10 Bioavailability and Bioequivalence

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10.1 INTRODUCTION

To provide therapeutic benefit, pharmaceutical agents must gain access to their intended sites of pharmacological effect. Although pharmacologic effect is governed by interactions between drug and molecular targets such as cellular receptors or enzymes, drug transfer from the site of absorption and subsequent distribution to extravascular tissue also contributes to the extent and timing of drug effect. Thus, it follows that the magnitude of effect will generally be related to the amount of drug available to the site of action, whereby insufficient systemic drug levels may result in therapeutic failure and excessive levels could lead to toxicity.

To define safe and effective dosage regimens, an understanding of drug concentrations and their relationship to drug pharmacodynamics (PD) is required. Although some drugs may be administered directly to the site of action, such as by topical, pleural, or peritoneal routes, a majority of drug substances must enter the general circulation and be distributed to tissue before reaching their intended site of action. Therefore, the extent of drug transfer to the general circulation can be a function of drug distribution across successive membrane and cellular barriers. As a dose is transferred between the site of administration and its site of measurement in the circulation, the serial arrangement of physiological membranes and cellular elements may lead to cumulative drug loss. Thus, bioavailability is the pharmacokinetic (PK) parameter that describes the proportion of a dose to successfully reach the bloodstream as parent drug [1].

Bioavailability studies are largely concerned with determining systemic drug availability after oral administration, as the oral route is the intended mode of administration for the majority of drug substances. However, absorption of an oral dosage form is a complex process that is influenced by gastrointestinal (GI) physiology as well as the physicochemical properties of a drug. Following oral administration, drug must pass

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from the GI lumen, across the gut wall, and into the hepatic circulation before systemic distribution. In addition to GI physiology, transfer of an oral dose to the systemic circulation also depends on drug solubility in aqueous and lipid environments as well as the potential for recognition by membrane transporters and metabolizing enzymes. From a PK perspective, bioavailability studies relate systemic exposure to drug absorption, while helping to understand mechanisms underlying drug distribution, transport, and metabolism.

Bioavailability studies are also conducted to evaluate the performance of one or more drugs or drug products against a previously defined reference. Such studies are referred to as *comparative bioavailability* studies and are designed to demonstrate bioequivalence of test and reference formulations for both new drug as well as generic drug products. While comparative studies are important elements of drug development, they may also be conducted after drug product registration in support of manufacturing changes [2]. Early in the drug development process, oral bioavailability of an initial dosage form might be determined relative to drug administered by the intravenous (IV) route or relative to an oral solution. As drug development progresses toward registration, improvements in oral delivery frequently lead to formulation changes and comparative bioavailability studies may be required in order to confirm equivalent PK and reestablish drug safety and efficacy of the reformulated dosage form relative to the earlier form. For marketed drug products, studies to demonstrate bioequivalence could be necessary following major changes in components, composition, and/or method of manufacture made after approval.

Determining bioequivalence is also a critical component for abbreviated new drug application (ANDA) submissions of generic drug products. Approval of generic drug products relies on the bioequivalence between the generic and innovator formulations. In order to be considered bioequivalent, drug products must contain the same active ingredient and display comparable bioavailability when studied under similar experimental conditions [3]. Thus, it is assumed that two bioequivalent drug products will be therapeutically equivalent and may be substituted for one another.

This chapter provides an introduction to the interrelated concepts of bioavailability and bioequivalence and their application to characterize drug disposition. While calculating bioavailability provides an assessment of the amount of drug reaching the systemic circulation relative to a reference dose, demonstration of bioequivalence for two drug formulations further permits an important assumption of therapeutic equivalence and potential for product substitution in clinical practice. As drug products are frequently designed for administration by the oral route, potential sources of drug loss that could contribute to incomplete oral bioavailability are also reviewed.

10.2 BIOAVAILABILITY

Bioavailability is characterized by the rate of drug absorption, how fast a drug enters the systemic circulation, and its extent of absorption, the quantity of dose to enter the body. These essential features of a nonvascular dosage form define the time course of circulating drug concentrations and their relationship to therapeutic effect, where the onset of drug effect is often a function of absorption rate and effect duration is typically related to the extent of absorption. Thus, the PK parameter bioavailability is

used to describe the proportion of a dose transferred to the site of drug measurement that becomes available for tissue distribution.

In experimental terms, drug bioavailability is determined by comparing the exposure levels of intact drug of a test dose to the exposure levels of a reference dose. When the entire reference dose is available at the site of sampling, as assumed for drugs administered by the IV route, then the systemic availability of a test dose is referred to as *absolute bioavailability*.

Drug concentrations are commonly measured in venous blood or plasma and therefore intravenously administered drugs are considered to be completely available within the systemic circulation. Although this is a valid assumption when intact drug reaches the arterial bloodstream without loss, experimental verification of the assignment of 100% bioavailability is not common. On IV administration, the entire dose becomes available to the pulmonary circulation during exchange to the arterial blood supply. While contributions to first-pass metabolism by the lung are difficult to quantify, the activities of several cytochrome P450 (CYP) enzymes are present in human lung [4,5]. In addition, recent evidence suggests that CYP activities localized in the right ventricle of the human heart may also play a role in pulmonary first pass [6]. Although the potential for pulmonary first-pass extraction exists for an IV dose, equivalent extraction is assumed for an oral dose during exchange to the arterial blood supply before sampling and therefore the net effect on estimating bioavailability is thought to be negligible.

Frequently, however, drug administration by the IV route cannot be considered, as the majority of drugs are not approved for IV use nor have they been monitored in a clinical development setting following IV administration. In order to evaluate human IV PK for absolute bioavailability determination, supportive toxicology and manufacturing data are also required to demonstrate drug safety associated with IV dosing. Additionally, dosing formulations developed for a nonvascular route may not be suitable for human IV use and thus identification of a formulation compatible with IV administration may also be required. Although the study of human PK following IV administration would generate valuable exposure data for future bioavailability characterization, approval to conduct clinical PK studies is not always sought.

When IV dosing is not plausible, both the reference dose and test dose of the same drug substance are introduced by nonvascular route(s) of administration. Consequently, systemic exposure following a test dose will be compared to a reference dose for which complete availability at the site of measurement cannot be assumed. The parameter *relative bioavailability* is used to characterize the availability of a test dose relative to a nonvascular reference dose.

Bioavailability studies to compare drug formulations dosed by nonvascular routes of administration are frequently undertaken to demonstrate generic drug bioequivalence to an innovator reference formulation. The calculation of relative bioavailability may also contribute to understand exposure–effect relationships for drug products when measurements of drug levels occur directly at the site of action. For instance, concentrations of the anticancer agent temozolomide were determined in brain interstitium by intracerebral microdialysis after oral temozolomide administration. Using this approach, temozolomide bioavailability within the central nervous system was determined to be 17.8% relative to systemic temozolomide concentrations after oral dosing [7]. Similarly, external γ-scintigraphy was used to determine relative bioavailability directly at a local site of drug action following nasal spray application of cromolyn sodium [8]. Although these specialized techniques for drug monitoring are not routine

approaches to determine drug bioavailability, knowledge of drug disposition at the site of therapeutic activity may further provide an understanding of exposure–effect relationships.

In other cases, however, alternate routes have been employed to facilitate drug application directly to the site of action when drug loss that may occur after oral dosing has not supported clinical use or when systemic exposure is to be minimized by design. Nonoral drug administration by topical [9], inhalation [10,11], and intranasal [12] routes are common. However, comparative bioavailability studies based on systemic exposure are not always possible. For instance, dermatological agents administered by topical application to the skin effectively maximize drug concentration at the site of action with minimal drug present in circulation. Consequently, systemic availability does not necessarily reflect local tissue exposure. Comparisons of drug formulations for bioequivalence determinations have instead utilized measures of *in vivo* tissue exposure such as tape stripping and microdialysis [9,13]. Recent efforts have also attempted to relate measures of *in vitro* permeability from an excised human skin model for *in vivo* efficacy predictions [14].

10.2.1 Calculation of Absolute Bioavailability

Following oral dose administration, maximum plasma concentration of drug (C_{max}) is commonly used to evaluate the rate of absorption, while total area under the plasma concentration versus time curve (AUC) is used to evaluate the extent of absorption. However, in terms of estimating drug bioavailability, the proportion of dose reaching the systemic circulation as intact drug is determined by comparison of AUC exposures. When plasma drug concentrations are compared after administration of an extravascular test dose and IV reference dose, absolute bioavailability (F) is calculated as the AUC ratio of extravascular (AUC_{ev}) and IV (AUC_{iv}) exposures. As the IV route of administration is assumed to result in complete systemic drug availability, reference to AUC_{iv} provides an estimate of absolute bioavailability. Often the occurrence of high initial drug concentrations after IV dosing precludes evaluation of a matched dose by a nonvascular route. Accordingly, dose levels are taken into account when calculating absolute bioavailability [1]:

$$F = \frac{AUC_{ev}}{AUC_{iv}} \times \frac{(Dose)_{iv}}{(Dose)_{ev}} \tag{10.1}$$

Bioavailability may also be calculated from urinary excretion data for drugs that undergo renal elimination. When renal clearance accounts for the same proportion of total drug clearance after IV and extravascular administrations, absolute bioavailability can be estimated from urinary excretion data. This approach is feasible as the fraction of drug excreted unchanged in urine is independent of the route of administration within a linear dose range. However, a requirement for this approach is the complete collection of dose excreted in urine. Thus, by comparing the total amount of unchanged drug excreted in urine (Au,inf) after extravascular and IV dosing, the fraction of extravascular dose available in the systemic circulation can be determined by

$$F = \frac{(Au,inf)_{ev}}{(Au,inf)_{iv}} \times \frac{(Dose)_{iv}}{(Dose)_{ev}} \tag{10.2}$$

Inherent in Equations 10.1 and 10.2 is the assumption that drug clearance remains constant and independent of the route of administration. Clearance of drug from the systemic circulation is defined by the rate of drug elimination and drug concentration in plasma [15]. Therefore, in terms of mass balance, systemic clearance (CL) may be expressed as a ratio of amount of bioavailable dose and total AUC exposure in plasma such that

$$\mathrm{CL} = \frac{F \times \mathrm{Dose}}{\mathrm{AUC}} \tag{10.3}$$

Equation 10.3 can be simplified for an IV dosing situation when complete bioavailability is assumed (i.e., $F = 1$). Thus, expressing an AUC ratio following extravascular and IV administrations in terms of bioavailable dose and systemic clearance, it follows that absolute bioavailability may be calculated by the following:

$$F = \frac{\mathrm{AUC_{ev}}}{\mathrm{AUC_{iv}}} \times \frac{\mathrm{CL_{ev}}}{\mathrm{CL_{iv}}} \times \frac{(\mathrm{Dose})_{\mathrm{iv}}}{(\mathrm{Dose})_{\mathrm{ev}}} \tag{10.4}$$

When the assumption of constant clearance holds after IV and PO dosing, Equation 10.4 may be simplified to Equation 10.1.

