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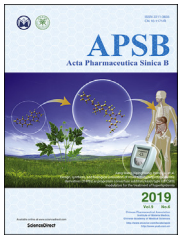

ANNUAL REVIEW

Current trends in drug metabolism and pharmacokinetics

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Abstract Pharmacokinetics (PK) is the study of the absorption, distribution, metabolism, and excretion (ADME) processes of a drug. Understanding PK properties is essential for drug development and precision medication. In this review we provided an overview of recent research on PK with focus on the following aspects: (1) an update on drug-metabolizing enzymes and transporters in the determination of PK, as well as advances in xenobiotic receptors and noncoding RNAs (ncRNAs) in the modulation of PK, providing new understanding of the transcriptional and posttranscriptional regulatory mechanisms that result in inter-individual variations in pharmacotherapy; (2) current status and trends in assessing drug−drug interactions, especially interactions between drugs and herbs, between drugs and therapeutic biologics, and microbiota-mediated interactions; (3) advances in understanding the effects of diseases on PK, particularly changes in metabolizing enzymes and transporters with disease progression; (4) trends in mathematical modeling including physiologically-based PK modeling and novel animal models such as CRISPR/Cas9-

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based animal models for DMPK studies; (5) emerging non-classical xenobiotic metabolic pathways and the involvement of novel metabolic enzymes, especially non-P450s. Existing challenges and perspectives on future directions are discussed, and may stimulate the development of new research models, technologies, and strategies towards the development of better drugs and improved clinical practice.

1. Introduction

Pharmacokinetics (PK) is defined as the quantitative study of drug absorption, distribution, metabolism, and excretion (ADME)—*i.e.*, the ways the body processes a drug[1] while the drug exerts its actions in the body. The scope of PK not only covers studies on healthy subjects but also includes broad research on variations under a variety of physiologic or pathologic conditions and the underlying mechanisms, potential drug−drug interactions (DDI), and possible strategies such as dose adjustment to achieve precision medication. Collectively, these aspects of PK allow customization of drug dosage regimens to enhance therapeutic outcomes[1]. Therefore, PK study is a prerequisite to establish the relations and the underlying mechanisms of a drug to its activities and clinical benefits. The information obtained is crucial for lead identification and optimization in drug discovery, as well as dosage regimen design and adjustment in clinical practice[2]. The complexity of PK has evolved, largely in relation to the rapid developments in analytical chemistry, computer science, molecular biology and biochemistry. Although much is known with regard to the PK of many drugs, and many technologies have been established for PK research, recent studies are revealing the existence of new mechanisms by which how drugs are metabolized and how PK is regulated. New experimental models and computational modeling algorithms are arising for an improved understanding of the significance of PK in a whole-body system; nonetheless, many challenges remain.

This review will provide a comprehensive overview of recent developments in the areas of PK research. First, we will provide an update of findings on drug-metabolizing enzymes and transporters in the control of PK, as well as advances in nuclear receptors and noncoding RNAs (ncRNAs) in the modulation of PK, which will provide new insights into understanding the transcriptional and posttranscriptional regulatory mechanisms behind inter-individual variations in pharmacotherapy. Second, we will review the current status and trends in assessing DDIs, especially the interactions between drugs and herbs, between drugs and therapeutic biologics, and microbiota-mediated DDIs and HDIs. Third, we will summarize recent advances in disease−drug interactions, in particular, regulation of metabolizing enzymes and transporters and alteration of PK by different diseases or physiological states. Fourth, we will summarize the trends in mathematical modeling including physiologically-based PK, which could be applied to support clinical investigations. In addition, we will discuss novel animal models such as CRISPR/Cas9-based animal models for DMPK research and overview some interesting non-classical biotransformation pathways including those utilizing novel drug-metabolizing enzymes. Existing challenges and future perspectives are also discussed. It is expected that this review will provide an update on recent advances in PK fields and may stimulate the establishment of new research models, technologies, and strategies towards the development of better drugs and improvements in clinical practice.

2. Determinants of PK

Drug-metabolizing enzymes and transporters play a very important role in the control of PK. Furthermore, transcriptional and posttranscriptional factors such as nuclear receptors and noncoding RNAs (ncRNAs) are critical in the modulation of PK and provide in-depth insight into understanding regulatory mechanisms to solve problems in PK. These mechanism-driven PK studies can improve the success of drug development related to its efficacy and safety and improve the rational use of medication in clinical practice.

2.1. *Drug-metabolizing enzymes in the control of PK*

Drug-metabolizing enzymes mediate the metabolism of exogenous and endogenous substances. Most drugs lose their pharmacological activities mainly through metabolic transformation, yielding metabolites with high water solubility that are readily excreted. Hence, metabolizing enzymes play an extremely important role in the control of drug PK. The biotransformation of xenobiotics by xenobiotic-metabolizing enzymes (XMEs) may be classified into Phase I and Phase II reactions. Advanced characterizations of enzymes involved in human drug metabolism are urgently needed, which help to avoid severe adverse drug reactions. Advances are being made in understanding the role of drug-metabolizing enzymes in the control of PK, including individual isoforms of many enzymes such as cytochrome P450s (CYPs) and UGTs, and their selective substrates, inducers and inhibitors. Other non-P450 oxidative enzymes and conjugative enzymes are also discussed in this section since an increasing number of drugs are metabolized *via* these enzymes[3].

2.1.1. *CYPs critical for PK*

CYPs can oxidize foreign substances, enhance the water solubility and make drugs easier to be eliminated from the body. Most drugs are metabolized by CYPs, which mainly are located in the inner membrane of mitochondria or the endoplasmic reticulum of cells[4]. There are a total of 57 human CYP genes in 18 families. The members of CYP1 to CYP4 families oxidize thousands of exogenous and endogenous substrates (Table 1); whereas all members of CYP5 family and higher principally metabolize endogenous substrates in a highly substrate-specific manner[5].

