DRUG METABOLISM IN DISEASES

Edited by

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I am very happy to present you with this special topic book titled *Drug Metabolism in Diseases*.

Drug metabolism is essential for both drug therapy and drug safety. In addition to the genetic and environmental factors, diseases and physiological states of patients have profound effects on drug metabolism and disposition. Examples of diseases known to affect drug metabolism include liver and kidney diseases, inflammation and sepsis, cardiovascular diseases, and diabetes. Physiological conditions that can affect drug metabolism exist in pediatric and pregnant populations, and patients in critical care, among others. Understanding the pathophysiologic effect on drug metabolism will help to guide better and safer use of clinical drugs. Armed with the knowledge of disease effects on drug metabolism, the use of drugs can be tailored to specific diseases or physiological conditions, which is highly relevant to personalized medicine and precision medicine. For these reasons, there is a great need for a book in this area. The topics mentioned earlier have been systemically covered in this book.

Another key feature of this book is the mechanistic insights by which diseases and physiological states affect drug metabolism. Examples of the mechanistic insights throughout the chapters include pharmacogenetics, xenobiotic receptors, nuclear receptors, transcriptional regulation of genes encoding drug metabolizing enzymes and transporters, crosstalk between xenobiotic metabolism and endobiotic metabolism, as well as the reciprocal effect of the expression and activity of drug-metabolizing enzymes and transporters on the clinical outcome of diseases. Understanding these mechanistic insights is paramount in harnessing the benefits of the knowledge communicated in this book and to improve the effective and safe use of drugs in the clinic.

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CHAPTER 1

Introduction of Drug Metabolism
and Overview of Disease Effect on Drug Metabolism

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List of Abbreviations

AAG Alpha-1 acid glycoprotein
ADME Absorption, distribution, metabolism, and elimination
AUC Area under the curve
BCRP Breast cancer resistance protein
CHF Chronic heart failure
CL_int Intrinsic clearance
COPD Chronic obstructive pulmonary disease
CYP Cytochrome P450
ER Extraction ratio
FcRn Neonatal Fc-receptor
GIT Gastrointestinal tract
IFN Interferon
IL Interleukin
INTRODUCTION

In today’s contemporary medical arena, medical practitioners have at their disposal numerous drugs to treat acute and chronic diseases. Furthermore, many patients take multiple drugs daily to treat one or more illnesses, in particular, the increasing elderly population. This combination of events naturally leads to an increased number of drug–drug interactions. Although drug–drug interactions are becoming more predictable due to our understanding of the effect drugs can have on drug-metabolizing enzymes (expression and inhibition), our understanding of the effect of disease states on drug metabolism and disposition is less well comprehended. Many diseases are accompanied by one or more physiological and biochemical changes that affect the absorption, distribution, metabolism, and elimination (ADME) of drugs. These physiological/biochemical changes can lead to changes in the clearance, exposure, and distribution of drugs. There are often multiple changes that occur simultaneously during the course of a disease; therefore the overall effect of a disease on the pharmacokinetics of a drug can be complex. In addition, the degree of disease severity will also impact these changes, i.e., increasing severity of disease leading to increased physiological/biochemical changes.

The most significant organs related to absorption, drug metabolism, and elimination include the liver, intestine, and kidneys. Changes in the normal function of these organs due to disease will have the greatest impact on drug exposure and efficacy. For example, celiac disease and changes in drug absorption (Tran et al., 2013) or irregular drug absorption in Parkinson’s disease (Nyholm and Lennernas, 2008). Liver disease has been shown to have an effect on the expression of drug-metabolizing enzymes and elimination of some drugs, as well as significant interindividual variations in drug elimination in the case of nonalcoholic fatty liver disease or cirrhosis (Merrell and Cherrington, 2011; Palatini et al., 2010). Lastly, kidney disease has been shown to have effects beyond the expected reduction in renal clearance with effects on drug-metabolizing enzymes and drug transporters in the kidney and liver (Nolin et al., 2003; Yeung et al., 2014).

In regards to drug interactions, the pharmaceutical industry has concentrated on the ability to predict and eliminate drug–drug interactions, but is less likely to incorporate the effects of various diseases on drug ADME disposition (drug–drug interactions) in the development process. This is particularly true during the drug discovery stage where the emphasis is bringing forward compounds that have appropriate potency to the target, reasonable overall ADME properties, and a satisfactory safety profile. It is not until clinical development that particular attention is given to how a drug may need to be tailored (changes in dose or dosing regimen) when administered while treating certain diseases or concomitant diseases associated with the primary disease.
This chapter begins with a survey of general drug metabolism principles, such as biotransformation reactions, major drug-metabolizing enzymes, drug absorption and elimination, and enzyme expression. For a more comprehensive review of drug metabolism principles, the reader is referred to Parkinson et al. (2013). The remainder of the chapter describes major physiological and biochemical changes that occur in various disease states and the impact they have on the ADME characteristics of drugs. These changes and their effects are illustrated throughout the chapter using pharmacokinetic principles and literature examples from animal and human studies. The majority of the chapter is devoted to drug metabolism and the effects that naturally occurring or inherited diseases can have on drug exposure. However, keeping in mind that metabolism is only one element that controls drug exposure, the remaining portion of the chapter describes how disease can affect the absorption and distribution of drugs, as well as physiological changes like blood flow, and how disease affects the expression of drug transporters.

