Drug Metabolism in the Liver

Omar Abdulhameed Almazroo, MSc, CCTS[a], Mohammad Kowser Miah, PhD[a], Raman Venkataramanan, PhD[b,c,*]

KEYWORDS

• Drug metabolism • Cytochrome P450 • Conjugation • Drug transporters
• Liver metabolism • Phase I, II, and III metabolism enzyme

KEY POINTS

- Drug metabolism typically results in the formation of a more hydrophilic compound that is readily excreted by the liver, kidney, and/or gut.
- Drug metabolism involves chemical biotransformation of drug molecules by enzymes in the body; in addition, drug transporters facilitate movement of drugs and metabolites in and out of cells/organs.
- In rare cases, a metabolite formed from a drug can cause hepatotoxicity.
- Several disease states and altered physiologic conditions can affect the efficiency of the drug metabolic or transport processes.
- Certain pathophysiologic conditions and use of certain concomitant medications can alter the metabolism or transport of drugs and metabolites and result in altered pharmacokinetic and/or pharmacodynamics of certain drugs.

INTRODUCTION

Drugs are typically small molecules that are generally classified as xenobiotics, which are foreign to the human body. Several endogenous molecules, however, such as steroids and hormones, are also used for the treatment of certain disease conditions and are also referred to as drugs. The term, *metabolism*, refers to the process of transformation of chemicals from one chemical moiety to another by an enzyme. The most well-known drug-metabolizing enzymes are cytochrome P450s (CYP450s), which

The authors have nothing to disclose.
[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, 731 Salk Hall, 3501 Terrace Street, Pittsburgh, PA 15261, USA; [b] Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, 718 Salk Hall, 3501 Terrace Street, Pittsburgh, PA 15261, USA; [c] Department of Pathology, University of Pittsburgh Medical Center, Thomas Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, USA
* Corresponding author. Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, 718 Salk Hall, 3501 Terrace Street, Pittsburgh, PA 15261.
E-mail address: RV@pitt.edu

Clin Liver Dis 21 (2017) 1–20
http://dx.doi.org/10.1016/j.cld.2016.08.001 **liver.theclinics.com**

are mainly oxidases, reductases, and hydrolases. The primary purpose of metabolism is to clear endogenous and/or exogenous molecules from the body. Typically, the process of metabolism converts lipophilic chemicals to hydrophilic products to facilitate elimination.[1,2] In certain instances, however, drug-metabolizing enzymes convert substances into their pharmacologically active form. For example, prodrugs (pharmacologically inactive) are synthesized to overcome absorption/bioavailability issues and they are converted to activate drug after being absorbed into the body. To overcome the low bioavailability of ampicillin, pivampicillin is synthesized as a prodrug, which can be hydrolyzed into ampicillin after being absorbed into the blood stream. Another important example of a prodrug is the use of mycophenolate mofetil to increase the oral bioavailability of mycophenolic acid.[3] Mycophenolic acid is used as immunosuppressant in transplant recipient to prevent acute rejection.[4]

The by-products of metabolism are known as metabolites; they can be either pharmacologically active or inactive.[5] CYP450 enzymes that play a major role in drug elimination are mainly present on the smooth endoplasmic reticulum (ER) and mitochondria of the hepatocytes and small intestinal epithelia and to a lesser extent in the proximal tubules of the kidneys.[6] The contribution and importance of conjugating enzymes and drug transporters are increasingly appreciated.[7] These pathways interplay during the absorption, distribution, metabolism, and excretion of drugs, and any alterations may result in changes in the pharmacokinetics and pharmacodynamics of a drug.

This article discusses the major drug-metabolizing/eliminating pathways: phase I, phase II, and phase III (**Table 1**). Additionally, the contribution of the primary organs (liver, gut, and kidneys) involved in drug metabolism is reviewed. In the last part, major factors that could affect these pathways are summarized.

DRUG METABOLISM PATHWAYS

Phase I Pathway

The most common phase I drug-metabolizing enzymes are represented by CYP450 superfamily. CYP450s are the major group of enzymes that chemically modify drugs

Table 1
Summary of main role of liver, gut, and kidneys in the 3 drug metabolism pathways

	Liver	Gut	Kidneys
Pathway I	Hepatic CYP450s are very important in metabolism of xenobiotics and endogenous molecules.	Enterocytes contain enzymes that can metabolize xenobiotics.	Minimal metabolism activity but important in steroid metabolism
Pathway II	Liver expresses UGTs and other conjugation enzymes; UGTs metabolize approximately 40%–70% of the xenobiotics.	Intestinal enterocytes participate in phase II drug metabolism as well.	Kidney also makes significant contribution in phase II drug metabolism, but GST is the main conjugating enzyme in kidney.
Pathway III	Drug transporters uptake compounds into hepatocytes and efflux into bile.	P-gp is well known to decrease the bioavailability of several drugs because of efflux mechanism into the gut.	They are important to actively efflux drugs into the urine.

into their water-soluble products to facilitate the excretion by kidney and/or liver.[1] In the late 1980s, Nebert developed and reported a nomenclature system for CYP450 enzymes. Human CYP450 genes comprise more than 115 gene and pseudogene members and are among the most extensively annotated mammalian genes, starting from CYP1A1 and currently ending with CYP51P3.[8,9] In humans, CYP450s are distributed throughout various tissues and organs, including peripheral blood cells, platelets, aorta, adrenal glands, adipose tissues, nasal tissue, vaginal tissues, seminal vesicles, brain, lung, kidneys, gut, and liver. Of all the various tissues, liver and small intestine contribute to the maximum extent to the overall metabolism and elimination of drugs. Among all the CYP450 enzymes in human liver, CYP3A4 is the most abundant, followed by CYP2E1 and CYP2C9, representing approximately 22.1%, 15.3%, and 14.6% of the total CYP450s (based on protein content), respectively (**Fig. 1**).[10] CYP450 enzymes also may be classified based on their major substrates, such as sterols, xenobiotics, fatty acids, eicosanoids, vitamins, and unknown substrates.[8]

CYP450 enzymes catalyze several reactions, including oxidation, sulphoxidation, aromatic hydroxylation, aliphatic hydroxylation, N-dealkylation, O-dealkylation, and deamination. Among all, oxidation is the primary reaction, which leads to addition of 1 or more oxygen atom(s) to the parent drug.[2] The CYP450-mediated oxidation process is chemically represented in the following scheme:

$$NADPH + H^+ + O_2 + RH \xrightarrow{CYP450} NADP^+ + H_2O + R\mathbf{O}H \ ,$$

where, *NADPH*, *RH*, and *ROH* are nicotinamide adenine dinucleotide phosphate (a cofactor), any oxidizable substrate, and the oxidized metabolite, respectively.

Reduction of the parent compounds is another pathway of phase I drug metabolism. This type of reaction is coupled with secondary enzymatic system that is known to be either NADH cytochrome-b_5 reductase system or NADPH cytochrome-*c* reductase. This route is important for metabolizing aromatic nitro, nitroso, azo, and N-oxide compounds. Hydrolysis of the parent compounds is also carried out by certain CYP450s, particularly in case of esters and amides.