Most known chemical carcinogens, including aromatic amines and polycyclic aromatic hydrocarbons (PAHs), are substrates of CYP1 family, and their metabolism often results in the formation of active carcinogenic metabolites. In 2018, CYP1B1 was found in the mitochondria of cancer cells, where it reportedly metabolizes melatonin to form the metabolite *N*-acetylserotonin (NAS), which has antitumor effects[6]. CYP2D6, another important metabolic enzyme, is involved in the metabolism of many anti-cancer drugs, such as cyclophosphamide, tamoxifen, and gefitinib[7]. Recent research has found that in brain, CYP2D6 can metabolize both m-tyramine and p-tyramine into dopamine[8]. The CYP4 family has gained increasing attention for its potential to generate interesting metabolites and dispose of endogenous substrates in recent years. CYP4F11, together with CYP4F2, plays an important role in the synthesis of 20-hydroxyeicosatetraenoic acid (20-HETE) from arachidonic acid, and participates in the metabolism of vitamin K[9]. *Cyp2a5*, the mouse correlate of human *CYP2A6*, encodes an enzyme that exhibited circadian regulation[10]. The other CYP1 to CYP4 subfamilies are involved in metabolism of different endogenous and exogenous substrates, as listed in Table 1.

Understanding variation in mechanism-based enzyme activity is crucial for improving the clinical use of drugs. Highly selective inducers and inhibitors of CYPs have been cited in Guidance for Industry by FDA (https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers). Recent studies have revealed new chemicals and herb products as inducers or inhibitors of CYPs. For example, CYP7A1 is upregulated by an intestinal HIF-2α inhibitor called PT2385[17]. The ketene intermediate of erlotinib can inactivate CYP3A4 and CYP3A5, which can result in liver injury[18]. Due to the complexity of components in the extract of herbs it is common that herb products exhibit different effects on the regulation of multiple enzymes. *Sophora flavescens* can inhibit CYP2B6, CYP2C8, CYP2C9, and CYP3A activities, while catalpol can inhibit the activity of CYP3A4, CYP2E1 and CYP2C9[19,20]. Other regulatory factors can also alter the expression of CYPs. For example, tumor suppressor p53 can regulate *Cyp2b10* directly and thereby attenuate APAP-induced hepatotoxicity[21].

Herbs may be used singly or in combination in the treatment of diseases[22]. It is very important to understand how drug exposure alters molecular mechanisms underlying many complex drug interactions. For example, data show that ellagic acid from pomegranate peel guava leaf extract can significantly increase the AUC of warfarin with concomitant use. A significant reduction in CYP2C8, 2C9, and 3A4 activity was the main reason for this interaction[23].

Based on recently available data, new information on the relative content of individual isoforms of P450 has been generated. Total CYP concentrations are significantly different between Chinese and Caucasian populations and the metabolic capabilities of CYPs in Chinese liver microsomes was significantly lower (<50%) in the CL_{int} for substrates of CYP1A2, CYP2C9, CYP2C19 and CYP2E1 than those of Caucasian populations[24]. Large variations in protein content, mRNA levels, and intrinsic activities of ten P450s (CYP3A4, 1A2, etc) have been revealed and some single nucleotide polymorphisms had significant impact on P450 expression; for example, CYP2C19 activity varied more than 600-fold[25]. A recent human PK study further evaluated CYP1A2 content in Chinese compared with Caucasian populations, enhancing the confidence in pharmacokinetic prediction of CYP1A2 content using two substrates (caffeine and theophylline)[26].

Other organs like kidney and intestine also have significant metabolic capacity. There is definitive evidence for CYP2B6 and 3A5 expression in human kidney, while multiple CYPs are expressed in intestine[27,28]. The role of renal and intestinal enzymes in herbal product metabolism has been uncovered. Aminoglycoside antibiotics are leading causes for nephrotoxicity; combination with herbs or dietary supplements at reduced dosage is possible to reduce the risk of drug-mediated renal toxicity. A recent study revealed that moringa oleifera seed oil could limit gentamicin-induced oxidative nephrotoxicity[29]. Additional herbs have been identified as having effects on intestinal metabolism, such as the extracts of Yin-Chen-Hao Tang (YCHT), a very popular hepatoprotective three-herb formula in China and Japan[30]. These findings contribute to the understanding of the metabolic characteristics of renal and intestinal metabolism.

2.1.2. *Non-P450 oxidative enzymes*

The contribution of non-P450 enzymes to drug metabolism can be significant and affect the overall development of drugs. Non-CYP enzymes can be divided into four general categories: namely oxidative, reductive, conjugative, and hydrolytic. Non-CYP oxidative enzymes include flavin-containing monooxygenases (FMOs), monoamine oxidases (MAOs), peroxidases, xanthine oxidases (XO), aldehyde oxidase (AO), alcohol dehydrogenase (ADHs) and aldehyde dehydrogenase (ALDHs)[31].

Very little is known about the regulation of content and activity of non-P450 oxidative enzymes. Recently, some selective substrates and inhibitors of non-P450 enzymes have been identified in natural products and other sources. FMOs are involved in the metabolism of a wide array of xenobiotics. Well-known inhibitors of FMOs include indole-3-carbinol and methimazole, and 2-mercaptobenzimidazole[32]. Classified into two different isoforms (MAO-A, MAO-B), MAOs are enzymes involved in the catabolism of monoamines. Benextramine and its derivatives were identified as novel human monoamine oxidases inhibitors, which could be considered as candidate drugs for the treatment of neurodegenerative diseases[33]. In addition, 3-(3-(dimethylamino)propanoyl)-7-hydroxy-5-methyl-2*H*-chromen-2-one hydrochloride has been found to function as a novel selective hMAO-B inhibitor, which is expected to be a promising multifunctional Parkinson's disease treatment agent[34]. XO and AO are involved in the oxidation of aldehydes and heterocycles, and carbazeran was used as a selective probe substrate of AO in hepatocytes[35]. Allopurinol and *S*-allyl cysteine (SAC) are XO inhibitors used in the treatment of gout and hyperuricemia[36]. A single-nucleotide polymorphism of human cytochrome P450 oxidoreductase (POR) in the Chinese population can regulate the content of POR and P450 isoforms[37]. Identifying specific inhibitor compounds will greatly facilitate investigation of enzyme-mediated drug disposition and drug interactions.