**OVERVIEW OF DRUG METABOLISM**

Once administered, drugs can be eliminated from the body by one of three major pathways or routes. Polar drugs are often eliminated intact in urine via the kidneys, and both polar and nonpolar drugs can be eliminated directly through bile. Lastly, and most importantly, drugs are most often metabolized by endogenous enzymes and then eliminated in urine or bile. Drug metabolism or drug biotransformation is the process by which xenobiotics are enzymatically modified to make them more readily excretable and eliminate pharmacological activity. In most cases, this involves modifications to the parent drug or addition of functional groups that make the parent drug molecule more hydrophilic and more amenable to elimination. The liver is the major organ where drug metabolism occurs followed in significance by the intestine and kidneys and a variety of additional organs to a lesser degree (e.g., blood, skin, and brain).

Drug-metabolizing enzymes are generally categorized into two classes (phases 1 and 2) based on the type of biotransformation they perform. Phase 1 enzymes are considered oxidative or hydrolytic enzymes. They typically increase the polarity of molecules by the addition of a hydroxyl group (oxidative) or the unmasking of a more polar functionality (hydrolytic), such as the cleavage of an amide or ester bond to expose the free amine or carboxylic acid, respectively (both being more polar than the original amide or ester). Typical phase 1 oxidative enzymes include: cytochrome P450 (CYP) enzymes, flavin monooxidases, monoamine oxidases, alcohol/aldehyde oxidoreductases, and aldehyde/xanthine oxidases. Phase 1 hydrolytic enzymes include: epoxide hydrolases, esterases, and amidases. The CYP family of enzymes is by far the predominant biotransformation pathway or elimination process for the majority of marketed drugs. The second class of drug-metabolizing enzymes is called phase 2 conjugative enzymes. Phase 2 enzymes are responsible for conjugating parent drug molecules or metabolites derived from phase 1 metabolism by enzymatic addition of a polar group. Typical phase 2 enzymes and their conjugates include: UDP-glucuronosyltransferases (UGTs) that conjugate UDP-glucuronic acid, sulfotransferases that conjugate a sulfate group, glutathione S-transferases that conjugate the tripeptide glutathione, N-acyltransferases that conjugate an acetyl group to an amine, and methyltransferases (MTs) that conjugate a methyl group.
group. All of these reactions create larger and more polar metabolites, making them easier for the body to eliminate in bile or urine. A notable exception is the MTs, which generally make the parent drug or metabolite less water soluble (less hydrophilic); fortunately MTs constitute a minor phase 2 pathway for drugs. Of all the phase 2 pathways, the UGT family of enzymes metabolizes the greatest number of drugs and metabolites.

The systemic exposure of the drug depends on its bioavailability. Following an oral dose, the drug moves through the lumen of the gastrointestinal tract (GIT) and is absorbed by the epithelial cells. The fraction that is absorbed from the lumen \( (f_a) \) can be metabolized by the enzymes in the gut wall. The fraction that escapes gut wall metabolism \( (f_g) \) then moves through the portal vein to the liver, where it undergoes first-pass metabolism. The bioavailability of a drug is the product of the fraction absorbed \( (f_a) \), the fraction that escapes gut wall metabolism \( (f_g) \), and the fraction that escapes hepatic clearance \( (f_h) \). Fig. 1.1 describes the processes a drug goes through before it reaches the systemic circulation (drug exposure). Disease states can influence one or more of these processes ultimately influencing the bioavailability of the drug and these will be discussed throughout the chapter.

**FIGURE 1.1** Overview of factors affecting oral bioavailability. Bioavailability depends on the fraction of dose absorbed \( (f_a) \), the fraction that escapes gut wall metabolism \( (f_g) \), and hepatic metabolism. The inset illustrates the route a drug may take once in the liver: from blood to hepatocyte, back to blood or into bile (either as parent drug or metabolite).
Changes to the expression (or activity) of these phase 1 and 2 enzymes, in particular the CYPs and UGTs, due to disease can have a significant impact on the rate of metabolism and systemic clearance of many drugs. Hepatic or liver clearance (CL\textsubscript{h}) is related to liver blood flow (Q\textsubscript{h}) and intrinsic clearance (CL\textsubscript{int}) as shown in Eq. (1.1).

\[
CL\textsubscript{h} = Q\textsubscript{h} \left( \frac{CL\textsubscript{int}}{Q\textsubscript{h} + CL\textsubscript{int}} \right)
\]

CL\textsubscript{int} represents the ability of the liver to clear unbound drug from the blood when there is no flow limitation demonstrating the inherent enzyme activity of the drug-metabolizing enzyme. Any increase or decrease in the CL\textsubscript{int} of an enzyme results in a commensurate change in the hepatic clearance of a drug that is significantly metabolized by that particular enzyme.

