion" method and measure the disappearance of an NCE over time in an *in vitro* system, and express this as a disappearance half-life, and then calculate intrinsic clearance from this [6–8]. The *in vitro* intrinsic clearance parameter can then be scaled up to the whole liver using various scaling parameters as suggested by a number of investigators [9, 10]. Furthermore, the latter can be extrapolated to predict an expected *in vivo* hepatic clearance value for that particular drug in humans. This can be done using a number of different models for the liver, including the *dispersion, parallel-tube*, or *well-stirred model*. The latter two are currently most often utilized because of their simpler mathematical basis. They differ from each other with regard to their assumption on how drug concentrations decline within the liver: the *well-stirred* model assumes the concentration to be homogeneous throughout the organ and to be reflected by the outflow concentration; whereas the parallel-tube model considers an exponential decline in drug concentration through the organ. The dispersion model is intermediate between those two with respect to drug mixing within the organ but is mathematically more complex. Further details on these models can be found in Refs. 9–11. In a comprehensive comparison of the three models, Ito and Houston [12] observed minimal differences between the models for the prediction of the *in vivo* hepatic clearance and suggested that the well-stirred model was adequate in a screening process to identify an NCE with a high or low clearance. The extrapolation of *in vitro* data to predict *in vivo* hepatic clearance has been extensively reviewed over recent years [13–15].

Acceptable correlations between *in vitro* and *in vivo* clearance values have been reported for a few lipophilic drugs, demonstrating the utility of these models/methods, but in general the quantitative predictions of *in vivo* clearance from *in vitro* metabolism data using existing models has been poor [11, 16]. It has been suggested that this is due to the lack of understanding of the interplay between the intrahepatocyte concentration and plasma and tissue binding, and the part played by drug influx and efflux by hepatocyte transporters [11]. Also, it is increasingly being recognized that many drugs exhibit "atypical" enzyme kinetics *in vitro* that cannot be adequately modeled by the basic Michaelis–Menten equation [17]. In order to improve the prediction of the relevant *in vivo* pharmacokinetic parameters, more complex models need to be applied to accommodate (1) more complex

enzyme kinetics (e.g., autoactivation, substrate inhibition, interactions between several binding sites on the enzyme); (2) incorporation of active transport mechanisms into, and within, the liver; and (3) tissue binding to both blood and liver components. It has been suggested that a physiologically based pharmacokinetic model might be more appropriate, as such models can incorporate more complex physiological and biochemical processes and are capable of predicting drug disposition quantitatively in different tissues in humans [17]. With the increased understanding of the mechanistic processes of ADME from *in vitro* studies, physiologically based pharmacokinetic modeling may prove to be a more valuable tool in the future [18].

In summary, in preclinical drug development, the prediction that a particular NCE has a high hepatic clearance forewarns that (1) it is likely to be subject to flow-dependent elimination (and may require dose reduction in situations of decreased blood flow such as in congestive heart failure), (2) is subject to high first-pass metabolism and thus will have low oral bioavailability (see Section 30.2.2), and (3) it may be more prone to presystemic drug or food metabolism interactions on oral dosing. In contrast, an NCE with a low hepatic clearance is more susceptible to protein binding changes (but note that the unbound concentration does not change, and thus no change in dose rate is required) and changes in metabolizing enzyme activity due to induction or inhibition.

30.2.2 Oral Bioavailability (*F*)

Orally administered drugs must be absorbed and pass sequentially from the gastrointestinal lumen through the gut epithelia and liver, before entering the systemic circulation. The proportion of an orally administered drug that reaches the systemic circulation intact is referred to as its bioavailability. Hence, bioavailability usually refers to the biologically available portion of the drug dose that may exert its pharmacological actions. Oral bioavailability is a function of the fraction of the dose absorbed, and the fraction of the drug that escapes extrusion back into the gut lumen by active efflux transporter systems and/or metabolism by intestinal and liver enzymes. The latter is referred to as the first-pass metabolism. Although metabolism is largely carried out in the liver, for some drugs, such as cyclosporine, there is evidence that a significant amount also occurs in the enterocytes in the gut wall and contributes to the overall first-pass effect [19, 20]. If a large proportion of a drug is metabolized by the epithelial cells of the gut or by the liver before it reaches the systemic circulation, this will result in a low bioavailability, and consequently, a much greater dose must be administered to compensate for this loss. If we assume all the drug is absorbed intact (i.e., fraction absorbed = 1), then the bioavailability (F) depends on the fraction escaping the first-pass extraction, and thus