2.1.3. *Importance of UDP-glucuronyltransferases (UGTs) in PK*

UDP-glucuronyltransferases (UGTs) are a family of endoplasmic reticulum-bound enzymes which are responsible for the process of glucuronidation, a major part of phase II metabolism[38]. Human UGTs include 22 different functional enzymes and are classified into four gene families, UGT1, UGT2, UGT3 and UGT8[39]. The UGT1 and UGT2 families are primarily enzymes involved in drug glucuronidation, while the contribution of the UGT3 and UGT8 families to drug metabolism is minimal[40].

Recently, UGT1A3 was found to be involved in the glucuronidation of alpinetin[41]. UGT1A4 is involved in the glucuronidation of metizolam[42]. Other UGT isoforms involved endogenous and exogenous substrates are listed in Table 2[23,43–46].

Table 1 Endogenous and exogenous substrates of CYPs and ligands of transcription factors.

Family	Enzyme	Endogenous substrate	Xenobiotic substrate	Transcription factor
CYP1	CYP1A1	Steroid (especially estrogen), aromatic amines, polycyclic aromatic hydrocarbons	Benzo[*a*]pyrene	AhR CAR
	CYP1A2		Phenacetin[11]	AhR, CAR
	CYP1B1	Steroid (especially estrogen), melatonin[6]	Aromatic amines, polycyclic aromatic hydrocarbons	AhR
CYP2	CYP2A6	Steroid	Nicotine, cotinine, coumarin[12,13]	PXR, NFE2L2, ER, GR, PXR, HNF4α
	CYP2A13	Unknown	Nicotine, coumarin, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)[14], naphthalene[15]	FOXA2
	CYP2B6	Synthesis of cholesterol, steroids and other lipids.	Bupropion[11], efavirenz	CAR, PXR, HNF4α
	CYP2C8	Arachidonic acid[16]	Paclitaxela, repaglinide, AZD9496, Taxol	CAR, PXR, ROR, VDR
	CYP2C9	Serotonin, polyunsaturated fatty acids, arachidonic acid.	Warfarin, phenytoin, tolbutamide	PXR, CAR, VDR, HNF4α
	CYP2C18	Arachidonic acid, linoleic acid, docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA).	Tolbutamide, cyclophosphamide, ifosfamide	Unknown
	CYP2C19	Arachidonic acid	*S*-Mephenytoin	PXR, CAR, FOXA3
	CYP2D6	Hydroxytryptamines, neurosteroids, *m*-tyramine, *p*-tyramine[8]	Tamoxifen, gefitinib, cyclophosphamide, bufuralol	HNF4α
	CYP2E1	Arachidonic acid	Chlorzoxazone (CHZ), acetaminophen	LXR, HNF1α, NRF2
	CYP2F1	3-Methylindole (3MI)	Naphthalene, benzene, 1,1-dichloroethylene	Unknown
	CYP2J2	Arachidonic acid, vitamin D3	Astemizole	Unknown
	CYP2R1	Vitamin D3	Unknown	Unknown
	CYP2S1	Prostaglandin G(2)/H(2), thromboxane A(2), oxygenated eicosanoids	Benzo[*a*]pyrene-7,8-diol	Unknown
	CYP2U1	Arachidonic acid, docosahexaenoic acid (DHA)	Debrisoquin sulfate	Unknown
	CYP2W1	Fatty acids, lysophospholipids,retinoic acid	Canduocarmycin	Unknown
CYP3	CYP3A4	Steroid (including testosterone), vitamin D3	Midazolam, rivaroxaban, 3-acetyl-11-keto-β-boswellic acid (AKBA)	CAR, PXR, FXR, HNF4α, LXR, VDR
	CYP3A5	Steroid (including testosterone), progesterone, Rostenedione	Diltiazem, cyclosporine, 3-acetyl-11-keto-β-boswellic acid (AKBA)	PXR, LXR, HNF4α
	CYP3A7	Steroid (including testosterone)	3-acetyl-11-keto-β-boswellic acid (AKBA)	Glucocorticoid receptor (GR), PXR
	CYP3A43	Androgen	Alprazolam	Unknown
CYP4	CYP4A11 CYP4A22	Arachidonic acid, fatty acid, lauric acid	Unknown	PPARα
	CYP4B1	Furan pro-toxin 4-ipomeanol	Pneumotoxin, 4-ipomeanol, aromatic amines, 2-aminofluorene	Unknown
	CYP4F2	Arachidonic acid, vitamin K menaquinone, leukotrienes, prostaglandins	Pafuramidine, fingolimod	Unknown
	CYP4F3	Arachidonic acid, prostaglandins, leukotriene-B4	Pafuramidine	Unknown
	CYP4F8	Arachidonic acid, prostaglandins, eicosanoids, dihomo-γ-linolenic acid, leukotrienes, 19-hydroxylase of prostaglandin endoperoxides (PGEs)	Unknown	Unknown
	CYP4F11	Arachidonic acid, vitamin K menaquinone[9], prostaglandins, leukotrienes	Benzphetamine, ethylmorphine, chlorpromazine, imipramine, erythromycin	RXR
	CYP4F12	Arachidonic acid, docosahexaenoic and eicosapentaenoic acids, prostaglandins, leukotrienes	Ebastine, terfenadine	PXR
	CYP4F22	Arachidonic acid, eicosanoids, prostaglandins, leukotrienes	Unknown	Unknown
	CYP4V2	Medium chain fatty acids	Unknown	PPARγ
	CYP4X1	Arachidonic acid, anandamide	Unknown	PPARα
	CYP4Z1	Lauric acid, myristic acid	Unknown	Unknown

Highly selective substrates and selective inhibitors of UGTs have been found in natural products and from other sources. Resveratrol can activate UGT1A8 expression, and is used for breast cancer treatment[47]. Different doses of emodin can inhibit the activity of UGT2B7[48].