The regulation or expression of drug-metabolizing enzymes is controlled by two main mechanisms. The most common mechanism of enzyme expression is mediated by nuclear receptors or transcription factors, such as the aryl hydrocarbon receptor, pregnane X receptor, or constitutive androstane receptor (Xie, 2009). Combined, these three transcription factors control the normal expression of most drug-metabolizing enzymes and drug transporters. These receptors can be activated through binding of drugs to the receptor leading to an increase in the expression of drug-metabolizing enzymes (increased CL\textsubscript{int} and CL\textsubscript{h}). The second less common mechanism of enzyme regulation is stabilization of the mRNA or protein (Koop and Tierney, 1990). By decreasing the degradation of mRNA or protein (with no change in expression level), the pool of active enzyme accumulates and can be responsible for enhanced metabolism and greater elimination of drug.

Most often, diseases cause suppression of expression of drug-metabolizing enzymes (Gandhi et al., 2012; Shah and Smith, 2015). The suppression of CYPs is predominately attributed to decreases in transcription; however, it can also be due to decreased translation or posttranslational modification of CYPs (Riddick et al., 2004). This suppression leads to a reduction in enzyme expression and pool of active enzyme (decreased CL\textsubscript{int} and CL\textsubscript{h}), leading to decreases in drug elimination. Cytokines (small proteins associated with cell signaling) have been shown to suppress CYP expression and enzyme activity in human hepatocyte cultures, and the effect has been shown to be gene specific. For example, interleukin 1 (IL-1) has been shown to downregulate CYP2C8 and CYP3A4 mRNA expression by 75–95%, with no effect on CYP2C9 or CYP2C19 (Aitken and Morgan, 2007). Additional studies have shown that an increase in IL-6 concentration results in suppression of CYP3A4 in primary human hepatocytes (Dickmann et al., 2011).

**DISEASE EFFECTS ON DRUG METABOLISM**

Although aging is not considered a disease, studies on the liver changes that occur with age do provide insight into how diseases may affect the liver and its ability to metabolize drugs. As we age, our ability to metabolize drugs decreases; this is thought to be due to several physiological changes that occur in the liver, such as an ~40% decrease in liver volume, an ~40% decrease in liver blood flow, and a decline in the expression of CYP enzymes (Tajiri and Shimizu, 2013). Not surprisingly, acute or chronic diseases of the liver
Liver Disease

Liver cirrhosis is associated with several different effects that can influence drug exposure. A common phenomenon associated with liver cirrhosis is portal-systemic shunting, a physiological change that diverts blood drained from the intestine to the systemic circulation consequently bypassing its normal route to the liver. This shunting of blood away from the liver has the effect of increasing the bioavailability of orally administered drug as they do not enter the liver to be metabolized. In addition, a lack of oxygen (oxygen is an obligatory cofactor for all CYP enzymes) could lead to decreased enzyme function (Verbeeck, 2008). It has also been shown that CYP levels (reduced activity) in patients suffering from cirrhosis change with the severity of the disease. In the early stages of the disease, CYP3A4 and CYP2C19 are affected, whereas in the later stages of the disease CYPs 1A2, 2D6, and 2E1 are also affected (Verbeeck, 2008). The effects of liver cirrhosis appear to have a greater impact on the CYP enzymes than on phase 2 enzymes, such as glucuronidation, although in severe states of cirrhosis, glucuronidation is also affected.

Hypoxia is a deficiency in the amount of oxygen reaching tissues. Hypoxia due to cardiorespiratory disease has been shown to affect the metabolism and exposure of several drugs. The elimination of theophylline (CYP1A2 substrate) and tolbutamide (CYP2C substrate) was shown to change in patients with hypoxia. The elimination of theophylline was decreased, whereas the elimination of tolbutamide was increased (du Souich and Fradette, 2011). The clearance of both drugs is blood flow independent, so the changes in elimination are likely due to changes in mRNA expression or enzyme activity of CYP1A2 and CYP2C.

Influence of Infection and Inflammation

Infection and inflammation are known to have effects on the hepatic and extrahepatic metabolism of drugs, and many chronic diseases are often associated with inflammation (Gandhi et al., 2012; Morgan, 2001, 2009; Shah and Smith, 2015). Woolfes and Borzelleca (1966) demonstrated that inflammation led to a decrease in drug metabolism activity in mice. After a decade, it was shown that infection along with inflammation led to an increase in quinine exposure (due to reduced metabolism) in patients suffering from malaria (Trenholme et al., 1976). Inflammation is the result of a tissue’s attempt to adapt to cellular stress by releasing inflammatory cytokines IL-1β, IL-6, tumor necrosis factor alpha (TNF-α), and interferons (IFNs) α/γ, all of which are thought to affect (suppress) the expression of CYP enzymes (Feghali and Wright, 1997).

In patients with rheumatoid arthritis, chronic inflammation results in downregulation of CYP3A4 activity. In clinical studies with tocilizumab, an anti-IL-6 antibody approved for use in rheumatoid arthritis, an increase in the CYP3A4 function was observed by way of increased clearance of simvastatin, a substrate of CYP3A4 (Schmitt et al., 2011). Infusion with the anti-IL-6 antibody results in restoring the CYP3A4 levels to normal levels, leading to a significant decrease in simvastatin exposure [fourfold decrease in the area under the curve...
(AUC), 1 week post dose] and a twofold increase in simvastatin clearance. Interestingly, there was no change in the pharmacokinetics of other concomitant medication, such as omeprazole (CYP2C19 substrate) or dextromethorphan (CYP2D6 substrate) (Evers et al., 2013).