CYP450s expression is regulated in different compartments of the cell, nuclei, or cytosol by many factors. Nuclear receptor–mediated regulation of gene expression occurs in the nucleus, which is the most critical regulatory pathway, resulting in

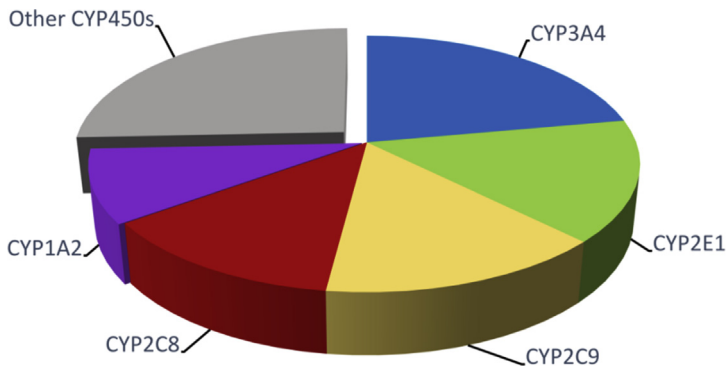

Fig. 1. Pie chart showing the expression of various CYP450 enzymes in the liver in the human. (*Data from* Achour B, Barber J, Rostami-Hodjegan A. Expression of hepatic drug-metabolizing cytochrome p450 enzymes and their intercorrelations: a meta-analysis. Drug Metab Dispos 2014;42(8):1352.)

differential gene transcription. Aryl hydrocarbon receptor is a receptor activated by several endogenous and exogenous ligands, which activates the gene translation and synthesis of various CYP450s.[11] Both pregnane X receptor (PXR) (NR1I2 [nuclear receptor subfamily 1, group I, member 2]) and constitutive androstane receptor (CAR) (NR1I3 [nuclear receptor subfamily 1, group I, member 3]) play similar roles in the regulation of expression of several important CYP450s.[12–16]

In the cytosol, cofactors, such as NADPH-Cytochrome P450 reductase; cytochrome-b_5 reductase; and/or cytochrome-*c* reductase, are essential to carry out the biotransformation reactions. Iron is important for CYP450s synthesis and is present in the center of the binding site between the enzyme and substrate.[2,17] Thus, the different statuses of all these regulators affect the functional activity of CYP450s, resulting in interindividual and intraindividual variability in the metabolic capacity in a population. Consequently, differences in the pharmacologic responses to the same dose of a drug may result due to differences in metabolism and elimination of drugs.[18]

Apart from CYP450 enzymes, other phase I enzymes can contribute to the clearance of many drugs. Some examples of non-CYP450 enzymes that could metabolize endogenous molecules and xenobiotics include flavin-containing monooxygenases, monoamine oxidases, molybdenum hydroxylases, alcohol dehydrogenases, aldehyde dehydrogenases, aldo-keto reductase, NADPH:quinone reductases, and hydrolytic enzymes.[17]

The expression and activity of CYP450 enzymes can be modulated by several factors. Increased mRNA expression leads to increased protein synthesis and a corresponding increase in the activity of enzymes. Induction of CYP450 enzymes leads to increased clearance of certain drugs, leading to decreased drug exposure and response. On the other hand, CYP450 inducers could decrease the risk of hepatotoxicity of certain drugs. Examples of inducers are rifampin and phenobarbital. Calcineurin inhibitors, such as tacrolimus and cyclosporine, and mammalian target of rapamycin inhibitors, such as sirolimus and everolimus are substrates for CYP3A.[19] Induction of CYP3A by rifampin increases their metabolism and decreases their exposure, requiring an increase in their dose.

Inhibition of CYP450 enzymes by endogenous or exogenous compounds leads to a decreased ability of the enzyme to clear the drug. CYP450 inhibitors can drastically increase the blood levels of various substrates of CYP450 enzymes, leading to toxicity. Inhibitors of CYP450 enzymes include azole antifungals; HIV protease inhibitors, such as ritonavir; and certain hepatitis C virus (HCV) drugs.[19,20] Coadministration of azole antifungals, such as ketoconazole, and protease inhibitors, such as ritonavir, lead to decrease in the clearance of certain drugs due to inhibition of CYP enzymes requiring a decrease in drug dosing. A typical dose of tacrolimus in a transplant patient not on ritonavir is 3 mg, twice a day. In patients on lopinavir and ritonavir (Kaletra, North Chicago, IL), it is sufficient to give less than 1 mg once a week to achieve comparable trough blood concentrations of tacrolimus.[21] (More information about substrates, inhibitors, and inducers is discussed later and in **Table 2**).

Phase II Pathways

During phase II drug metabolism, the drugs or metabolites from phase I pathways are enzymatically conjugated with a hydrophilic endogenous compound with the help of transferase enzymes. The most common phase II drug-metabolizing enzymes are UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), *N*-acetyltransferases (NATs), glutathione S-transferases (GSTs), thiopurine S-methyltransferases (TPMTs), and catechol O-methyltransferases (COMTs).

Table 2
List of well-known substrates, inhibitors, and inducers for phase I, II, and III metabolism pathways

Enzymes	Substrates	Inhibitors	Inducers
Phase I			
CYP3A	Midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, triazolam	Ketoconazole, clarithromycin, itraconazole, saquinavir, fluconazole, grapefruit juice, tipranavir/ritonavir	Phenytoin, rifampin, St. John's wort, efavirenz, etravirine, nafcillin, prednisone
1A2	Alosetron, caffeine, duloxetine, melatonin, ramelteon, tacrine, tizanidine	Ciprofloxacin, enoxacin, fluvoxamine, oral contraceptives, phenylpropanolamine,	Montelukast, phenytoin, smoking components of cigarettes
2C8	Repaglinide, paclitaxel	Gemfibrozil, fluvoxamine, ketoconazole, trimethoprim	Rifampin
2C9	Celecoxib, warfarin, phenytoin	Amiodarone, fluconazole, miconazole, oxandrolone, capecitabine, etravirine, fluvastatin, metronidazole, sulfinpyrazone, tigecycline	Carbamazepine, rifampin, aprepitant, bosentan, phenobarbital, St. John's wort
Phase II			
UGTs	Bilirubin, phenols, estradiols, opiates, and carboxylic acids	Paclitaxel, midazolam, cyclosporine A, ketoconazole, phenobarbital, and phenytoin	Bilirubin, phenobarbitone, rifampin
SULTs	Phenols, alcohols, and amines	Flavonoids, mefenamic acids, salicylic acids, clomiphene, and danazol	Retinoic acid, methotrexate
NATs	Para-aminobenzoic acid, para-aminosalicylic acids, para-aminoglutamate, sulfamethazine, isoniazid, hydralazine, and sulfonamides	Caffeic acid, esculetin, qurcetin, genitin, scopoletin and coumarin	Androgens, aminophylline
GSTs	Epoxides, quinone, sulfoxides, esters, and peroxides	Phenols, quinone, vitamin C derivatives, dopamine, and *trans* retinoic acid	Extracts of broccoli, cabbage, Brussels sprouts, and grapefruits
Phase III			
P-gp	Digoxin, loperamide, vinblastine, talinolol	Amiodarone, azithromycin, cyclosporine, diltiazem, dronedarone, erythromycin, itraconazole, ketoconazole, lopinavir/ritonavir, quinidine, verapamil	Avasimibe, carbamazepine, phenytoin, rifampin, St John's wort, tipranavir/ritonavir

Uridine 5′-diphospho-glucuronosyltransferases

Glucuronidation is the major phase II drug metabolism pathway, with approximately 40% to 70% of human endogenous and exogenous compounds conjugated to glucuronidated end products.[22] Conjugated products are more hydrophilic and are readily excreted from body. In the cytoplasm, glucose-1 phosphate reacts with uridine triphosphate to form uridine diphosphate glucuronic acid (UDPGA), a cosubstrate, and this is transferred into the ER by transmembrane proteins. In the ER, UGT attaches UDPGA to the appropriate substrate by nucleophilic attack, forming glucuronidated compounds. UGTs are members of a superfamily of protein, having a molecular weight in the range of 50 kDa to 60 kDa, and structurally they have a catalytic domain and a C-terminal anchoring domain. Until now, 4 families of UGTs in human have been identified: UGT1, UGT2, UGT3, and UGT8. UGT2s have been subdivided into UGT2A and UGT2B.[23]

UGTs metabolize a wide range of compounds and their substrates also overlap with each other. Based on current knowledge, UGT1A1 is the highly expressed phase II enzyme in human, which preferentially metabolizes bilirubin; UGT1A1 also metabolizes certain phenols and estradiols.[24] Whereas UGT2B7 metabolizes opiates,[25] UGT1A3, UGT1A9, and UGT2A1 metabolize carboxylic acids. Various organs express UGTs; however, UGTs are normally highly expressed in the liver and gut (see **Table 1**).

