21 ADME of Cardiovascular Drugs

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21.1 SUMMARY OF CARDIOVASCULAR DRUG METABOLISM

Effective drug therapy for cardiovascular diseases (CVDs) is determined by systemic exposure of the therapeutic agent and specificity for the target. Drug concentration at the target site is dependent on the distributional characteristics of the drug (e.g., membrane permeability and unbound fraction) and by its concentration in blood or plasma. Since the systemic exposure of a drug is usually directly related to maximize desired effect and minimize undesired effects, the factors that influence the concentration of the agent over time determine the success of the therapy. Accordingly, those factors that control clearance and elimination of the drug ultimately control safety and effectiveness.

Clearance of most cardiovascular drugs is mediated by one or more enzymatic or transport processes. Isoforms of cytochrome P450 (CYP) make up the most prevalent oxidative or the so-called phase I clearance pathway. Some cardiovascular (CV) drugs are metabolized by primary or secondary conjugation, forming glucuronides, sulfates,

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or other conjugates, the so-called phase II pathway. A few drugs, notably angiotensin-converting enzyme (ACE) inhibitors are metabolized by hydrolysis pathways. Although the human CYP superfamily is composed of more than 50 members, CV drugs interact primarily with CYP3A4, 2D6, or 2C9. Given that CYP3A4 is the most highly expressed CYP in the liver and has the largest active site, the association with CV drug metabolism is not surprising. However, liver CYP2D6 abundance is relatively low (about 2% of total) and the active site more restricted, yet it is the primary clearance mechanism for a number of CV drugs.

The most notable CV adverse events have stemmed from drug interactions due to changes in the enzymatic clearance process. Enzymatic processes are subject to saturation, inhibition, induction, and genetic polymorphism, each of which can change systemic exposure and therapeutic efficacy. In the case of the thienopyridine platelet inhibitors, CYP2C19 directly controls effectiveness by virtue of the activation pathway for generation of the active thiols. Other oxidative or hydrolytic enzymes also control drug clearance, particularly in the case of ACE inhibitors.

The enzymatic and transporter processes that control drug clearance can be capacity-limited processes, subject to variation in activity or expression due to competing substrates or regulation by intrinsic and external factors. Each of these attributes has the potential to influence effectiveness of cardiovascular drugs as well as cardiotoxic effects of noncardiovascular therapeutics.

21.2 DRUG-METABOLIZING ENZYMES

Drug-metabolizing enzymes (DMEs) play an important role in cardiovascular health and disease. These enzymes, especially CYP, control the clearance of many small-molecule cardiovascular drugs and are involved in the metabolism of endogenous substances (such as leukotrienes, steroids, and bile acid) that are in turn implicated in several cardiovascular-related pathways, including inflammation, lipid metabolism, and blood pressure regulation. In general, drugs can be metabolized by oxidation, reduction, hydrolysis, or conjugation, generally accepted pathways for excretion of drugs from the body. The enzymes involved in metabolism are present in many tissues but are most concentrated in the liver. These enzymes are divided into two groups. Phase I metabolizing enzymes, including CYP450s and flavin monooxygenases (FMOs), catalyze the introduction of an oxygen atom into substrate molecules often resulting in hydroxylation, dealkylation, and hydrolysis. Phase II reactions involve conjugation with an endogenous or exogenous substance. Phase II conjugating enzymes include the UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), *N*-acetyltransferases (NATs), and glutathione transferases (GSTs). Metabolites formed from either or both processes are more polar and more readily excreted by renal and/or hepatobiliary processes. Among these enzymes, CYPs often play a critical role in the disposition of cardiovascular drugs.

21.3 CYTOCHROME P450

CYP is a superfamily of hemoprotein found in human liver and other tissues such as small intestine that play an important role in the metabolism of exogenous as well

TABLE 21.1 Cardiovascular Drugs as CYP Substrates

Substrates	CYP3A4/5	CYP2C19	CYP2C8	CYP2C9	CYP2D6	CYP1A2
Lipid lowing agents						
Simvastatin	×	—	×	—	—	—
Lovastatin	×	—	—	—	—	—
Atorvastatin	×	—	×	—	—	—
Fluvastatin	×	—	×	×	—	—
Pravastatin	×	—	—	—	—	—
Rosuvastatin	×	×	—	×	—	—
Pitavastatin	—	—	—	×	—	—
Gemfibrozil	—	—	×	—	—	—
Diuretics						
Torsemide	—	—	×	×	—	—
Antithrombotics						
Warfarin	—	—	—	×	—	×
Antiarrhythmics						
Quinidine	×	—	—	×	—	—
Procainamide	—	—	—	—	×	—
Lidocaine	×	—	—	—	—	×
Propafenone	×	—	—	—	×	×
Carvedilol	—	—	—	—	×	
Propranolol	—	—	—	—	×	×
Metoprolol	—	—	—	—	×	
Amiodarone	×	—	×	—	—	—
Debrisoquine	—	—	—	—	×	—
Flecainide	—	—	—	—	×	—
Mexiletine	—	—	—	—	×	—
Verapamil	×	—	—	—	—	×
Diltiazem	×	—	—	—	—	—
Antiplatelet						
Clopidogrel	×	×	—	×	—	×

Data were collected from drug interaction database from the University of Washington.

as endogenous molecules. CYP enzymes are so named because they absorb light at a wavelength of 450 nm when carbon monoxide binds to the enzymes at their reduced state. There are more than 50 CYP enzymes in human, although many of these enzymes have the capacity to metabolize drugs, the majority of CYP-mediated drug metabolism in human is catalyzed by subfamily CYP3A, 2C, 2D6, and 1A enzymes (Table 21.1).

21.3.1 Cytochrome P450 3A Subfamily

CYP3A is the most highly expressed isoform in human, representing ∼30% of the spectroscopically detectable CYP in the liver [1] and have an important role in the oxidative, peroxidative, and reductive metabolism of endogenous steroids, many procarcinogens, and at least 50% of all drugs. The most abundant CYP3A isoform expressed in liver and gut is CYP3A4 [2]. Interindividual expression of CYP3A4 can vary by 50-fold among individuals and functionality (i.e., drug clearance) by about 20-fold. High interindividual variation can be associated with genetic and environmental factors [3].

Despite extensive efforts to identify genetic variation within the CYP3A4 gene associated with altered enzyme activity, multiple groups have failed to identify common CYP3A4 genotypes that can explain variable CYP3A4 expression [4,5]. Although the substrate specificity of CYP3A5 is similar to CYP3A4, it is thought to be less important for drug metabolism because it is expressed at only 10–30% of CYP3A4 levels. A unique characteristic of CYP3A is the autoactivation or heteroactivation, which is associated with the increased catalytic activity of a particular substrate by the addition of another xenobiotic or same substrate. This positive cooperativity or activation of CYP3A occurs with midazolam by α-naphthoflavone, alprazolam, flunitrazepam, and triazolam by testosterone and so on [6]. The clinical significance of activation is not clear, complicating the interpretation of *in vitro* studies and extrapolation to human pharmacokinetics (PK).

CYP3A4 is often implicated in drug–drug interactions (DDIs) as a result of inhibition or induction by drugs or dietary components, sometimes leading to adverse drug reactions. Azole antifungal agents, ketoconazole, itraconazole, and fluconazole are potent competitive CYP3A4 inhibitors with K_i values below 1 μM. Macrolides such as erythromycin, and troleandomycin and antidepressant such as fluoxetine and nortriptyline are CYP3A inhibitors acting via the formation of metabolite–intermediate complexes. HIV protease inhibitors such as ritonavir and 17-α-ethinyl-substituted steroids have been found to be mechanism-based CYP3A4 inhibitors. Rifampin, carbamazepine, phenytoin, and phenobarbital are reported as potent CYP3A inducers. Terfenadine, a well-known early example of drug interactions, was approved in the United States in 1985 for the treatment of seasonal allergic rhinitis. The drug is primarily eliminated by CYP3A4-mediated metabolism. Coadministration with potent CYP3A4 inhibitors such as ketoconazole and erythromycin markedly elevated terfenadine plasma level to produce serous cardiac arrhythmias leading to death in some instances [7]. Terfenadine was removed from the market when fexofenadine, the active metabolite of terfenadine, was approved and marketed. Fexofenadine does not block cardiac potassium channels. Prolonged QT interval and syncope were also reported for nonsedating antihistamine, astemizole, when coadministered with ketoconazole, itraconazole, or erythromycin [8]. Nevertheless, most clinically significant drug interactions result agents with a narrow therapeutic index coadministered potent inhibitors or inducers. More modest interactions are generally of lesser concern since the consequences are within the window of normal variability.

21.3.2 Cytochrome P450 2D6 Subfamily

CYP2D6 accounts for about 2% of total hepatic CYP but is responsible for about 25% of the metabolism of known drugs, including many of those used in the treatment of CVD. Genetic variants of CYP2D6, which result in varying levels of metabolic activity, are of clinical importance in some settings. Currently, more than 75 allelic variants have been identified; all variant alleles are presented at the home page of the human CYP allele nomenclature committee (http://www.imm.ki.se/cypalleles/cyp2d6.htm). The variant CYP2D6 alleles result from point mutations, deletion, or duplication of the entire gene and result in an abolished, decreased, normal, increased, or qualitatively altered catalytic activity. Establishing which phenotype an individual has often depends on the administration of a probe drug that is a substrate of CYP2D6 (e.g., dextromethorphan, debrisoquine, or sparteine) and measuring the metabolite–parent

ratio. However, there are some limitations for estimating *in vivo* enzyme activity using urinary metabolite to parent ratio [9]. For dextromethorphan, there is not a linear correlation between enzyme activity and urinary parent/metabolite ratio, probably a result of pH dependence and renal or hepatic transport. Dextromethorphan is also a substrate of CYP3A. In addition, cost and time effectiveness of urine analysis have precluded widespread, routine phenotyping determinations. As a practical alternative, genotyping of the CYP2D6 gene is increasingly used in clinical practice to assess the risk of undesired drug effects or therapeutic failure for individual patient and eventually to apply a dose adjustment or change in therapeutic strategy. Considering the growing number of CYP2D6 alleles and corresponding ranges of activity, prediction of a subject's phenotype based on their genotype is a significant challenge. Currently, standard process of translating genotype data into a phenotype prediction is not established. Several different genotype–phenotype systems for CYP2D6 have been proposed, including the CYP2D6 "activity score" (AS) system [9,10]. In this system, over 25 CYP2D6 allelic variants were genotyped in 672 subjects of Caucasian and African-American heritage. For each variant CYP2D6 allele, a value relative to the fully functional CYP2D6*1 reference allele is assigned based on published literature on *in vivo* and *in vitro* activity toward commonly used probe drug, such as dextromethorphan (DM), debrisoquine, or sparteine. The AS of a genotype is the sum of values assigned to each allele with the assumption of both alleles contributing equally to overall CYP2D6 activity. A total 95 genotypes are categorized into six AS groups. A final model (AS-model A) is generated to relate AS groups to CYP2D6 activity based on urinary metabolite data (DM/dextrorphan ratios). The authors also emphasize that the probability of belonging to a particular phenotype group using AS-model A may be more accurately predicted if ethnicity is considered. Although there are some limitations of using dextromethorphan urinary ratios to estimate *in vivo* enzyme activity, AS system is a simplified tool for translating vast genotype data into CYP2D6 activity prediction.