When plasma drug levels fall below the lower limit of assay quantitation soon after dose administration, insufficient concentration–time data may lead to unreliable estimates of AUC exposure and inaccurate bioavailability determinations. In such cases, parent drug bioavailability could be determined indirectly by monitoring metabolites present in circulation. Accordingly, determinations of plasma metabolite AUC ($\mathrm{AUC_m}$) after extravascular and IV dosing can be used to calculate parent drug bioavailability when presystemic first-pass metabolism does not play a role in metabolite formation [16]:

$$F = \frac{\mathrm{AUC_{m,ev}}}{\mathrm{AUC_{m,iv}}} \times \frac{(\mathrm{Dose})_{\mathrm{iv}}}{(\mathrm{Dose})_{\mathrm{ev}}} \tag{10.5}$$

10.2.2 Variability in Bioavailability Estimates

Variation in oral bioavailability may arise from potential differences in drug absorption or first-pass elimination from the gut and liver across a patient population. However, it is generally accepted that variability will be greatest for drugs characterized by low bioavailability due to high first-pass extraction [17]. Accordingly, when variability in drug PK among individuals is large, predictions of PD effect at a given dose level are met with uncertainty. Moreover, PK variability between individuals is of particular concern for drugs with a narrow therapeutic index, as relatively small differences exist in drug concentrations associated with PD effect and toxicity or therapeutic failure.

Individual differences in drug PK can arise from physical differences in age, sex, and body size as well as disease state. In addition, there is increasing evidence that genetic differences in enzyme and transporter activities and expression also contribute to PK variability. In some cases, such as for anticancer agents, attempts to individualize treatment have involved calculating dose in terms of body surface area. Alternatively, bioavailability studies conducted in healthy subjects commonly employ a crossover design within the same group of individuals to minimize potential sources of PK

variability. This testing paradigm ensures all treatments evaluated in the study can be compared for each individual across the same panel of subjects. Thus, a crossover design avoids potential bias in comparisons of AUC exposure and decreases the effects of variability in drug elimination between subjects (intersubject). However, crossover studies do not account for PK variability within the same individual (intrasubject). Differences exhibited in systemic clearance within the same individual from one dose administration to the next can be difficult to distinguish experimentally and effects on bioavailability cannot readily be predicted.

Although identification of intrasubject differences in clearance would reduce variability in the estimation of bioavailability, determination of clearance differences in the same individual from one treatment to another is not possible using routine approaches without assumptions regarding other PK parameters. However, changes in elimination half-life ($t_{1/2}$) are measurable and may reflect changes in clearance when apparent volume of distribution is assumed to be constant between dose administrations. Expressing clearance in terms of elimination half-life and volume of distribution ($\mathrm{CL} = 0.693 \times V_\mathrm{d}/t_{1/2}$) and substituting into Equation 10.4 yields

$$F = \frac{\mathrm{AUC_{ev}}}{\mathrm{AUC_{iv}}} \times \frac{t_{1/2,\mathrm{iv}}}{t_{1/2,\mathrm{ev}}} \times \frac{(\mathrm{Dose})_\mathrm{iv}}{(\mathrm{Dose})_\mathrm{ev}} \tag{10.6}$$

Thus, in some circumstances, the accuracy of bioavailability estimates may be improved by incorporating half-life estimates to accommodate changes in clearance. However, experimentally derived estimates of half-life are a subjective measure of drug elimination that can be confounded by multiphasic concentration–time profiles as well as relatively slow rates of drug absorption that cannot be distinguished from the rate elimination.

The use of stable isotope methodologies provides an additional approach to decrease potential sources of variability in the calculation of bioavailability. To address potential within-subject differences in drug clearance that may arise from dosing on two separate occasions, stable labeled and nonlabeled drug have been administered simultaneously by two different dosing routes or by the same dosing route as two different formulations. These studies use drug substances prepared by incorporation of stable isotopes in order to take advantage of mass spectrometric analytical methods to distinguish between different isotopic forms of the drug in the same sample. Drug bioavailability studies have commonly employed the stable isotopes of hydrogen (deuterium, [^{2}H]), carbon (carbon-13, [^{13}C]), and nitrogen (nitrogen-15, [^{15}N]). In this way, human PK for two simultaneous dose administrations can be determined from concentrations of stable labeled and nonlabeled drug quantified from the same plasma samples. Implicit to an assumption of similar PK is an expectation that both isotopically labeled and nonlabeled drug substances will distribute identically in the systemic circulation and be metabolized identically.

Stable isotope techniques have been used to determine the absolute oral bioavailability of many drugs, including ribavirin, an antiviral agent, the calcium channel blocker verapamil, and the anticonvulsant phenytoin. In the case of ribavirin, prolonged systemic exposure caused by intracellular sequestration of drug would require a long washout period between ribavirin dosing. A stable isotope study was planned with ribavirin in order to avoid the risk of insufficient washout between the treatment phases of a crossover design. To estimate absolute bioavailability, the study design involved

IV administration of [^{13}C]-ribavirin followed 1 h later by an oral dose of unlabeled drug [18]. The use of stable isotope methodology provided a robust estimate of ribavirin bioavailability (51.8%) without a period bias or a washout effect to confound the data. The absolute oral bioavailability of verapamil was also investigated using isotopically labeled drug. Deuterium-labeled verapamil was dosed orally to compare first-pass metabolism differences in patients with liver cirrhosis and healthy subjects. Dosage adjustments for patients with liver disease were recommended as a result of a nearly twofold increase in bioavailability associated with a reduction in oral clearance [19]. A stable isotope approach was also employed to estimate absolute bioavailability of phenytoin in patients with epilepsy. Conventional approaches to determine bioavailability are complicated by the slow absorption rate, extensive plasma protein binding, nonlinear PK, and narrow therapeutic window of phenytoin. Mean estimates of absolute bioavailability after simultaneous administration of an oral formulation and parenteral stable labeled phenytoin ranged from 86.4% in younger patients to 92.5% in older patients [20].

The use of stable isotopes has also facilitated relative bioavailability determinations in order to establish the bioequivalence of highly variable drugs. The oral bioavailability of the anti-arrhythmic moricizine was studied by simultaneous administration of nonlabeled tablet and [^{13}C]-moricizine oral solution. Initial studies determined that high variability within subjects due to the extensive first-pass metabolism of moricizine precluded a conventional crossover study design. It was estimated that a large number of study subjects would be required for a crossover study in order to pass average equivalence criteria with the desired statistical power. Instead, fewer subjects were studied by a stable isotope approach in order to confirm the bioequivalence of a moricizine oral solution to the marketed commercial tablet [21].

Although the use of stable label techniques for the study of bioavailability has afforded opportunities to minimize variability within subjects, inherent limitations regarding use in human studies have curtailed the broad application of this approach. Stable isotope use in evaluating bioavailability has been limited by requirements for custom syntheses of highly purified material conducted in accordance with good manufacturing practices (GMP), challenges associated with preparation of stable labeled formulations that are representative of clinical formulations and the need for a compliment of nonclinical safety studies necessary to gain approval for IV dosing of stable labeled drug.

Advances in mass spectrometry have provided new opportunities for determining drug bioavailability with the use of trace levels of radioactive isotopes, obviating some of the drawbacks associated with the use of stable label approaches outlined above. Accelerator mass spectrometry (AMS) is a relatively new technique with high sensitivity, used to measure low concentrations of radiolabeled drug and metabolites in multiple matrices. The analytical sensitivity of AMS is derived from the fact that individual drug molecules are being detected by the mass spectrometer. Conventional radiolabel studies rely on detection methods such as scintillation counting to monitor the radioactive decay of the labeled drug. In contrast, AMS measures the ratio of individual radioactive atoms, commonly carbon-14 for human studies, and the naturally abundant carbon-12 atoms in samples after drug administration. A comparison of carbon-14/carbon-12 ratios in samples collected from subjects who received drug versus the same ratio in pretreatment samples allows very low levels of drug to be quantified in the femtomole to attomole concentration range.

The high sensitivity of AMS has made this technology amenable to mass balance studies and microdose scale PK studies [22,23]. Human bioavailability studies have also employed AMS technology to monitor low circulating plasma concentrations following subtherapeutic amounts of radiolabeled drug. Study designs to assess absolute bioavailability have involved either simultaneous IV administration of a microdose of radiolabeled drug with oral administration of nonlabeled drug or administration of a microdose of radiolabeled drug on two occasions separated by a suitable washout period between IV and extravascular dosing.

Although microdose studies represent an efficient approach to estimate absolute bioavailability in the clinic and are supported by an abbreviated safety dossier, drug PK after a microdose must accurately represent the kinetics at higher therapeutic dose levels. Accurate bioavailability determinations require that the PK are independent of drug concentrations across a range of dose levels. Therefore, studies have occasionally been conducted in nonclinical species to confirm linear PK at microdose and therapeutic dose levels before dosing to human subjects [24,25].

The bioavailabilities of several drugs following microdose administrations have also been conducted in human subjects in order to evaluate microdosing study designs. Results from a study in human volunteers indicated concordance between subtherapeutic and therapeutic dose PK for erythromycin, midazolam, and an experimental compound ZX253 [26]. Estimates of absolute bioavailability were determined for each compound by simultaneous administration of an IV carbon-14-labeled microdose plus a pharmacological oral dose and compared with estimates obtained in a conventional crossover study at therapeutic dose levels. Results of the study indicated relatively good agreement (i.e., differing by less than twofold) in the estimates of bioavailability obtained from microdose and conventional studies for midazolam and ZX253. The estimate of erythromycin bioavailability from a conventional crossover study at therapeutic dose levels was greater than twofold larger than the estimate of bioavailability obtained from a microdose—pharmacologic dose paring (35% vs 14%, respectively). However, the difference in absolute bioavailability was attributed in part to the acidic conditions of the upper GI tract and potential degradation of erythromycin following oral administration.

10.3 BIOEQUIVALENCE

The assessment of bioequivalence of two or more drug products offers important information regarding the safe and efficacious use of therapeutic agents. When measures of systemic exposure relate to drug efficacy and safety, bioequivalence confers therapeutic equivalence. As such, a common goal of all bioequivalence studies is to demonstrate that one drug product can be substituted for a second.

Studies to test for bioequivalence involve comparisons of bioavailability between two drug formulations that are either pharmaceutical equivalents or pharmaceutical alternatives of one another. That is, drug products that contain the same active ingredient and administered in the same dosage form (pharmaceutical equivalents) or in similar dosage forms prepared for the same route of administration (pharmaceutical alternatives). For pharmaceutical equivalents or alternatives to be considered bioequivalent, the active ingredient in a test product must not exhibit a significantly different rate and extent of absorption as that of the reference product when administered at the same

molar dose and under similar experimental conditions [3]. Inherent in the definition of bioequivalence is an assumption that once absorbed into the circulation, distribution, metabolism, and excretion of the active ingredient will be the same irrespective of formulation.

Two drug products are considered to be therapeutically equivalent if they are pharmaceutical equivalents and bioequivalent, thus leading to the same profile of clinical efficacy and safety. However, if test product levels in plasma are substantially lower than those of the reference product, concerns of a potential lack of therapeutic efficacy are raised. Alternatively, when test product levels are higher than those of the reference then concerns shift to a focus on safety. Although therapeutic equivalence is generally not tested directly, it is presumed based on tests of bioequivalence. When bioequivalent drug formulations share the same clinical effect and safety profile in patients, then they are considered to be therapeutically equivalent and may be used interchangeably.