The functional activity of the UGTs is controlled by the amount of enzymes available and the amount of cosubstrate available to conjugate the drug or the metabolite. There are some drugs, such as phenobarbital and rifampin, which are known to increase the expression of UGTs and decreased drug exposure. On the other hand, competition for UGTs may lead to inhibition of metabolism and increased drug exposure.

Sulfotransferases

SULTs are another important superfamily of phase II drug-metabolizing enzymes. Although they are not as highly expressed in the body as UGTs, they are essential for metabolism of several endogenous compounds. They catalyze the reaction between 3′-phosphoadenosine 5′-phosphosulphate (PAPS) and N, O, or S atoms in targeted compounds. A wide range of endogenous compounds (steroids, catecholamine, serotonin, eicosanoids, retinol, and so forth) as well as exogenous compounds are metabolized by SULTs. An endogenous compound like dopamine is almost entirely metabolized by SULTs. Expression of SULTs in human occurs almost in every organ, most commonly found in liver, gut, breast, lung, adrenal glands, kidney, blood cells, brain, and placenta. SULTs have 2 forms; one is metabolically very important and presents in cytosol, the other one is membrane bound and metabolically less important. The function of the membrane-bound isoforms is to synthesize housekeeping substances rather than to metabolize endogenous or exogenous compounds.

Until now, 13 SULTs have been identified in humans and they have been divided into 4 families: SULT1, SULT2, SULT4, and SULT6. There are 9 members in SULT1 family, which could be further subdivided into 4 subfamilies (1A1, 1A2, 1A3, 1A4; 1B1; 1C1 and 1C2; and 1E1). On the other hand, SULT2 family has been divided into 2 subfamilies, SULT2A and SULT2B. Additionally, the SULT4 and SULT6 family contain only 1 member in each group, SULT4A1 and SULT6B1, respectively.

So far, SULT1A1 is the most extensively studied sulfation enzyme, which metabolizes phenols, alcohols, and amines. SULT1A2 and SULT1A3 also metabolize amines; aromatic amines are the primary substrates for both of these isoforms. SULT1B1 is restricted to the metabolism of thyroids hormones and some small phenolic compounds. SULT1C1 metabolizes iodothyronines, and SULT1C2 metabolizes

4-nitrophenols. Furthermore, SULT1E1 has special preference for the metabolism of estrogens, even though it has affinity for other compounds as well. There are various compounds that are reported to be inhibitors of SULTs. Curcumin is a potent inhibitor of SULT1A1. Additionally, SULT1A1 and SULT1A3 can be inhibited by various fruits juices, such as grape, orange, green tea, and black tea. Nonsteroidal anti-inflammatory drugs also have been reported to be inhibitors of SULT1A1 and SULT1E1.[26,27] Mefenamic acid, salicylic acid, clomiphene, and danazol are also potent inhibitors of SULTs.[26] There are several medications that can induce the expression of SULTs in human cells. Retinoic acid induces SULT1A1, SULT2A1, and SULT1E1 in hepatic carcinoma cells as well as in Caco-2 cells.[28] Methotrexate has been shown to have induction capability for various SULTs enzymes in human cells.

N-acetyltransferases

Unlike other enzymes, products of NATs are sometimes more lipophilic instead of being more hydrophilic (metabolites of sulfonamides). In certain situations, the metabolite itself can become more toxic than the parent compound. There are several enzymes that increase the hydrophilicity of the metabolites as well as perform housekeeping activity, such as histone acetyltransferase that regulates the expression of genes in human and other animals. Acetyltransferases in human are classified into 2 subfamilies: NAT1 and NAT2. All NATs are cytosolic enzymes and they use acetyl coenzyme A as a cofactor for metabolic reaction. Until now, 25 members of NAT1 and 27 members of NAT2 alleles have been identified in humans. NAT1 is ubiquitous enzyme, expressed in almost all tissues.

NATs have different substrate specificity and they do not overlap like other metabolizing enzymes. Para-aminobenzoic acid, para-aminosalicylic acid, and para-aminoglutamate are the main substrates for NAT1 in human. On the other hand, sulfamethazine, isoniazid, hydralazine, and sulfonamides are the common substrates for NAT2. Polyphenolic compounds are believed the main inhibitors of NATs. Compounds like caffeic acid, esculetin, qurcetin, and genitin inhibit NAT1, whereas scopoletin and coumarin are the known inhibitors of NAT2.[29] Additionally, major components of garlic, diallyl sulphide and diallyl disulphide, are the common inhibitors for both enzymes.[30]

Glutathione S-transferases

GSTs are ubiquitously present isozymes, found in almost all animal species. GSTs are also important phase II drug-metabolizing enzymes, involved in the metabolism of exogenous and endogenous compounds. Additionally, GSTs are crucial for the detoxification of endogenously produced free radicals, hence protecting the body from oxidative stress. There are 2 superfamilies of GSTs, and members of both the groups have transferases activity. One group is called soluble GSTs; they are found mostly in the cytosol, and recent studies have shown that mitochondria also contains soluble forms of GSTs. The second group of GSTs are called microsomal transferases, also called membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG).[31] Soluble GSTs are again divided into 8 families based on their degree of sequence identity, designated α, μ, π, σ, θ, ζ, ω, and κ. On the other hand, there are 6 members in the MAPEG family. These enzymes are distributed throughout the human body; the most heavily expressed organs are liver, kidney, brain, heart, lung, and gut.

Almost all types of compounds are substrate for GSTs, which are capable of reacting with the thiol moiety in glutathione; common compounds are epoxides, quinone, sulfoxides, esters, and peroxides. Up-regulation of GSTs in cancer is one of the

reasons for therapeutic failure of certain anticancer drugs (melphalan and doxorubicin). Inhibition of GSTs over expression remains one of the treatment strategies in cancer. Until now, however, there has been no report available showing significant improvement in cancer treatment due to inhibition of GST. The most commonly used inhibitors of GST include phenols, quinone, certain vitamin C derivatives, dopamine, and *trans* retinoic acid. Induction of GSTs may also be beneficial in certain situations, which would help remove oxidative molecules that are generated in the body. There are reports suggesting that broccoli, cabbage, and Brussels sprouts induce GSTs.[32] Acetaminophen-induced hepatic toxicities are well known in toxicology. A glutathione precursor (*N*-acetylcysteine) as well as glutathione itself is used as an antidote to protect the liver from acetaminophen-induced hepatic toxicity.[33]

Thiopurine S-methyltransferases

TPMT is an important enzyme, particularly in cancer chemotherapy; this enzyme catalyzes S-methylation of aromatic heterocyclic sulfhydryl compounds, including anticancer and immunosuppressive medications. Thiopurines 6-mercaptopurine, 6-thioguanine, and azathioprine are prodrugs. These drugs have to be metabolically converted to the active form by hypoxanthine phosphoribosyl transferases. The metabolites of hypoxanthine phosphoribosyl transferases are cytotoxic and exert anticancer activity; however, there has to be a delicate balance between cytotoxicity for anticancer action in cancer cells and normal cells. TPMT metabolizes those compounds to the nontoxic forms by methylation. TMPT is a cytosolic enzyme mostly found in the liver, kidney, and lung; additionally, human red blood cells also have a significant level of TMPT expression. Thiopurines are good substrates for the TPMT, which make them important for cancer chemotherapy. Inhibition of TPMT activity may lead to the accumulation of toxic metabolites in the human body, causing other conditions, such as myelosuppression after azathioprine treatment. Naproxen, mefenamic, and tolfenamic acid are known to inhibit TPMT in noncompetitive manner.[34]