It is important to establish a more quantitative system to precisely predict CYP2D6 activity *in vivo* and transform this information to dose adjustment for individual patient for drugs that are primarily metabolized by CYP2D6, such as the antiarrhythmics flecainide and propafenone or β-blocker metoprolol.

21.3.3 The Cytochrome P450 2C Subfamily

The CYP2C subfamily of P450 enzymes metabolize ~20% of clinically used drugs; CYP2C8, 2C9, and 2C19 are of clinical importance. Next to CYP3A4, CYP2C9 is the most predominantly expressed CYP enzyme in the human liver and the most highly expressed of the 2C subfamily. It metabolizes many clinically important drugs, including the diabetic agent tolbutamide, the anticonvulsant phenytoin, the *S*-enantiomer of the anticoagulant warfarin, D1-tetrahydrocannabinol, and numerous anti-inflammatory drugs such as ibuprofen, diclofenac, piroxicam, tenoxicam, and mefenamic acid [11], the antihypertensive losartan [12], the antidiabetic drug glipizide, and the diuretic torsemide [13]. Defective alleles of CYP2C9 [14,15] have the potential to affect the safety of those CYP2C9 drugs with smaller therapeutic indices such as warfarin, phenytoin, and certain antidiabetic drugs. CYP2C9*2 and 2C9*3 are known to affect catalytic function. CYP2C9*2 has a frequency of ~8–10% in Caucasians but has a lower frequency in African-Americans and appears to be virtually absent in Asians

[16,17]. CYP2C9*3 has a frequency of ~6% in Caucasians (the frequency of homozygous CYP2C9*3 is 0.3%); the frequency in Asians is probably 2%. Results from the human studies published to date have concluded that CYP2C9 genotype contributes significantly to the variability in disposition and dose–response of substrates such as warfarin, phenytoin, tolbutamide, glipizide, losartan, candesartan, irbesartan, ibuprofen, flurbiprofen, and celecoxib. Individuals carrying the CYP2C9*2 and *3 alleles also have lower warfarin and phenytoin daily dose requirements and appear more susceptible to adverse events during the initiation of therapy. CYP2C9 genotype-guided dosing may be clinically useful and warrants prospective investigation [18].

CYP2C19 is the principle catalyst for metabolism of several well-known drugs such as omeprazole, diazepam, and mephenytoin; it is also responsible for activation of proguanil to cycloguanil. Genetic variation attributable to CYP2C19 was first described in the 1980s [19], about the same time, the thienopyridine antiplatelet drug ticlopidine was brought to market. Although clopidogrel is the current standard of care for coronary artery disease patients undergoing a percutaneous coronary intervention (PCI), ~25% of the patients experience a subtherapeutic antiplatelet response [20]. Clopidogrel is a prodrug, about 85% of the clopidogrel dose is hydrolyzed by esterase to an inactive metabolite, and the remaining clopidogrel is available for the conversion to the active metabolite via two sequential metabolism-mediated steps [21]. The role of CYP2C19 in its metabolism was unknown until several years after its approval that a report suggested possible polymorphism in CYP2C19 might be a cause of variable pharmacodynamic response [22]. This was confirmed in a number of subsequent reports, thoroughly reviewed by Farid *et al*., pointing to the conclusion that CYP2C19 is the major contributor to 2-oxo-clopidogrel (active metabolite) formation from clopidogrel. CYP2C19 variant allele carriers exhibit a significantly lower capacity to metabolize clopidogrel and inhibit platelet activation and are therefore at higher risk of adverse cardiovascular events. Finally resulting in a US Food and Drug Administration (FDA) recommendation that patients be genotyped for CYP2C19 PM to avoid inadequate platelet inhibition [20].

21.3.4 Cytochrome P450 1A2 Subfamily

CYP1A2 accounts for ~13% of total hepatic CYP expression [1], CYP1A2 shows up to 40-fold variability between individuals and corresponding variability of enzyme activity and drug metabolism. Although there is evidence that genetic factors have major influence on observed enzyme activity [23], the role of polymorphisms identified to date is unclear. Apart from smoking, coadministration of drugs that are inhibitors, inducers, or competing substrates for CYP1A2 can influence CYP1A2 activity [24]. Phenotyping represent a suitable method to shed light on the actual enzyme activity. Caffeine is the most commonly used substance for CYP1A2 phenotyping. The first step in its metabolism is almost exclusively mediated by CYP1A2, and concentrations in biological matrices can be determined using a simple HPLC method. An epidemiological study examining the role of diet and caffeine in cardiovascular health found an association of CYP1A1*1F allele with nonfatal myocardial infarction (MI) [25]. Carriers of *1F allele are "slow metabolizers" of caffeine compared to wild type, probably due to reduced expression of the enzyme. Overall, the involvement of CYP1A2 in cardiovascular drug metabolism is relatively low.

21.3.5 Other Cytochrome P450s and Phase II Enzymes

Other CYP enzymes such as CYP2B6, CYP1A1, UGT, or SULT are generally not critically involved in the metabolism of CV drugs, although it should be noted that 1-*O*-glucuronide of gemfibrozil was found to be a potent mechanism-based CYP2C8 inhibitor. Several reported drug interactions between gemfibrozil and CYP2C8 substrates such as antidiabetic agents rosiglitazone and pioglitazone are probably due to CYP2C8 inhibition by 1-*O*-glucuronide of gemfibrozil [26,27].

21.4 DRUG-METABOLIZING ENZYMES EXPRESSED IN THE CARDIOVASCULAR SYSTEM

Although significant efforts have been focused on hepatic CYP-mediated cardiovascular drug interactions and genetic polymorphism of hepatic CYPs leading to adverse drug events, limited information is available regarding cardiac metabolism [28,29]. Differences in cardiac drug metabolism may also influence pharmacological efficacy or adverse drug effects. For example, lack of efficacy for ACE inhibitors in treating right-ventricular hypertrophy was attributed to metabolic inactivation of drug in the right ventricle but not in the left ventricle [30]. Further, endogenous CYP metabolites, epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acid (HETEs), prostacyclin (PGI2), aldosterone, and sex hormones have pivotal roles in the maintenance of cardiovascular health.

Evidence for expression or at least transcription has been observed for a number of CYPs in cardiac tissue. CYPs identified in mammalian species include CYP1A1, 1B1, 2A1/2, 2A6/7, 2B6/7, 2C8/9, 2D6, 2E1, 2 J2, 3A4, 4A1/2, 4B1, and 4F1 [29].

CYP1A1 mRNA was detected in the right ventricle, pulmonary aorta, ascending aorta, and the left atrium of patients with dilated cardiomyopathy and in the left ventricle of healthy subjects [31]. CYP1A2 mRNA is absent in healthy human heart [32]. CYP1B1 mRNA expression was observed in the normal human heart in relatively high abundance [32]. CYP1A1 mRNA and CYP1B1 are inducible in vascular smooth muscle cells (SMCs) [33].

In humans, CYP2B, 2C, 2D6, 2E1, and 2 J2 were detected in heart tissues [30,34]. CYP2B6/7, 2C8-19, and 2D6 were predominantly expressed in the right ventricle; the unilateral expression of the 2D6 gene in right-ventricular tissue [30], whereas CYP2E1 mRNA was expressed in various parts of the heart, including the right and left atria, right and left ventricle, aorta, and the ventricular septum. CYP2E1 protein was found in the endocardium and coronary vessels of human autopsy samples [35]. CYP2C8, 2C9, and 2 J2 mRNA and protein are present in various amounts in the human heart, coronary artery, and aorta tissues [34]. CYP2J2 mRNA levels in the heart were ~10 times higher than those of CYP2C9 or 2C8. CYP2J2 and 2C8 protein were shown to be predominate CYP expressed in human heart, CYP2J2 is also expressed in blood vessels. Beside higher expression in heart tissue, CYP2J2 is most abundant in small intestine [36,37]. In fact, CYP2J2 was shown to be responsible for the first-pass metabolism of ebastine and astemizole [38]. In a recent screening of CYP2J2 substrates using 139 marketed therapeutic agents, several novel substrates including terfenadine, astemizole, amiodarone, albendazole, danazol, thioridazine, tamoxifen, cyclosporine, nabumetone, and mesoridazine were identified [39]. Interestingly, the substrates identified for CYP2J2

are also metabolized by CYP3A4. Although CYP2J2 active site can accommodate large molecules such as CYP3A4, CYP2J2 metabolism was more restricted to a single site of molecule, whereas CYP3A4 commonly metabolizes compounds at multiple sites of molecules. Little information is available on the expression of CYP3A family in cardiovascular tissues. CYP3A4/5/7 mRNA was not found in human heart tissues [30]. CYP4F3/12 mRNA was detected in the human and dog heart [40,41]. The role and regulation of CYP expression in the heart is not clear but it is unlikely that it plays any meaningful role in drug clearance.

21.5 ROLE OF CYP METABOLITES IN CARDIOVASCULAR HEALTH

Metabolism of eicosanoids in the cardiovascular system is well described and partly dependent on CYPs. There are three major metabolic pathways involved in arachidonic acid metabolism [42]. First, hydroperoxyeicosatetraenoic acids (HPETEs) and dihydroxyeicosatetraenoic acid (DiHETE) are formed by lipoxygenase and subsequently converted to HETEs by peroxidases; in the second pathway, cyclooxygenase catalyzes arachidonic acid to prostaglandin G2, in a subsequent peroxidase reaction, PGG2 undergoes a two-electron reduction to PGH2. PGH2 can be further metabolized to other eicosanoids such as PGI2 and thromboxane A2 (TXA2). Third, CYP metabolizes arachidonic acid to regio- and stereospecific EET and HETEs.