In some cases, herbal products are metabolized by multiple UGTs. Linoleic acid and glutaric acid can inhibit the glucuronidation of berberrubine, a lipid-lowing metabolite of berberine, as well as the activities of UGT isoforms, such as UGT1A7, 1A8, 1A9[49]. Glucuronidation of catalposide, an active component of *Veronica* species, was catalyzed by gastro-intestine-specific UGTs 1A8 and 1A10[50].

Gene polymorphisms are a key factor in the regulation of the content and activity of UGTs. UGT1A and UGT2B genetic variation can alter nicotine and nitrosamine glucuronidation in European and African American smokers[51]. In addition, the *UGT1A4*3* genetic polymorphism is associated with low posaconazole plasma concentrations in patients with hematological malignancies[52]. *UGT1A1*6* polymorphisms are correlated with irinotecan-induced neutropenia in cancer patients[53].

2.1.4. *Other conjugative enzymes important for PK studies*

In addition to UGTs, sulfonyl transferases (SULTs) and glutathione *S*-transferases (GSTs) are also important conjugative enzymes mediating phase II reaction.

SULTs catalyze the transfer of the water-soluble sulfonate group from 3′-phosphoadenosine 5′-phosphosulfate to drugs or endogenous molecules that contain hydroxy or amine group(s)[54]. At present, four families of human SULTs have been discovered, namely SULT1, SULT2, SULT4 and SULT6. SULT1E1 plays an important role in the metabolism and detoxification of estrogens and flavonoids[55]. SULT2 enzymes, mainly SULT2A and SULT2B, are primarily responsible for catalyzing the sulfation of hydroxysteroids[56]. A recent study found that tumor suppressor p53 could regulate the expression of SULTs[57].

GSTs are a group of phase II drug-metabolizing enzymes that catalyze the binding of glutathione to various electrophilic compounds. In humans, cytosolic GST isoenzymes of the alpha, zeta, theta, mu, pi, sigma and omega classes have been found. GSTA1 plays a significant role in the metabolism of acetaminophen[58]. GSTA4 metabolizes electrophilic and carcinogenic substances such as endogenous carcinogen 4-hydroxy-2-nonenal[59]. The detailed substrates of SULTs and GSTs are listed in Table 3.

2.1.5. *Updates on the nuclear receptor-mediated regulation of xenobiotic-metabolizing enzymes*

The human nuclear receptors comprise a family of 48 ligand-regulated transcription factors that in turn regulate target genes involved in metabolism and other physiological functions. Some of these receptors (*e.g.*, peroxisome proliferators-activated receptor (PPAR), liver X receptor (LXR), hepatocyte nuclear factor (HNF)) are of particular interest in regard to drug metabolism and disposition as they have been found to regulate many XMEs in recent years.

PPARα induces the expression of CYP4A in response to a heterogeneous group of peroxisome proliferators. PPARγ also regulates the expression of CYP4V2, a fatty acid metabolizing enzyme, in human tetrahydropyranyl 1 (THP1) macrophages[60]. LXR controls the transcription of *Cyp7a1* and *Cyp27a1*, *Cyp3a11* and *Cyp2e1*[61–63].

Traditional transcriptional factors can bind directly to specific DNA sequences and thus control the gene expression. However, epigenetic regulation like histone modification and DNA methylation modulates transcription of UGTs or CYPs mainly by changing chromatin architecture. For example, the *UGT1A* gene can be repressed by the recruitment of histones in females[64]. Several studies determined that microRNAs (miRNAs), could down-regulate the expression of metabolizing enzymes, which will be further reviewed in Section 2.3.

In summary, the expression and activity of metabolizing enzymes can be regulated by multiple factors, including drugs, nuclear receptors, gene polymorphisms, and even ethnic categories. Non-P450 enzymes and other conjugative metabolizing enzymes have gained attention in drug metabolism in recent years. It is desirable to illustrate the key factors responsible for variable expression and activity of drug metabolizing enzymes, as it may be beneficial in the prediction of potential therapeutics, drug−drug interactions, and in modifying the PK of drugs.

2.2. *Transporters in the control of PK*

2.2.1. *Introduction of transporters*

Transporters are membrane-bound proteins expressed on the cell membrane in most tissues with varying abundance. They can transport a variety of endogenous or exogenous substrates (such as drugs and their metabolites) in and out of cells. For drugs, transporters are the gatekeepers for cells and control the uptake and efflux of drugs. Transporters are involved in the ADME process of drugs. Therefore, transporters play critical roles in the pharmacokinetics, efficacy and toxicity of drugs. Alteration of transporter function or expression may significantly change the blood and/or tissue exposure of drugs, leading to significant changes in pharmacokinetics. Furthermore, the induction or inhibition of transporters by co-administered drugs can change PK and pharmacodynamics of therapeutic drugs and produce DDI.

There are more than 400 membrane transporters belonging to two major superfamilies: adenosine triphosphate (ATP)-binding cassette (ABC) and solute carrier (SLC) transporters. They utilize the energy that is released by ATP hydrolysis or an electrochemical ion gradient to translocate drugs across the membrane.