Catechol O-methyltransferases

COMTs are the enzymes responsible for the transfer of a methyl group from S-adenosylmethionine to its substrate. This methylation is one of the major pathways for the metabolism of catecholamines and catechol estrogens, including neurotransmitters, such as dopamine, epinephrine, and norepinephrine as well as drugs that have catechol functional groups attached to their structure.[35] COMT is mostly expressed in the postsynaptic neurons in the mammalian cells. There are 2 forms of COMT: the soluble form, called S-COMT, and the membrane-bound form, called MB-COMT. Structurally, both S-COMT and MB-COMT share almost similar sequences, however, their substrate affinity and specificity vary significantly. For example, MB-COMT has approximately 10 times more affinity for dopamine and noradrenaline compared with S-COMT. There are several inhibitors of COMT but the most commonly used are entacapone, tolcapone, and flavonoids (found in green tea). Inhibition of COMT leads to the accumulation of its substrate, which is used as treatment strategies for Parkinson diseases.[36]

Phase III Pathways

Drug transporters are generally transmembrane proteins that facilitate the transport of large and/or ionized molecules in and out of the cells. Phase III pathway is classified into 2 main superfamilies: ATP-binding cassette (ABC) and solute carrier (SLC) transporters. ABC transporters are dependent on the energy (ATP) consumption to actively

uptake or efflux the drug from one side of the cell membrane to another, whereas SLCs facilitate the passage of certain solutes (eg, sugars and amino acids) across the membrane and actively transport other solutes against their electrochemical gradients by coupling the process with other solute or ion. They are present in many locations, such as liver, kidney, intestine, and brain. Conceptually, uptake transporters help in transferring the molecules into the cells and efflux transporters pump them outside the cell. In the liver, the main uptake transporters are Na^+-taurocholate cotransporting polypeptide (NTCP) (SLC10A1), organic cation transporter 1 (OCT1) (SLC22A1), organic anion transporter 2 (OAT2) (SLC22A7), and organic anion-transporting polypeptides (OATP1B1, OATP1B3, OATP2B1; SLCO1B1, SLCO1B3, and SLCO2B1, respectively). The hepatic efflux transporters are multidrug resistance protein 1 (MDR1) (also known as P-glycoprotein [P-gp] and ABC subfamily B member 1 [ABCB1]), bile salt export pump (BSEP) (also known as ABC subfamily B member 11 [ABCB11]), and multidrug resistance-associated protein (MRP) 2 (also known as ABC subfamily C member 2 [ABCC2])[37–39] (Fig. 2).

SITES OF DRUG METABOLISM

Liver

The liver is the major organ for phase I and phase II drug metabolic processes. Nevertheless, drug transporters also play an important role in facilitating the entry of molecules into the hepatocytes and out into the bile. Large and charged compounds are normally transported by drug transporters. Fig. 2 demonstrates the interplay between phase I, II, and III pathways in the hepatocyte. Some drugs secreted in the bile are reabsorbed back from the intestine; some metabolites secreted in the bile can be converted back to the drug by β-Glucuronidase in the gut and can be reabsorbed. This phenomenon is known as enterohepatic circulation, a process that prolongs the residence of a drug in the body.

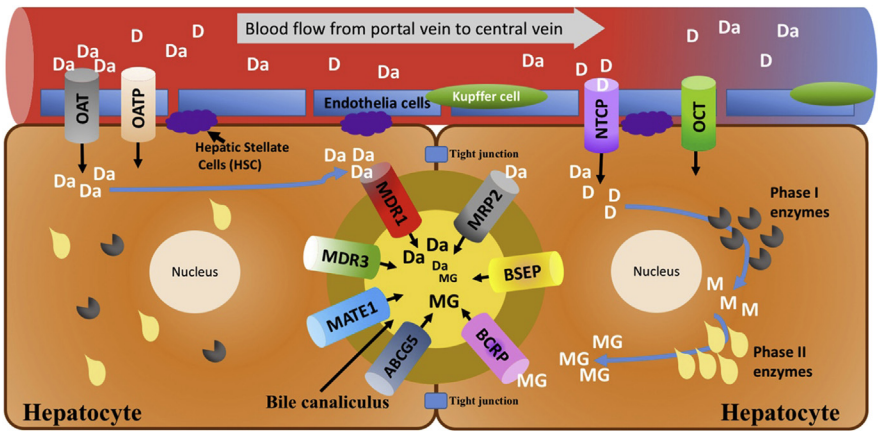

Fig. 2. Hepatocyte showing the main components and transporters. D and Da are 2 different drugs that have different clearance pathways. (1) Drug D is uptaken by transporter then metabolized by phase I pathway to M (main metabolic product) followed by conjugation process by phase II enzymes and finally effluxed into biliary system by transporter. (2) In this scenario, drug Da is transported into the hepatocyte through OAT then pumped out by MDR1 without any chemical modification to the drug molecule. D, drug; Da, drug a; M, metabolite; MG, metabolite glucuronide.

Liver and gut play a dominant role in the first-pass metabolism of orally administered medications.[40] The oral bioavailability of various drugs is increased with liver diseases. In patients with liver cirrhosis there is an increase in the severity of drug-induced liver injury. In HCV patient population, it is advised to closely monitor drug dosing after liver transplantation, especially when HCV therapies are used to prevent any recurrence.[19]

Gut

The primary function of intestinal tract is to serve as an absorptive organ for nutrients, electrolytes, and drug molecules. The gut also has metabolic functions, however, which can have an impact on the systemic bioavailability of certain therapeutic agents. Any medication that is taken orally has to be absorbed from gut lumen into the enterocytes, then move into the portal circulation, then to the liver, and finally into the systemic circulation. The systemic availability (*F*) of a drug is the product of fraction absorbed (*Fab*), fractions escaping gut metabolism (*Fg*), and the fraction escaping hepatic metabolism (*Fh*) before reaching the systemic circulation; this can be expressed as, $F = Fab \times Fg \times Fh$.[41] Human gut expresses most of the phase I and phase II drug-metabolizing enzymes, including CYP450s and UGTs.

Human small intestine expresses appreciable amounts of various CYP450 enzymes, contributing to the oxidation of several xenobiotics. Among all the CYP450s, CYP3A4 is the more prominent CYP450 enzyme, affecting the bioavailability of several therapeutic agents. In addition to CYP3A, the human small intestine also expresses CYP2C9, CYP2C19, CYP1A1, CYP2D6, and CYP2J2. The amount of CYP450 expression in the intestine varies from 20 pmol/g to 210 pmol/g of the tissues, indicating a large interpersonal variability. Additionally, the expression of the enzymes also is not uniform throughout the intestine. The duodenum, jejunum, and ileum are the places where expression is maximum; the expression level goes down further down in the intestine. CYP3A (CYP3A3/CYP3A4, CYP3A5, CYP3A7, and CYP3A43) is the predominant CYP450 family enzymes, which represent almost approximately 70% to 80% of the total CYP450 enzymes expressed in the intestine.[42]

UGTs are one of the major phase II drug-metabolizing enzymes expressed in human intestine in addition to the liver and kidney. Human intestine expresses several UGTs, including UGT1A1, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10. Bioavailability of drugs could be affected by UGTs significantly; raloxifene is an estrogen receptor modulator, used for the treatment of osteoporosis and breast cancer. Unfortunately, oral bioavailability of raloxifene is only approximately 2%, where intestinal UGT1A8 and UGT1A10 play a significant role in lowering the systemic availability of the compound.[43]