Several CYPs can metabolize arachidonic acid to EETs, including CYP1A, 2B, 2C, 2E, and 2 J subfamilies [43,44]. CYP2J isoforms have been proposed as the predominant enzymes responsible for epoxidation of endogenous arachidonic acid pools in human and rat heart [36,45]. The induction of CYP2C in native porcine coronary artery endothelial cells by β-naphthoflavone enhances the formation of EET, as well as endothelium-derived hyperpolarizing factor (EDHF)-mediated hyperpolarization and relaxation. Transfection of coronary arteries with CYP2C antisense oligonucleotides results in decreased levels of CYP2C and attenuates EDHF-mediated vascular responses, indicating that the EDHF synthase in the porcine coronary vascular bed is a CYP2C isoform. EETs are important components of many intracellular signaling pathways involved in vasodilatory, anti-inflammatory, and cardioprotective responses in cardiovascular system [29].

In addition to generation of the vasorelaxant 11,12-EET, CYP2C9 was shown to be potential major source of reactive oxygen species (ROS) [46]. ROS generated in arteries reacts with NO to produce peroxynitrite ($ONOO^-$) resulting in a reduction of NO bioavailability that impairs endothelium-dependent vascular function in atherosclerosis [47]. Hunter *et al*. recently demonstrated that CYP2C inhibition during the peritransplant period prevented development of cardiac allograft vasculopathy (CAV) by inhibiting early SMC proliferation and intimal hyperplasia. This effect could also be a result of reduced postischemic oxidative damage, contributing to increased NO bioavailability and/or prevention of interferon c (IFN-c) production [48]. Interestingly, it was reported that concomitant pretreatment with 11,12- or 14,15-EET in combination with the free radical scavenger, 2-mercaptopropionyl glycine (2-MPG), completely abolished the cardioprotective effect of 11,12- and 14,15-EETs, suggesting part of the cardioprotective effects of EETs in rat hearts against infarction is the result of an initial burst of ROS and subsequent activation of both the sarcK_{ATP} and mitoK_{ATP} channel [49].

CYP ω-hydroxylases, such as CYP4A and 4F, biosynthesize 20-HETEs [50]. CYP1A1/2, 1B1, and 2E1 were reported to produce different regioisomers of HETE [28]. HET0016 is a potent and fairly selective inhibitor of 20-HETE formation with little effect on the activities of cyclooxygenase and other CYP enzymes [51]. The IC_{50} values of HET0016 for the formation of 20-HETE by human recombinant CYP4F2, 4F3, and 4A11 enzymes are 125, 100, and 42 n*M*, respectively [52]. In addition to CYP inhibitors, antisense of cDNA oligonucelotides (ODNs) has been developed to block the formation of 20-HETE. CYP4A1/4A2/4A3-specific antisense ODNs can inhibit protein expression and the corresponding catalytic activity [53]. The role of HETEs in the control of cardiovascular functions has been reviewed [54]. Altered expression and function of arachidonic acid ω-hydroxylases in models of hypertension, diabetes, inflammation, and pregnancy suggest that 20-HETE may be involved in the pathogenesis of these diseases [50].

21.6 DRUG–DRUG INTERACTIONS

Pharmacokinetic drug interactions can cause excess or insufficient drug exposure leading to, respectively, toxicity or ineffectiveness and in some cases to termination of drug development, severe prescribing restriction, and withdrawal of drugs from the market. The majority of DDIs stem from changes in enzymatic capacity. The existing guidance issued by the FDA covers mainly CYP450-mediated drug interactions, the importance of other mechanisms, such as transporter-based drug interaction, non-CYP-based interaction, and interactions involving therapeutic proteins, have been recognized more recently. Many cardiovascular drugs are metabolized in the liver; CYP3A4, 2D6, and 2C9 are primary enzymes mediating cardiovascular drug interactions. CYP inducers such as phenytoin, barbiturates, rifampin, and St John's wort can induce CYP3A4, 2B6, and 2C8/9 and increase metabolism of susceptible cardiovascular drugs (e.g., some statins, nifedipine, or warfarin), resulting in decreased plasma concentrations and reduced efficacy. On the other hand, systemic exposure of cardiovascular drugs can be increased by potent CYP inhibitors (e.g., erythromycin, grapefruit juice, or omeprazole).

Some cardiovascular drugs are transporter substrates or inhibitors. For example, most statins are hepatic organic anion transporting polypeptide (OATP)1B1/1B3 substrates; cyclosporine causes up to 20-fold increase in area-under-the-curve (AUC) values of statins in organ transplant patient via OATP1B1/1B3 inhibition leading to greater risk of myopathy [55]. Quinidine and verapamil can cause significant increase in digoxin absorption via gut P-glycoprotein (PGP) inhibition [56].

The interaction between clopidogrel, an antiplatelet drug, and omeprazole, a proton pump inhibitor, is illustrated in words from a regulatory agency. "New data show that when clopidogrel and omeprazole are taken together, the effectiveness of clopidogrel is reduced. Patients at risk for heart attacks or strokes who use clopidogrel to prevent blood clots will not get the full effect or this medicine if they are also taking omeprazole." The updated label acknowledges that "concomitant use of drugs that inhibit CYP2C19 (e.g., omeprazole) should be discouraged." The underlying mechanism for this drug interaction is that omeprazole inhibits the CYP2C19, which is the key enzyme for the conversion of clopidogrel into its active form [57]. Other examples of adverse pharmacokinetic drug interaction include increased risk of myopathy with

statins during the coadministration of gemfibrozil via CYP2C8 inhibition [58] and market withdrawals of mibefradil, a calcium channel blocker, because of severe interactions with multiple drugs via inhibition of CYP3A4 and PGP [59]. Detailed drug interactions of major classes of cardiovascular drugs are discussed in later sections.

Of course, noncardiovascular drug interactions may produce significant cardiovascular side effects. Coadministration of CYP3A4 inhibitors (such as ketoconazole, itraconazole, and erythromycin) with terfenadine, methadone, or cisapride have been reported to increase plasma concentration of these drugs and the risk of Torsade de Pointes [56].

21.7 TRANSPORTERS IN CV DRUG METABOLISM

Although more than 400 membrane transporters have been identified in humans, 7 transporters account for much of the apparent drug transport activity in humans: two efflux transporters [PGP and breast cancer resistance protein (BCRP)] and five uptake transporters (OCT2, OAT1, OAT3, OATP1B1, and OAT1B3) [60]. The efflux transporters PGP and BCRP are both members of the ATP-binding cassette (ABC) family and function on the apical or luminal side of the intestinal epithelia, kidney proximal tubules, hepatocytes (canalicular membrane), and blood–brain barrier (capillary endothelial cells). The five uptake transporters are members of the solute carrier (SLC) family. OATP1B1 and OATP1B3 function on the sinusoidal side (blood side) of hepatocytes. OCT2, OAT1, and OAT3 function as uptake transporters on the basolateral side (blood side) of kidney proximal tubule cells and function by selectively pumping substrates from the blood. In this way, transporters serve a protective role by pumping substrates out of the cells. Examination of recent publications on drug transporters shows considerable involvement of cardiovascular drugs [60]. Indeed, the field of membrane transporters has grown in part because of the need to understand the role of uptake and efflux transporters in the disposition of the statin drugs. Of the five major drug transporter families, DDIs with four are characterized primarily by cardiovascular drugs [60]. Although the discovery and understanding of transporters in drug disposition and interactions has lagged that of CYPs, progress in recent years has provided a much more complete understanding of the situation to the point where guidelines for evaluation of DDI has been proposed and the role of transporters in disposition of drugs is expected.

The efflux transporter PGP (MDR1 and ABCB1) appears to serve as a protective mechanism by pumping its substrate back into the lumen of the small intestine, out of the brain, liver, and kidney. It is a member of the ABC family of transporters, characterized by ATP-dependent directional flow. It has broad substrate specificity and plays an important role in limiting the absorption of many drugs through the gut wall and blood–brain barrier. It also facilitates excretion of many drug substrates from the liver and kidney. The cardiac glycoside digoxin is a prototypic inhibitor of PGP.

BCRP is a related ABC transporter expressed in tissues similar to PGP and functioning in similar manner but with different overlapping substrate specificity. Although it might play a role in the disposition of some statins, it appears to have few interactions with cardiac drugs.

The OATP family represents a family of SLC transporters with substrate specificity for amphiphilic compounds. Notable are OATP1B1 and OATP1B3, both of which

appear to be involved in hepatic uptake of statins and might be important in controlling PK and toxicity of some statins.

Two additional transporters that have limited drug substrates but nevertheless important for disposition of ACE inhibitors (e.g., zofenopril and fosinopril) are PEPT1 and PEPT2. Both are members of the SLC family, functioning as uptake transporters on the apical membrane (i.e., luminal side) of intestinal epithelia and kidney proximal tubule cells.

Although some drugs are cleared solely by metabolism involving one or more enzymes and a few are cleared only by transporters, many are cleared by a combination of transporters and DMEs. This can result in vectorial transport of some drugs or complex disposition interactions for others [61].

Bosentan, a dual antagonist of endothelins A and B, is used in treatment of pulmonary artery hypertension. Although its clearance is dependent on the CYP-mediated metabolism, the hepatic uptake via OATP1B1 and 1B3 becomes rate determining in the overall elimination [62].

21.8 BIOMARKERS AND METABOLOMICS

CVD is one of the major causes of human mortality. Methods to minimize CVD risk is a major challenge in the prevention and treatment of CVD. Clinical assessment is the keystone of patient management; however, such evaluation has its limitations [63]. Biomarkers could provide an important approach to understand the spectrum of CVD with applications in at least five areas: screening, diagnosis, prognostication, prediction of disease recurrence, and therapeutic monitoring of CVD [64]. However, close scrutiny of a range of biomarker studies shows shortcomings of some biomarker hopes [65].

The so-called metabolomics is the study of complex metabolite profiles in biological samples, such as urine, plasma, cerebral spinal fluid, or tissue biopsies. Disease changes the concentration and fluxes of small biochemicals that may be captured by metabolite fingerprint in the cells, tissues, and biofluids. In contrast to classical biochemical approaches that often focus on single metabolites or reactions, metabolomics involves the collections of quantitative data on broad series of metabolites in an attempt to gain an overall understanding of metabolism and/or metabolic dynamics associated with disease status and drug action [66]. The metabolome consists of a variety of chemicals with different physicochemical properties such as amino acids, peptide, glucose, lipids, organic acid, nucleotides, ATP, and biogenic amine neurotransmitters. The large numbers of metabolites present in human (over 20,000) and their wide concentration range spreading over nine orders of magnitude make it impossible to achieve with a single analytical method. The two most important tools employed presently in metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) [67–69].