$$F = 1 - ER_{\mathrm{I+H}} \tag{30.10}$$

where $ER_{\mathrm{I+H}}$ represents the combined extraction ratio for the intestines and the liver. If we consider a drug with a low extraction ratio, which is poorly metabolized by the liver (and the gut wall), nearly all the drug escapes the first-pass process, and bioavailability is essentially complete (as long as it is well absorbed from the gut). For example, if 3% of a theoretical drug is extracted by the liver, the resulting

bioavailability will be 97%. However, any major alteration in this extraction ratio by induction (e.g., a doubling of ER_{I+H} from 3% to 6%) or inhibition (e.g., ER_{I+H} halved from 3% to 1.5%) will have very little overall effect on bioavailability with a reduction from 97% to 94% on induction, and an increase from 97% to 98.5% with inhibition. Such changes in bioavailability would be insignificant in the clinical situation. In contrast, if a drug has a high ER_{I+H} , most of the dose is extracted on the first pass through the liver, so that only a minor proportion reaches the systemic circulation intact. Thus, although induction or inhibition of the metabolizing enzymes would have only a small effect on ER_{I+H}, it would have a significant effect on the proportion escaping extraction and on the bioavailability. For example, a theoretical drug with a high ER_{I+H} of 90% would have a bioavailability of 10%. However, if induction of the metabolizing enzymes increased this ER_{I+H} to 95%, this would result in the halving of the bioavailability from 10% to 5%. Similarly, if the metabolizing enzymes were inhibited, decreasing the ER_{I+H} to 80%, a doubling of the bioavailability would result. For many drugs, such changes in bioavailability would certainly be considered significant. Thus, for drugs with high extraction ratios, hepatic and intestinal enzyme activity are the major determinants of first-pass metabolism, and consequently their oral bioavailability. (However, note that after intravenous administration, hepatic blood flow is the major determinant of the systemic clearance of high extraction drugs.) After oral administration, the plasma concentrations of high extraction drugs, such as propranolol and verapamil, are more variable, both within and between individuals compared to the plasma concentrations of low ER_H drugs, and small changes in the metabolism capacity can cause large changes in oral bioavailability [5, 20]. Obviously, if an NCE experiences a very high first-pass metabolism (i.e., $ER_{I+H} \approx 1$) after oral administration, an alternative route of administration may have to be found or the molecule redesigned to increase its metabolic stability and reduce its first-pass metabolism.

30.2.3 Volume of Distribution (V)

Along with plasma clearance, the volume of distribution is the other major independent pharmacokinetic parameter. The concept of distribution volume is used to relate the plasma drug concentration to the dose administered (assuming no elimination has occurred), or to the amount of drug in the body. For most drugs, it does not relate to a real physiological or anatomical space, but provides an estimate of the extent of the drug's distribution through body-fluid compartments and its uptake by tissues. A major determinant is the relative avidity of the drug for tissue components as compared to blood. Obviously, the failure of an NCE to distribute to the site of action in a tissue or a cellular compartment *in vivo* will render the compound inactive and necessitate the redesign of the compound to overcome this problem.

Several volume of distribution terms are used in pharmacokinetics and can be determined in a number of ways. The simplest method is to regard the body as a simple one-compartment model and find the plasma concentration at zero time by back extrapolation of the log concentration–time plot after an intravenous (IV) bolus injection. Many drugs and NCEs adhere to this simple model. However, other two- and three-compartment models may be necessary for an adequate description of the concentration–time profile of some drugs. Alternatively, a steady-state volume of distribution term (V_{ss}) can be derived from pharmacokinetic analysis based on

statistical moments [21]. The V_{ss} term essentially corresponds to the plasma and tissues volumes in which a drug is distributed when steady-state conditions have been achieved. Further discussion on compartmental and noncompartmental pharmacokinetic approaches can be found in Section 30.3.

The volume of distribution is a useful parameter for determining the size of the loading dose, in order to get to the steady-state situation more rapidly after a constant rate infusion or repetitive dosing, but has no influence on the magnitude of the steady-state concentration achieved. This is solely determined by the rate of drug administration and the systemic clearance. Probably of more importance with regard to desirable pharmacokinetic characteristics is the influence of volume of distribution on a drug's elimination half-life, as discussed next.