In addition to the drug-metabolizing enzymes, the gut expresses a wide range of transporters including both influx and efflux transporters (**Fig. 3**). The intricate interplay between drug-metabolizing enzymes and transporters could significantly alter overall systemic availability of some drugs. Among all the efflux transporters, human gut expresses P-gp (MDR1), MRPs, and BCRP (breast cancer resistance protein), which are known to be responsible for most of the efflux function. Overexpression of the efflux transporters at the luminal site could lead to lower bioavailability of the drug, whereas overexpression at the abluminal site may increase systemic availability.[44,45] Transporters are mainly gate keepers at the gut, preventing toxins or unwanted substances from entering into blood. Most of the influx transporters are expressed at the luminal site, playing an important role in drug uptake. OATP3A1, OATP4A1, OCT1, and OCT2 are expressed at the abluminal site as well.[46,47] P-gp expressed luminally limits the absorption of cyclosporine, and the expression of P-gp is inversely correlated with the

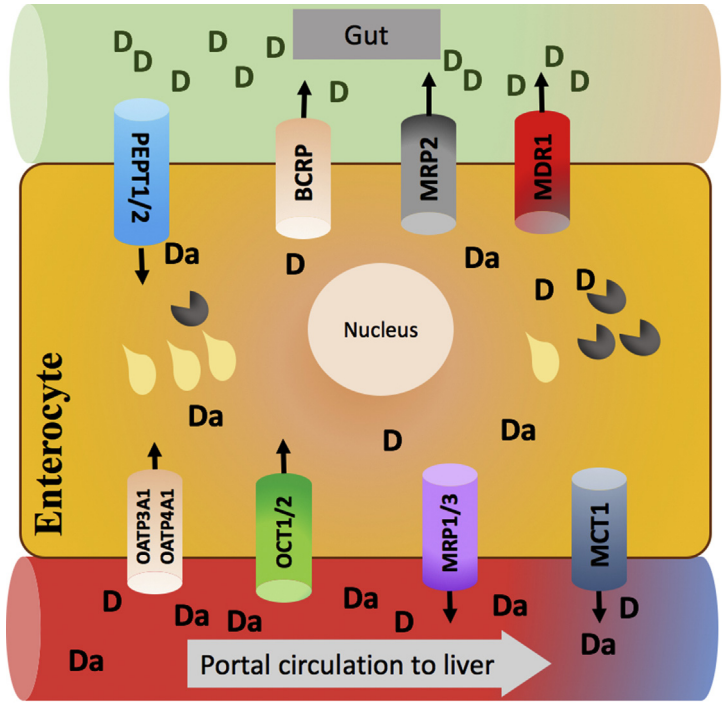

Fig. 3. Drug-metabolizing enzymes and transporters in gut, enterocyte. The drug (D) is transported from gut into enterocyte and then to the circulation. Metabolite (Da) also can be affected by transporters either by efflux or uptake transports.

oral bioavailability of its substrates. Ironically, the efflux transporters may be responsible for the development of drug resistance as well as causing therapeutic failure.

Kidney

Kidneys play an important role in clearing toxins but contribute to a lesser extent in terms of overall drug metabolism. Nephrons are the main functional unit of kidney and drugs are normally filtered excreted based on glomerular filtration, secreted at the proximal tubules, and reabsorbed by the tubules. Phase III transporters play a critical role in actively secreting drug molecules against their electrochemical gradients.[1] Kidneys do contribute to the metabolism of some endogenous compounds and xenobiotics. For example, kidneys activate 25-hydroxyvitamin D to the hormone 1,25-dihydroxyvitamin D_3 by CYP27B1, then deactivate it by CYP24A1.[48] Cyclosporine is converted to its metabolites in the kidney by CYP3A enzymes in the kidney and this may contribute to cyclosporine mediated nephrotoxicity.[49,50]

FACTORS AFFECTING THE METABOLISM OF DRUGS

Metabolism of drugs in human is not constant or immune to various internal and external factors. Metabolism could be affected by age, gender, pregnancy, different disease states, solid organ transplantation, medications, and genetic polymorphism (Fig. 4).

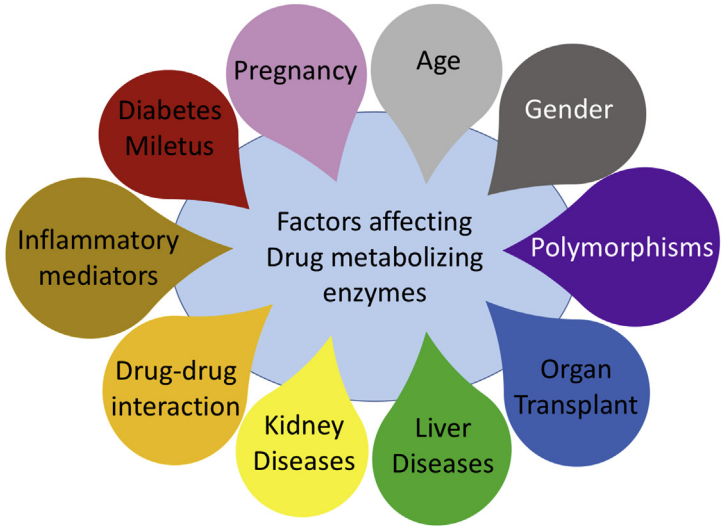

Fig. 4. Factors affecting the drug-metabolizing enzymes expression and functions.

Age

Age is known to have an impact on hepatic drug metabolism. In infants, lower expression of the enzyme may lead to decreased drug metabolism. The fetal liver expresses CYP3A7, which metabolizes endogenous compounds and some exogenous substrates of CYP3A4. Typically, the neonatal expression of CYP2C, CYP2E1, and CYP1A2 are approximately 10 times lower than adults. The activity of CYP450 enzymes in neonates is less than half that in adult human, which results in the inability of neonates to clear most of the medication at earlier life. The clearance of caffeine is approximately 6 times lower in newborn compared with adults. As neonates mature, the drug metabolic capacity increases. Young children tend to have higher clearance of certain drugs compared with young adults. Conjugation reactions can also be affected by age. In neonates, the clearance of bilirubin is low because UGTs are not well expressed at that age and in some cases can lead to neonatal jaundice. Overall, the maturation of the conjugative enzymes is slower than other biotransformation pathways. The expression and function of the enzymes are not significantly affected in elderly subjects compared with adults. The function of most CYP450 enzymes does not decline with advanced age, except CYP2D6 and CYP1A2. In the elderly, however, a combination of disruptive hepatic blood flow and use of multiple drugs (polypharmacy) may modify drug metabolism. With age, the liver volume decreases at a rate of 0.5% to 1% per year. Additionally, decrease in hepatic blood flow and oxygenation and increase in the fat deposition could lead to reduced overall metabolism of drug in elderly.[51–53]

Gender

Adverse drug reaction is 1.5 to 2 times more common in women compared with men.[54] This difference in side effects of drugs may be due to impact of sex hormones, body weight, body fat composition, and volume of distribution. It has been reported that while the activity of certain enzymes is higher in men than women, other enzymatic pathways are increased in women. The activities of CYP2D6 and CYP1A2 have been reported to be higher in men than women. CYP3A4 activity has been reported to be higher in women than in men.[55]

Pregnancy

Pregnancy is associated with several physiologic changes. These changes in body water, fat content, and hormones can potentially alter absorption, distribution, metabolism, and elimination of drugs.[56,57] In the United States, more than 60% pregnant women take at least 1 medication and many of these are metabolized.[58] The activities of CYP2D6, CYP3A4, CYP2B6, and CYP2C9 increase during pregnancy. As a result, the half-life of drugs those are substrate for these enzymes are shorter compared with nonpregnant women (phenytoin, midazolam, and metoprolol). On the other hand, the activity of CYP1A2 and CYP2C19 decreases during pregnancy, which leads to decrease in the clearance of drugs, which are substrates for the enzymes (caffeine-CYP1A2). Additionally, the activity of conjugating enzyme is altered during pregnancy. There are reports suggest that the activity of UGT1A4 is significantly increased during pregnancy, whereas the activity of UGT2B7 remains unaffected, and NATs are reduced.