Cancer is another disease associated with inflammation and inflammation-associated cytokines. Patients with cancer often exhibit higher levels of the cytokines IL-6 and TNF-α than normal healthy individuals (Filella et al., 1996). These elevated levels of cytokines are likely to downregulate the expression and enzyme activity of CYP3A4, which will affect the pharmacokinetics of many drugs, including anticancer drugs such as docetaxel, erlotinib, and vemurafenib.

Immunotherapy is a rapidly advancing field for the treatment of cancer. Ipilimumab is an anti-CTLA-4 (cytotoxic T-lymphocyte associated protein 4) antibody that has been approved for use in advanced melanoma. Inhibition of the CTLA-4 protein results in upregulation of T-cell activity and proliferation. Nivolumab is an anti-PD1 antibody, approved for use in non-small cell lung cancer and melanoma. PD1 is an immune checkpoint that dampens the immune response to prevent damage to tissues. Production of TNF-α, IL-6, and interferon γ is increased following anti-CTLA-4 and anti-PD-1 treatment (Curran et al., 2010; Dulos et al., 2012). In addition to the inflammation already inherent to cancer, these immunomodulators also have the potential to further impair CYP-mediated metabolism (Harvey and Morgan, 2014).

A study by Dostalek et al. found that the activity and expression level of CYP3A4 in patients with diabetes mellitus are significantly reduced. Human liver microsomes isolated from diabetic livers were shown to have decreased expression and activity of CYP3A4, which corresponds with observations from animal models of diabetes (Dostalek et al., 2011; Wang et al., 2007). The downregulation of CYP3A4 was attributed to elevated levels of IL-6 and TNF-α found in diabetic patients (Morgan, 1997).

**Chronic Kidney Disease**

The kidney contributes to the elimination and metabolism of a wide variety of drugs; it is highly distinct from other organs of metabolism since it has specific regions of cellular activity. The CYP enzymes are found in the renal tubular epithelial cells of the renal cortex. Studies in animal models of renal failure show a decrease in protein expression and activity of CYP2C11 and CYP3A2 (Leblond et al., 2002). Rat hepatocytes treated with uremic serum from patients with advanced chronic renal failure led to a 35% reduction in the protein and mRNA expression of CYP2C6, 2C11, 3A1, and 3A2, as well as a reduction in CYP enzyme activity (Dreisbach and Lertora, 2008). As in Eq. (1.1), hepatic clearance depends on intrinsic clearance, as well as drug-free fraction [Eq. (1.4)]. A decrease in CYP expression results in a decrease in intrinsic clearance, thus increasing the bioavailability. In renal failure, along with a decrease in enzyme expression, there is also a decrease in serum albumin levels, increasing the free fraction of the drug. The relative magnitude of changes in free fraction and intrinsic clearance will determine the net effect on the bioavailability. Moreover, phase 2 drug-metabolizing enzymes are also affected in patients with kidney disease as observed by the increase in morphine exposure (due to reduced glucuronidation of morphine) in patients as compared with control subjects (Osborne et al., 1993).
Disease Effects and Genetic Disorders

Some genetic disorders can lead to physiological/biochemical changes that may have profound effects on endogenous components, as well as drugs. Crigler–Najjar syndrome and Gilbert syndrome are disorders affecting the metabolism of bilirubin. In both Gilbert syndrome and Crigler–Najjar syndrome, activity of the phase 2 enzyme UGT1A1 is diminished or absent. Therefore drugs that are substrates of UGT1A1 cannot be metabolized/eliminated and can cause potential toxicity. A good example of this is irinotecan, an anticancer prodrug that is converted to its active form SN-38, which has a narrow therapeutic index. SN-38 is mainly inactivated by UGT1A1. The lack of the UGT1A1 enzyme leads to toxicity in oncology patients treated with irinotecan (Cecchin et al., 2009). Ethinylestradiol is also a substrate for UGT1A1 (Ebner et al., 1993). Therefore women using ethinylestradiol as a component of an oral contraceptive and who have Gilbert or Crigler–Najjar syndrome are at increased risk for hyperbilirubinemia and/or estrogen-related adverse effects. Glucuronidation and the impact of these genetic disorders have been reviewed in detail by Strassburg (2010) and de Wildt et al. (1999).

DISEASE EFFECTS ON ABSORPTION PARAMETERS

Absorption of drugs takes place by passive diffusion, paracellular transport, or carrier-mediated transport. Paracellular transport (i.e., movement of compounds through fenestrations in the membrane) depends on the size of the molecule (<500 g/mole) and largely occurs with water-soluble compounds that cannot move through cells. Active transport (carrier-mediated transport) is carried out by transporters that may exist on the luminal or abluminal side of cell membranes. Additional discussion of drug transporters and disease effects are described later in the chapter.

Changes in Permeability

A major mechanism of drug absorption through the GIT as well as transport through membranes is by passive diffusion. The rate of diffusion/transport in turn depends on the concentration gradient of the solute across the membrane, the surface area available for absorption, and the permeability of the compound as described by Eq. (1.2).