2.2.1.1. The ABC family of drug transporters. ABC transporters mainly act as exporters and pump drug molecules out of cells by utilizing the energy released by the hydrolysis of ATP. According to the organization and sequence of ATP-binding domains, 49 ABC transporters are classified into seven subfamilies: ABC1/ABCA, multidrug resistance (MDR)/TAP/ABCB, MRP/ABCC, ALD/ABCD, OABP/ABCE, GCN20/ABCF and White/ABCG[65]. Among them, P-glycoprotein (P-gp, MDR1, ABCB1), MRPs/ABCCs, breast cancer resistance protein (BCRP/ABCG2) and bile salt export pump (BSEP/ABCB11) are recognized for their importance in drug disposition[66]. P-gp, which is expressed at a high level in the intestine, liver, kidney, brain and placenta, is the most studied ABC transporter. Many substrates of P-gp including antibiotics, statins, immunosuppressants, anticancer drugs and a broad spectrum of drugs overlap with the substrates of CYPs. The expression of P-gp is regulated by several transcription factors including PXR, CAR, vitamin D receptor (VDR) and CCAAT/enhancer binding protein (C/EBP) and some microRNA such as miR-451, miR-27a and miR-145[67,68]. Furthermore, P-gp is usually overexpressed in cancer cells and plays a critical role in MDR. For example, during chemotherapy, P-gp may be an obstacle for drug exposure if the therapeutic drugs are P-gp substrates[69]. Besides its

Table 2 Endogenous and exogenous substrates of UGTs and ligands of transcription factors.

Family	Enzyme	Endogenous substrate	Xenobiotic substrate	Transcription factor
UGT1A	UGT1A1	Bilirubin, estradiol, fatty acids	SN-38, leonurine, bergenin, axitinib	CAR, PXR, PPARα, AhR[43], NRF2
	UGT1A3	Bile acid, arachidonic	Polyaromatic amines, non-steroidal anti-inflammatory drugs, statins, ahydroxygenkwanin, genkwanin, ursolic acid[44], fimasartan[45], alpinetin[23]	PPARα, HNF1, AhR, LXR, PXR
	UGT1A4	Eicosanoids	Imipramine, lamotrigine, clonazolam, deschloroetizolam, etizolam, flubromazolammetizolam	HNF1, PPARα, PXR, CAR, AhR, HNF1α
	UGT1A6	Serotonin	1-Naphthol 4-nitrophenol	AhR, CAR, PXR, PPARα
	UGT1A7	Unknown	Icaritin, carcinogens	AhR, HNF1, HNF4α, NRF2
	UGT1A8	Fatty acids	Retinoids, catechol estrogens, opioids, coumarins, flavonoids, anthraquinones, phenols, raloxifene	HNF1, HNF4α, AhR, NRF2
	UGT1A9	Steroids, fatty acids	Bulky phenols, propofol, mycophenolic acid, niflumic acid, psoralidin	CAR, HNF1, HNF4α, PPARα, AhR, NRF2
	UGT1A10	Estrogens	Nitrosamine, flavonoids, polycyclic aromatic hydrocarbons, raloxifene, dopamine	HNF1α, HNF4α, AhR, NRF2
UGT2A	UGT2A2/3	Hyodeoxycholic acid	Tobacco carcinogen	HNF1, LXR
UGT2B	UGT2B4	Arachidonic acid	Naftopidil, deoxynivalenol	PPARα, AhR, FXR
	UGT2B7	Sex-steroid hormones, glucocorticoids, mineralocorticoid, bile acids	Naftopidil, deoxynivalenol, mirabegron, efavirenz, zidovudine, codeine, morphine	HNF1α[46], CAR, PXR, FXR, PPAR, NRF2
	UGT2B10	Eicosanoids	Amitriptyline, imipramine, clomipramine, trimipramine	CAR, FXR, AR
	UGT2B11	Unknown	3a-Hydroxyandrogens, 3a-pregnanes, Hydroxylestrogens	ER, AR
	UGT2B15	Sex-steroid hormones	Oxazepam, lorazepam, sipoglitazar, bisphenol-A	AR, ER, HNF3α, FXR
	UGT2B17	Sex-steroid hormones	Coumarins, anthraquinones flavonoids, chlorantraniliprole	HNF1α, HNF4α, HNF3α, AR, ER
	UGT2B28	Sex-steroid hormones	Unknown	ER, AR
UGT3	UGT3	Unknown	*N*-Acetylglucosamine	Unknown
UGT8	UGT8A1	Bile acids	Unknown	LXR

role in MDR induction, P-gp plays a critical role in pharmacokinetics, pharmacology and toxicology. Through pumping multiple drugs out of cells, P-gp decreases the bioavailability of oral drugs and increases drug efflux into urine or bile. Furthermore, P-gp also plays a vital role in the maintenance of the blood−brain barrier by pumping drugs or toxins out of the CNS[70]. Another important ABC transporter group is the MRP family that consists of 9 MRP proteins (MRP1−MRP9). Among them, MRP2 is important in drug pharmacokinetics. MRP2, once known as the canalicular multispecific organic anion transporter, is highly expressed in liver, intestine and kidney. Chemotherapeutics such as methotrexate, melphalan, and statins are the classical substrates of MRP2. Since co-expressed in the liver, many liver-enriched transcription factors such as LXR, farnesoid X receptor (FXR), HNF and C/EBP regulate the transcription of MRP2. Another efflux transporter BCRP is a half transporter and is expressed at a high level in a wide variety of tissues such as intestine, kidney, liver, testis and brain. BCRP is modulated by the progesterone receptor B (PRB) and estrogen receptor (ER). Another ABC family drug transporter, BSEP, is primarily expressed in the liver and pumps bile acids and non-bile acid drugs such as pravastatin into bile.