Liver Diseases

Various liver diseases are known to affect the metabolism of drugs as well as endogenous compounds. There are several reasons for the observed changes in drug metabolism in patients with liver disease. Altered hepatic blood flow, altered expression of drug-metabolizing enzymes, altered availability of cosubstrates, and altered binding of drugs to plasma proteins can account for the observed changes in drug metabolism in patients with liver disease.

Plasma concentrations of midazolam are more than 2-fold higher in patients with nonalcoholic steatohepatitis (NASH) compared with normal health subjects. The observed increase in plasma concentration of midazolam is due to NASH-mediated decrease in hepatic drug metabolism. Additionally, more than 50% reduction in the plasma concentration of 4β-hydroxycholesterol, which is used as an endogenous biomarker for CYP3A4 activity, was reported in patients with simple steatosis.[59] The clearance of several drugs that are metabolized in the liver is decreased in cirrhosis. Liver cirrhosis decreases the clearance of voriconazole, a drug that is completely metabolized in the liver. Patients with hepatic insufficiency must be closely monitored when dosed with voriconazole to prevent any drug-associated toxicities[60,61] (**Table 3**) includes a list of hepatic diseases and their effect on drug-metabolizing enzymes.[62–66]

Table 3
List of hepatic diseases and their common effect on drug-metabolizing enzymes and drugs

Liver Diseases	Affected Enzymes	Selected Drugs Affected
Cirrhosis	CYP2E1, CYP2C9, CYP3A, CYP1A2, and UGTs	Isavuconazole, tacrolimus, sirolimus cyclosporine, chlorzoxazone, midazolam, dacarbazine, enflurane, diazepam
Hepatitis	CYP2E1, CYP3A, and CYP2C19	Isavuconazole, sirolimus, tacrolimus, cyclosporine, chlorzoxazone, midazolam, dacarbazine, omeprazole, phenytoin, lansoprazole
NASH	CYP2E1, CYP3A, UGTs, and SULTs	Isavuconazole, sirolimus, cyclosporine, tacrolimus, chlorzoxazone, midazolam, dacarbazine, midazolam
Cholestasis	CYP1A2, CYP2E1, CYP3A4, and UGTs	Acetaminophen, caffeine, verapamil, sirolimus, Cyclosporine, tacrolimus, midazolam, isavuconazole

Data from Refs.[62–66]

Kidney Diseases

Multiple molecular mediators and mechanisms can cause a reduction in the functionality of the nonrenal excretion pathways, mainly hepatic metabolism in patients with kidney diseases. As an example, the systemic exposure of intravenously given midazolam, a CYP3A4 probe, in chronic kidney disease before hemodialysis had increased 6-fold in comparison to healthy subjects.[67,68] There is a direct association between accumulation of circulating uremic toxins and reduction in the levels of mRNA, protein, and activity of some CYP450s. Two months after successful kidney transplant or directly after hemodialysis, however, the enzymatic activity appears to be restored. Most of the effect seems correlated with the accumulation of parathyroid hormone and proinflammatory cytokines (eg, interleukin [IL]-6, tumor necrosis factor [TNF], and IL-1β). The mechanism is believed to be reduced binding of the nuclear receptor PXR complex to CYP450 promoter region and, indirectly, by activation of the nuclear factor κB that depresses CYP450 transcription.[68] Furthermore, alteration of drug transporters activity as measured half-life of fexofenadine (3 times longer), which is a nonspecific transporter probe, has been documented in patients with glomerulonephritis compared with control subjects.[69]

Diabetes Mellitus

Diabetes mellitus has been an increasingly recognized problem worldwide and affects several systems in the body, such as the cardiovascular and nervous systems. Drug metabolism plays a major role in the regulation of glucose, lipoproteins, and lipid metabolism. Drug-metabolizing enzymes have been reported to be altered in diabetes. Dostalek and colleagues[70] investigated the effect of diabetes on the expression and activity of CYP3A4 and CYP2E1. They have shown that the protein levels and mRNA expressions in liver tissue were significantly decreased in diabetic patient compared with healthy individuals. In addition, CYP3A4 activity was tested using midazolam and testosterone as probes in human liver microsomes. It was concluded that diabetes significantly decreased CYP3A4 activity, resulting in lower metabolite(s) production (1'-hydroxymidazolam, 4-hydroxymidazolam, and 6β-hydroxytestosterone).[70] Patients with diabetes have a greater risk of having side effects and toxicities due to decreased metabolism. Therefore, modification of the dose of hepatically metabolized medications with narrow therapeutic index should be considered in diabetic population.

Solid Organ Transplantation

Liver transplantation is the only cure for patients with end-stage liver disease. Many factors that are inherent after liver transplantation can affect all 3 pathways. In cases of living donor liver transplantation, the size of transplanted graft is much smaller than normal livers and livers in deceased donor liver transplantation. Consequently, the intrinsic metabolic capacity is significantly reduced. The hepatic blood flow is higher per unit weight of liver in the liver donor and transplant recipient.[71]

In the deceased donor liver transplantation population, liver grafts are more susceptible to ischemia/reperfusion injury because of the longer duration of cold preservation and lack of oxygen and nutrients. Additionally, this injury is worsened when the hepatic blood flow is re-established. Ischemia/reperfusion injury is also associated with higher proinflammatory cytokines that lead to lower activity of drug-metabolizing enzymes. Metabolizing enzymes and transporters can be altered when graft rejection occurs, either acutely or chronically. It is documented that the expression of P-gp (MDR1) transporter and CYP3A4 in the intestine is decreased when there is chronic rejection

of the liver graft with an increase in proinflammatory cytokines (cyclooxygenase-2, IL-2, IL-6, IL-8, IL-10, and TNF-α).[72]

Commonly used immunosuppressants in solid organ transplantation are cyclosporine, tacrolimus, and mycophenolic acids.[73] Cyclosporine A is a well-known inhibitor of P-gp and can potentially alter the bioavailability of drugs that are P-gp substrate.[74]

Medication (Drug-Drug Interactions)

Concomitant administration of medications can affect metabolism of each other. The Food and Drug Administration requires all drugs under development to be tested for any possible interaction as substrate, inhibitors, and/or inducers.[75] Inhibitors mainly work on the enzyme levels where they block or compete at the site of metabolism. Types of inhibitors are competitive (binds to the active site of free enzyme), uncompetitive (binds to the drug-enzyme complex to inhibit), noncompetitive (binds to different site other than site of the metabolism), and mixed. Inducers act by increasing the gene transcription that result in higher enzyme content. Some drugs can increase and stabilize the enzyme to be more active. Both of these processes increase the overall metabolic rate. **Table 2** lists different inhibitors and inducers that are clinically relevant. Usually in the presence of inhibitors drug exposure increases and that requires a decrease in the dose, dosing interval, or both and vice versa for the inducers.[76–78]

Polymorphism

Polymorphism in drug-metabolizing enzymes and transporters is known to influence the kinetics of several drugs. For example, CYP3A has several polymorphisms, including CYP3A4*1G, CYP3A5 6986A > G, and CYP3A5*1. Also, MDR1 (P-gp) has been identified as having several polymorphisms that affect the clearance of many drugs.[71] Warfarin is an oral anticoagulant used to treat thrombotic disorders. The effective dose of warfarin varies from 0.5 mg to 60 mg daily; this wide range is mainly because of interindividual variability in metabolism of warfarin. Warfarin is primarily metabolized by CYP2C9, and genetic polymorphism in this enzyme leads to variation in the metabolism of the drug.[79] Patients who carry various alleles of CYP2C9, such as CYP2C9*2 and CYP2C9*3, tend to have lower metabolism of warfarin leading to higher plasma concentrations.[80,81] Both of these alleles have impaired hydroxylation activity of S-warfarin when tested in vitro. Voriconazole metabolism is mainly affected by CYP2C19 polymorphisms. Patients can be either homozygous poor metabolizer, heterozygous extensive metabolizer, or homozygous extensive metabolizer; voriconazole levels can be 4 to 5 times higher in poor metabolizers in comparison to extensive metabolizers. Poor phenotype varies based on ethnicity from less than 7% to 20%.[61]