Rate of transport = Permeability × Surface area × Concentration gradient  

The permeability of a drug depends on its intrinsic properties such as size, lipophilicity, and charge. Small, lipophilic, and unionized molecules are able to cross membranes more easily than larger hydrophilic molecules, such as peptides and proteins. Permeability also depends on the thickness of the membrane. Patients with irritable bowel syndrome typically have damaged, thinner intestinal mucosa, leading to increased gut permeability (Spiller et al., 2000). In addition to changes in permeability, delayed gastric emptying observed in patients with irritable bowel syndrome is likely to change the rate and extent of absorption of drugs.

The distribution of compounds can also be influenced by permeability into tissues. The blood–brain barrier is vital in protecting the brain from xenobiotics. The endothelial cells
in the brain capillaries have tight junctions as well as glial processes that limit the permeability of most polar drugs. In addition, a range of efflux transporters are expressed on the luminal side of brain capillaries and actively pump compounds out of the brain. Growth of certain brain tumors can cause changes in the vasculature of tumor microvessels leading to development of fenestrations in the endothelial layer (Loscher and Potschka, 2005). Preclinical tumor models have demonstrated that the permeability of certain drugs such as methotrexate is enhanced when tumors are present, allowing more drugs to reach the brain (Groothuis, 2000).

**Changes in Gut and Lung Surface Area**

Patients with celiac disease show atrophy of the villi in the small intestine, decreasing the surface area available for absorption. A study in patients with hypothyroidism showed that patients with celiac disease required higher doses of levothyroxine than patients without celiac disease (Tran et al., 2013). Interestingly, patients who were treated for celiac disease, who would presumably have repaired/improved small intestines over time, required lower doses of levothyroxine, supporting the hypothesis that the small intestine atrophy limited the absorption of levothyroxine.

The pulmonary route of administration is useful because absorption through the lungs can bypass first-pass metabolism in the liver. In addition, the lungs are highly vascularized and have a high surface area, allowing for efficient absorption. Several anesthetics and other drugs, such as nicotine, antibiotics, and protein therapeutics are administered via the lungs, resulting in good systemic exposure. Pulmonary disorders that result in airflow obstruction or airway inflammation due to asthma or chronic obstructive pulmonary disease (COPD) could potentially alter the extent of systemic absorption. However, in the case of inhaled corticosteroids, such as fluticasone, it has been shown that lowered systemic absorption of the steroids in patients with COPD and asthma actually reduces undesired suppression of the adrenal and pituitary glands as compared with healthy volunteers (Singh et al., 2003). In this situation, the limited systemic absorption is beneficial as it decreases drug exposure to the adrenal and pituitary glands thereby decreasing the undesired side effects.

**Changes in Gastric Emptying Time**

Gastric emptying and intestinal transit time determine the available time that a drug is in contact with the absorptive surfaces in the GIT. According to the pH-partition hypothesis, only unionized drugs can cross cellular membranes. Even weakly acidic drugs, more likely to be unionized in the stomach, are mostly absorbed from the small intestine due to its larger surface area. For example, for a weakly acidic drug such as paracetamol, the rate of absorption is directly related to the rate of gastric emptying; faster absorption is observed in subjects with faster gastric emptying (Prescott, 1974). On the other hand, the absorption of drugs that have slow dissolution characteristics could be enhanced by a decrease in gastric motility and increased intestinal transit time. The following examples help illustrate how these changes affect the absorption of drugs.

The treatment of Parkinson’s disease depends on oral administration of the dopamine precursor levodopa. Levodopa is primarily absorbed from the small intestine; therefore
emptying is the rate-limiting step for onset of activity. Irregular gastric emptying time is common in patients with Parkinson’s disease. Delays in gastric emptying cause the levodopa to be metabolized by amino acid decarboxylase enzyme in the gastric mucosa, decreasing the fraction absorbed by the gut \( F_g \), thus decreasing the bioavailability and limiting efficacy (Nyholm and Lennernas, 2008). Patients with celiac disease often exhibit faster gastric emptying than normal subjects. Using aspirin as a test substrate, Parsons et al. (1977) found that the time to reach maximal plasma concentration \( T_{\text{max}} \) is shorter in patients with celiac disease than in normal subjects.

Absorption of drugs also depends on their dissolution profile. Ketoconazole is an antifungal agent administered orally for the treatment of oral thrush. The oral absorption of ketoconazole is decreased in patients with AIDS, who exhibit achlorhydria, or decreased secretion of gastric acid (Chin et al., 1995). Ketoconazole is practically insoluble in water, except at a pH below 3. The decrease in gastric secretions due to achlorhydria or ingestion of food results in a stomach pH greater than 3, limiting the solubility of ketoconazole, resulting in incomplete absorption (Mannisto et al., 1982).

**CHANGES IN BLOOD FLOW TO ORGANS**

In all tissues perfused with blood, the vascular system acts as a transportation system delivering and removing substances. If the movement of a drug through a membrane (permeability) occurs readily, then the perfusion of the tissue is the rate-limiting step for drug distribution. The net rate of movement of drug in tissue (extravasation) is the difference between the rate of entry and the rate of exit of the drug and can be described by Eq. (1.3).

\[
\text{Rate of extravasation} = Q \times (C_A - C_V)
\]

where \( Q \) is the blood flow, \( C_A \) is the arterial blood concentration, and \( C_V \) is the concentration in venous blood. Therefore the rate of blood flow can determine the extent to which a drug can distribute into tissues and decrease systemic exposure.