Bioequivalence studies can be integral to the development of new drugs, as improvements in drug formulation may be required during clinical development. For instance, drug formulations used in clinical trials are often not suitable for commercialization. Thus, demonstrating bioequivalence of a drug product intended for market use will allow its substitution for the experimental formulation. Bioequivalence trials are also conducted to evaluate the effects of food, changes in the marketed formulation, or dose route of administration. Recent efforts to reformulate the antifungal agent amphotericin B represent attempts to reposition a previously approved drug that suffers from infusion-related side effects and renal toxicity. Amphotericin B has low solubility and permeability that result in negligible oral absorption. Reformulation of amphotericin B for oral administration would significantly lower treatment costs and improve its accessibility [27]. In contrast to oral formulations, therapeutic equivalence of drug products formulated for IV administration may be established by demonstrating pharmaceutical equivalence and does not require *in vivo* testing [28].

Bioequivalence studies are also conducted as components of an ANDA for a generic drug product. Because generic drugs are intended to be interchanged with innovator products, a demonstration of bioequivalence between formulations is essential for safe and efficacious use of the generic formulation. Thus, therapeutic equivalence to the innovator drug product is accepted on demonstration of bioequivalence of a pharmaceutically equivalent generic drug product.

The rules that govern generic drug substitution were defined by the Drug Price Competition and Patent Term Restoration Act of 1984 (Hatch–Waxman Amendments). Since adopting Hatch–Waxman, more than 11,000 generic drug products have been approved by the Food and Drug Administration (FDA). In order to judge the relative success of these approvals, a recent survey was conducted by FDA of generic drugs approved over a 12-year period spanning from 1996 to 2007 [2]. The survey revealed that in 2070 bioequivalence trials conducted over a 12-year period through 2007, the average difference in rate and extent of drug absorption between generic and innovator products was 4.35% and 3.56%, respectively [2].

In order to avoid complications that could arise from product substitution, the FDA maintains a list of generic drugs that can be safely and appropriately substituted for brand products. These drugs are listed in a federal publication entitled Approved Drug Products with Therapeutic Equivalence Evaluations, commonly known as the *Orange Book* [29]. To permit substitution of a brand drug, a generic equivalent must demonstrate bioequivalence as part of the approval process. However, the process by which a

brand drug is approved differs from the process required for generic product approval. A summary of the similarities and differences between new drug application (NDA) and ANDA dossiers are shown in Table 10.1. Both innovator and generic products must contain the same active ingredient in the same strength. Moreover, drug chemistry, manufacturing details and quality control measures must be documented for both products. However, after this point, the two processes begin to diverge. The Hatch–Waxman Amendments do not require generic drug applicants submitting an ANDA to provide nonclinical or clinical studies to establish the safety and efficacy of the active ingredient, as these characterizations have been established previously for the innovator product. Required of the generic product is demonstration of bioequivalence to the innovator product.

10.3.1 Study Design

Bioequivalence studies are generally conducted in a small number of healthy volunteers to determine the relative bioavailability of one or more test formulations following single dose administration. In most studies, the reference formulation is defined as the historical or innovator drug product and all individuals participating in the trial typically receive reference as well as test formulations. There are three basic study designs employed for determination of drug product bioequivalence—randomized crossover design, parallel design, and partial-block crossover design.

10.3.1.1 Randomized Crossover Design. A two-period randomized crossover design is one of the most commonly employed study designs to test the equivalence of two treatments. In accordance with this design, subjects are divided into two equal groups on random assignment to either a test or reference treatment group. After the first dosing period, a sufficient washout period equivalent to five or more drug half-lives is allowed to elapse before dosing resumes. For the second dosing period, subjects are crossed over into the opposite treatment group and receive either the test or reference dose depending on their dosing history in the first period. A randomized crossover study is intended to minimize intersubject variability by employing a Latin square design to allow each individual serves as a control subject. Bioequivalence studies to assess multiple regulatory requirements might employ a randomized

TABLE 10.1 Requirements of the New Drug Application (NDA) and Abbreviated New Drug Application (ANDA) Review Processes

NDA Requirements	ANDA Requirements
Chemistry	Chemistry
Manufacturing	Manufacturing
Controls	Controls
Labeling	Labeling
Testing	Testing
Animal studies	Bioequivalence
Clinical studies	
Bioavailability	

Source: Adapted from Ref. 30.

crossover of multiple treatments with a Latin square design. For instance, a study to compare test and reference treatments in fed and fasted subjects would generally be conducted as a single dose, randomized, four-period crossover design.

10.3.1.2 Parallel-Group Design. In a parallel study, subjects are divided into equal groups on random assignment to either a test or reference treatment group. Subjects receive the assigned treatment during the study and plasma concentrations are measured. A comparison of results allows an assessment of relative bioavailability. In contrast to the crossover design, higher intersubject variability is anticipated with a parallel design and larger numbers of enrolled subjects will be required. Consequently, testing for formulation differences could be insensitive due to a potential for larger statistical error. However, in some circumstances, a parallel-group study design may be a preferred option. For instance, parallel-group studies might be used to evaluate the therapeutic equivalence of two formulations in patients if treatment changes during the course of the study are precluded by consideration of patient welfare [31,32]. In addition, parallel designs might also be preferred for studying drugs with long half-lives or those requiring lengthy follow-up periods, thus making crossover designs impractical options.

10.3.1.3 Partial-Block Crossover Design. A partial-block design is an extension of the crossover design concept in which subjects receive a subset of formulations being tested. This design might be considered when a comparison of several formulations is planned for each subject in a time-consuming trial that potentially risks high numbers of subject dropouts. A partial-block crossover design could also be employed when studying a long half-life drug. For drugs with a long half-life, an extended duration of a washout period between treatment arms could potentially add several weeks to a complete crossover trial. Using a partial-block crossover design, each subject would experience fewer treatment washout periods, while maintaining within-subject comparisons that are intended to minimize variability. In general, the number of individuals enrolled in a partial crossover study is less than what is required for a parallel design but greater than that required for a complete crossover design.

As an example, a partial-block crossover design was employed to study the 5-α-reductase inhibitor dutasteride in a combination capsule with the α_1-adrenoceptor antagonist tamsulosin due to the markedly different half-lives of these two compounds [33]. Dutasteride has a half-life of 7–9 days, much longer than the 9–11 h for tamsulosin, which required at least 28 days of washout between treatment periods. If conducted as a single four-way crossover study, the three long washout periods would have resulted in an excessively long study of approximately five months in duration. To avoid lengthy washout periods, a three-way partial crossover design was selected to evaluate two formulations in fed (groups A and B) and fasted (groups C and D) healthy volunteers, shown in Table 10.2. This design attempted to minimize the time spent on study for the subjects, while maintaining within-subject comparisons.

10.3.2 Statistics

The number of individuals enrolled in a bioequivalence study will be determined by the PK variability of the reference dose and based on prior experience. In general, the study size must be sufficient to detect a 20% difference in the measured exposure parameters

TABLE 10.2 Partial-Block Randomized Crossover Bioequivalence Study Design Used to Study Dutasteride–Tamsulosin Combination in Fed and Fasted Subjects

Subject No.	Drug Product[a]		
	Period 1	Period 2	Period 3
1	A	B	C
2	A	C	B
3	B	A	C
4	B	C	A
5	C	A	B
6	C	B	A
7	A	B	D
8	A	D	B
9	B	A	D
10	B	D	A
11	D	A	B
12	D	B	A

[a]Treatments: (A) AVODART (dutasteride) plus Flomax (tamsulosin hydrochloride) in the fed state; (B) dutasteride–tamsulosin combination product in the fed state; (C) AVODART plus Flomax in the fasted state; and (D) dutasteride–tamsulosin combination product in the fasted state.
Source: Ref. 33.

with 80% certainty. The dose selected for study is often the highest strength being considered, as comparisons are most discriminating between treatments when exposures increase proportionally or greater than proportional to changes in dose level. When exposure increases are less than dose proportional, the lowest strength dose should be compared [2].

Data resulting from bioequivalence studies are evaluated statistically to analyze two one-sided confidence intervals. The first of two one-sided tests determines if a test product is 20% less bioavailable when substituted for the reference. A second test determines whether the reference is 20% less bioavailable when substituted for the test product. A difference of more than 20% for each of the statistical tests is considered significant, resulting in a lack of bioequivalence. In the case of the two one-sided tests, the null hypothesis is one of nonequivalence. Thus, incorrectly concluding bioequivalence for two nonequivalent drug products (type I error) places risk on the consumer. Alternatively, an incorrect conclusion of bioinequivalence for two equivalent drug products (type II error) places risk on the sponsor.

In practice, the two one-sided tests are each carried out at a 0.05 level of significance, with the combined tests yielding a 10% total error and 90% confidence interval of 80–125%. Therefore, the bioequivalence of two drug preparations can be concluded when the 90% confidence intervals of the geometric mean test/reference ratios of $C_{\max}$ and AUC fall within the limits of 80–125%. In other words, bioequivalence will be concluded when the test product differs from the reference product by $-20\%/+25\%$ or less in terms of the rate and extent absorption. Use of the $-20\%/+25\%$ interval was based on a medical decision that differences in systemic drug concentrations that fall within the -20%/+25% range would not likely be clinically significant [34].

10.3.3 Approaches to Demonstrate Bioequivalence

Bioequivalence studies are designed to determine whether a test formulation may be substituted for an existing reference formulation. Successful substitution requires that the two drug products will provide equivalent therapeutic effects. For two oral drug products to be considered bioequivalent, each product must exhibit the same rate and extent of absorption. Thus, bioavailability must be measured by appropriate *in vivo* or *in vitro* methods in order to document bioequivalency. In accordance with accepted FDA methods, studies to determine the bioequivalence of two drug products may be conducted with PK, PD, clinical, or *in vitro* endpoints [3]. Thus, experimental methods to demonstrate bioequivalence will vary case by case, as the selection of appropriate study design and outcome measures will depend on the characteristics of drug products to be compared.

10.3.3.1 Pharmacokinetic Studies. Studies to quantify systemic exposure and characterize drug PK provide opportunities to compare drug bioavailabilty. Inherent to these studies is the assumption that systemic exposures will reflect drug concentrations at the site of action and thus will provide some assurance of comparable therapeutic effects. For orally administered drugs, the parameters of C_{max} and AUC provide measures related to the rate and extent of drug absorption, respectively. Bioequivalence studies commonly enroll healthy volunteers to receive a single dose of each formulation. A crossover study design is employed in order to minimize interindividual variability influences on bioavailability, as exposure measurements are assumed to have less interoccasion variability compared to the variability associated with formulation performance [3]. Differences in bioavailability will therefore likely reflect true differences in formulation.

Establishing bioequivalence is most often based on parent drug concentrations, as the parent compound is generally the entity most sensitive to formulation differences. However, when safety or efficacy is considered a function of all drug-related species present in circulation, then monitoring metabolite plasma concentrations to determine the bioequivalence of two drug products could be warranted. Metabolite exposure might be appropriate to monitor if parent drug is rapidly and extensively metabolized, pharmacological activity is correlated with metabolite levels, or if both parent drug and metabolite account for therapeutic effect [35].