SUMMARY

Drug metabolism is an important process for the removal of unwanted substances from the body. Abnormal drug metabolism profile could lead to life-threatening complications. Both phase I (mainly CYP450s) and phase II (mainly UGTs) enzymes play a significant role in drug metabolism. Although metabolites in general are expected to be not active and not toxic, certain metabolites can cause hepatotoxicity. Various diseases may potentially change the metabolic profile of a drug by altering the expression and function of key enzymes. Additionally, coadministration of multiple drugs may also lead to drug-drug interaction and adverse reaction due to competitive binding to the same metabolizing enzyme. Care must be exercised while prescribing multiple

medications to patients with certain diseases, which can alter drug pharmacokinetics/pharmacodynamics profiles.

REFERENCES

1. Benedetti MS, Whomsley R, Poggesi I, et al. Drug metabolism and pharmacokinetics. Drug Metab Rev 2009;41(3):344–90.
2. Ionescu C, Caira MR. Drug metabolism: current concepts. Dordrecht (Netherlands): Springer; 2005.
3. Lee WA, Gu L, Miksztal AR, et al. Bioavailability improvement of mycophenolic acid through amino ester derivatization. Pharm Res 1990;7(2):161–6.
4. Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet 1998;34(6):429–55.
5. Olsen L, Oostenbrink C, Jorgensen FS. Prediction of cytochrome P450 mediated metabolism. Adv Drug Deliv Rev 2015;86:61–71.
6. Mittal B, Tulsyan S, Kumar S, et al. Cytochrome P450 in cancer susceptibility and treatment. Adv Clin Chem 2015;71:77–139.
7. Terada T, Hira D. Intestinal and hepatic drug transporters: pharmacokinetic, pathophysiological, and pharmacogenetic roles. J Gastroenterol 2015;50(5):508–19.
8. Ortiz de Montellano PR. Cytochrome P450: structure, mechanism, and biochemistry. 3rd edition. New York: Kluwer Academic/Plenum Publishers; 2005.
9. Nelson DR. The cytochrome p450 homepage. Hum Genomics 2009;4(1):59–65.
10. Achour B, Barber J, Rostami-Hodjegan A. Expression of hepatic drug-metabolizing cytochrome p450 enzymes and their intercorrelations: a meta-analysis. Drug Metab Dispos 2014;42(8):1349–56.
11. Guenthner TM, Nebert DW. Cytosolic receptor for aryl hydrocarbon hydroxylase induction by polycyclic aromatic compounds. Evidence for structural and regulatory variants among established cell cultured lines. J Biol Chem 1977;252(24):8981–9.
12. Kliewer SA, Moore JT, Wade L, et al. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 1998;92(1):73–82.
13. Bertilsson G, Heidrich J, Svensson K, et al. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. Proc Natl Acad Sci U S A 1998;95(21):12208–13.
14. Forman BM, Tzameli I, Choi HS, et al. Androstane metabolites bind to and deactivate the nuclear receptor CAR-beta. Nature 1998;395(6702):612–5.
15. Honkakoski P, Zelko I, Sueyoshi T, et al. The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. Mol Cell Biol 1998;18(10):5652–8.
16. Kohle C, Bock KW. Coordinate regulation of human drug-metabolizing enzymes, and conjugate transporters by the Ah receptor, pregnane X receptor and constitutive androstane receptor. Biochem Pharmacol 2009;77(4):689–99.
17. Khojasteh SC, Wong H, Hop CECA, et al. Drug metabolism and pharmacokinetics quick guide. New York: Springer Science+Business Media, LLC; 2011. Available at: http://dx.doi.org/10.1007/978-1-4419-5629-3.
18. Dvorak Z, Pavek P. Regulation of drug-metabolizing cytochrome P450 enzymes by glucocorticoids. Drug Metab Rev 2010;42(4):621–35.
19. Parikh ND, Levitsky J. Hepatotoxicity and drug interactions in liver transplant candidates and recipients. Clin Liver Dis 2013;17(4):737–47, x–xi.
20. Tischer S, Fontana RJ. Drug-drug interactions with oral anti-HCV agents and idiosyncratic hepatotoxicity in the liver transplant setting. J Hepatol 2014;60(4):872–84.

21. Jain AB, Venkataramanan R, Eghtesad B, et al. Effect of coadministered lopinavir and ritonavir (Kaletra) on tacrolimus blood concentration in liver transplantation patients. Liver Transpl 2003;9(9):954–60.
22. Wang Q, Jia R, Ye C, et al. Glucuronidation and sulfation of 7-hydroxycoumarin in liver matrices from human, dog, monkey, rat, and mouse. In Vitro Cell Dev Biol Anim 2005;41(3–4):97–103.
23. Iyer KR, Sinz MW. Characterization of Phase I and Phase II hepatic drug metabolism activities in a panel of human liver preparations. Chem Biol Interact 1999;118(2):151–69.
24. Iyer L, King CD, Whitington PF, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 1998;101(4):847–54.
25. Coffman BL, King CD, Rios GR, et al. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). Drug Metab Dispos 1998;26(1):73–7.
26. Wang LQ, James MO. Inhibition of sulfotransferases by xenobiotics. Curr Drug Metab 2006;7(1):83–104.
27. Harris RM, Waring RH. Sulfotransferase inhibition: potential impact of diet and environmental chemicals on steroid metabolism and drug detoxification. Curr Drug Metab 2008;9(4):269–75.
28. Maiti S, Chen X, Chen G. All-trans retinoic acid induction of sulfotransferases. Basic Clin Pharmacol Toxicol 2005;96(1):44–53.
29. Kukongviriyapan V, Phromsopha N, Tassaneeyakul W, et al. Inhibitory effects of polyphenolic compounds on human arylamine N-acetyltransferase 1 and 2. Xenobiotica 2006;36(1):15–28.
30. Lin JG, Chen GW, Su CC, et al. Effects of garlic components diallyl sulfide and diallyl disulfide on arylamine N-acetyltransferase activity and 2-aminofluorene-DNA adducts in human promyelocytic leukemia cells. Am J Chin Med 2002; 30(2–3):315–25.
31. Jakobsson PJ, Morgenstern R, Mancini J, et al. Common structural features of MAPEG – a widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. Protein Sci 1999; 8(3):689–92.
32. Perez JL, Jayaprakasha GK, Valdivia V, et al. Limonin methoxylation influences the induction of glutathione S-transferase and quinone reductase. J Agric Food Chem 2009;57(12):5279–86.
33. Saito C, Zwingmann C, Jaeschke H. Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. Hepatology 2010;51(1):246–54.
34. Oselin K, Anier K. Inhibition of human thiopurine S-methyltransferase by various nonsteroidal anti-inflammatory drugs in vitro: a mechanism for possible drug interactions. Drug Metab Dispos 2007;35(9):1452–4.
35. Zhang J, Kulik HJ, Martinez TJ, et al. Mediation of donor-acceptor distance in an enzymatic methyl transfer reaction. Proc Natl Acad Sci U S A 2015;112(26): 7954–9.
36. Antonini A, Abbruzzese G, Barone P, et al. COMT inhibition with tolcapone in the treatment algorithm of patients with Parkinson's disease (PD): relevance for motor and non-motor features. Neuropsychiatr Dis Treat 2008;4(1):1–9.
37. International Transporter Consortium, Giacomini KM, Huang SM, et al. Membrane transporters in drug development. Nat Rev Drug Discov 2010;9(3):215–36.