Perfusion of metabolizing organs, like the liver, also drives the clearance of a drug, as described by the well-stirred model of hepatic metabolism, Eq. (1.4).

\[
CL_h = Q_h \times ER = Q_h \times \left( \frac{f_u \times CL_{\text{int}}}{Q_h + f_u \times CL_{\text{int}}} \right)
\]

where \( Q_h \) is the hepatic blood flow and \( CL_{\text{int}} \) is the intrinsic clearance. Therefore hepatic clearance/elimination depends on several factors, such as blood flow \( (Q_h) \), enzyme activity \( (CL_{\text{int}}) \), and protein binding \( (f_u) \). ER is the hepatic extraction ratio, a measure of the organ’s relative efficiency in eliminating drug from blood. For example, an ER of 0.8 would be considered high as 80% of the drug is cleared from the blood as it passes through the liver.

Chronic heart failure (CHF) is associated with hypoperfusion (decreased \( Q_h \)) to the sites of drug clearance leading to decreased clearance of drugs. Decreased perfusion of tissues can also lead to a decrease in volume of distribution. This decrease in volume of
distribution can be up to 40% of normal values, necessitating a decrease in loading doses (Woosley et al., 1986). In this situation, the half-life of a drug remains nearly unchanged due to the simultaneous decreases in volume and clearance. As described in Eq. (1.5), the half-life \( t_{1/2} \) of a drug is directly proportional to the volume of distribution \( (V) \) and inversely proportional to its clearance \( (CL) \).

\[
t_{1/2} = \frac{0.693 \times V}{CL} \tag{1.5}
\]

Therefore a simultaneous decrease in both the volume of distribution and clearance will minimize any changes in half-life.

Stenson et al. reported that patients with low cardiac output and therefore low hepatic blood flow exhibited higher exposures of lidocaine. They also observed that patients with CHF were more likely to have incidences of lidocaine toxicity and recommended that doses could be lowered while still maintaining therapeutic concentrations (Stenson et al., 1971). Quinidine, an antiarrhythmic, is another drug that shows altered kinetics in patients with CHF. The decreased perfusion of blood limits the absorption of quinidine, when administered orally, possibly due to the decreased blood flow to the intestine. The volume of distribution and the clearance of quinidine are also decreased, leading to no change in the elimination half-life (Crouthamel, 1975). Thus diminished blood perfusion to absorption sites, such as the GIT and muscle (intramuscular route of administration), can result in altered absorption of drugs.

As described previously, in liver cirrhosis, a fraction of the blood in the portal vein does not come in contact with the hepatocytes, thus decreasing the hepatic blood flow. This shunting of liver blood flow decreases the hepatic blood flow by 20−40% of normal (Moreno et al., 1967). As seen in Eq. (1.4), the hepatic clearance of a drug is directly proportional to the blood flow to the liver as well as intrinsic clearance. For a drug with high ER, a decrease in blood flow can decrease hepatic clearance, thus improving bioavailability. On the other hand, for drugs with low ERs, the clearance depends more on the intrinsic clearance \( (CL_{int}) \) and free fraction \( (f_u) \), than on blood flow. Therefore a change in blood flow should not greatly affect the hepatic clearance of low-ER drugs. However, in liver disease, both blood flow to the liver and enzyme activity are decreased, and it is not possible to distinguish between the influences of these two physiological changes, highlighting the complexity of effects that can occur even within a single disease.

### CHANGES IN PROTEIN BINDING

The distribution of drugs within the body depends on its reversible binding to blood cells, plasma proteins, and tissues. The fraction of drug not bound to plasma proteins is available to distribute to tissues or sites of action. The protein–drug complex acts as a transport system to carry drugs to their site of action. This form of transport is particularly important for drugs with poor solubility. Protein-bound drug is also unavailable for clearance by hepatic/renal metabolism as only the free (unbound) drug is available for clearance. Therefore protein binding is an important factor that influences the distribution and clearance of drugs.
Small Molecules

The majority of small-molecule drugs bind to serum albumin, alpha-1 acid glycoprotein (AAG), lipoproteins, or erythrocytes. Albumin is the most abundant protein in human serum with a concentration of 35–50 g/L and a long half-life of about 20 days. Albumin binds to acidic drugs as well as endogenous compounds such as bile acids. AAG is another plasma protein that is present in the plasma but at much lower concentrations than albumin. Due to its acidic nature, AAG is much more likely to bind to basic drugs. The concentration of AAG in plasma is highly variable in healthy as well as diseased individuals. Increases in AAG concentration can result from chronic renal failure, inflammatory diseases, trauma, cancer, and acute myocardial infarction, but reduced in liver cirrhosis (Edwards et al., 1982; Paxton and Briant, 1984).