30.2.4 Elimination Half-life ($T_{1/2}$)

The elimination half-life ($T_{1/2}$) is defined as the time taken for half the amount of drug present in the body to be eliminated by either metabolism or excretion. It has been suggested that the half-life of an NCE is a more important pharmacokinetic parameter than either plasma clearance or volume of distribution, with regard to guiding decisions in the drug discovery and development process [10]. Half-life is important because it is an indicator of duration of action for many drugs and thus determines how often a drug needs to be administered. Dosing frequency of most drugs depends on how long the active drug remains in the circulation, which is determined by its half-life. With a knowledge of half-life, the dosing frequency required to avoid large fluctuations in plasma concentrations over the dosage interval may be determined. For example, if a drug is rapidly absorbed and is given every half-life, the peak-to-trough concentration ratio will be ~2. However, if the half-life is short (e.g., less than 4 hours) and the drug exhibits a narrow therapeutic index (e.g., twofold), it is often difficult to administer frequently enough to avoid toxicity at the peak concentration, or loss of effect at the low concentrations before the next dose. In addition, significant compliance issues occur with a dosing frequency greater than once or twice a day [22].

For most drugs, the half-life is independent of concentration and is a function of its clearance (assuming first-order elimination kinetics) and volume of distribution as follows:

$$T_{1/2} = 0.7V/CL \tag{30.11}$$

where 0.7 is an approximation of the natural logarithm of 2.

Manipulation of an NCE's volume of distribution has not commonly been used to achieve a more favorable "longer" half-life. For example, it could be suggested that the volume of distribution might be enhanced by increasing the overall lipophilicity of an NCE, thus leading to a longer half-life. However, this improvement in volume of distribution might be offset by increased metabolism and clearance, as the binding sites of the metabolizing enzymes are generally lipophilic in nature and more readily accept lipophilic substrates. Thus, any gains in $T_{1/2}$ time due to a larger volume of distribution may be canceled out by increased metabolic activity. More commonly in drug development, strategies are employed to enhance metabolic stability by structural modifications of the NCE in order to decrease hepatic

clearance, and thus prolong the half-life, and yet maintain the optimal level of potency [23]. A longer half-life should allow lower and less frequent dosing, thus enhancing patient convenience and promoting better compliance. Additional advantages arising from enhanced metabolic stability of the NCE might also include increased oral bioavailability, reduction in the number (and perhaps significance) of possible active metabolites, and lower intra- and interpatient variability in plasma concentration–time profiles (which largely result from differences in metabolic capacity). However, such chemical modifications to improve metabolic stability may in some cases give rise to additional problems with properties such as reduced absorption or increased renal excretion, resulting in a lack of overall improvement in desirable pharmacokinetic properties [24].

An alternative and perhaps easier strategy for reducing dosing frequency for a drug with a short $T_{1/2}$ would be the development of a slow-release formulation [25]. This would slow the rate of drug absorption into the body, so that the magnitude of the concentration fluctuations during a dosing interval would be determined by the absorption rate rather than the elimination rate. A similar approach to lengthen the duration of drug action can make use of a prodrug. The synthesis of a prodrug with a relatively slow metabolic conversion to the active species would result in this conversion step being the rate-determining step in the disappearance of the active species, and would determine its plasma concentration–time profile and thus require less frequent dosing [26].

The elimination half-life of a drug is also useful in predicting the time required to achieve steady-state conditions during a continuous intravenous infusion or repetitive dosing. The maximum effect of many drugs might be expected once this steady-state equilibrium had been achieved during chronic dosing. This is generally accepted to occur after 4–5 half-lives. Similarly, when drug dosing is terminated, it takes 4 half-lives for more than 90% of the drug to be eliminated.

30.3 APPROACHES TO PHARMACOKINETIC ANALYSIS

30.3.1 Compartmental Analysis

The traditional approach to pharmacokinetic analysis involves the use of compartmental analysis, which focuses on the rates of absorption, distribution, and elimination, usually in terms of first-order rate constants (linear kinetics) [27]. This is known as the model-dependent approach. When the exchange of a drug between the plasma and tissue proceeds rapidly compared with the rate of elimination, the whole body may be considered mathematically as a single compartment. This one-compartment model is the simplest model and regards the body as a single, kinetically homogeneous unit, and the decline in plasma concentration after a single bolus intravenous injection can be described by a single exponential function. If the distribution of the drug out of the plasma is so slow that it cannot be disregarded, a model must then be considered, which contains a central compartment and at least one other peripheral compartment. Although these compartments lack physical or anatomical reality, for many drugs, the central compartment may correspond to the plasma or blood volume, together with the extracelular fluid of highly perfused tissues such as the heart, lungs, liver, kidneys, and endocrine glands. It is also pre-