Sabatine *et al*. [70] reported the application of metabolomics to acute myocardial ischemia. Metabolite profiles in plasma were obtained before and after exercise stress from 18 patients who demonstrated inducible ischemia (cases) and 18 of whom did not (control). Lactic acid and metabolites involved in skeletal muscle adenosine monophosphate (AMP) catabolism increased after excise in both cases and controls. Statistically

significant change in six members of the citric acid pathway were found in myocardial ischemic group but remained unchanged in controls.

Metabolomic studies of adipose tissues have been demonstrated to be useful in discovery of new biomarkers related to CVD. Several hormones and factors produced in this tissue have been associated with obesity, insulin resistance, and CVD, particularly, the accumulation of visceral adipose tissue increases the risk of developing metabolic disease and CVD [69]. In addition, alteration in lipid metabolism has been linked to several diseases such as atherosclerosis, diabetes, obesity, and stroke [69,71]. Altered lipoprotein particle composition has been detected by NMR in patients with hypertension [68].

21.9 LIPID-LOWERING AGENTS

21.9.1 Statins

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) are widely used and well-tolerated drugs in the treatment of hypercholesterolemia, lowering the risk of CVD. However, myopathy is a potential adverse effect of these agents. Cerivastatin was withdrawn from world markets as a result of the relatively high rate of fatal rhabdomyolysis that raised concerns on the safety of statins [72]. The mechanism of statin-caused myopathy is unclear; however, it appears to be dose dependent, the risk increases significantly when high dose is used and in particular when statins are prescribed in combination with agents that are also myotoxic or that increase the plasma concentration of statins [73,74]. Statins are highly selective inhibitors of HMG-CoA reductase inhibitors, showing almost no relevant affinity toward other enzymes or receptors [75], making pharmacodynamic interaction less likely. Clinically significant DDIs with statins are thought to result from altered clearance and distribution of statins. It was reported that the rate of myopathy is twofold greater (0.22%) in statin combination therapy with potential inhibitors than statins alone (0.12%) [76].

21.9.1.1 Drug Interaction by CYP Enzymes. Drug-specific interactions with each statin are dependent on metabolic pathway of individual statins (Tables 21.2 and 21.3). Simvastatin and lovastatin are administered as lipophilic lactone prodrugs, whereas other statins are given as active acid forms. Simvastatin, lovastatin, and atorvastatin are predominantly metabolized by CYP3A4 [77–79] and fluvastatin is metabolized primarily by CYP2C9 and to a lesser extent by CYP2C8 and 3A4 [80]. Rosuvastatin shows minimal metabolism, being excreted largely unchanged via the biliary route, and almost no meaningful pharmacokinetic interactions [81,82]. Pravastatin is partly degraded to 3′α-isopravastatin and 6′-epipravastatin in the stomach and partially metabolized by CYP enzymes or excreted as the parent compound into urine and bile [81].

Potent CYP3A inhibitors used with simvastatin or lovastatin significantly increased the exposure of these statins, probably a result of inhibited first-pass metabolism. Strong CYP3A inhibitors, such as itraconazole and ketoconazole, can increase the AUCs of simvastatin and lovastatin up to 20-fold [83–85]; however, only a slight AUC increase was observed with pravastatin, atorvastatin [86,87], rosuvastatin [88], and fluvastatin. Combination of saquinavir with ritonavir as a boosting agent increased AUC of simvastatin acid 30-fold; similarly, the AUC of atorvastatin was increased 3.5-fold [89].

TABLE 21.2 Pharmacokinetic Properties, Metabolizing Enzymes, and Transporters of Statins

Parameter	Simvastatin	Lovastatin	Atorvastatin	Fluvastatin	Pravastatin	Rosuvastatin	Pitavastatin
Lactone prodrug	Yes	Yes	No	No	No	No	No
Lipophilicity	++++	++++	+++	+++	+	++	+++
Absorption (%)	60–85	30	30	98	35	50	80
Bioavailability (%)	<5	5	12	30	18	20	60
Hepatic extraction (%)	≥ 80	≥ 70	70	≥ 70	45	63	—
Protein binding (%)	>95	>98	>98	>98	50	90	96
Half-life (h)	2–5	2–5	7–20	1–3	1–3	20	10–13
Metabolism	Extensive	Extensive	Extensive	Extensive	Mainly unchanged	Mainly unchanged	Limited
Metabolizing enzymes	CYP3A4, CYP2C8*	CYP3A4	CYP3A4, CYP2C8*, UGT*	CYP2C9, CYP3A4*, CYP2C8*	CYP3A4*	CYP2C9, CYP2C19*, CYP3A4*	CYP2C9*
Uptake transporters	OATP1B1	OATP1B	OATP1B1, OATP2B1	OATP1B1*, OATP1B3, OATP2B1	OATP1B1, OATP1B3, OATP2B1	OATP1B1, OATP1B3, OATP2B1	OATP1B1, OATP1B3, OATP2B1
Efflux transporters	PGP, BCRP (lactone)	PGP	PGP, BCRP	BCRP	BCRP, PGP, MRP2	BCRP, MRP2	BCRP*, PGP, MRP2

Source: Adapted from Ref. 55.

TABLE 21.3 Percent AUC Increase in Statins Following Coadministration of CYP and/or Transporter Inhibitors

Inhibitor/inducer	Simvastatin	Lovastatin	Atorvastatin	Fluvastatin	Pravastatin	Rosuvastatin	Pitavastatin
Itraconazole	900	1380, 3540	47, 150	—	48, 71	26, 37	—
Clarithromycin, erythromycin	521, 895	—	32–345	—	111	—	179
Verapamil, diltiazem	99–381	26	—	—	31	—	—
Cyclosporine	155, 696	406	645	89–254	1077, 2183	608	351
Grapefruit	37–1513	91, 1426	—	—	—	—	—
Gemfibrozil, fenofibrate	43	—	24	—	22–102	88	25, 36
PI or PI combination	507	—	74–836	—	90	36–212	31

Data collected from drug interaction database (University of Washington).

Grapefruit juice, an intestinal CYP3A4 inhibitor, can increase lovastatin and simvastatin AUC up to 15-fold and atorvastatin by 3.3-fold but no change for pravastatin. The extent of interaction depends on the amount of grapefruit juice; <1 quart daily consumption appears to have minimal effect [90]. Weak or moderate CYP3A4 inhibitors, such as verapamil, diltiazem, erythromycin, and clarithomycin, increase the AUC of simvastatin acid by about three- to ninefold [87,91]. There are few reports of clinically relevant DDI between fluvastatin and CYP2C9 inhibitors, even potent CYP2C9 inhibitor fluconazole appears to only slightly increase the plasma concentration of fluvastatin [92]. CYP2C8 contributes to the metabolism of simvastatin and lovastatin acid. Gemfibrozil and its glucuronide metabolites are CYP2C8 inhibitors. Gemfibrozil markedly increase the AUCs of the active simvastatin acid and lovastatin acid [93,94]. Generally, induction-mediated statin interaction is less clinically significant than that caused by potent inhibitors. CYP and transporter inducers, such as rifampin and carbamazepine, reduced the AUCs of simvastatin by 80–90% [95,96], rifampicin decreased fluvastatin by 50% and that of pravastatin by 30% [80,97].

21.9.1.2 Drug Interaction by Transporters. Many statins are substrates of efflux or uptake transporters expressed in the intestine and liver, for example, PGP, MRP2, bile salt export pump (BSEP), BCRP, OATP1B1, OATP1B3, and OATP2B1 (Table 21.2). Since most statins are eliminated by metabolism or biliary excretion, their active hepatic uptake, metabolism, and biliary excretion can regulate their total clearance. The interplay between intracellular CYP3A4 and the efflux transporter PGP in the intestinal wall may contribute to the high presystemic extraction of simvastatin and lovastatin, as well as mediate DDI [55].

The significance of BCRP in the pharmacokinetics of different statins has been shown in several studies [98,99]. Individuals with ABCG2c.421AA variant allele showed AUCs 70–144% higher for atorvastatin, rosuvastatin, fluvastatin, and simvastatin than mean AUC values for individuals with the c.421cc genotype.

OATP1B1, 1B2, and 1B3 are expressed on the sinusoidal membrane of hepatocytes and can facilitate liver uptake of their substrate drugs. All statins are OATP1B1 substrates; some are also substrates of other uptake transporters. OATP1B1 appears to be the most important liver-specific uptake transporter of statins [100]. It is believed that OATP1B1 inhibitors decrease the liver uptake of statins and increase plasma to liver concentration ratio, potentially leading to increased risk of skeletal muscle toxicity and attenuated cholesterol-lowering effect. In addition, genetic variability in the gene encoding OATP transporter can result in marked interindividual differences in pharmacokinetics. In one study, SLCO1B1c.521CC participants had a 91% and 74% larger pravastatin AUC than those with the c.521TT or c.521TC genotype, respectively [101]. The plasma concentrations of active simvastatin acid, pitavastatin, atorvastatin, and rosuvastatin have been 221%, 162%, 144%, and 65% higher in c.521CC homozygotes than c.521TT homozygotes; however, no significant effect was observed for fluvastatin [100].

Several drugs including cyclosporine, rifampicin, clarithromycin, and ritonavir act as OATP1B1 inhibitor *in vitro*. Cyclosporine is a potent OATP1B1 inhibitor ($K_i = 0.242\,\mu M$), it also inhibits several influx and efflux transporters, such as OATP1B3, OATP2B1, MDR1, MRP2, and CYP3A4. Cyclosporine causes a 2- to 20-fold increase in AUC values of statins in organ transplant patient [102–109]. Since fluvastatin, pitavastatin, pravastatin, and rosuvastatin are not significantly

metabolized by CYP3A4, plasma concentration increases in these statins may be primarily due to OATP1B1 inhibition. A study by Bergman *et al*. [110] in pigs explored the mechanism of cyclosporine–rosuvastatin interaction. Intravenous infusion of cyclosporine markedly decreased the hepatic extraction of a single intrajejunal rosuvastatin dose, causing a 9.1-fold increase in AUC in hepatic vein with a 2.1-fold decrease in bile exposure. It was concluded that strong effect of cyclosporine resulted from the inhibition of OATP1B1-mediated sinusoidal transport, rather than canalicular transporters.

21.9.2 Gemfibrozil

Gemfibrozil [5-(2,5-dimethylphenoxy)-2,2-dimethyl-pentanoic] is an oral lipid-lowering agent, which is classified as a fibric acid derivative. Gemfibrozil decreases serum triglycerides, very low density lipoprotein, and increases high density lipoprotein cholesterol.