Circulating levels of pharmacologically active metabolites are frequently monitored in comparative studies in order to estimate the relative bioavailability between formulations of high clearance drugs. For example, the anthelmintic agent albendazole undergoes rapid and essentially complete first-pass metabolism to a sulfoxide metabolite. Suspension and solution formulations were found to provide 4.3- and 9.7-fold improvements in oral bioavailability relative to tablets, respectively, as demonstrated by comparing AUC exposures of the sulfoxide metabolite [36]. Similarly, the potent desmethyl metabolite of tramadol, a centrally acting analgesic, was monitored to compare the PK of an extended-release tablet relative to an immediate-release reference product [37]. However, in the case of the antiplatelet agent clopidogrel, neither parent drug nor pharmacologically active metabolite is present in circulation at high concentrations following oral administration. Therefore, circulating levels of an inactive carboxylic acid metabolite of clopidogrel were monitored to compare different formulations in a randomized crossover study [38,39].

10.3.3.2 Pharmacodynamic Studies. PD studies are designed to establish bioequivalence of drug products based on measures of pharmacological effect. PD studies differ from PK studies in that circulating drug levels may or may not reflect concentrations at the site of action or correlate with therapeutic effect. PD endpoints are used to determine bioequivalence more often for drug products applied directly to the site of action where they may exert their desired effect, such as topical cream formulations [40,41]. In such cases, drug products are not intended to be absorbed into the systemic circulation. Therefore, bioavailability may be assessed by measurements intended to reflect the rate and extent to which drug becomes available at the site of action. Frequently, however, PD measurements are obtained in conjunction with PK measures in order to relate circulating drug concentrations with drug effect. Thus, the bioequivalence of drug products would typically be based on a measure of dose–response equivalence, where an E_{max} model is commonly assumed. Drug product comparisons might be made in terms of maximal effect, minimal effect, or average effect measures as well as the area under the effect versus time curve (AUEC). When PK data are available, drug concentrations associated with half-maximal effect (EC_{50}) may be compared when calculated from the model:

$$E = \frac{E_{max} \cdot C}{EC_{50} + C} \tag{10.7}$$

where E is the effect and E_{max} the maximal effect [39,42].

10.3.3.3 Clinical Studies. Bioequivalence may also be based on a comparison of clinical outcomes between treatment groups. However, clinical assessments are generally not the sole determinant of equivalence when plasma drug concentrations or measures of PD effect are also available. Although clinical endpoints may be useful to conclude bioequivalence when quantitative measurements are not feasible, clinical assessments are also subjective and difficult to reproduce. Additionally, clinical studies frequently require large numbers of patients in order to ensure adequate statistical power to differentiate drug products.

Most clinical endpoint bioequivalence studies are limited to determination of whether a given treatment failed or succeeded. In a recent trial with the atypical antipsychotic clozapine, a subgroup of patients was randomly assigned to switch to a generic formulation or remain on the initial formulation. Both patient groups were monitored in parallel during the study and clinical outcomes determined from psychiatric tests administered before the switch and at time points after the switch were compared across treatments. A lack of significant differences in score results between the two treatment groups allowed investigators to conclude bioequivalence of generic clozapine based on therapeutic equivalence [31]. Clinical endpoints were similarly used to test the bioequivalence of two formulations of the antifungal agent amphotericin B. On the basis of comparison of clinical cure rates in patients with visceral leishmaniasis treated with each formulation, therapeutic equivalence of the test liposomal formulation of amphotericin B was concluded. In the trial, a single infusion of liposomal amphotericin B was determined to be noninferior to conventional therapy with amphotericin B deoxycholate [32].

10.3.3.4 **In Vitro *Studies.*** A fourth approach to characterize drug formulation bioequivalence incorporates knowledge of the key determinants of oral absorption. The biopharmaceutical classification system (BCS) provides a scientific approach to classify drug substances based on *in vitro* measurements of aqueous solubility and intestinal permeability [43]. Using BCS, drugs may be categorized into four classes according to *in vitro* estimates of aqueous solubility and permeability to predict *in vivo* bioavailability, as shown in Fig. 10.1. On the basis of this classification scheme, high solubility drugs may be characterized as having either high permeability (class I) or low permeability (class III), and low solubility drugs are similarly characterized as either high (class II) or low (class IV) permeability. Subsequently, BCS classification was adopted by FDA to allow waiver of *in vivo* bioavailability and bioequivalence testing of immediate-release solid dosage forms for drugs characterized by high solubility and high permeability (class I) that exhibit rapid dissolution [44]. Thus, characterization of drugs in terms of the fundamental properties governing oral absorption seeks to ensure similarities in systemic availability.

The utility of the BCS to classify drugs in terms of solubility and permeability has also provided a framework to predict the effects of food on oral absorption [46]. Physiological changes induced by the presence of food in the GI tract were predicted to similarly influence the oral absorption of drugs of the same BCS class. A relationship between drug disposition and BCS classification was later recognized, as drug substances characterized by high permeability (classes I and II) were eliminated primarily by metabolism and low permeability compounds (classes III and IV) were primarily excreted in urine and bile. On the basis of these observations, a modification was proposed to replace the permeability criterion of BCS with a designation of the major route of drug elimination [45]. Hence, the biopharmaceutics drug disposition classification system (BDDCS) was proposed with metabolism as an alternate measure of drug permeability (Fig. 10.1). BDDCS has also been used to predict the mechanistic effects of food on drug absorption [47].

In general, the *in vitro* determination of aqueous solubility is relatively straightforward and has led to accurate characterization of most drugs. As such, waiver of *in vivo* bioavailability and bioequivalence testing of rapidly dissolving drugs belonging to BCS class I is well accepted. A similar waiver for drugs belonging to BCS class III (high solubility and low permeability) that exhibit rapid dissolution has also been proposed [48,49]. However, *in vitro* predictions of intestinal drug permeability do not share the same level of consensus as do measures of aqueous solubility. This has led to increased acceptance of alternatives to the BCS measures of permeability, such as the

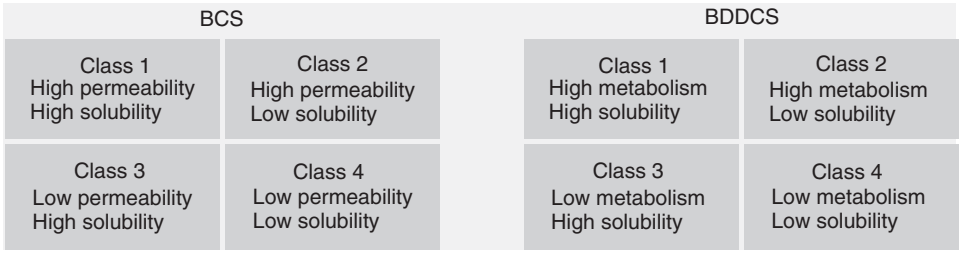

Figure 10.1 Comparison of biopharmaceutics classification system (BCS) and biopharmaceutics drug disposition classification system (BDDCS). *Source*: Refs. 43 and 45.

incorporation of drug elimination by the BDDCS [45,50]. Although contrasting features of the BCS and BDDCS suggest differences in predictions of *in vivo* absorption should be anticipated, a recent comparison of drug classifications indicated that the two systems were largely in agreement. Concordance in the BCS and BDDCS classification of drugs with low solubility (classes 2 and 4) was observed with 87–92% agreement across 164 compounds. The level of concordance was slightly lower for high solubility drugs (classes 1 and 3) ranging from 64% to 72% being identically classified. The distinguishing feature leading to differences between the two systems was the selection of the BCS permeability reference drug. Overall agreement between BCS and BDDCS predictions was best when cimetidine was the reference permeability drug and weakest when metoprolol was used [51].

10.4 DETERMINANTS OF ORAL BIOAVAILABILITY

Given that the majority of drugs are administered as an oral dosage form, the role of the GI tract is significant in the disposition of many drugs. The GI tract serves many important functions involved in the digestion of food, absorption of dietary nutrients, exchanges of liquids, and excretion of waste products as well as providing a protective barrier to the entry of xenobiotics and other foreign matter such as bacteria [52]. Consequently, absorption into the portal circulation requires the physicochemical properties of a drug that govern solubility and permeability to be balanced with respect to physiology of the GI tract such as transit time, luminal content, pH environment, and absorptive surface area. The serial arrangement of the small intestine and liver also provide sequential barriers for drug transit to the systemic circulation.

For orally administered drugs, the amount of dose transferred from the site of administration to the systemic circulation will be determined by the fraction of absorbed dose as well as the available fraction not subjected to intestinal or hepatic first-pass elimination. Although some degree of passive permeability becomes a requisite feature for drug transfer across the gut wall, a component of bioavailability is also determined by drug affinity for transporters and metabolizing enzymes localized in the epithelial lining of the gut. Moreover, hepatocytes in the liver express a full complement of transporters and enzymes that may further limit drug transit to the systemic circulation. Thus, absorption from the GI lumen, metabolism by enzymes, and active transport processes localized in enterocytes, along with hepatic uptake and metabolism, are considered to be the major sources of drug loss following oral administration.

Oral bioavailability is a measure of the fraction of dose absorbed as intact drug from the GI lumen (F_a), the fraction transferred to the portal bloodstream without intestinal extraction (F_g), and the fraction not extracted on hepatic first pass (F_h) [1]. Thus, drug bioavailability can be estimated as a product of dose fractions that escape loss at each site during the sequential transit of intact drug from the GI tract to the liver and the systemic circulation. Accordingly, the determinants of oral drug bioavailability are related by the following expression:

$$F = F_a \times F_g \times F_h \tag{10.8}$$

The utility of this simple mathematic relationship can be illustrated by considering the example of raloxifene, a selective estrogen receptor inhibitor that exhibits low

oral bioavailability. After oral administration, ~2% of a raloxifene dose enters the systemic circulation as parent drug with the balance lost to malabsorption and first-pass elimination. Prior knowledge of the fraction of dose absorbed as intact raloxifene (63%), plus the fraction of dose that evaded hepatic extraction (59.3%), allows an estimate of the fraction of dose transferred across the gut mucosa. Using the above expression to solve for F_g, in which $F_g = (63\% \times 59.3\%)/(2\%)$, ~5.4% of the fraction of absorbed dose escaped intestinal extraction, suggesting that first-pass gut extraction is the major limitation to raloxifene oral bioavailability [53].

The bioavailability of orally administered drugs is determined by drug absorption and intestinal and hepatic first-pass elimination, as described by Equation 10.8. To understand the mechanisms responsible for incomplete drug bioavailability, limitations to absorption as well as first-pass elimination by the gut and liver must be determined separately. Experiments conducted in cannulated animal models have offered an approach to study drug loss in a physiologically intact system. Frequently, drug extraction or loss is measured by infusing drug at multiple sites of input (i.e., intra-arterial, IV, duodenal, and intraportal) and sampling from a single venous site in a rodent model. Alternatively, extraction can be determined by infusing drug at a single catheter site and sampling at multiple sites [54]. A five-catheter chronic rat model has also been used to assess drug bioavailability in animals that have fully recovered from surgery and anesthesia associated with catheter placement. This model records drug extraction under normal physiologic conditions as GI and liver functions are returned to presurgery status before experiments are initiated [55]. Although surgical approaches have frequently been adopted to elucidate the determinants of oral bioavailability in preclinical animal models, the use of invasive techniques is not common in human subjects.