38. Hediger MA, Romero MF, Peng JB, et al. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteinsIntroduction. Pflugers Arch 2004;447(5):465–8.
39. Morrissey KM, Wen CC, Johns SJ, et al. The UCSF-FDA TransPortal: a public drug transporter database. Clin Pharmacol Ther 2012;92(5):545–6.
40. Ding X, Kaminsky LS. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. Annu Rev Pharmacol Toxicol 2003;43:149–73.
41. Peters SA, Jones CR, Ungell AL, et al. Predicting drug extraction in the human gut wall: assessing contributions from drug metabolizing enzymes and transporter proteins using preclinical models. Clin Pharmacokinet 2016;55(6):673–96.
42. Pavek P, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. Curr Drug Metab 2008;9(2):129–43.
43. Mizuma T. Intestinal glucuronidation metabolism may have a greater impact on oral bioavailability than hepatic glucuronidation metabolism in humans: a study with raloxifene, substrate for UGT1A1, 1A8, 1A9, and 1A10. Int J Pharm 2009; 378(1–2):140–1.
44. Lown KS, Mayo RR, Leichtman AB, et al. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin Pharmacol Ther 1997;62(3):248–60.
45. Fricker G, Drewe J, Huwyler J, et al. Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation. Br J Pharmacol 1996; 118(7):1841–7.
46. Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administered drugs. Pharm Res 2009;26(9):2039–54.
47. Chan LM, Lowes S, Hirst BH. The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. Eur J Pharm Sci 2004;21(1):25–51.
48. Jovicic S, Ignjatovic S, Majkic-Singh N. Biochemistry and metabolism of vitamin D. J Med Biochem 2012;31(4):309–15.
49. Dai Y, Iwanaga K, Lin YS, et al. In vitro metabolism of cyclosporine A by human kidney CYP3A5. Biochem Pharmacol 2004;68(9):1889–902.
50. Zheng S, Tasnif Y, Hebert MF, et al. CYP3A5 gene variation influences cyclosporine A metabolite formation and renal cyclosporine disposition. Transplantation 2013;95(6):821–7.
51. Wynne HA, Cope LH, Mutch E, et al. The effect of age upon liver volume and apparent liver blood flow in healthy man. Hepatology 1989;9(2):297–301.
52. Woodhouse KW, Wynne HA. Age-related changes in liver size and hepatic blood flow. The influence on drug metabolism in the elderly. Clin Pharmacokinet 1988; 15(5):287–94.
53. Wynne H. Drug metabolism and ageing. J Br Menopause Soc 2005;11(2):51–6.
54. Kando JC, Yonkers KA, Cole JO. Gender as a risk factor for adverse events to medications. Drugs 1995;50(1):1–6.
55. Zhou S, Yung Chan S, Cher Goh B, et al. Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs. Clin Pharmacokinet 2005;44(3):279–304.
56. Zhao Y, Hebert MF, Venkataramanan R. Basic obstetric pharmacology. Semin Perinatol 2014;38(8):475–86.
57. Feghali M, Venkataramanan R, Caritis S. Pharmacokinetics of drugs in pregnancy. Semin Perinatol 2015;39(7):512–9.

58. Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy: how does drug disposition change during pregnancy and what are the mechanisms that cause such changes? Drug Metab Dispos 2013;41(2):256–62.
59. Woolsey SJ, Mansell SE, Kim RB, et al. CYP3A Activity and expression in nonalcoholic fatty liver disease. Drug Metab Dispos 2015;43(10):1484–90.
60. Weiler S, Zoller H, Graziadei I, et al. Altered pharmacokinetics of voriconazole in a patient with liver cirrhosis. Antimicrob Agents Chemother 2007;51(9):3459–60.
61. Pasqualotto AC, Xavier MO, Andreolla HF, et al. Voriconazole therapeutic drug monitoring: focus on safety. Expert Opin Drug Saf 2010;9(1):125–37.
62. Weltman MD, Farrell GC, Hall P, et al. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology 1998;27(1):128–33.
63. Schuck RN, Zha W, Edin ML, et al. The cytochrome P450 epoxygenase pathway regulates the hepatic inflammatory response in fatty liver disease. PLoS One 2014;9(10):e110162.
64. Tsunedomi R, Iizuka N, Hamamoto Y, et al. Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. Int J Oncol 2005;27(3):661–7.
65. Hardwick RN, Ferreira DW, More VR, et al. Altered UDP-glucuronosyltransferase and sulfotransferase expression and function during progressive stages of human nonalcoholic fatty liver disease. Drug Metab Dispos 2013;41(3):554–61.
66. Chen J, Zhao KN, Chen C. The role of CYP3A4 in the biotransformation of bile acids and therapeutic implication for cholestasis. Ann Transl Med 2014;2(1):7.
67. Thomson BK, Nolin TD, Velenosi TJ, et al. Effect of CKD and dialysis modality on exposure to drugs cleared by nonrenal mechanisms. Am J Kidney Dis 2015; 65(4):574–82.
68. Ladda MA, Goralski KB. The effects of CKD on cytochrome P450-mediated drug metabolism. Adv Chronic Kidney Dis 2016;23(2):67–75.
69. Joy MS, Frye RF, Nolin TD, et al. In vivo alterations in drug metabolism and transport pathways in patients with chronic kidney diseases. Pharmacotherapy 2014; 34(2):114–22.
70. Dostalek M, Court MH, Yan B, et al. Significantly reduced cytochrome P450 3A4 expression and activity in liver from humans with diabetes mellitus. Br J Pharmacol 2011;163(5):937–47.
71. Li M, Zhao Y, Humar A, et al. Pharmacokinetics of drugs in adult living donor liver transplant patients: regulatory factors and observations based on studies in animals and humans. Expert Opin Drug Metab Toxicol 2016;12(3):231–43.
72. Masuda S, Goto M, Kiuchi T, et al. Enhanced expression of enterocyte P-glycoprotein depresses cyclosporine bioavailability in a recipient of living donor liver transplantation. Liver Transpl 2003;9(10):1108–13.
73. Liu Z, Chen Y, Tao R, et al. Tacrolimus-based versus cyclosporine-based immunosuppression in hepatitis C virus-infected patients after liver transplantation: a meta-analysis and systematic review. PLoS One 2014;9(9):e107057.
74. Liow JS, Lu S, McCarron JA, et al. Effect of a P-glycoprotein inhibitor, Cyclosporin A, on the disposition in rodent brain and blood of the 5-HT1A receptor radioligand, [11C](R)-(-)-RWAY. Synapse 2007;61(2):96–105.
75. Food and Drug Administration-Center for Drug Evaluation and Research. Guidance for industry: drug interaction studies — study design, data analysis, implications for dosing, and labeling recommendations. Rockville, MD: Food and Drug Administration; 2012. p. 79.
76. Gubbins PO, Amsden JR. Drug-drug interactions of antifungal agents and implications for patient care. Expert Opin Pharmacother 2005;6(13):2231–43.

77. Bruggemann RJ, Alffenaar JW, Blijlevens NM, et al. Clinical relevance of the pharmacokinetic interactions of azole antifungal drugs with other coadministered agents. Clin Infect Dis 2009;48(10):1441–58.
78. Lin JH, Lu AY. Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet 1998;35(5):361–90.
79. Daly AK, Day CP, Aithal GP. CYP2C9 polymorphism and warfarin dose requirements. Br J Clin Pharmacol 2002;53(4):408–9.
80. Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 2001;52(4):349–55.
81. Furuya H, Fernandez-Salguero P, Gregory W, et al. Genetic polymorphism of CYP2C9 and its effect on warfarin maintenance dose requirement in patients undergoing anticoagulation therapy. Pharmacogenetics 1995;5(6):389–92.