2.2.1.2. The SLC family of drug transporters. The SLC family consists of 55 gene subfamilies and more than 360 family members. SLC transporters mainly utilize the energy stored in the ion gradients across membranes, but do not depend directly on ATP hydrolysis[71]. Several SLC family transporters play important roles in drug disposition including organic anion-transporting proteins (OATPs/SLC21/SLCO), organic anion and cation transporters (OATs and OCTs/SLC22), peptide transporters (PEPTs/SLC15) and sodium-dependent bile acid transporters (NTCP/SLC10A1). The OATP family consists of 11 members. Among them, four transporters including OATP1A2 (SLCO1A2), OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3) and OATP2B1 (SLCO2B1) are involved in drug transport[72]. OATP1A2 is expressed in the intestinal epithelium, renal epithelium and brain capillary endothelial cells, while OATP1B1, OATP1B3 and OATP2B1 are expressed predominantly in hepatocytes. Statins and anti-cancer drugs like paclitaxel, sorafenib and methotrexate are known as the substrates of OATPs. The SLC22 family consists of 23 members, including OCTs, zwitterion/cation transporters (OCTNs) and OATs. Among OCTs, OCT1 (SLC22A1) is mainly expressed in the liver, OCT2 (SLC22A2) is located at a high level in proximal tubular cells, and OCT3 (SLC22A3) has a broader expression range. Several drugs have been identified as OCT substrates including anesthetic drugs, the anti-diabetic drug metformin, antidepressants, β-blockers and anti-cancer chemotherapeutics. Among OATs, OAT1 (SLC22A6) and OAT3 (SLC22A8) have a broader expression range with the highest expression in

Table 3 Endogenous and xenobiotic substrates for GSTs and SULTs that are also ligands of particular transcription factors.

Enzyme		Endogenous substrate	Xenobiotic substrate	Transcription factor
GSTs		Steroids, bilirubin, heme, fatty acids	1-Chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), 4-nitrobenzyl chloride (pNBC), ethacrynic acid (ETHA), aflatoxin B1 (AFB1), 4-hydroxynonenal (4HNE), acrolein, *N*-acetyl-*p*-benzoquinone imine (NAPQI), cisplatin, busulfan, dichloroacetate, cyclophosphamide, azathioprine (AZA)	PXR, CAR, steroidogenic factor 1 (SF-1), RXR
SULT1	SULT1A1	4-Methylphenol, iodothyronines	4-Nitrophenol	PXR, CAR
	SULT1A2	Dopamine, estrogens, catechol estrogens	4-Nitrophenol, 2-naphthol, naloxone, minoxidil	PXR, CAR, FXR, HNF4α
	SULT1A3	Dopamine, norepinephrine, iodothyronines	6-Hydroxydopamine, hydromorphone	
	SULT1B1	Thyroxine	3-OHB[*a*]P,1-naphthol	
	SULT1C1	Thyroxine	*N*-Hydroxyarylamines	
	SULT1E1	Iodine thyroxine, pregnenolone	1-Naphthol, naringenin, genistein, 4-hydroxytamoxifen	
SULT2	SULT2A	Dehydroepiandrosterone (DHEA), bile acid, cholesterol, estrone	Tibolone, budesonide	
	SULT2B	Dehydroepiandrosterone (DHEA), bile acid, cholesterol, estrone	3β-Hydroxysteroids	

kidney, while OAT2 (SLC22A7) is primarily expressed in the liver. OAT1 substrates include antiviral drugs, antibiotics, diuretics and angiotensin-converting enzyme (ACE) inhibitors. For the SLC15 subfamily, PEPT1 and PEPT2 are the most studied transporters. Both mediate oligopeptide uptake. PEPT1 is highly expressed in the intestine and mediates drug absorption, while PEPT2 is mainly expressed in kidney and affects renal reabsorption.

2.2.2. *Transporters are critical for PK*

The ADME process determines the blood and tissue concentration of drugs, as well as subsequent pharmacological or toxicological effects. The intestine and liver, both of which tightly regulate the entry of drugs into the blood circulation, are important organs in determining the bioavailability of oral drugs. Elimination of drugs or their active metabolites occurs either by metabolism to inactive metabolites that are excreted, or by direct excretion of drugs or active metabolites in the kidney. The transporters expressed in intestine, liver and kidney are involved in the absorption, distribution and excretion processes of drugs, and are the major determinant in blood and tissue concentration of drugs.

2.2.2.1. Transporter-mediated oral drug absorption. Oral drug absorption primarily occurs in the intestine, which is the major determinant of drug bioavailability, together with the first-pass extraction in the liver. Drug molecules pass through the membranes in the intestine through two pathways: passive diffusion and transporter-mediated absorption.

The process of transporter-mediated oral drug absorption consists of two parallel transport processes including transporter-mediated uptake and transporter-mediated efflux[73,74] (Fig. 1A). In general, net drug absorption depends on multiple uptake and efflux transporters in the intestine. Uptake transporters such as OATP2B1, PEPT1 and sodium-dependent bile acid transporter (ASBT/SLC10A2) are involved in the intestinal uptake of drugs across the brush border membrane[75]. For example, PEPT1 transports di/tripeptides-like anticancer drugs such as bestatin and β-lactam antibiotics into enterocytes[76–78]. Efflux transporters expressed on the brush border membrane of the intestine, are considered as the barriers for intestinal drug absorption. P-gp, MRP2 and BCRP are three major efflux transporters in the intestine. P-gp, the most studied efflux transporter, has broad substrate specificity and significantly limits the bioavailability of many oral drugs[79]. For example, co-treatment with verapamil, a P-gp inhibitor, increases the intestinal absorption of afatinib or bestatin due to P-gp inhibition in the intestine[80,81]. On the contrary, rifampin, a P-gp inducer, decreases the oral absorption of cyclosporine and tacrolimus through the induction of P-gp in the intestine[82]. BCRP is another efflux transporter expressed in the intestine and suppresses the intestinal absorption of drugs[83]. Due to only one ATP binding site and six putative transmembrane helices, BCRP is considered a "half-transporter". The substrates of BCRP include statins (pitavastatin, rosuvastatin), antiviral drugs (lamivudine, zidovudine, abacavir), anticancer drugs (methotrexate, SN-38, irinotecan, gefitinib, imatinib, erlotinib) and antibiotics (nitrofurantoin, ciprofloxacin)[84]. The efflux transporter MRP2 is also expressed on the brush border membrane of the intestine and transports a variety of substrates conjugated with sulfate, glutathione and glucuronide, as well as various unmodified drugs. Previous studies showed that resveratrol inhibited MRP2 and thereby increased the intestinal absorption of methotrexate[85].