Gemfibrozil is completely absorbed after oral administration in human [111]; ~70% of an orally administered dose is excreted in urine, mostly as glucuronide conjugates, with <2% excreted as unchanged gemfibrozil. Six percent of the dose is accounted for the feces. Three major metabolic pathways of gemfibrozil were characterized. The first metabolic pathway is oxidation of *meta*-methyl group, yielding a benzyl alcohol that is further oxidized to benzoic acid metabolite. The second pathway is the hydroxylation of aromatic ring to phenol. The third pathway is glucuronide conjugation of gemfibrozil and its oxidative metabolites. Mano *et al*. [112] demonstrated that UGT2B7 is the primary isozyme responsible for gemfibrozil glucuronidation, although other UGT isoforms such as UGT1A1, 1A3, 1A9, and 2B15 also possess catalytic activity [113]. Also, Ogilvie *et al*. [58] showed human liver microsomes and recombinant CYP2C8 both convert gemfibrozil glucuronide to a hydroxylated metabolite. Gemfibrozil is shown to be a CYP2C9 inhibitor (IC_{50} of 30 μM), whereas its glucuronide was found to be a more potent mechanism-based CYP2C8 inhibitor (IC_{50} of 1.8 μM). Coadministration of gemfibrozil with the CYP2C9 substrate warfarin does not increase the plasma concentrations of warfarin [114]. However, gemfibrozil can increase the plasma concentration of CYP2C8 substrates. The AUCs of antidiabetic agents rosiglitazone and pioglitazone and μ-opioid receptor agonist loperamide were increased to over twofold [115–118] and the AUC of repaglinide can be increased up to eightfold [26,27].

In addition, gemfibrozil caused a sixfold increase in the mean AUC value of cerivastatin [119] and moderate AUC increase in active simvastatin acid [93], lovastain acid [94], atorvastatin [120], pravastatin [121], and rosuvastatin [122]. Gemfibrozil–statins interaction may be based on the dual inhibition of both OATP1B1 and CYP2C8; the extent of interaction may depend on the relative importance of OATP1B1 and CYP2C8 in the pharmacokinetics of the statin. Gemfibrozil and its 1-*O*-glucuronide appear to be relatively weak inhibitors of OATP1B1 ($K_i \sim 22$–$25\,\mu M$) [123]. *In vitro* experiments showed that gemfibrozil (200 μM) reduced OATP1B1-, 2B1-, and 1B3-mediated fluvastatin transport from 62% to 97% [124].

Combination treatment of statins and fibrates is a potentially useful strategy to improve lipid and lipoprotein profiles and reduce cardiovascular risk in patients with diabetes mellitus and metabolic syndrome. However, statin–fibrate combination regimens have potential adverse effects on skeletal muscles including myopathy. The

mechanism of statin-related or statin-fibrate-related muscle toxicity are not clear, may result from both pharmacodynamic and pharmacokinetic mechanism [125]. Since other fibrates, such as fenofibrate and bezafibrate, do not have significant effects on OATP1B1, and CYP2C8 showed only minor pharmacokinetic interaction [126], it is expected that treatment with fenofibrate in combination with statin might be less likely to cause adverse muscle effects than gemfibrozil–statin regimes on the base of pharmacokinetic interaction.

21.10 DIURETICS

The loop diuretic torsemide is well absorbed and yields a bioavailability of 80% in healthy individuals [127]. Torsemide undergoes extensive hepatic metabolism, only 20% of the parent drug is recovered unchanged in the urine. The major portion of renal excretion of torsemide occurs via tubular secretion. Renal failure seems to have minimal effect on total plasma clearance, whereas ~50% decrease in plasma clearance was observed in patients with liver disease and an increase in elimination half-life. Hydroxylation of the methyl group of the phenyl ring, M1, and further oxidized metabolite, M5 (carboxylic acid), are major metabolite detected in urine. The metabolite M5 is pharmacologically inactive, metabolite M1 may be ~10% as active as the parent drug. M1 formation is primarily mediated by CYP2C9 with an apparent K_m value of 11–23 μM [128]. CYP2C8 contributed the M1 formation to a minor extent [129]. A clinically significant drug interaction of torsemide involving the CYP450 system has not been reported.

Thiazide diuretics, indapamide and xipamide, undergo extensive hepatic metabolism; however, the underlying enzymes are poorly characterized [130]. Other loop diuretics, such as furosemide, bumetanide, ethacrynic acid, and thiazide diuretics, including chlorothiazide, cyclothiazide, hydrochlorothiazide, and trichlormethiazide, are primarily eliminated in urine via tubular secretion. These diuretics, which carry a common chemical characteristic of a sulfamoyl group, are weak organic acids. It has been reported that organic anion transporters are involved in the tubular secretion of diuretics [131]. OAT1 is suggested to play an important role in the basolateral uptake of thiazides, whereas OAT3 is mainly responsible in the uptake of loop diuretics, it is also suggested that bumetanide taken up by OAT3 and/or OAT1 is excreted into the urine by OAT4. In rats, OAT1 contributed to the renal tubular secretion of thiazides and loop diuretics [132].

Concomitant administration of probenecid and furosemide results in a ~2.5-fold increase in furosemide AUC [133] and 60% decrease in furosemide oral clearance, suggesting probenecid inhibits furosemide secretion, probably by the organic anion transporter (OAT) system.

21.11 ANTITHROMBOTICS

21.11.1 Warfarin

A widely prescribed oral anticoagulant for the treatment and prevention of thrombotic diseases, warfarin has a narrow therapeutic range and a >10-fold interindividual variability in the dose required to attain a therapeutic response [134]. The most common

adverse event associated with warfarin overdose is bleeding. Warfarin initiation doses are often prescribed empirically and typically range from 2.5 to 10 mg/day with peak effects usually observed after ~5 days [135]. It usually takes several weeks to establish a patient's optimal maintenance dose by intense International Normalized Ratio (INR) monitoring and dose adjustments based on multiple influencing factors, such as diet, disease state, concomitant use of other medications, and genetic factors. At least 30 genes have been associated with the metabolism and action of warfarin [136]; single nucleotide polymorphisms in CYP2C9 and VKORC1 are strongly associated with warfarin dose requirements [134,135,137]. In August 2007, the FDA revised the prescribing information for warfarin to encourage, but not require, pharmacogenomics testing when initiating warfarin therapy [138].

Warfarin tablets contain a racemic mixture of R- and S-isomers. The S-isomer exhibits three to five times more anticoagulant activity than the R-isomer in humans. Under steady-state conditions, *S*-warfarin accounts for 60–70% of warfarin anticoagulant effect and *R*-warfarin for 30–40% [139]. Warfarin is completely absorbed after oral administration with peak concentration generally attained within the first 4 h. Elimination of warfarin is almost entirely by metabolism. The warfarin isomers undergo stereoselective metabolism; *S*-warfarin is metabolized primarily by CYP2C9 to 7-hydroxywarfarin, R-isomer is metabolized primarily by CYP1A2 to 6- and 8-hydroxywarfarin, by CYP3A4 to 10-hydroxywarfarin, and by carbonyl reductase to diastereoisomeric alcohol [14]. CYP2C9 tends to have more clinical significance than CYP3A4 or carbonyl reductase. Two common variants CYP2C9*2 and *3 have 12% and 5% reduced catalytic activity compared to wild type (*1) [136]. A systematic review and meta-analysis of nine studies has showed that patients carrying at least one variant allele of CYP2C9*2 have a 17% reduction of daily warfarin dose and a 37% reduction of daily warfarin dose for patients carrying CYP2C9*3 [140]. Patients carrying at least one copy of variant alleles have a increased relative risks of bleeding as compared to wild type (relative risk of 1.9 for CYP2C9*2 and 1.77 for CYP2C9*3).

Vitamin K epoxide reductase (VKOR) is involved in making vitamin K-dependent clotting factors and is the target enzyme for warfarin. Multiple variants in the genes encoding VKOR have been identified and also correlate with warfarin dose requirements. Several studies suggest that variations in CYP2C9 and VKOR can potentially account for 5–22% and 6–37% of the interindividual variability of warfarin dose [134]. A pharmacogenetic algorithm was developed by the International Warfarin Pharmacogenetics Consortium using genotypes from VKORC1 and CYP2C9 and clinical variables to predict the stable therapeutic dose [141]. The data are from a large and diverse cohort of patients (4043 patients) and validation cohort (1009 patients). The algorithm appears to predict the stable therapeutic dose of warfarin better than a fixed dose approach and better than a clinical algorithm built from the same large data set. The pharmacogenetic was more accurate at identifying patients who required low doses (21 mg or less per week) and those requiring high doses (49 mg or more per week). In January 2010, the FDA updated the warfarin label specifying that "a patient's CYP2C9 and VKORC1 genotype information when available, can assist in selection of the starting dose." The agency also provides initial dosage recommendations for patients with different variant combinations. However, the FDA does not require that genetic testing be done before prescribing warfarin.

21.11.2 Antiplatelet Drugs

Coronary artery disease, one of the leading causes of mortality in the United States, can be effectively managed by administration of various antithrombotic strategies. The most widely used is treatment with one of several antiplatelet regimens. There are three marketed thienopyridine antiplatelet prodrugs that function by inhibition of ADP $P2Y_{12}$, resulting in diminished platelet aggregation and lessened risk of thrombosis.

The thienopyridine ticlopidine, clopidogrel, and prasugrel are prodrugs that require biotransformation to produce their respective active metabolites. Ticlopidine is the first compound of this class, introduced to the market in 1979 for prevention of thrombotic stroke. After a single oral dose of [^{14}C]ticlopidine to human, about 60% of the administered radioactivity was recovered in the urine and 23% in the feces [142,143]. 2-Chlorohippuric acid was the main metabolite detected in urine, whereas ticlopidine was present in trace amount in urine. Other lower level metabolites such as 2-oxo-ticlopidine, ticlopidine-*N*-oxide, and *o*-chlorohippuric acid were also detected. The pharmacologically active metabolite was not detected or identified. The structure of the active metabolite was first deduced based on the structures of prasugrel and clopidogrel active metabolites and later on characterized after *in vitro* incubation of 2-oxo-ticlopidine with homogenates prepared from phenobarbital-induced rat livers [144]. CYP2C19 and 2B6 were shown to contribute to the formation of 2-oxo-ticlopidine [145,146]; however, the CYPs involved in the metabolic transformation of 2-oxo-ticlopidine to active metabolite are unknown.