sumed that the drug is eliminated solely from this central compartment. Drugs distribute within a few minutes through this compartment and equilibrium between plasma and tissues is rapidly established. The peripheral compartment is then formed by less perfused tissue such as muscle, skin, or adipose tissue into which the drug may enter more slowly. This combined effect of two compartments gives rise to a biphasic concentration–time curve after an intravenous bolus injection, with two distinct linear portions when drawn on a semilog ordinate scale. Although drug disposition to the peripheral compartment is slow, it is usually much faster than the elimination rate of the drug. The two-compartment model may be expanded to contain additional compartments, which can be described mathematically as the sum of as many individual exponential functions as there are relevant compartments. In practical terms, the sensitivity of the available assay technique for the determination of the drug concentration usually allows the definition of a maximum of three compartments. This approach to pharmacokinetic analysis allows mathematical simulations and the prediction of drug concentrations in plasma (representing the central compartment) and in other deep compartments. These models are usually robust for a variety of conditions, such as altered physiological or pathological conditions and during chronic medication [27].

30.3.2 Noncompartmental Analysis

An alternative and perhaps simpler approach to pharmacokinetic analysis is the use of noncompartmental analysis (the model-independent approach), where the drug pharmacokinetic parameters are determined using statistical moments and are derived from relationships involving the area-under-the-zero-moment-curve (AUC) and the area-under-the-first-moment-curve ($AUMC$) of the plasma drug concentration–time curve [27]. No complicated modeling of the disposition of the drug is required, but consequently it lacks the predictive and/or simulative power for drug concentration in the plasma and other tissue compartments [16]. Further discussion and a comparison of these two approaches to pharmacokinetic analysis can be found in Atkinson et al. [21].

30.3.3 Physiologically Based Analysis

Yet another more complex approach to pharmacokinetic analysis involves using physiologically based pharmacokinetic models. This method attempts to take into account the fundamental anatomical and physiological factors (e.g., blood flow to the different organs/tissues, organ uptake and active transport, and tissue binding) that influence drug uptake and disposition [18, 27]. Individual organs or tissue groups are linked in an anatomically correct fashion via the circulatory system, with drug elimination permitted from the relevant organs. This is by far the most realistic view of what is happening to the drug in the body but requires extensive computing power and data inputs (e.g., blood flow, partitioning or tissue binding data for all species). Examples of physiologically based pharmacokinetic models of varying complexity can be found in the review by Gerlowski and Jain [28]. Due to their complexity, these models have only had a limited application to clinical studies to date, but they are expected to have greater importance in the future [17, 27].

30.4 PREDICTING PHARMACOKINETIC PARAMETERS IN HUMANS

As mentioned earlier, very often *in vitro* drug metabolism information in isolation fails to scale up and provide robust predictions of the pharmacokinetics of an NCE in the human population. An alternative strategy for predicting plasma pharmacokinetic parameters of NCEs in humans involves the use of allometric scaling methods plus preclinical animal pharmacokinetic data [29].

30.4.1 Allometric Scaling

Interspecies allometric scaling is based on empirically observed relationships between physiological and biochemical parameters (e.g., basal metabolic rate, creatinine clearance, and cytochrome weight) and body weight among mammals [30]. As pharmacokinetic parameters are a function of biochemical and physiological processes, these have also been shown to scale with body weight across species [31, 32]. This has allowed the prediction of pharmacokinetic parameters, such as CL and V, by using a simple power function of the form

$$Y = aB^n \tag{30.12}$$

where Y is the dependent variable (e.g., clearance) and B is the body weight, with a and n being the allometric coefficient and exponent, respectively. Allometric scaling from preclinical animal studies has proved to be useful for the prediction of the volume of distribution in humans, particularly if plasma protein differences between species are taken into account [33, 34]. However, allometric scaling has not proved to be particularly successful at predicting the hepatic clearance of drugs eliminated mainly by metabolism, especially those with low hepatic clearance. Adjustments to the basic allometric equation using maximum life span potential and brain weight have been proposed, but with limited success [34–36]. More recently, a combination of allometric scaling with *in vitro* human microsomal data has been proposed [36–38]. However, in a recently published study on the comparison of five different models (including allometric scaling with *in vitro* data input) using a dataset of 22 extensively metabolized compounds, Zuegge and colleagues [39] concluded that the most cost-effective and accurate approach for the prediction of hepatic clearance by metabolism was based on *in vitro* data alone, and that the inclusion of *in vivo* preclinical data and allometric scaling did not significantly improve prediction accuracy. They found that the prediction accuracy of the allometric models was at the lower end of all methods compared.