2.2.2.2. Transporter-mediated drug distribution. Transporters also affect the tissue distribution and contribute to the selective distribution of drugs to specific tissues. For example, OATP1B1 and OATP1B3 are the major uptake transporters in the liver for cilostazol, and MRP2, BCRP, P-gp pump cilostazol out of the liver into bile[86]. These transporters assist the liver-specific distribution of cilostazol. Another example is pravastatin, which enters into the liver through OATP1B1 and OATP1B3. After being excreted into the bile, pravastatin is reabsorbed in the intestine to the portal vein and taken up by the liver, and effectively undergoes enterohepatic circulation[87]. Therefore, the liver concentration should be higher

than that in the circulating blood, leading to a high pharmacological effect at a relatively low plasma concentration. Transporters are also expressed on the blood−brain barrier and play critical roles in restricting the distribution of drugs into the brain. Increasing evidence has demonstrated that P-gp on the blood−brain barrier can suppress the distribution of drugs into the CNS[88,89]. Also, BCRP is recognized as an efflux transporter on the blood−brain barrier suppressing drug entry into the brain. Except for efflux transporters, uptake transporters are also expressed on the blood−brain barrier and play key roles in the uptake of neuroactive drugs. OAT3 is highly expressed on the basolateral membrane of brain capillaries[90], and OCT2 is expressed in neurons and the choroid plexus. OCT2 is involved in the reabsorption of many drugs such as serotonin, norepinephrine, dopamine, choline and histamine from the cerebrospinal fluid.

2.2.2.3. Transporter-mediated drug excretion. Drug elimination primarily occurs in the liver and kidney. Hepatobiliary elimination processes can be summarized as follows: (1) the uptake of drugs into hepatocytes *via* uptake transporters or passive diffusion; (2) drug metabolism in hepatocytes including CYP metabolism (phase I metabolism) and conjugation (phase II metabolism); (3) excretion from hepatocytes into bile or portal blood *via* efflux transporters. Hepatobiliary transport of drugs is attributable to transporters located on the basolateral (sinusoidal) or canalicular (apical) membrane of hepatocytes (Fig. 1B). SLC superfamily transporters are responsible for drug uptake from the portal blood into hepatocytes. Among them, OAT2, OCT1, OATPs and NTCP are major uptake transporters. Efflux transporters such as P-gp, BCRP, MRP2 and BSEP are responsible for the hepatobiliary excretion of drugs and their metabolites. In addition, the efflux transporter MRP3, 4 and 6 expressed on the basolateral membrane are responsible for the basolateral efflux of drugs from the liver into the blood circulation. The hepatic transporters OAT2, OATP1B1/1B3 and OCT1 are highly expressed in the liver and are considered to be of particular importance for hepatic drug elimination, PK and efficacy. Much like the interplay of transport and metabolic enzymes at the intestinal barrier, these transporters also have a "gatekeeper" function in the drug movement from the blood into hepatocytes; they regulate both the number of drugs available for metabolism by liver enzymes and the subsequent biliary excretion. Efflux transporters including P-gp, BCRP, MRP2 and BSEP are responsible for the biliary excretion of endogenous and exogenous molecules. Many studies have shown that P-gp transports amphiphilic cationic drugs such as doxorubicin, digoxin and vinblastine into bile[91]. BCRP is involved in the biliary excretion of sulfated conjugates of steroids and drugs such as doxorubicin, mitoxantrone and daunorubicin, while BSEP transports drugs including vinblastine and taxol, et al. Due to their important roles in hepatobiliary efflux, the inhibition of BSEP, BCRP and MRP2 may lead to cholestasis. Therefore, the effects of chemicals on transporter-mediated hepatobiliary excretion must be determined in drug discovery[92].

The kidney is the major organ of drug excretion. Renal clearance of drugs consists of glomerular filtration, tubular secretion and reabsorption. The proximal tubule region is responsible for the active secretion and reabsorption of drugs. Many transporters are located at the renal tubular epithelial cells and are involved in the proximal tubular secretion and reabsorption (Fig. 1C). These transporters include OCTs, OATs, multidrug