Ticlopidine is dosed at 250 mg BID and although generally safe is characterized by a number of side effects, including gastrointestinal disturbances, skin rash, and in rare cases hepatoxicity, agranulocytosis, neutropenia, and aplastic anemia [143,147]. The later could be due to formation of reactive metabolites and high dose [147,148]. Clopidogrel and prasugrel were introduced at much lower dose of 75 and 10 mg QD and are characterized by generally better safety.

Metabolism of clopidogrel proceeded with two competitive pathways with the primary pathway leading to the formation of inactive acid metabolite by carboxyesterase I (hCE1) [149,150]. The minor pathway proceeded with the formation of 2-oxo-clopidogrel, an intermediate to further ring opening active metabolite. Several studies suggested CYP1A2, 2B6, and 2C19 contribute to 2-oxo-clopidogrel formation; the active metabolite was formed from the thiolactone by CYP2B6, 2C9, 2C19, and 3A4. Several clinical trials have shown that efficacy of clopidogrel is associated with CYP2C19 functionality such that subjects who were carriers of the reduced-function CYP2C19 allele showed significantly poorer clinical response and lower AUC of active drug [143]. The FDA has added a black-box warning to the clopidogrel label, indicating that mutations in the CYP2C19 gene render certain patients unable to respond to the drug, which places them at increased risk for heart attack and stroke.

Prasugrel is the newest thienopyridine antiplatelet drug approved for use in Europe and the United States for the reduction of thrombotic cardiovascular events in patients with acute coronary syndrome, who are to be managed with PCI. Prasugrel is rapidly absorbed and extensively metabolized such that it is not detected in plasma. It is rapidly hydrolyzed by hCE2, primarily an intestinal enzyme, to form the thiolactone, which is further metabolized to the active metabolite primarily by CYP3A4 and 2B6 and to a lesser extent by CYP2C9 and 2C19 [143]. The active metabolite is the major metabolite circulating in plasma, representing 26% of the AUC_{0-12h} of the radioactivity [143].

In vitro, ticlopidine was a potent mechanism-based inhibitor of CYP2C19 slowing the clearance of omeprazole and phenytoin, resulting in toxicity of the latter [151]. Clopidogrel showed similar though less pronounced inhibition of CYP2C19 and both drugs showed inhibition of CYP2B6 [146]. Prasugrel did not meaningfully inhibit either isoform due to its activation via noncup processes. This series of drugs presents an illustrative example of progressive drug discovery.

21.12 ANTIARRHYTHMICS

Antiarrhythmic agents are a group of pharmaceuticals that are used to suppress fast rhythms of the heart such as atrial fibrillation, atrial flutter, ventricular tachycardia, and ventricular fibrillation. Antiarrhythmic drugs comprise many different drug classes with different mechanisms of action. The Vaughan–Williams classification is one of the most widely used classification schemes for antiarrhythmic agents. This classification scheme is based on the primary mechanism of its antiarrhythmic effect. There are five main classes in the Vaughan–Williams classification of antiarrhythmic agents. Class I agents interfere with the sodium channel, class II agents are β-blockers, class III agents affect potassium efflux, class IV agents affect calcium channel and the atrioventricular (AV) node, and class V agents work by other or unknown mechanisms.

21.12.1 Class I Agents

Quinidine is the oldest clinically used antiarrhythmic agent. In human liver microsomes, two major metabolites of quinidine are identified: (3*S*)-3-hydroxy-quinidine (3-OH-Q) and quinidine-*N*-oxide (Q-N-OX) [152]. Formation of 3-OH-Q is mediated exclusively by CYP3A4 with the K_m value of $74\,\mu M$. CYP3A4 is also a primary enzyme catalyzing Q-N-OX formation with a K_m of $76\,\mu M$; CYP2C9 and 2E1 catalyze minor proportions of the N-oxidation. *in vivo* studies to assess the effect of diclofenac, disulfiram, itraconazole, grapefruit juice, erythromycin, and fluvoxamine on the pharmacokinetics of quinidine confirm the important role of CYP3A4 in the oxidation of quinidine *in vivo*, particularly in the formation of 3-OH-Q [153,154]. It has been suggested that the formation of 3-OH-Q may serve as an indicator of CYP3A4 activity *in vivo* [155]. Quinidine is also a potent competitive CYP2D6 inhibitor with K_i value in the range of $0.027–0.4\,\mu M$ and a PGP substrate with an efflux ratio of 3 in caco2 assay [156].

Quinidine has been involved in numerous DDIs. Coadministration of quinidine with cimetidine, itraconazole, verapamil, and diltiazem led to a moderate increase in quinidine plasma concentration and a 30–60% decrease in total plasma clearance. The effect appears to be mediated through inhibition of CYP3A4 and/or inhibition of renal tubular secretion of the parent drug [157–160]. Plasma concentration of quinidine can be significantly decreased by CYP inducers such as rifampicin, phenytoin, and phenobarbital [161]. Coadministration of metoprolol, a CYP2D6 substrate, with quinidine resulted in a fivefold increase in the metoprolol AUC [162]. Similar quinidine cotherapy with dextromethorphan led to a 40-fold increase in AUC values of dextromethorphan, a CYP2D6 substrate [163]. Quinidine was associated with >threefold increases in plasma digoxin concentrations when coadministration with quinidine. Several studies suggested that inhibition of PGP-mediated digoxin efflux by quinidine leading to

increased absorption and decreased renal and biliary clearance may be the underlying mechanism [98,164].

Procainamide is an antiarrhythmic medication that is primarily used to treat ventricular tachydysrhythmias. Oral doses of procainamide are rapidly and nearly completely absorbed [165]. At therapeutic doses, 48% of dose is excreted unchanged in urine. *N*-acetyl procainamide (NAPA) was the major metabolite in the urine (15% of the dose). The C_{max} of NAPA was 50% of that of procainamide in the 10 human subjects; however, plasma concentration of NAPA was higher than that of procainamide in 2 patients. The formation of NAPA was mainly mediated by the cytosolic polymorphic NAT. The NAPA to procainamide ratios in urine and plasma were found to be higher in rapid than in slow acetylator [166–168]. Genetic variant NAT2*4, NAT2*6A, and NAT2*7B were shown to metabolize procainamide with K_m values in the range of 2–3 mM [169]. NAPA is an active metabolite with ~70% of the antiarrhythmic activity of the parent drug [170]. It is important to consider both procainamide and NAPA levels when evaluating the status of a patient. In addition to N-acetylation, Uetrecht and coworkers [171] demonstrated that procainamide was metabolized to the reactive *N*-hydroxylamine-procainamide (NOH-PA), further formed highly reactive nitroso procainamide (NO-PA), NO-PA may covalently bind to macromolecules, leading to the drug-induced lupus erythematosus syndrome observed during the procainamide chronic therapy. *In vitro* chemical inhibition and correlation analysis suggested that CYP2D6 was the major human CYP isozyme involved in the formation of the reactive metabolite of procainamide, namely, *N*-hydroxyprocainamide [172]. It was reported that coadministration with amiodarone, antibacterials trimethoprim and ofloxacin, propranolol, and cimetidine increased the plasma concentration of procainamide and NAPA [161].

Lidocaine is a widely used local anesthetic and antiarrhythmic drug that undergoes extensive metabolism in the liver [173,174]. Following a single oral dose of 250 mg to two healthy volunteers, the major urinary metabolite was 4-hydroxy-2,6-dimethyaniline and its conjugating metabolites (72% of the dose). Monoethylglycinexylidide (MEGX) and other aromatic hydroxylated metabolites represented <5% of the dose in urine. In human liver microsomes, lidocaine was mainly oxidized to N-deethylated metabolite, MEGX, whereas 3-hydroxylation on the aromatic ring was a minor metabolic pathway [175]. Both CYP1A2 and 3A4 were capable of catalyzing the formation of MEGX and 3-hydroxyl lidocaine [93], CYP1A2 was the major isoform catalyzing lidocaine N-deethylation at low substrate concentration (5 μM), CYP3A4 contributed more at higher lidocaine concentration (800 μM), and 3-hydroxylation was primarily catalyzed by CYP1A2. MEGX appeared to have similar antiarrhythmic and convulsant activities to lidocaine and may be synergistic to the effectiveness and toxicity of lidocaine [176]. CYP3A4 inhibitors, erythromycin and itraconazole, increased lidocaine peak plasma concentration by 40–70% [177]. Isohanni and coworker [178] further studied the effect of fluvoxamine (CYP1A2 inhibitor) and erythromycin on the pharmacokinetics of lidocaine, fluvoxamine alone increased the mean AUC of lidocaine to 305% and the C_{max} to 220% compared to the placebo phase, but there was no significant effect on the T_{max} or half-life of lidocaine. The combination of fluvoxamine and erythromycin increased the mean AUX of lidocaine to ~360% and the C_{max} of lidocaine to 250%. It was concluded that inhibition of CYP1A2 by fluvoxamine considerably reduces the presystemic metabolism of oral lidocaine and may increase the risk of lidocaine toxicity if lidocaine is ingested. Lidocaine disposition is significantly affected by changes in hepatic blood flow [179]. β-Blocker propranolol has

been shown to decrease lidocaine clearance secondary to decreased hepatic blood flow [161], subsequently elevated lidocaine plasma concentration has been associated with central nervous system (CNS) toxicity. Similar interaction may be expected with other β-blockers since most appear to decrease hepatic blood flow. In addition, coadministration with amiodarone, cimetidine, and HIV protease may also alter plasma concentration of lidocaine.