30.4.2 Microdosing

With the development of ultrasensitive analytical technologies (such as accelerator mass spectrometry (AMS), and positron emission tomography (PET)) with the capability of measuring drug and metabolite concentrations in the low picogram to femtogram range, human microdosing has been proposed as a new approach to obtaining crucial human pharmacokinetic data for comparing and selecting drug candidates earlier in their development [40–42]. Both technologies have the necessary sensitivity to follow the fate of trace amounts of radiolabeled drug in the body

after very small doses (e.g., 1% of a therapeutic dose, maximally <100 μg), which would be expected to have no biological activity. PET can provide real-time data on the distribution of a radiolabeled drug in the body and its availability at the site of action; whereas AMS combined with high performance liquid chromatography (HPLC) can quantitate extremely low concentrations of a drug and metabolites in body fluids at time intervals after dosing [43]. The essential aim of microdosing studies is to obtain early human pharmacokinetic information for new drug candidates to assist in the candidate selection process, rather than acquiring efficacy or safety data. The major question with regard to human microdosing is whether the pharmacokinetic parameters after microdoses replicate those observed at therapeutic doses. In a small trial of five diverse drugs eliminated by metabolism, Garner [44] reported a 70% correspondence between the pharmacokinetics after a microdose and a therapeutic dose. However, more in-depth studies are required to judge the predictive abilities of microdosing in humans, and whether it will play a significant role in drug development in the future is not known at present. The exquisite sensitivity and specificity of the combination of HPLC and AMS also raises the possibility of the "cocktail" or cassette-dosing approach, where several NCEs are administered simultaneously in microdoses, and their pharmacokinetics are followed concurrently. This allows for a larger number of compounds to be investigated, and more efficient and rapid identification of the compound with the most desirable pharmacokinetics in humans.

30.5 METABOLISM FEATURES THAT MAY CONVEY PHARMACOKINETIC DISADVANTAGES

As previously indicated, metabolism is responsible for determining the major pharmacokinetic parameters of clearance, bioavailability, and half-life for most drugs. These pharmacokinetic characteristics in turn are very important in defining the pharmacological and toxicological profile of an NCE and indicating to drug developers whether favorable pharmacokinetics can be expected in patients. Desirable pharmacokinetics in patients would include a suitable half-life to allow a convenient dosing frequency of once or twice daily, and low hepatic/intestinal metabolism to allow acceptable and reproducible oral bioavailability. However, it is also important to realize that, although the preclinical *in vitro* and *in vivo* data might predict favorable pharmacokinetics in humans, there are a number of factors (discussed later) that may have a significant impact on an NCE's metabolism, leading to undesirable pharmacokinetic characteristics in patients and ultimately compound failure.

30.5.1 Nonlinear Pharmacokinetics

In the preceding discussion on pharmacokinetics, it has been assumed that the disposition systems operating on the drug obey first-order kinetics. This means that clearance, volume of distribution, bioavailability, and elimination half-life are independent of drug concentration (and dose). Under these conditions, drug accumulation is a linear function of dose, and any change in dose rate will produce a proportional change in the steady-state concentration. Some drugs do not have such linear characteristics. The most common cause of nonlinearity is believed to be the

limited capacity of certain elimination processes, such as the drug-metabolizing enzyme systems. Most drugs are presented to the metabolizing enzymes at concentrations well below their maximal capacity, and they display first-order kinetics. However, for some drugs to be effective, concentrations are required at sufficiently high levels to cause saturation of the eliminating enzyme system, and the elimination occurs at a constant maximal rate, that is, zero-order kinetics. The anticonvulsant drug phenytoin and ethanol are probably the best known and studied examples of such compounds, where saturation of metabolism occurs with pharmacologically active concentrations [5, 45]. For such compounds, drug elimination rate will depend on the plasma concentration (or the magnitude of the dose), and drug accumulation with repetitive dosing will not be readily predictable. Once saturation of the eliminating system has been reached, the plasma concentration will increase disproportionately with subsequent dosing, and the time required to reach steady state will increase with the increasing half-life. If saturation of the elimination mechanisms is observed at pharmacologically active concentrations in the preclinical development of an NCE, structural modification would be recommended to remove this property, as this compound would be very difficult to use safely in patients, especially if it also had a low therapeutic index.