10.4.1 Oral Drug Absorption

10.4.1.1 Approximation of F_a. In clinical studies, an estimate of F_a is frequently provided by monitoring the cumulative excretion of a single dose of radiolabeled drug. For drugs in which both parent and metabolites are eliminated by renal excretion, the proportion of total radioactive dose excreted into urine [(Au,rad)/dose] can be used to approximate the fraction of absorbed dose. Comparison of total radioactivity excreted after oral (po) and IV dose administrations provides an estimate of F_a according to the following expression:

$$F_a \cong \frac{(\text{Au,rad})_{po}}{(\text{Au,rad})_{iv}} \times \frac{(\text{Dose})_{iv}}{(\text{Dose})_{po}} \tag{10.9}$$

Although Equation 10.9 requires determination of the proportion of IV dose eliminated in urine, IV data are frequently not available. However, in some cases, F_a can be estimated from oral dose data only, such as with the cyclooxygenase-2 inhibitor rofecoxib [56]. In the case of rofecoxib, F_a could be approximated from radioactivity excreted after oral dosing (Eq. 10.10) because the fraction of total radioactivity in urine represented a relatively large proportion of overall rofecoxib elimination:

$$F_a \cong \frac{(\text{Au,rad})_{po}}{(\text{Dose})_{po}} \tag{10.10}$$

Modest improvements in estimates of the fraction of absorbed dose have also been achieved by application of Equation 10.10 plus consideration of metabolite levels excreted in feces as a percent of dose. In the case of the antipsychotic drug olanzapine, the fraction of absorbed dose was approximated by the sum of total radioactive dose recovered in urine plus the fraction represented by excretion of the 10-*N*-glucuronide metabolite in feces [57]. However, a caveat to this approach assumes that metabolite was excreted into feces and not formed by biotransformation of either unabsorbed dose or excreted parent drug returned to the GI tract.

10.4.1.2 Dissolution and Solubility. The fraction of an oral dose absorbed from the GI tract is a function of the physicochemical properties of a drug substance, as absorption can be limited by incomplete dissolution of solid drug as well as low intrinsic solubility. When administered as a tablet or capsule, partial dissolution of solid drug or a slow rate of dissolution relative to transit times within the absorptive regions of the GI tract may limit drug absorption. The intestinal environment may also influence drug solubility. Because the luminal content of the small intestine is mostly water, aqueous solubility plays an important role in drug absorption. However, drug solubility and oral absorption can also be influenced by changes in gastric pH as well as the heterogenous composition of the intestinal lumen.

Most drug substances are either weak acids or weak bases that can become ionized under physiological conditions of the GI tract that ranges from low pH of the stomach (pH 0.8–1.8) to near neutral pH in the distal GI (pH 6.5–7.2) in the fasted state [58]. The degree to which a weak acid or weak base exists in an ionized or unionized state depends on pH of the local environment and the pK_a of the drug. Therefore, regional differences in pH along the GI tract can have an effect on the absorption of weak acids and weak bases caused by pH-dependent changes in solubility. The antifungal ketoconazole is an example of a weak base characterized by pH-dependent solubility and absorption. Owing to a low pK_a, weak bases such as ketoconazole have improved solubility under acidic conditions. Ketoconazole has two pK_a values (2.9 and 6.5) and is relatively insoluble near neutral pH, but solubility is dramatically improved at low pH conditions. Experimental evidence of the pH dependence of ketoconazole absorption was demonstrated in healthy volunteers. Oral ketoconazole bioavailability was increased when dosed in an acidic solution and decreased when gastric pH was raised by coadministration with the proton pump inhibitor cimetidine [59].

In addition to pH-dependent effects on solubility, bile salts in the intestinal lumen may also influence drug solubility and absorption. The aggregation of bile salts present in the small intestine form micelles that may enhance the solubility and absorption of some poorly water soluble drugs. The importance of bile salts to the dissolution and solubility of the antifungal agent griseofulvin and the steroid danazol have been proposed to explain enhancements in postprandial bioavailability of these agents [60]. Oral absorption of the cyclooxygenase-2 inhibitor rofecoxib is also believed to be facilitated by bile salts in the GI tract. Following oral [^{14}C]rofecoxib administration, the fraction of absorbed rofecoxib dose was lower in cholecystectomy patients than in healthy volunteers, suggesting enhanced rofecoxib intestinal solubility in the presence of bile [56].

10.4.1.3 Food Effects. The presence of food within the GI tract may further complicate processes leading to oral bioavailability, as changes in drug absorption and

systemic exposure may result from physiological changes caused by food. Following a meal, digestive processes initiate transient increases in gastric pH accompanied by slower gastric emptying as well as longer overall GI transit times. Changes in intestinal fluid composition which result from stimulated secretions from the pancreas, liver, and gallbladder may also affect drug absorption. Additionally, constituents of a meal such as fats, carbohydrates, and minerals, may interact directly with drug substances to influence absorption as well as with membrane transport proteins or enzymes responsible for drug disposition.

Predictions of the effect of food on drug absorption and systemic exposure have been afforded from estimates of relative solubility and permeability characteristics of different drug substances. Thus, grouping compounds based on BCS [43] and BDDCS [47] criteria provide a framework to predict the effects of a meal on bioavailability. However, although changes in physiology resulting from food intake are known, the mechanisms by which food may change the bioavailability of a given drug are not always straightforward. Moreover, the magnitude of effect due to individual mechanisms makes predictions of food effects even more challenging. For instance, the nonsteroidal anti-inflammatory drug ibuprofen is a weak acid characterized by low solubility and high permeability. With a meal, greater oral absorption of ibuprofen due to increased solubility at higher pH and prolonged gastric emptying time would be expected to improve bioavailability relative to fasting. However, following ingestion of a meal, ibuprofen bioavailability was decreased by ~20%, potentially due to drug binding to viscous chyme generated from the meal and rendering it unavailable for absorption in the proximal intestine [61].

The effects of a meal on oral absorption may result in an increase in drug exposure (positive food effect), a decrease in exposure (negative food effect), or an insignificant change in exposure. Although systemic exposures of many commonly prescribed drugs used in clinical practice can be altered when taken with a meal, dosing recommendations to instruct use with or without food are relatively uncommon. A representative sample of drugs having product inserts that contain dosing recommendations related to food intake are shown in Table 10.3. For many drugs that exhibit negative food effects, food restrictions are recommended in order to maximize systemic exposure. An extreme example is the bisphosphonate alendronate that requires dosing with water before the first food or beverage after an overnight fast in order to achieve ~1% oral bioavailability. For drugs that exhibit positive food effects, many are recommended with a meal as a means to improve bioavailability and achieve therapeutic concentrations. In many instances, variability in systemic exposure is decreased by food as the fraction of absorbed dose can be increased in the fed state. However, in the case of the tyrosine kinase inhibitor lapatinib, a positive food effect was associated with equivalent or slightly increased PK variability and therefore lapatinib dosing is recommended without food. The product insert of another tyrosine kinase inhibitor nilotinib also recommends a food restriction, although a high fat meal was shown to increase AUC exposure by 50% in patients and 82% in healthy volunteers [62]. Unique among the drugs with food interactions listed in Table 10.3 is the black box QTc safety warning of nilotinib that includes a description of food effects on drug exposure [63].

Interactions between drugs and multivalent cations present in foods and antacids can also lead to changes in drug absorption. The oral bioavailability of some drugs may decrease as a result of chelation, the formation of a complex between polar groups

TABLE 10.3 Representative Drugs with Package Inserts that Include Dosage and Administration Instructions Related to Food Intake

Drug	% F (Fasting)[a]	Package Insert Comments and Dosing Recommendations[b]
Drugs with Positive Food Effects		
Efavirenz	NA	Increased exposure when tablet was dosed with high fat meal; C_{max} (+79%; 1.79-fold) and AUC (+28%; 1.28-fold). Dosing recommended on an empty stomach. Dose: 600 mg q24h
Isotretinoin	40% (fed)	Owing to high lipophilicity, absorption is increased when dosed with a high fat meal; C_{max} (+170%; 2.7-fold) and AUC (+186%; 2.86-fold) for 80 mg dose. Dosing recommended with food
Lapatinib	NA	AUC is increased approximately threefold (low fat) to fourfold (high fat) with food. C_{max} increased ~2.5- to 3-fold. Dosing recommended at least 1 h before or after a meal. Dose: 1200 mg q24h
Lovastatin	≤ 5%	Increased exposure when dosed with food. Dosing recommended with meals. Dose: 10–80 mg q24h or 5–40 mg q12h
Nelfinavir Mesylate	— 20–80%	— Increased exposure and decreased PK variability with food. AUC is increased 2.2- to 5.2-fold with food and C_{max} is increased 2.0- to 3.8-fold with food. Dosing recommended with meals. Dose: 750 mg q8h or 1250 mg q12h
Nilotinib	NA	Black box safety warning to avoid food intake for at least 2 h before dosing due to associated QTc risk. Observed AUC increases of 50–82% when administered with a high fat meal. Dose: 400 mg q12h
Ziprasidone Hydrochloride	— 59% (fed)	— Increased bioavailability up to twofold when dosed with food. Dosing recommended with food. Dose (initial): 20 mg q12h
Drugs with Negative Food Effects		
Alendronate Sodium	— <0.7%	— Owing to poor solubility, absorption is decreased with food or beverages other than plain water. Dosing recommended a least 30 min before first food or beverage of the day
Lansoprazole	81%	Decreased exposure when dosed 30 min after meals; C_{max} (−50%) and AUC (−70%). Dosing recommended before a meal. Dose: 15–30 mg q24h
Riluzole	64%	Decreased bioavailability when dosed with food; C_{max} (−45%) and AUC (−20%). Dosing recommended at least 1 h before or 2 h after a meal. Dose: 50 mg q12h
Tegaserod Maleate	— 11%	— Decreased bioavailability when dosed with food; C_{max} (−20% to 40%) and AUC (−40% to 65%). Dosing recommended before a meal. Dose: 6 mg q12h
Valsartan	23%	Decreased exposure when dosed with food; C_{max} (−50%) and AUC (−40%). Dosing recommended with or without food. Dose: 80/160/320 mg q24h
Voriconazole	96%[b]	Decreased C_{max} (−34%) and AUC (−24%) with high fat meal. Dosing recommended at least 1 h before or 1 h following a meal. Dose: 200 mg q12h

Abbreviation: NA, not available.
[a] *Source*: Ref. 64.
[b] *Source*: Ref. 65.

of a drug molecule and multivalent metal ions, such as aluminum, calcium, and magnesium. Chelates formed between drug substances and meal ions may impair tablet or capsule dissolution or may cause solubilized drug to precipitate in the gut lumen. The oral bioavailabilities of several antibiotic agents, such as the tetracyclines, oral cephalosporins, and quinolones, have been decreased by the formation of stable chelate complexes in the gut lumen. For example, decreased absorption of the quinolone antibiotic ciprofloxacin due to interaction with multivalent ions from dietary supplements, antacids, and phosphate-binding agents have been associated with 38–54% decreases in bioavailability [66,67].