Propafenone is a class 1C antiarrhythmic drug with local anesthetic effects and a direct stabilizing action on myocardial membranes. The metabolism of propafenone was studied in human after a single oral dose [180]. Propafenone is completely absorbed and metabolized, $<1\%$ of the dose was recovered as parent drug. The major metabolites in excreta are conjugates of 5-hydroxypropafenone (5-OH-PF) and hydroxy-methoxy-propafenone and propafenone glucuronide. Conjugates of hydroxylated derivatives of propafenone are predominant components in the plasma. Propafenone is administered as a racemate of S(+)- and R(-)-enantiomers. (R, S)-propafenone was mainly metabolized to 5-OH-PF and N-dealkylated propafenone (NDPF) in human liver microsomes [181]. The formation of 5-OH-PF is primarily mediated by CYP2D6; CYP3A4 and 1A2 mediated the formation of NDPF. The 5-OHPF formation from racemic propafenone and from its individual enantiomers followed one-enzyme Michaelis–Menten kinetics with a mean K_m of $0.12\,\mu M$. Stereoselectivity in V_{max} and K_m values was observed, with (S)-propafenone displaying higher K_m and V_{max} values. N-Depropylpropafenone (N-DPP) was monophasic with a mean K_m of $116\,\mu M$. No stereoselectivity in propafenone N-dealkylation was observed [182]. Large interindividual variations in plasma concentration observed in human can be explained by genetically determining metabolism of propafenone via CYP2D6. A clinical study in 28 patients with chronic ventricular arrhythmias (22 extensive metabolizers (EMs) and 6 poor metabolizers (PMs)) concluded that propafenone metabolism was polymorphic and cosegregated with that of debrisoquine 4-hydroxylation [183]. The data also suggested that PMs are at higher risk of developing CNS side effects presumably due to the high plasma concentration achieved in these patients. Therefore, monitoring of the plasma concentration of propafenone may be clinically useful for the prediction of CNS side effects. It was proposed to use urinary excretion of intact glucuronides of propafenone to determine the metabolic phenotype of propafenone [184]. Propafenone is also a potent CYP2D6 competitive inhibitor with a K_i value of 34 nM [185] and a weak CYP1A2 inhibitor with the K_i value of $21\,\mu M$ [186]. Moreover, propafenone was not a substrate of PGP, whereas propafenone and its metabolites 5-OH-PF and NDPF inhibited PGP-mediated digoxin transport with IC_{50} values of 6.8, 19.9, and 21.3 mM, respectively, therefore contributing to the digoxin–propafenone interaction observed in human [161,187–189]. Propafenone increased the plasma concentration of metoprolol, propranolol, mexiletine, and warfarin [141,190–192] and led to adverse effects. Thus, the dose of these drugs should be adjusted when coadministration with propafenone.

21.12.2 Class II Agents: β-Adrenergic Blockade

β-Adrenoreceptor blockers are widely prescribed for the treatment of CVD, including hypertension, coronary heart disease, and heart failure. Over 30 agents are available worldwide and are distinguished by the presence of β1 selectivity, partial agonism, membrane stabilizing effect, the presence of β-receptor antagonism, direct vasodilating

properties, and so on. From a pharmacokinetic perspective, these drugs can be divided into two general categories [193]: those primarily metabolized by the liver and those that are predominantly excreted unchanged by the kidney. The former include propranolol, metoprolol, carvedilol, and so on and the latter include atenolol, nadolol, sotalol, and so on.

Carvedilol is metabolized primarily to glucuronide conjugates in human (22% of total plasma radioactivity and 32% of total urine radioactivity) [194,195]. Three UGT isoforms including UGT1A1, 2B4, and 2B7 can catalyze the reaction with similar K_m values in the range of $20-50\,\mu M$ [196]. Stereoselective metabolism of racemic carvedilol by UGT1A1 and 2B7 was observed [197]. Oxidative metabolites 4 or 5-hydroxy carvedilol were also observed in urine with a small portion of dose (6.4%). CYP2D6 was mainly involved in the formation of these two oxidative metabolites [198]. A clinical study evaluating the effects of polymorphisms in UGTs and CYP2D6 on pharmacokinetics of carvedilol in Japanese suggested that genetic polymorphisms of UGT2B7 and VYP2D6 may be partially responsible for interindividual variation in carvedilol clearance [199].

Propranolol is cleared almost entirely by metabolism with <1% of the dose recovered as unchanged drug [200]. The drug is metabolized through three primary pathways: direct glucuronidation (17% of dose), aromatic hydroxylation (mainly 4-hydroxylation, 42% of dose), and side-chain oxidation. Four major metabolites are characterized as propranolol glucuronide, naphthyloxylactic acid, glucuronide, and sulfate conjugates of 4-hydroxyl propranolol. *In vitro* studies indicate that CYP2D6 is the predominant propranolol 4-hydroxylase; however, CYP1A2 may also significantly contribute propranolol 4-hydroxylation, especially in the 2–10% of the Caucasians who are poor metabolizers of CYP2D6 [201]. Both CYP1A2 and 2D6 catalyze the side-chain oxidation [202]. UGTs 1A9, 1A10, 2B4, and 2B7 catalyze propranolol glucuronidation [203]. On average, the contribution of CYP2D6 to the overall elimination of propranolol is insufficient to cause differences in its pharmacokinetics between phenotypes [204]. However, coadministration of several CYP2D6 inhibitors, such as quinidine, propafenone, or fluoxetine, resulted in significant increases in propranolol plasma level and altered the clinical response [190,205,206]. Rifampicin is a potent inducer of propranolol metabolism, resulting in two- to fourfold increase in propranolol clearance [207,208]. Propranolol is also an inhibitor of CYP2D6 and 1A2, the major isoforms catalyzing its metabolism. Coadministration with theophylline (CYP1A2 probe substrate) resulted in the increased plasma level of these drugs [209].

Metoprolol undergoes extensive hepatic metabolism with <5% of the dose recovered unchanged in urine [210]. Three major metabolic pathways were identified: O-desmethylation (65% of dose), deamination (10% of dose), and α-hydroxylation (10% of dose). CYP2D6 phenotype has a significant effect on metoprolol plasma concentration with PMs having sixfold higher steady-state concentration than EMs [204,211]. Coadministration of CYP2D6 inhibitors profoundly affects metoprolol plasma level and may result in phenotype change from EM to PM. Quinidine coadministration causes a 60% decrease in metoprolol plasma clearance [212], paroxetine increased mean metoprolol AUC about fourfold [213]. Although the expected effects of CYP2D6 genotype on pharmacokinetics of metoprolol were observed, the effect of CYP2D6 genotype on efficacy and toxicity of metoprolol is controversial, most studies suffer from small numbers of CYP2D6 PMs [204,214]. A recent clinical study with 1533 patients demonstrated metoprolol subject homozygous for the CYP2D6*4 allele had a significantly

lower heart rate and diastolic blood pressure than those with the wild-type genotype [214].

21.12.3 Class III Agents: Potassium Channel Blockade

Class III antiarrhythmic drugs, including amiodarone, bretylium, dofetilide, ibutilide, and sotalol, are effective for the management of various types of cardiac arrhythmias both atrial and ventricular in origin. Among the class III antiarrhythmic drugs, bretylium and sotalol are primarily excreted unchanged in urine. Ibutilide is extensively metabolized in the liver to primarily ω-oxidative metabolites followed by sequential β-oxidation of the heptyl side chain of ibutilide. These reactions are not mediated by CYP3A4 and 2D6, but possibly by CYP4 families. No significant pharmacokinetic drug interactions for these agents have been identified [161,215]. Dofetilide is mainly cleared in the kidney via the cationic transport system. Cimetidine, ketoconazole, trimethoprim, prochlorperazine, megestrol, and thiazide diuretics can inhibit tubular secretion. Thus, drugs that inhibit CYP3A4 and/or the renal transport system may interact with dofetilide [215].

Amiodarone has a large volume of distribution and is distributed extensively to extravascular tissues such as liver, lung, and adipose [216]. High dose amiodarone has been associated with pulmonary toxicity. A recent study suggests that amiodarone is a OATP2B1 substrate and that amiodarone uptake via OATP2B1 might lead to accumulation of amiodarone in the lung and admiodarone-induced pulmonary toxicity [186]. Amiodarone undergoes extensive metabolism in the liver, the primary and principle metabolite is desethylamiodarone [217], this metabolite is found to parallel amiodarone concentration in plasma but has variable concentration in tissues. Desethylamiodarone has higher concentration than that of amiodarone in most tissues but lower concentration in adipose. Desethylamiodarone may also contribute to amiodarone-induced pulmonary toxicity. Desethylamiodarone metabolite is pharmacologically active and may be synergistic to the pharmacological effect of amiodarone [176]. Multiple CYP enzymes including CYP1A1, 3A4, 1A2, 2D6, 2C8, and 2C19 catalyzed amiodarone N-deethylation. However, CYP2C8 and 3A4 were significantly involved in amiodarone N-deethylation in human liver microsomes. CYP2C8 has been suggested to be a predominant isoform at clinically relevant concentrations of amiodarone [218,219]. Amiodarone weakly inhibits CYP2C9, 2D6, and 3A4 with K_i values of $45-271\,\mu M$. Desethylamiodarone inhibits CYP2D6, 2A6, 2B6, 1A1, 1A2, 2C9, and 2C19 with lower K_i values of $2.3-15.7\,\mu M$ [220]. Amiodarone and desethylamiodarone markedly inhibited the basal–apical transport (renal secretion) of [^{3}H]digoxin and increased the apical to basal transport (reabsorption) with IC_{50} of 5.48 and $1.27\,\mu M$, respectively. The clinically significant DDI between digoxin and amiodarone could be due to the increased digoxin bioavailability via inhibition of PGP present in the gastrointestinal tract [215,221]. Numerous drug interactions have been reported between amiodarone and other drugs. Coadministration with phenytoin, warfarin, and cyclosporine increases the plasma concentrations of these drugs through inhibitions of CYP2C9, 2C9 and 1A2, and 3A4, respectively [161,215]. The magnitude of interaction appears to be dependent on the dose of amiodarone. Plasma concentration of these drugs should be monitored and dosage adjusted accordingly. Other antiarrhythmic agents, such as dofetilide, flecainide, lidocaine, ibutilide, lidocaine, procainamide, and sotalol, showed drug interaction with amiodarone [215].

21.12.4 Class IV Agents: Calcium Channel Blockade

Verapamil is a class IV L-type calcium channel blocker of the phenylalkylamine chemical class. It has been used in the treatment of hypertension, angina pectoris, cardiac arrhythmia, and most recently, cluster headaches. Verapamil is administered as a racemic mixture of S- and R-enantiomers. The two enantiomers possess different pharmacokinetic and pharmacodynamic properties. The pharmacological properties (i.e., the negative dromotropic effect) are up to 20 times more potent for *S*-verapamil than *R*-verapamil [222]. In human, the metabolism of verapamil is stereoselective, the clearance of S-enantiomer is fourfold more rapid than that of R-enantiomer [223] after oral administration of racemic mixture. AUC ratio of *R*-verapamil to *S*-verapamil is ~5. However, when administered intravenously, the AUC of *S*-verapamil is ~50% lower than that of *R*-verapamil [224]. Verapamil is extensively metabolized; only 3–4% of dose is excreted in urine as the unchanged drug [225]. N- and O-dealkylation are prominent metabolic pathways, producing a secondary amine (D-617) and norverapamil [226]. The N-dealkylation of verapamil in human liver microsomes is not stereoselective. CYP3A and 1A2 are the primary enzymes responsible for N-dealkylation and N-demethylation [227], whereas O-demethylation is mainly mediated by CYP2C family [228]. Grapefruit juice, a moderate CYP3A inhibitor, increases verapamil AUC by 40%; smokers had significantly lower AUC and $C_{max,ss}$ values than nonsmokers by (means) 0.61- to 0.85-fold for verapamil and norverapamil enantiomers, respectively [229]. While the CYP3A inducer rifampin causes a 90% reduction in verapamil AUC, *in vitro* studies suggest that verapamil is a PGP substrate with an efflux ratio in the range of 2–5 [230–232]. Lovastatin and atorvastatin increase verapamil AUC by 40–60% in healthy Korean subjects, possibly via inhibiting both PGP and CYP3A [233,234]. Verapamil is a moderate CYP3A4, 2D6, 1A2, and 2C19 inhibitor *in vitro* [235–238]. Verapamil increases the AUCs of CYP3A4 probe substrates, midazolam, buspirone, and simvastatin by three- to fivefold [87,239,240]. Coadministration with verapamil only slightly increases theophylline AUC [241,242].