30.5.2 Drug–Drug Interactions

Drug–drug interactions are a major problem for the pharmaceutical industry and have resulted in drugs being withdrawn after being launched onto the worldwide market [46]. For example, terfenadine (a nonsedating antihistamine used for the treatment of rhinitis) was withdrawn after the occurrence of potentially fatal cardiac arrhythmias due to a substantial increase in its bioavailability when given with ketoconazole or grapefruit juice [46, 47]. Both ketoconazole and grapefruit juice are potent inhibitors of CYP3A4, which is the major enzyme involved in the metabolism of terfenadine and the termination of its activity. Indeed, the most common cause of clinically significant drug–drug interactions is primarily associated with the oxidation reactions catalyzed by the cytochrome P450 (CYP) enzyme family [48]. The CYPs in families 1–3 catalyze the metabolism of approximately 60–70% of all clinically used drugs. Of these, CYP3A4 is probably the most important, as it is involved in the metabolism of half of all drugs. Other important CYP isozymes involved in drug metabolism include CYP1A2, CYP2C9, CYP2C19, and CYP2D6. Inhibition of metabolism usually has a rapid onset and most commonly results in a dramatic increase in plasma concentration and an exaggerated response, with increased risk of toxicity and fatalities in extreme cases. The awareness of these adverse consequences has resulted in the development of a battery of automated screens to identify potent inhibitors of the CYP systems [8]. Some examples of drugs most commonly involved in inhibition of the CYP family include the azole antifungal drugs (e.g., ketoconazole), the quinolone and macrolide antibiotics (e.g., erythromycin), the selective serotonin reuptake inhibitors (e.g., fluoxetine, paroxetine, and fluvoxamine), the antiviral protease inhibitors (e.g., ritonavir and indinavir), and the antiulcer drug cimetidine [49].

The potent inhibition of the CYP enzyme family would be a property to be avoided in the development of an NCE, as it could lead to clinically significant drug–drug interactions. However, the decision to withdraw a drug from the market

after evidence of a drug–drug interaction depends on several factors, including the severity of the interaction, the disease being treated, and whether alternative drugs are available. For example, the calcium channel blocker mibefradil, which is a substrate and potent inhibitor of CYP3A4, was withdrawn as there were equally effective drugs available that did not possess this undesirable characteristic. In contrast, a number of protease inhibitors (e.g., indinavir, saquinavir, and ritonavir), which are also potent inhibitors of CYP3A4, were not withdrawn, as there was a lack of effective alternatives in the treatment of HIV infection [50].

Significant drug–drug interactions are not confined to the CYP metabolizing family, as illustrated by the inhibition of dihydropyrimidine dehydrogenase by the antiviral drug sorivudine. Dihydropyrimidine dehydrogenase is a key enzyme in the metabolism (inactivation) of the anticancer drug 5-fluorouracil, and its inhibition by sorivudine caused a reduction in 5-fluorouracil's clearance, leading to elevated concentrations and toxicity. The latter mechanism was believed to have resulted in a number of fatalities in Japan, and consequently sorivudine was withdrawn from the marketplace [51].

Induction of drug-metabolizing enzymes by an NCE may also be problematic in drug development. In contrast to inhibition, induction has a less rapid onset, taking 4–5 days to achieve a peak effect. Most commonly, this results in lower plasma concentrations and treatment failure of comcomitant drugs. Well-known inducing agents include the anticonvulsants (e.g., phenytoin, phenobarbital, and carbamazepine), the antibiotic rifampicin, and the anti-inflammatory agent dexamethasone. Both first-pass metabolism by the intestines and the liver can be dramatically increased by enzyme-inducing agents, resulting in decreased bioavailability and loss of the therapeutic response. The discovery that many compounds induce drug-metabolizing enzymes via an interaction with the nuclear hormone receptors, constitutive androstane receptor (CAR), and the pregnane-X-receptor (PXR) has prompted the development of enhanced throughput systems for identification of CYP inducers, using both PXR radioligand binding assays and also reporter gene technology [52, 53]. The availability of high resolution human PXR ligand-binding domain crystal structures also raises the possibility of *in silico* testing of NCEs for their potential to bind to PXR, as an indication of their induction ability [54].

Drugs likely to be the subject of clinically relevant drug–drug interactions tend to be drugs with a steep concentration–response curve or a narrow therapeutic window [20]. Thus, a relatively small increase in concentration can quickly lead to toxicity, or conversely a decrease can lead to the disappearance of the therapeutic effect. For most drugs, the absence of significant drug–drug interactions may be a crucial determinant of the ultimate clinical success of a drug. Thus, to avoid the potential of drug–drug interactions, it is desirable to develop a new drug candidate that is not a potent CYP inhibitor or inducer, and whose metabolism is not readily inhibited or induced by other drugs. In order to eliminate unfavorable compounds early in development, high throughput *in vitro* systems for identifying drug metabolism pathways, the enzymes involved, and inhibitor/induction potential of new drug candidates are a rapidly evolving area of drug development [8, 55]. However, the information from both induction and inhibition *in vitro* technologies must be integrated with *in silico* approaches to maximize the potential of both [55].