A decrease in plasma binding could result in an increase in the volume of distribution of certain drugs. In patients with cirrhosis of the liver, serum albumin concentrations are lowered; this can potentially increase the free fraction of drugs. Unbound drug is available to cross into tissues; therefore an increase in free fraction will increase the tissue distribution of a drug. Increased tissue distribution will in turn result in lower plasma concentrations, leading to a higher apparent volume of distribution. For example, the apparent volume of distribution for cefodizime is three times larger in patients with cirrhosis than in healthy individuals (el Touny et al., 1992). Also, patients with ascites (accumulation of fluid in the peritoneal cavity) have a significant increase in volume of distribution of some drugs due to the additional fluid possibly requiring the use of larger loading doses (Verbeeck, 2008).

A good example of the influence of protein binding on clearance is naproxen. Naproxen is a low-ER drug with high plasma binding, and thus has a very small volume of distribution (0.15 L/kg). The plasma binding of naproxen is decreased in patients with alcoholic cirrhosis, resulting in unbound free fractions that are two- to fourfold higher than in healthy subjects (Williams et al., 1984). However, there is no large change in the volume of distribution. This is because for drugs with a small volume of distribution, a large change in the unbound fraction will not result in major changes in the volume of distribution. Williams et al. (1984) did not find a difference in the total clearance of naproxen; however, the clearance of unbound drug (CL × f_u) was decreased by about 60% in patients with cirrhosis.

Hypoalbuminemia can result from burns, malnutrition, or cirrhosis of the liver and patients with chronic kidney disease show decreased serum albumin levels (Liumbruno et al., 2009; Klammt et al., 2012). In patients with renal failure, the unbound fraction of cerivastatin is increased but the total exposure is more than doubled due to the decrease in hepatic uptake of the statin by organic anion transporter proteins (OATPs) (Vormfelde et al., 1999). This example once again illustrates the complex nature (i.e., multiple simultaneous changes) of pharmacokinetic modifications that occur in disease states sometimes leading to unpredictable changes.

Protein Therapeutics

Therapeutic proteins are extensively used in the treatment of cancer, HIV, and other diseases. Monoclonal antibodies, IFNs, and cytokines are examples of some of the macromolecular therapeutic proteins. Proteins are not good substrates for CYP enzymes and are generally
cleared by renal filtration or degraded to smaller peptides or amino acids in several tissues by circulating phagocytic cells or by their target antigen-containing cells (Keizer et al., 2010). In addition, antibodies and endogenous immunoglobulins can sometimes be protected from degradation by binding to protective receptors [the neonatal Fc-receptor (FcRn)], which explains their long elimination half-lives (up to 4 weeks).

FcRn recycles both albumin and IgG thereby circumventing each from being degraded and extending their half-life (Sand et al., 2014). The fusion of a therapeutic protein with albumin enhances the half-life of the therapeutic protein by taking advantage of the recycling of human albumin by FcRn receptors. A deficiency in FcRn receptors due to a mutation (β2-microglobulin gene) can result in decreased plasma concentrations of IgG and albumin (Wani et al., 2006). This disorder is known as familial hypercatabolic hypoproteinemia, and the hypercatabolism (high clearance) of IgG and albumin can result in lower concentrations of albumin fusion proteins or therapeutic antibodies (Kim et al., 2007). Disorders such as this are likely to influence the pharmacokinetics of therapeutic antibodies and albumin-fused therapeutic proteins.

DISEASE EFFECTS ON TRANSPORTER EXPRESSION

ATP-binding cassette transporters (ABC transporters) play a major role in the maintenance of nutrient uptake and elimination of waste products, energy generation, and cell signaling. The normal function of some human ABC transporters is to secrete cytotoxic compounds (dietary cytotoxics and therapeutic drugs) from cells. These transporters [P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein (MRP) 1] are highly expressed in the gut, liver, and kidneys, where they restrict the bioavailability of administered drugs. P-gp and BCRP in particular are also expressed in the epithelia of sensitive tissues (e.g., the brain and placenta) and in stem cells, where they perform a barrier function.

The transporters in the kidney are located in the plasma membranes of epithelial cells of the proximal tubules and actively facilitate the movement of drug into the cells from the plasma against the membrane negative charge. The drug transporters are broadly divided into organic anion transporters, organic cation transporters, and ATP-dependent active transporters, e.g., P-gp. The renal and nonrenal clearances of many drugs are significantly reduced in patients with chronic renal failure. Animal models of chronic renal failure indicate that renal failure modulates the efflux transporter, Mrp2 in the liver and kidneys with an increase of 70–200% in protein and mRNA expression (Laouari et al., 2001). In contrast, a decrease in the expression and function of intestinal P-gp was found in a rat model of renal failure (Veau et al., 2001).