21.13 ACE INHIBITORS

ACE inhibitors are important therapies in the treatment of hypertension, congestive heart failure, postmyocardial infarction, and diabetic nephropathy. By inhibition of ACE, the drugs reduce angiotensin II synthesis in the circulation and tissues. The ACE inhibitors are currently classified into three classes based on their molecular structures. The first class represents sulfhydryl-containing agents, such as captopril (the first ACE inhibitor) and zofenopril. The second class, the dicarboxylate-containing agents, are the largest group including enalapril, lisinopril, ramipril, perindopril, trandolapril, quinapril, benazepril, imidapril, temocapril, spirapril, and moexipril. The third class represents phosphorus-containing inhibitors; fosinopril is the only member of this group.

Captopril is the first ACE inhibitor introduced to the market for the treatment of hypertension and congestive heart failure. After oral administration of captopril, ~70% of dose is absorbed with a bioavailability of 60% [203]. The compound is primarily cleared by urine. For patients with renal impairment, the dose may be reduced based on the degree of renal failure. The most common adverse effects associated with captopril use is skin rash, taste disturbance, neutropenia, and more severe forms of cutaneous

disorders, such as toxic epidermal necrolysis, lichenoid eruption, or pemphigus. The latter have been attributed both to a sulfhydryl substituent and overdosage [203,243]. Second-generation ACE inhibitors lacking the sulfhydryl group do not show the cutaneous adverse events. All dicarboxylate-containing agents except lisinopril are prodrugs designed to improve oral absorption from gastrointestinal tract and are metabolized by esterase in the liver and/or small intestine to the active components. CYP is not involved in the metabolism of ACE inhibitors. The predominant elimination pathway of most ACE inhibitors including enalapril, benazepril, quinapril, trandolapril, moexipril, and imidapril is via the kidney [203,244]. For other ACE inhibitors, such as fosinopril, ramipril, lisinopril, spirapril, and temocapril, the proportion of a dose recovered in feces increased. For most ACE inhibitors, AUC and elimination half-life increase by three- to sixfold in patient with $CL_{cr} < 30$ mL/min. A dose reduction is often recommended in patients with moderate to severe impairment of renal function. Dosage justification is not needed in patients with hepatic disease for most ACE inhibitors except for moexipril and spirapril. A 30–100% lower C_{max} and AUC were observed in patients with hepatic disease compared with healthy young volunteers.

Most ACE inhibitors resemble Ala-Pro dipeptide or Xaa-Ala-Pro tripeptide structures. It is known that di- and tripeptides are taken up into intestinal cells by the low affinity H^+/peptide cotransporter PEPT1. In the kidney tubule, di- and tripeptides are reabsorbed by PEPT1 and by the high affinity H^+/peptide cotransporter PEPT2 [245]. The involvement of PEPT1 and PEPT2 in the transport of ACE inhibitors is controversial. A recent investigation on the interaction of ACE inhibitors with PEPT1 and PEPT2 by Knutter and coworker suggests that most ACE inhibitors have low affinity with peptide transporters with the K_i values in the range of $0.3–3\,mM$; zofenopril and fosinopril show stronger affinity with K_i values of $13–30\,\mu M$. Further studies measuring transport current show low or no measurable electrogenic transport activity for all tested ACE inhibitors. Knutter *et al*. conclude that peptide transporters do not control intestinal absorption and renal reabsorption of ACE inhibitors. *In vitro* data suggests that enalapril is a substrate of OATP1B1/1B3 and MRP2 [246] and quinapril is a substrate of renal hOAT3 [247].

21.14 ANGIOTENSIN RECEPTOR BLOCKERS

The angiotensin receptor blockers (ARBs) are a group of antihypertensive drugs that act by blocking the effects of the hormone angiotensin II (Ang II) in the body, thereby lowering blood pressure. Their main indications are mild to moderate hypertension, chronic heart failure, and secondary stroke prevention and diabetic nephropathy. Saralasin, the first Ang II blocker, is an octapeptide analog of Ang II. This compound is not orally bioavailable, has short biological half-life, and shows partial agonist activity; therefore, it is not suitable as a drug [248]. However, early studies with saralasin provided the foundation for the therapeutic potential of Ang II receptor blockade. Great efforts by a group at DuPont and research investigators at Takeda focused on developing a smaller nonpeptide substance with similar binding affinity and acceptable oral bioavailability. The first successful Ang II blocker, losartan, was approved in 1995. Since then, five other ARBs including candesartan cilexetil, eprosantan, irbesartan, telmisartan, and valsartan and three combinations with hydrochlorothiazide (irbesartan, losartan, and valsartan) have been approved as antihypertensive agents. All ARBs

have a carboxylic acid group except for irbesartan. Candesartan cilexetil, a racemic prodrug, is rapidly and completely metabolized by esterase in the gastrointestinal tract to the active candesartan [249]. Candesartan is not metabolized by CYP system; after oral administration, candesartan is excreted mainly unchanged in feces via bile and in urine.

Losartan is rapidly and completely absorbed in healthy adult volunteers [250]. Losartan undergoes extensive first-pass metabolism with oral bioavailability of ~33%. It is converted to aldehyde intermediate E-3179 and further oxidized to a carboxylic acid metabolite (E-3174) that is responsible for most of the angiotensin II receptor antagonism. About 14% of an oral dose is converted to this active metabolite. *In vitro* and *in vivo* studies employing chemical inhibition, inhibitory antibody, and recombinant human hepatic CYP isoforms indicate that CYP2C9 is the predominant enzyme in losartan metabolism to its active metabolite D-3174 [251]. Relatively modest changes in the concentration of losartan and E-3174 were observed when the CYP2C9 inhibitor fluconazole [83,252] or inducer phenobarbital [12] was coadministered with locartan. Fluconazole decreased the E-3174 concentration by 70% and increased losartan AUC by 27%. Coadministration of phenytoin significantly reduced the oral clearance of losartan and $AUC_{(0-24)}$ only in wild-type CYP2C9 individuals [245]. This result suggests that DDI studies with losartan should be evaluated in the context of CYP2C9 genotype of the study subjects. Losartan is a PGP substrate [253]; however, it does not alter pharmacokinetics of digoxin [254]. To date, DDIs have not been reported to influence the blood-pressure-reducing ability of losartan.

Valsartan is primarily eliminated by biliary excretion in feces (83% of the dose) with a small portion in the urine (13%) [255]. Unchanged drug in the excreta accounted for 81% of the dose with about 20% of dose recovered as metabolites including 4-hydroxyvaleryl metabolite (9%). *In vitro* studies show that CYP2C9 is the only form responsible for 4-hydroxylation of valsartan [256]. Valsartan is significantly taken up into OATP1B1 and OATP1B3 expressing HEK293 cells. Saturable ATP-dependent transport into membrane vesicles expressing human MRP2 has been observed. Taken together, these results suggest that OATP1B1 and OATP1B3 as the uptake transporters and MRP2 as the efflux transporter are responsible for the efficient hepatobiliary transport of valsartan [257]. No clinically meaningful pharmacokinetic drug interactions are observed when valsartan is coadministered with simvastatin, amlodipine, atenolol, digoxin, furosemide, glyburide, hydrochlorothiazide, indomethacin, and warfarin [249,258].

Similar to valsartan, telmisartan is minimally metabolized by CYP; a small amount of acyl glucuronide is found in circulation. Most of the administered dose is eliminated unchanged in feces via biliary excretion (>97%) [259]. Telmisartan has been shown to be an OATP1B3 substrate *in vitro* [61], and MDR1, MRP2, and BCRP are involved in the hepatic export of telmisartan acyl glucuronide [61]. A recent study shows that telmisartan is a potent inhibitor of PGP with an IC_{50} of $0.38\,\mu M$ and a less potent BCRP and MRP2 inhibitor (IC_{50}: 16.9 and $25.4\,\mu M$, respectively). MRP2 genetic polymorphisms appear to strongly influence interindividual variation in telmisartan pharmacokinetics in Japanese renal transplant recipients [260]. Telmisartan causes variable increases in serum digoxin levels [261], it is therefore recommended that digoxin levels need to be monitored in patients taking this drug combination. There are no clinically significant DDIs of telmisartan with warfarin, acetaminophen, amlodipine, glibenclamide, ibuprofen, and hydrochlorothiazide [249].

21.15 THE FUTURE OF CARDIOVASCULAR DRUG METABOLISM

The enzymology of CYP and transporters is reasonably well understood and methodology for prediction of DDI is sufficiently mature so that there should be fewer, if any, postmarketing drug withdrawals due to single-point DDI-mediated adverse events, such as mibefradil and cerivastatin. The understanding of genetic variation on drug disposition has made considerable contributions to cardiovascular drug safety and knowledge will continue to grow. Although the influence of biomarkers on drug safety and efficacy has not been as dramatic as promised, it is likely that improvements in sensitivity, selectivity, quantitation, and lower cost will bring modest success. Given the successes of physiologically based pharmacokinetic (PBPK) and pharmacokinetic-pharmacodynamic (PKPD) modeling, it is likely the next big thing will be integration of multiple parameters into models resulting in better predictivity of efficacy and DDI. In particular, the ability to quantitatively combine several pathways for drug disposition, such as vectorial uptake, efflux, and metabolism by multiple active systems, and incorporating genetic population variation should be possible in the coming years. Similarly, recent years have shown steady improvement in development of drugs with larger safety margins and more directed actions, as seen with antiplatelet drugs and antithrombotics.

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