30.5.3 Polymorphic Drug Metabolism

Genetic differences in the expression of many metabolizing enzymes can have an impact on the pharmacokinetic characteristics of a drug in a population of patients and accounts for much of the interindividual variability associated with the efficacy and toxicity of a number of drugs [56]. There are many examples of polymorphisms in genes encoding drug-metabolizing enzymes, which can cause significant differences in drug response within a population (Table 30.1) [57, 58]. As with drug–drug interactions, most information is available for the CYP system, in particular, the polymorphic enzymes CYP2C9, CYP2C19, and CYP2D6, which catalyze the metabolism of approximately 40% of drugs undergoing phase I oxidation [59, 60]. In general, four phenotypes can be identified: poor metabolizers (PMs), who lack the functional enzyme; intermediate metabolizers (IMs), who are heterozygous for one deficient allele or carry two alleles that cause reduced activity; extensive metabolizers (EMs), who have two normal alleles; and ultrarapid metabolizers (UMs), who have multiple gene copies. Thus, the dosing requirement for a drug such as nortriptyline (which is a substrate for CYP2D6), may differ 10–20-fold among individuals. Consequently, using a "standard" dose in a European population would be expected to elicit no response in the 5% of the population who are UMs (due to too rapid metabolism of the drug), and perhaps elicit an adverse reaction in the 7% of the population who are PMs due to excessively high plasma concentrations [58]. The distribution frequency of these CYP2D6 phenotypes differs between ethnic groups. For example, 10% of Spaniards and 29% of Ethiopians have been reported to be UMs for the CYP2D6 polymorphism [61]. Similarly, polymorphisms in CYP2C19 have been shown to have clinical consequences resulting in toxicity, or lack of efficacy with many drugs, including *S*-mephenytoin, omeprazole, imipramine, and proguanil [58]. Population studies have indicated that CYP2C19 PMs occur in 3–5% of Caucasians and African Americans, 12–23% in most Asian groups, and 38–79% in Polynesian and Micronesian populations [59]. In a Japanese study, patients with peptic or duodenal ulcers were treated with a dual therapy of low dose omeprazole

TABLE 30.1 Examples of Genetic Polymorphisms Influencing Drug Metabolism

Gene[a]	Substrates
CYP2C9	Tolbutamide, diclofenac, warfarin, phenytoin
CYP2C19	Omeprazole, lansoprazole, diazepam, proguanil
CYP2D6	Antidepressants, codeine, β-blockers
CYP2A6	Coumarin
UGT1A1	Irinotecan
NAT2	Isoniazid, sulfonamide
TPMT	6-Mercaptopurine, 6-thioguanine, azathioprine
DPD	5-Fluorouracil
ALDH2	Acetaldehyde

[a]UGT1A1, UDP-glucuronosyltransferase 1A1; NAT2, N-Acetyltransferase 2; TPMT, thiopurine S-methyltransferase; DPD, dihydropyrimidine dehydrogenase; ALDH2, aldehyde dehydrogenase 2.

Source: Adapted from Ref. 56.

and amoxicillin [62]. Cure rates were highly dependent on CYP2C19 phenotype, with only 25% in EMs, rising to 60% in IMs, and 100% in PMs. The high cure rate in PMs was thought to be due to lack of metabolism, resulting in higher omeprazole plasma concentrations and a longer half-life. These examples illustrate some of the problems that arise with drugs that are eliminated mainly by polymorphic enzymes, especially their use in different ethnic populations. This is important for the global perspective of the pharmaceutical industry, and most companies now take the pharmacogenetic aspects of CYP metabolism into account during drug development. Obviously, an NCE that has a high affinity for a polymorphic metabolizing enzyme that plays a major role in its elimination would be less favorable than one that did not display this characteristic [63]. However, as costs decrease, predictive genotyping of patients may be justified for drugs subject to significant polymorphic metabolism, especially those with a narrow therapeutic index. For example, it has been suggested that the prospective use of the UGT1A1 genotype assay would identify patients with the UGT1A1*28 genotype, which is associated with reduced metabolism of SN-38 (the active metabolite of the anticancer drug irinotecan), and increased incidence of significant toxicity [64]. Such genotyping may help to ensure safer and more effective therapy in the future.