Inflammation modulates both the activity and expression of the CYP isozymes and transporters. For example, cytokines have been shown to decrease the mRNA expression of CYP 3A4 and increase the expression of MDRI A1 in a Caco2 cell model (Bertilsson et al., 2001). In a study by Ufer et al., P-gp mRNA and protein expression was decreased in patients with ulcerative colitis as compared with healthy volunteers. Expression of BCRP and P-gp in the intestinal tissue is reduced in patients with ulcerative colitis (Ufer et al., 2009). The effect of inflammatory mediators on expression of P-gp is not entirely understood and results from
different studies seem to vary. In a study by Bauer et al. (2007), a time-dependent change in P-gp activity postexposure to TNF-$\alpha$ was observed in capillary preparations from rat brain. They observed a transient reduction in activity, followed by a sustained increase. A similar observation was made by Seelbach et al. (2007) using a carrageenan-induced inflammatory pain model in rats. In a rat model of lipopolysaccharide-induced inflammation, the authors found that the expression of Mdr1a mRNA in the brain and liver was decreased. In addition to this, the authors also found a loss of CYP3A and Oatp2 mRNA in the liver (Goralski et al., 2003). Downregulation of P-gp and BCRP in the intestinal tissue may increase the exposure of orally administered substrates of P-gp and BCRP. Decreased expression of these transporters in the brain may increase the distribution of transporter substrates across the blood–brain barrier leading to higher CNS exposures. However, there have been no reports that directly connect the change in transporter function in disease states with changes in the pharmacokinetics of drugs. Dubin–Johnson and Rotor syndromes are two genetic disorders that cause an increase in conjugated bilirubin. Dubin–Johnson syndrome is caused by a defect in the multiple drug-resistance protein 2 gene (ABCC2/MRP2), whereas Rotor syndrome is due to mutations in $SLCO1B1$ (OATP1B1) and $SLCO1B3$ (OATP1B3) transporters (Strassburg, 2010). OATP1B1 and OATP1B3 are localized on the basolateral membrane of hepatocytes and mediate the uptake of drugs from the portal vein into hepatocytes. Deletion or decreased expression of ABC transporters and OATPs may affect distribution of substrates into various tissues and lead to increased GaN toxicity. Individuals with mutations in these genes could be more susceptible to toxicity of drugs and metabolites. For example, patients with a mutation in the $SLCO1B1$ gene exhibited higher concentrations (increased AUC) of repaglinide than those with the wild-type genotype (Kalliokoski et al., 2008).

**SUMMARY**

Table 1.1 provides an overview of the possible changes to pharmacokinetic parameters when changes in physiology, absorption, protein binding, or enzyme activity occur. As the table shows, a change in the extent of absorption should not influence the clearance and volume of distribution of a drug. However, the overall exposure of the drug could be affected. The clearance of a drug depends on the blood flow to the metabolizing organ ($Q$), intrinsic clearance ($Cl_{in}$), and drug free fraction ($f_u$) [Eq. (1.4)]. Therefore a change in blood flow or free fraction can affect drugs with high ER differently than drugs with low ER. The clearance of high-ER drugs is perfusion (blood flow) limited. A reduction in blood flow to organs of metabolism can affect drugs with a high ER by decreasing the clearance and extending the half-life, whereas the clearance of low-extraction drugs may not be affected. An increase in the free fraction of a drug can result in a higher volume of distribution and an increased half-life for high-ER drugs. For low-ER drugs, the increase in free fraction may increase both the clearance and the volume of distribution, minimizing any change in the half-life. Finally, changes in the level of enzymatic activity are more likely to influence the clearance of low-ER drugs compared with high-ER drugs.

As this chapter outlines, there are many physiological changes that take place in various disease states. Therefore the prediction of altered pharmacokinetics in the patient population
is not a trivial task. Fig. 1.2 outlines and illustrates the many ways in which different diseases can influence the pharmacokinetics of drugs.

A significant challenge in predicting appropriate dose adjustments due to physiological/biochemical changes are the multiple stages or degrees of severity a disease may have over the time course of disease progression or between patients. The time-dependent changes and spectrum of severity that occur within a disease state make it challenging to anticipate dose adjustments for an entire patient population. There are, however, tools that the pharmaceutical scientist can utilize to predict the pharmacokinetics of drugs in the clinic. First and foremost, one should understand the route of metabolism, route of elimination, protein binding, and physicochemical properties of the drug in the preclinical and clinical settings. Physiologically based pharmacokinetic (PBPK) models can then be built and the parameters that are altered in the diseased population (e.g., blood flow or enzyme activity) can be entered into the PBPK model to predict changes that may occur in the diseased state. Simcyp (Certara, Princeton, NJ, USA) is one such modeling software that can allow the user to predict pharmacokinetic profiles of drugs in the diseased state.

Although the pharmaceutical industry has not specifically focused on the effects of disease states on the metabolism and disposition of developing drugs until clinical development, this approach is changing. An earlier awareness of how disease can affect drug disposition is now recognized as a key to faster and more successful development of safe and efficacious drugs. As such, the physiological and biochemical changes that occur with major diseases are being evaluated in greater depth to bring about a better understanding of how these changes can ultimately affect drug metabolism and disposition.

### TABLE 1.1 Effect on Pharmacokinetic Parameters of Drugs due to Possible Changes in Physiology in Disease State for Drugs Eliminated by the Liver Administered Intravenously

<table>
<thead>
<tr>
<th>Pharmacokinetic Changes</th>
<th>ER Status</th>
<th>Clearance</th>
<th>Volume of Distribution</th>
<th>Half-Life</th>
<th>Area Under the Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased absorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Increased absorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Decreased blood flow to liver</td>
<td>High ER</td>
<td>↓</td>
<td></td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Low ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased fraction unbound in blood</td>
<td>High ER</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low ER</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased enzyme activity</td>
<td>High ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low ER</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Increased enzyme activity</td>
<td>High ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low ER</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

↓, decreased; ↑, increased; ↔, little or no change; ER, extraction ratio.
References