10.4.1.4 Stability. Oral absorption may also be curtailed for drug substances prone to instability within the GI tract. The fraction of an oral dose that is absorbed from the GI tract can be limited by drug loss in the lumen due to decomposition or chemical degradation (e.g., pH-dependent processes) as well as by microbial or enzyme-mediated degradation.

Acid-labile drugs such as the immunosuppressive agent erythromycin and the reverse transcriptase inhibitor didanosine are unstable in the acidic environment of the upper GI tract. Didanosine undergoes acid-induced hydrolysis in the stomach and has been formulated with antacid in order to improve its oral absorption profile and bioavailability. More recently, introduction of didanosine as an enteric coated capsule has improved drug stability by avoiding contact with the acidic environment of the stomach and eliminated the digestive intolerance associated with the previous buffered oral formulation [68].

In addition to pH-dependent stability, the fraction of absorbed dose may also be modulated for drugs susceptible to metabolism by GI microflora. Bacteria residing in the large intestine contribute to normal digestive function by fermentation of carbohydrates and proteins that escape digestion in the upper GI tract. Drug substances may also be metabolized by enzymes in the gut microflora that typically catalyze chemical reduction and hydrolysis reactions [52,69]. In addition, metabolism of drugs by microbial enzymes may also be a function of the amount of intact drug reaching distal regions of the GI tract, consistent with an increased prevalence of microflora within the large intestine.

Drugs with low solubility and/or low permeability have a sufficiently long residence time in the gut to allow access to the distal GI tract. For instance, the peripherally acting μ-opioid antagonist alvimopan is hydrolyzed by amidases in intestinal flora to an equipotent metabolite that may contribute to PD effect. Evidence of a role for gut flora in alvimopan metabolite bioavailability was supported by coadministration of ciprofloxacin with alvimopan. Although alvimopan bioavailability, ~6%, was not changed by ciprofloxacin coadministration, metabolite concentrations were decreased by ~99% relative to the levels after alvimopan treatment alone. These findings were consistent with formation of a presystemic metabolite of alvimopan by amidases in intestinal flora [70]. Additionally, β-glucuronidases located in the distal region of the GI tract may catalyze the hydrolysis of excreted drug conjugates, leading to increases in systemic exposure by rendering parent drug available for reabsorption. Examples of drugs that undergo enterohepatic recirculation include indomethacin [71] and mycophenylatemofetil [72]. Mycophenylatemofetil is a prodrug that is completely absorbed after oral dosing and subsequently hydrolyzed to mycophenolic acid (MPA), the active immunosuppressive agent (Fig. 10.2). The elimination of MPA occurs mainly

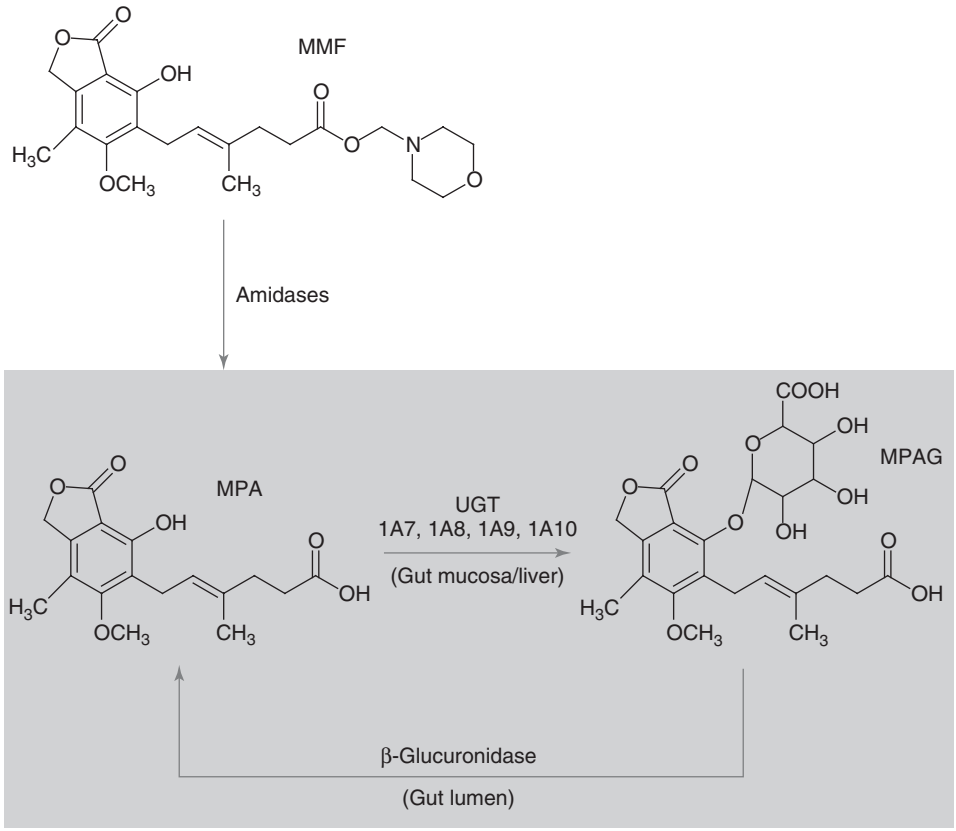

Figure 10.2 Proposed scheme of mycophenolate mofetil metabolism and enterohepatic recirculation of mycophenolic acid. Approximately one-third of MPA oral exposure is attributed to enterohepatic recirculation. *Abbreviations:* MMF, mychophenolate mofetil; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide.

by conjugation with glucuronic acid, which is partially excreted in bile. On excretion, the conjugated metabolite may by deglucuronidated by the gut microflora and reabsorption of the aglycone contributes to overall systemic exposure and immunosuppressive effects. A mean increase of ~37% in mycophenylatemofetil bioavailability was attributed to enterohepatic recirculation of MPA from a crossover study conducted with the bile acid sequestrant cholestyramine in healthy volunteers [72]. The extent of MPA reabsorption was similarly decreased by concurrent antibiotic therapy. In healthy volunteers, concomitant treatment with the antibiotic metronidazole was associated with a 36% decrease in oral AUC exposure of MPA [73]. Taken together, the interaction studies with cholestyramine or metronidazole suggested that ~35% of oral MPA exposure and bioavailability may result from enterohepatic recirculation of MPA.

10.4.1.5 Intestinal Transporters. The fraction of absorbed dose following oral administration is also a function of drug permeability across the intestinal wall. The permeability of a drug may be influenced by passive diffusion as well as active transport mechanisms. Passive permeability typically occurs by transcellular

or paracellular processes in which drugs diffuse across the intestinal wall from a region of relatively high concentration in the gut lumen to lower concentrations in the portal circulation. Depending on the characteristics of individual drug substances, recognition by membrane transporters lining the GI epithelium may also affect overall drug permeability.

Membrane transport proteins also have a role in intestinal drug disposition, as both efflux and uptake transporters may determine drug bioavailability. Efflux transporters are part of a superfamily of ATP-binding cassette (ABC) transporters that extrude drug in energy-dependent processes and may prevent drug absorption into the portal blood circulation when expressed in enterocytes in the gut. Uptake transporters responsible for drug disposition belong to the two solute carrier superfamilies, SLC and SLCO, involved in drug uptake via mechanisms of cotransport. SLC and SLCO proteins transport drugs according to a concentration gradient and may function to improve the degree of intestinal drug absorption. Together, the activities of many ABC transporters and solute carrier proteins expressed within intestinal epithelium have proven to affect drug oral bioavailability. A summary of clinical interaction studies implicating the role of individual transporters in oral drug bioavailability is shown in Table 10.4.

The efflux transporter P-glycoprotein (P-gp; MDR1), encoded by *ABCB1*, is expressed in multiple tissues important for drug disposition, including epithelia of the GI tract and hepatobiliary canaliculi as well as endothelia of the blood–brain barrier. In the gut, P-gp is localized at the apical membrane of enterocytes and may function as a barrier to absorption by transporting drug back to the lumen. Thus, recognition by P-gp has been considered a key determinant of oral bioavailability for an array of structurally diverse drugs. Substrates for P-gp transport generally tend to be hydrophobic and are represented by high molecular weight drugs such as paclitaxol and cyclosporine A (CsA) as well as smaller sized drugs such as talinolol [90]. Although *in vitro* and *in silico* models are frequently used to identify potential P-gp substrates, the impact of P-gp on oral drug bioavailability has been predicted with varying levels of success.

In addition to broad substrate recognition, the effects of P-gp on oral bioavailability are largely due to the expression profile of P-gp along the GI tract. P-gp is expressed in the luminal membrane (apical) of the small and large intestines where it plays a central role in the export of drug from epithelial cells such as enterocytes. Gradually increased from the proximal to distal regions of the human intestine, P-gp expression is heterogeneous along the GI tract, as mRNA levels measured in colon have been shown to be approximately sixfold higher than levels in the duodenum [91]. The significance of heterogeneous expression levels of intestinal P-gp and the effect on oral bioavailability was considered in an exploratory study with CsA. In human volunteers, drug absorption from different regions of the GI tract was studied by infusion of CsA into specific regions of the GI tract. Plasma concentrations of CsA revealed an absorbance profile of CsA with a rank order (stomach $>$ jejunum/ileum $>$ colon) that was consistent with changes in P-gp expression level (stomach $<$ jejunum/ileum $<$ colon) [92]. Differential expression of P-gp due to polymorphisms in *ABCB1* could be a further source of variation across individuals; however, the specific role of individual *ABCB1* polymorphisms in drug PK continues to be an area of active research [90].

A mechanistic role for P-gp in the absorption of some drugs has been assessed in mice deficient in *mdr1a/b* genes. Using the genetic mouse model, differences in oral

TABLE 10.4 The Role of Selected Human Transporter Proteins Implicated in the Bioavailability of Orally Administered Drugs

Drug	Modulator	Effect	References
P-glycoprotein (ABCB1)			
Digoxin	Talinolol	AUC_{po} (0–6 h) increased by 23%, due to P-gp inhibition	74
Docetaxel	OC144-093	Bioavailability increased from 8% without to 26% with OC144-093 cotreatment, due to P-gp inhibition	75
	Cyclosporin A	Absolute bioavailability was increased from 8% without to 90% with cyclosporine A cotreatment, due to P-gp and CYP3A4 inhibition	76
	Ritonavir	$AUC_{po}/AUC_{iv} = 1.31–1.61$ range: PO docetaxel (100 mg) + ritonavir (100 mg) compared with IV docetaxel (100 mg) alone	77
Paclitaxel	Cyclosporin A	Bioavailability was increased from 4% without to 28% with CsA cotreatment, due to P-gp and CYP3A inhibition	78
	GF120918	Bioavailability was increased to 30% with GF120918 cotreatment, due to P-gp inhibition	79
Talinolol	Carbamazepine	Bioavailability decreased from 62% without to 53% with CBZ cotreatment, due to P-gp induction	80
	SJW	Bioavailability decreased from 52% without to 39% with SJW, due to P-gp induction	81
	Digoxin	Digoxin had no effect on talinolol bioavailability; contrary to the observed decrease in digoxin exposure due to talinolol inhibition (see above)	74
BCRP (ABCG2)			
Topotecan	GF120918	Bioavailability increased from 40% without to 97% with GF120918 cotreatment, due to BCRP inhibition	82
Sulfasalazine	BCRP polymorphism	Sulfasalazine AUCpo was 2.4-fold greater in subjects with ABCG2 (34GG/421CA) variant than subjects with (34GG/421CC) genotype	83