30.5.4 Formation of Active/Reactive Metabolites

The rapid and extensive hepatic metabolism of an NCE may lead not only to pharmacokinetic disadvantages but also to issues with the identification of metabolites and evaluation of their biological activity. In general, *in vivo* metabolism leads to the formation of inactive and more readily excretable metabolites, but in some cases, metabolites with potent biological activity or toxic properties are produced, which may influence the outcome of therapy. The function and fate of these metabolites need to be studied, in order to gain a full insight into the potential biological consequences of exposure to the NCE. Metabolism to reactive intermediates is one of the major mechanisms by which drugs exert toxic effects. Despite a huge investment and many years of research, it is still not possible at present to accurately predict the potential for toxicity of a compound that has been shown to undergo metabolic activation [65]. It is important in drug development to investigate the metabolic fate of an NCE and evaluate the biological properties of possible metabolites. The relative merits of current and potential strategies for dealing with metabolite characterization have recently been reviewed by Nassar and Talaat [66]. The advancement of robotic systems using *in vitro* incubations of an NCE with human microsomes, hepatocytes, or liver slices, accompanied by the analysis of metabolites using techniques such as liquid chromatography–mass spectrometry (LC-MS) and/or liquid chromatography–nuclear magnetic resonance (LC-NMR) has made this more applicable in practice. In particular, significant advances in metabolite identification have been achieved using high performance liquid chromatography coupled with various mass analysers, such as triple quadrupole, quadrupole time-of-flight, ion trap, and quadrupole linear ion trap mass spectrometers [67, 68]. However, with the improved sensitivity and efficiency of NMR technology, the complementary nature of NMR and MS in definitive structure identification is being realized, and combined LC-MS-NMR systems will become crucial in the elucidation and confirmation of metabolite identification in the near

future. Combined LC-MS-NMR systems are in the midst of an evolutionary phase at present that will lead to greatly improved ability and efficiency in metabolite identification [66]. However, although structural information on metabolites can be a considerable asset for enhancing and streamlining the process of NCE development, in order to eliminate potentially toxic compounds early in the development process, it must be integrated with computational technologies to obtain maximum benefits [66, 69, 70]. Comprehensive databases of metabolism and toxicological information are available, and software packages can indicate possible metabolites and identify structural features that may confer adverse properties to molecules [4, 66, 67]. With the advent of improved algorithms and the availability of ever-increasing computing power, the future of drug metabolism and toxicity prediction is envisaged as an integration of structure–activity relationship models based on compound structure with "pattern" databases of tissue or organ response to drugs, and "systems biology" databases of metabolic pathways, genes, and regulatory networks [71, 72].

30.6 CONCLUSION

For many drugs, metabolism plays a central role in defining the concentration–time profile in the body and, consequently, the magnitude and duration of the biological response. We have only a very limited understanding of the relationships between a molecule's structure and its physicochemical properties and its likely metabolic fate in the body. Drug metabolism may occur in many different tissues in the body and may be catalyzed by a large variety of diverse enzymes, which can be influenced by innumerable factors. The capacity for the metabolism of a compound *in vivo* is usually measured by the hepatic clearance, which is arguably the most important pharmacokinetic parameter, with a major influence on the secondary parameters of half-life and oral bioavailability. Clearance, half-life, and bioavailability are key components in the design of a suitable oral dosage regimen, providing information on the magnitude and frequency of dosing. Thus, metabolism is the single most important factor contributing to inappropriate or undesirable pharmacokinetic characteristics in an NCE. This relationship between an NCE's metabolic profile (rate, extent, and multiplicity of resulting metabolites) and the overall systemic clearance in a patient is extremely complex and provides a formidable challenge for the prediction of this parameter in humans from preclinical *in vitro* human data and animal studies. There are many issues concerning the nonphysiological nature of the *in vitro* screening systems, interspecies differences in metabolizing enzymes and their regulation, the part played by active transporters in tissue distribution, and the complexities of *in vivo* pharmacokinetics and toxicity. Nevertheless, some progress has been made over the past ten years with the development of a more diverse range of investigative tools, including superior technology to measure and identify compounds and their metabolites at extremely low concentrations, the automation and expansion of various *in vitro* screening tests to allow more rapid understanding of the ADME fate of a compound, and *in silico* approaches based on molecular modeling, structure–activity relationships, and metabonomics. To fully realize the potential of these advances, there is the need to integrate these technologies into a single comprehensive environment for drug development. A small amount of prog-

ress has been made, but much is yet to be done to attain the ultimate goal of being able to predict human metabolism and pharmacokinetics (and toxicity) based solely on the structure of a novel NCE.

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