OATP1A2 (SLCO1A2)			
Fexofenadine	GFJ	Bioavailability decreased by 42% with GFJ compared to water, due to OATP1A2 uptake inhibition	84
OATP2A1 (SLCO2A1)			
Aliskiren	GFJ	AUC_{po} exposure was decreased by 61% with GFJ, due to enteric OATP2B1 uptake inhibition	85
OATP1B1 (SLCO1B1)			
Atorvastatin	Rifampin	AUC_{po} exposure of atorvastatin acid was increased by 6.8-fold with rifampin IV infusion, due to hepatic OATP1B1 uptake inhibition. Single rifampin dose unlikely to induce CYP3A4	86
Atorvastatin	SLCO1B1 polymorphism	Mean atorvastatin AUC_{po} was 1.44-fold (144%) greater in subjects with the *SLCO1B1* c.521CC genotype than in those with the c.521TT genotype	87
Simvastatin	SLCO1B1 polymorphism	Mean simvastatin AUC_{po} was 2.21-fold (221%) greater in subjects with the *SLCO1B1* c.521CC genotype than in those with the c.521TT genotype	88
OATP1B3 (SLCO1B3)			
MPAG	SLCO1B3 polymorphism	MPA enterohepatic cycling was decreased in patients with the OATP1B3 (334G-669A) haplotype, due to reduction in MPAG hepatic uptake	89

Abbreviations: GFJ, grapefruit juice; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide; SJW, Saint John's wart.

absorption of the cytotoxic agent paclitaxel confirmed the importance of P-gp recognition and efflux [93]. The contribution of P-gp to oral paclitaxel PK was implicated by an observed increase in absolute bioavailability from 11% in wild-type mice to 35% in genetically modified mice. These predictions made from the mouse model were later corroborated in humans by demonstrating increased oral paclitaxel exposures on coadministration of transport inhibitors selective for P-gp. Consistent with the effect of P-gp efflux to limit oral absorption, the bioavailability of paclitaxel was approximately eightfold higher when dosed in combination with the P-gp inhibitor CsA than after oral paclitaxel alone [78]. Although CsA also inhibits metabolism by CYP3A4, the effect of CsA coadministration was attributed to an increase in paclitaxel absorption due to inhibition of P-gp efflux. This conclusion was supported by findings from a subsequent study in which a change in paclitaxel bioavailability of similar magnitude was observed on coadministration of the P-gp inhibitor GF120918 with paclitaxel [79]. Clinical interaction studies have also implicated P-gp efflux as a mechanism to limit the absorbed fraction of other oral agents such as digoxin, talinolol, and docetaxel, as shown in Table 10.4.

Breast cancer resistance protein (BCRP; encoded by *ABCG2*) is also expressed at the apical membrane of the small intestine. Similar to P-gp, BCRP may facilitate secretion of drugs such as the topoisomerase inhibitor topotecan back into the gut lumen, thereby reducing the absorbed fraction of an oral dose [82]. The modulating effects on drug absorption by uptake of SLCO transporters such as OATP1A2 and OATP2B1 expressed in the human small intestine have also been demonstrated. Inhibition of OATP1A2 uptake of fexofenadine by oral grapefruit juice was associated with a decrease in fexofenadine bioavailability [86]. Because fexofenadine is not metabolized by CYP3A, it was concluded that inhibition of OATP1A2 by grapefruit juice led to an observed decrease in fexofenadine oral bioavailability. Similarly, the effects of oral grapefruit juice leading to a 61% decrease in the oral AUC exposure of the antihypertensive agent aliskiren were attributed to inhibition of OATP2B1 uptake in the gut [85]. Although aliskiren is metabolized by CYP3A4, the net effect of concomitant grapefruit juice was to reduce exposure presumably due to inhibition of OATP2B1 uptake (Table 10.4).

10.4.2 Intestinal Extraction

10.4.2.1 Approximation of F_g. Drug extraction across the intestinal wall may also contribute to incomplete bioavailability after oral dose administration. The presystemic removal of drug on transfer from the GI tract to the systemic circulation is referred to as *first-pass drug elimination* [94]. Although the liver is generally assumed to be the major site of presystemic metabolism, the importance of first-pass intestinal extraction after oral dosing has been acknowledged for an increasing number of therapeutic agents. However, the contributions to first pass from intestinal extraction and hepatic extraction are not easily differentiated in clinical studies and therefore direct estimates of F_g are not always obtained experimentally. Instead, as described in the raloxifene example above, F_g is frequently determined algebraically from estimates of F_a, F_h, and F by Equation 10.8.

Experimental estimates of human first-pass intestinal metabolism have been obtained occasionally, generally following more invasive techniques. The relative contributions of intestine and liver to first-pass metabolism of cyclosporine and midazolam were

shown directly in anhepatic patients during liver transplant procedures [95,96]. In the study with midazolam, intestinal extraction (i.e., $1 - F_g$) was determined from arterial and hepatic portal venous concentrations of 1′-hydroxymidazolam and compared after IV and intraduodenal infusions of midazolam. The intestinal extraction of midazolam by the interduodenal route (0.43) was more than fivefold greater than that by the IV route (0.08), implicating a role for the small intestine in midazolam first-pass elimination. Nifedipine extraction by the intestinal mucosa has also been evaluated in patients with a portocaval anastomosis as well as in healthy volunteers using a multilumen perfusion catheter [97,98].

More recently, oral grapefruit juice has been used in clinical studies to implicate the role of the intestine in first-pass elimination of several drugs metabolized by CYP3A. Grapefruit juice contains furanocoumarin constituents known to irreversibly inhibit CYP3A in the gut mucosa [99,100]. A study in human volunteers with grapefruit juice demonstrated that the oral bioavailability of felodipine, a calcium channel blocker, was limited by intestinal first-pass elimination [101]. Relative to coadministration of water, chronic oral grapefruit juice consumption was associated with a 3.1-fold increase in felodipine AUC exposure. The observed increase in felodipine bioavailability was presumed to result from inhibition of intestinal CYP3A4, as a lack of effect on hepatic CYP3A4 activity by grapefruit juice was determined by the IV erythromycin breath test. Grapefruit juice interaction studies have indicated that intestinal metabolism may also contribute to the first-pass elimination of saquinavir [102] and atorvastatin [103]. A representative group of drugs that undergo presystemic elimination and the proportion attributed to intestinal first-pass metabolism is shown in Table 10.5.

10.4.2.2 Intestinal Metabolism. Drug-metabolizing enzymes expressed in human intestinal epithelial cells create a barrier to the oral bioavailability of several highly metabolized drugs. Consequently, drugs may be subjected to phase I biotransformation reactions as well as phase II conjugation reactions on absorption from the GI lumen. Among the enzyme activities present in the gut mucosa that are frequently involved in the metabolism of drugs are various members of the UDP glucuronosyltransferase (UGT), sulfotransferase (SULT), and CYP enzyme families.

Although multiple CYP enzymes expressed in the intestinal mucosa may contribute to presystemic drug metabolism, CYP3A is the most abundant in human intestine. On average, CYP3A (CYP3A4 and CYP3A5) accounts for ~80% of total immunoquantified CYPs in the proximal small intestine, with the expression of CYP3A5 being highly variable across individuals. The second most abundant CYP enzyme in the human intestine is CYP2C9, accounting for ~15% of intestinal CYPs [110]. In addition to the differential expression of various CYP enzymes, their heterogeneous distribution within the GI tract may also affect the bioavailability of some drugs. CYP3A is localized in epithelial cells, enterocytes, which largely compose the mucosal lining of the gut. Microsomal CYP3A content and catalytic activities vary along the length of the human intestine, generally highest in the duodenum, and decline progressively toward the distal region of the GI tract.

Conjugation reactions within the intestine may also contribute to first-pass metabolism and subsequent detoxification of many xenobiotics as well as endogenous compounds. The bioavailabilities of drugs such as raloxifene [111] and the antidiabetic agent troglitazone [112] are limited by glucuronidation that occurs as a result first-pass gut metabolism. Similar to liver, multiple human UGT enzymes are expressed in

TABLE 10.5 Clinical Interaction Studies of Representative Drugs That Undergo First-Pass Metabolism by Human CYP3A

Drug	Modulator	Effect	References
First-Pass Metabolism			
Atorvastatin $F_g = 0.24$[a]	GFJ	Atorvastatin acid AUCpo increased by 2.5-fold with GFJ, due to gut CYP3A4 inhibition	103
Cyclosporin A $F_g = 0.41$	Ketoconazole	Bioavailability increased from 22% without to 56% with ketoconazole, due to gut and hepatic CYP3A4 inhibition	104
	Rifampin	Rifampin interaction showed CsA hepatic extraction (0.08) was much lower than intestinal extraction (0.60)	104
Felodipine $F_g = 0.45$[a]	GFJ	AUCpo increased by 3.1-fold with GFJ, due to gut CYP3A4 inhibition	101
	Erythromycin and GFJ	AUCpo was increased by 1.9 with GFJ (gut CYP3A4) and by 2.5-fold with erythromycin (gut and liver CYP3A4)	105
Midzolam $F_g = 0.57$	IV/PO	Mean oral bioavailability was 30% (range 12–50%) with complete absorption and $F_h \sim 0.56$	106
Nilotinib $F_g > F_h$	GFJ	AUCpo exposure was increased by 29% with GFJ, due to gut CYP3A4 inhibition	107
	Ketoconazole	AUC increased by threefold with ketoconazole treatment, due to CYP3A4 inhibition	108
	Rifampin	AUC decreased by 80% with rifampin treatment, due to CYP3A4 induction	108
Tacrolimus $F_g = 0.14$[a]	Ketoconazole	Bioavailability increased from 14% without to 30% with ketoconazole, due to gut and hepatic CYP3A4 inhibition	104
	Rifampin	AUC decreased twofold from 14% without to 7% with rifampin treatment, due to CYP3A4 induction	104
Saquinavir $F_g = 0.18$[a]	GFJ	Bioavailability increased from 0.7% without to 1.4% with GFJ, due to gut CYP3A4 inhibition	102

Abbreviations: GFJ, grapefruit juice.
[a] *Source*: Ref. 109.

the gut. Notably, the expression of two additional UGT enzymes, UGT1A8 and UGT1A10, is limited to the human small intestine [113,114].

Sulfate conjugation in the gut mucosa also constitutes a pathway of presystemic metabolism for endogenous compounds and drug substances. SULT expression levels in the small intestine are characterized largely by SULT1B1 and SULT1A3 [115,116]. For the adrenergic agonists albuterol, isoproterenol, and terbutaline, intestinal first-pass metabolism by sulfoconjugation is known to limit the bioavailability of each. Although these drugs are administered by inhalation to the lungs, a significant proportion of the dose is swallowed and subjected to gut first-pass metabolism. In the case of albuterol, enantioselective metabolism of the pharmacologically active (R) antipode indicated preferential bioavailabilty of the (S)-enantiomer from the intestinal tract [117]. In contrast to the higher expression levels of drug-metabolizing enzymes located in the liver, intestinal levels of SULT enzymes have been shown to exceed those of the liver [115].

10.4.2.3 Enzyme–Transporter Interplay. The efficiency of the human GI tract to limit the bioavailability of drug to the systemic circulation is due to a combination of factors that include GI physiology as well as the physicochemical attributes of drug substances. For many drugs, the extent of first-pass metabolism by CYP3A in the intestine is an important determinant of bioavailability. The relatively high expression level of intestinal CYP3A, ~80% of the total intestinal CYP content, localized at the epithelial layer of the gut mucosa make the proximal region of the small intestine well suited as a barrier to drug bioavailability. Additionally, the efflux transport protein P-gp functions to limit drug absorption from the gut lumen. Although CYP3A and P-gp are localized at the intestinal epithelium, the relative levels of each protein are differentially expressed in opposing direction along the GI tract. The expression pattern of CYP3A, highest in the proximal intestine and decreasing toward the distal regions, is complimentary to the progressive increase in P-gp levels toward the distal end of the GI tract.

In addition to the physiology of the human GI tract, bioavailability is also a function of the physicochemical properties of individual drug substances [118,119]. Although the active sites of CYP3A and P-gp may accommodate molecules that vary widely in size and chemical structure, substrates of these proteins share greater similarities with respect to the characteristics of hydrophobicity and permeability. Relative similarities in hydrophobic nature and membrane permeability that confer enzyme and transporter protein recognition are highlighted by the fact that most CYP3A substrates are also substrates or inhibitors of P-gp [120,121]. Moreover, the coordinate regulation of both CYP3A and P-gp by a single orphan nuclear receptor SXR has also been reported [122,123]. Taken together, the common physicochemical properties leading to overlapping substrate specificities and induction mechanism, plus coexpression in the epithelial layer of the gut mucosa with complimentary distributions along the GI tract, have led to a hypothesis that these proteins work together to coordinate an absorption barrier to drug transfer [104,124].

The relative importance of intestinal first-pass metabolism as a determinant of the bioavailability of some drugs has not been consistently predicted by CYP enzyme levels in human liver and intestine. Although estimates of total hepatic CYP content, ~25, 000 nmol [125], far exceed estimates of enteric CYP levels, estimated at 78 nmol [126], intestinal metabolism has been found to account for a large proportion of the first-pass elimination of CYP3A substrates such as CsA [104,127] and midazolam

[96]. The importance of intestinal extraction has been explained in part by potential differences in unbound drug concentration within the gut lumen and portal bloodstream that could account for restricted access to hepatic enzymes due to plasma protein binding. Additionally, the functional interplay between intestinal CYP3A and P-gp has also been proposed to explain efficiencies of the gut mucosa to limit oral bioavailability of some drug substances [104,124,128]. CYP3A4, like all CYP enzymes, has a finite capacity to metabolize drugs. Following oral tablet dissolution and solubilization, high intraluminal concentrations of drug available for absorption could lead to CYP3A saturation and increased uptake of intact drug. However, the enteric localization of P-gp with CYP3A suggests that drug efflux to the lumen could provide a mechanism by which intracellular drug concentrations are maintained below levels that would otherwise saturate metabolism.

A simplified scheme depicting the coordinated interaction between CYP3A and P-gp in the human intestine is shown in Fig. 10.3. In this simplistic model, intact parent drug (P) absorbed into the enterocyte is presented with three available outcomes—(a) transcellular diffusion into the portal circulation as intact drug, (b) biotransformation to metabolite (M) with subsequent diffusion to portal blood, and (c) extrusion by P-gp out of the enterocyte and returned to the intestinal lumen. Effluxed drug (P′) in the lumen may be reabsorbed by passive diffusion and the cycle begins anew. As luminal concentrations of drug are decreased along the GI tract, increasing proportions of absorbed drug are available for metabolism. In effect, the repeated cycle of absorption and efflux maintains low intracellular drug concentrations and thereby P-gp may control access of drug to intestinal CYP3A enzymes.

10.4.3 Hepatic Extraction and Uptake

10.4.3.1 Approximation of F_h. After incomplete dose absorption and first-pass metabolism within the gut mucosa, additional drug loss by hepatic extraction may further limit oral bioavailability. Once the absorbed fraction of parent drug enters the portal venous circulation, elimination by hepatic metabolism may occur on first passage through the liver. In clinical studies, the determination of drug clearance following

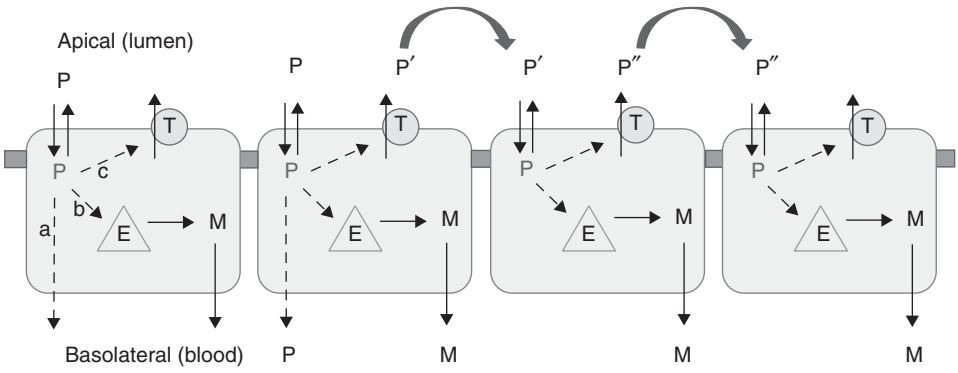

Figure 10.3 Proposed barrier to oral drug bioavailability involving P-glycoprotein and CYP3A localized at the intestinal epithelial cell layer. E, CYP3A4; M, metabolite; P, intact parent drug; P′, effluxed parent drug; T, P-glycoprotein transporter. *Source*: Adapted from Ref. 124. (See color insert.)

IV administration can provide a measure of hepatic availability. An estimate of F_h is provided by determining hepatic extraction (E_h) as the proportion of hepatic plasma flow ($Q_{h,pl}$) represented by systemic drug clearance after IV dosing, according to the following expression [15]:

$$F_h = 1 - E_h = 1 - \frac{CL}{Q_{h,pl}} \tag{10.11}$$

in which $Q_{h,pl}$ is calculated from the product of liver blood flow and (1 - hematocrit), and the estimate of liver blood flow calculated from the product of body weight and 0.0216 L/min/kg [106]. Assuming that drug loss due to metabolism in the intestinal epithelium is negligible after IV dose administration, hepatic availability can be estimated by Equation 10.11. Although the estimation of F_h may be influenced by clearance within the intestine following IV dosing, it is generally assumed to be a minor contributing factor. For instance, when measured in patients during the anhepatic phase of liver transplantation, midazolam extraction across the gut mucosa was ~8% following IV dosing [96]. However, an assumption of negligible enteric metabolism after IV dosing could lead to incorrect estimates of F_h and consequently the role of gut extraction in limiting oral bioavailability might be underestimated [129].

Many of the drug-metabolizing enzymes localized in the gut mucosa which contribute to first-pass drug elimination are also expressed in the liver at much higher levels. Metabolism by CYP3A4, the most abundant human P450 enzyme, contributes to intestinal as well as hepatic first-pass elimination of several drugs. Although CYP3A4 metabolism may occur in each tissue, the proportion of oral bioavailability limited by either intestinal metabolism or hepatic metabolism varies for different drugs. For instance, intestinal first-pass metabolism by CYP3A4 is more significant for drugs such as the immunosuppressive agents tacrolimus and cyclosporine and the protease inhibitor saquinavir ($F_g < F_h$). Conversely, hepatic first-pass metabolism predominates for the lipid-lowering agent simvastatin and calcium channel blocker nifedipine ($F_g > F_h$) [109].

Contributions to hepatic first-pass extraction by the polymorphic CYP2D6 have also been shown by comparing drug disposition in extensive and poor metabolizer individuals. For instance, the antitussive agent dextromethorphan is rapidly absorbed and undergoes first-pass metabolism catalyzed largely by CYP2D6 with a lesser contribution by CYP3A4. The bioavailability of oral dextromethorphan has been shown to range from ~1% to 2% in extensive metabolizers to ~80% in poor metabolizers, indicating the importance of CYP2D6 in dextromethorphan first-pass metabolism [130]. Similarly, for the antimuscarinic agent tolterodine and the *N*-methyl-D-aspartate (NMDA) receptor antagonist traxoprodil, determinants of oral bioavailability included the CYP2D6 phenotype of individual subjects. Tolterodine exhibits first-pass metabolism, and oral exposures in CYP2D6 poor metabolizer individuals were ~30-fold higher than in extensive metabolizers [131]. Traxoprodil also undergoes hepatic first-pass metabolism by CYP2D6 and absolute oral bioavailability was ~80% in poor metabolizer subjects. Oral bioavailability appeared to be linear and independent of dose in poor metabolizer subjects, in contrast to extensive metabolizers in which oral bioavailability appeared nonlinear and dose dependent [132].

Limitations in oral drug bioavailability due to hepatic first-pass extraction may also occur for substrates of membrane transport proteins. The effects of hepatic uptake

transporters on oral bioavailability can be exemplified by the role of OATP1B1 in statin disposition. Although all statins are substrates for OATP1B1, polymorphisms in *SLCO1B1* expression have been associated with differential effects on the bioavailabilities of individual statins. For some statins, such as simvastatin, pitavastatin, and atorvastatin, variability in systemic exposure has been related to *SLCO1B1* genotype [87,88]. However, for other statins, such as fluvastain, oral bioavailability appears to be independent of OATP1B1 genetics [133].

10.5 SUMMARY

The therapeutic use of a given drug is determined by factors associated with its pharmacological effect as well as safety. Inherent to both efficacy and safety is drug bioavailability, the proportion of dose to reach the systemic circulation, or site of pharmacological activity. For orally administered drugs, bioavailability may be limited by malabsorption from the GI tract as well as presystemic elimination by the gut mucosa and liver. Although the barriers to oral bioavailability have historically been considered as individual limitations, experimental research increasingly suggests that drug-metabolizing enzymes and transporters may provide a coordinated barrier to the portal circulation [124,134].

Estimation of bioavailability may be confounded by differences in drug PK, as low oral bioavailability has been associated with greater variability across patient populations [17]. Greater variability in PK can also affect bioequivalence testing as larger study groups are often required to provide sufficient statistical power to differentiate drug formulations. Although bioavailability studies frequently employ a crossover design within the same group of subjects in order to minimize PK variability between individuals, the use of isotopically labeled drug as well as microdosing approaches may also improve bioavailability estimation [22]. Moreover, accounting for sources of variability in enzyme as well as transporter expression and function may also improve the estimation of drug bioavailability.

While bioavailability studies relate systemic exposure to drug absorption and help understand mechanisms underlying drug distribution, transport, and metabolism, comparative bioavailability studies are conducted to evaluate the performance of one or more drugs or drug products against a previously defined reference. The bioequivalence of test and reference formulations for both new drug as well as generic drug products permits an assumption of therapeutic equivalence as well as the potential for product substitution.

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