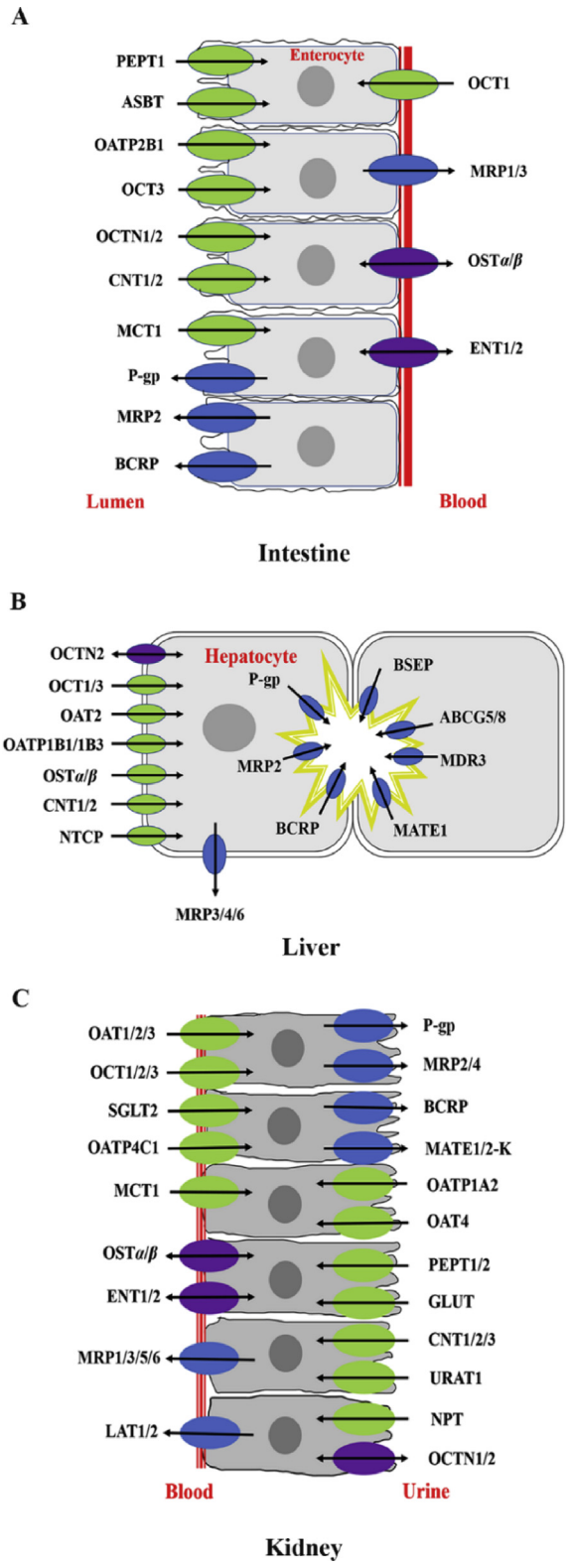

Figure 1 Drug transporter expression in tissues. Drug transporter expression in the intestine (A), liver (B) and kidney (C). The arrows indicate the general directions in which the substrates are transported.

and toxin extrusion proteins (MATE1 and MATE2-K), sodium-phosphate transporter (NPT/SLC17A1), OATPs and PEPTs, as well as equilibrium and concentration nucleoside transporters (ENTs and CNTs/SLC28A). Among them, OCTs, OATs and MATEs play critical roles in the active secretion of renal proximal tubule. These transporters work in concert with efflux transporters to transfer drugs into urine. OATs mainly transport anionic drugs such as beta-lactam antibiotics and anti-inflammatory drugs. The competitive inhibition of OATs may lead to a decrease in renal tubular secretion and an increase in the systemic concentration of drugs. For example, co-administration of probenecid, one OAT inhibitor, decreases renal secretion, leading to an increase in the plasma concentration of bestatin[93]. JBP485, a dipeptide with potential protective activity against kidney, liver and intestinal injury, has been demonstrated to be a substrate of OATs. Co-administration of JBP485 and cephalexin decreased the accumulative renal excretion and renal clearance of both compounds[77]. When JBP485 and lisinopril were co-administered, the competitive inhibition of OAT1 and OAT3 were also observed in OAT1/3-HEK293 cells[94]. In addition, acyclovir, an antiviral drug, was also a substrate of OAT1/3 and JBP485 can inhibit its renal excretion[95]. Furthermore, the DDIs between JBP485 and entecavir through the competitive inhibition of OAT1 and OAT3 significantly decreased the renal excretion of both compounds[96]. On the other hand, OATs are involved in drug-related nephrotoxicity. Probenecid, by inhibiting OAT1 and OAT3, reduced the accumulation of cephaloridine and subsequently nephrotoxicity[97,98].

Three OCT isoforms including OCT1, OCT2 and OCT3 have been found in the kidney. Among the three OCTs, OCT2 is the major transporter for renal secretion of a variety of drugs such as memantine, metformin and amantadine. DDIs may also occur through the competitive inhibition of OCTs. For example, through inhibiting OCT2, cimetidine decreases the renal excretion of metformin and increases its plasma concentration[99]. On the other hand, OCT2, by modulating the exposure of drugs to renal proximal tubule cells regulates the nephrotoxicity of anticancer drug cisplatin and its analogs[100]. Substrates taken up from the systemic circulation may subsequently undergo efflux across the brush border membrane of proximal tubule cells by various ABC efflux transporters such as P-gp and BCRP. For example, a probe P-gp substrate, methotrexate, is actively secreted into urine. Co-treatment with bestatin, another P-gp substrate, increases plasma concentrations and decreases the renal clearance of methotrexate[101]. MATE1 and MATE2-K are expressed on the brush border membrane of proximal tubular cells. MATE1 mediated the renal secretion of fluoroquinolones including gatifloxacin, ciprofloxacin, levofloxacin, enoxacin, pazufloxacin, norfloxacin and tosufloxacin.

In summary, the expression and activity of transporters can be regulated by drugs and competitive inhibition may occur after co-administration of more than one drug. Furthermore, species differences in transporters complicate pharmacokinetic scaling from preclinical species to humans. Additionally, the expression of transporters may also be regulated by disease progression[102]. Modulation of transporter expression by disease states can potentially modify the PK of drugs.

2.3. ncRNAs in the regulation of drug metabolism and pharmacokinetics

ncRNAs are genome-derived RNA molecules that are not translated into proteins. Indeed, the human genome is comprised of over 95% of noncoding sequences[103] that are transcribed into various forms of functional ncRNAs including miRs, transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nucleolar RNAs (snoRNAs), and long noncoding RNAs (lncRNAs). Among them, miRNAs usually lead to translation inhibition or enhance mRNA degradation in cells through complementary base pairing with target transcripts. Many miRNAs have been shown to modulate the expression of drug-metabolizing enzymes or transporters, and consequently alter cellular drug metabolism and transport capacity, as well as drug responses (see recent reviews[104−106]). For instance, miR-27b reduces CYP1B1 protein expression in human carcinoma cells and thus suppresses CYP1B1 enzymatic activity, as indicated by a P450-Glo™ luminescent assay[107]. Meanwhile, miR-27b modulates CYP3A4 expression through direct targeting of CYP3A4 3′-untranslated region (3′UTR) and "indirect" targeting of transcriptional factors such as NR1I1/VDR[108], which may significantly alter CYP3A4-mediated drug metabolism[109,110]. Furthermore, miR-27a/b regulates the expression of a number of transporters such as ABCB1/P-gp[111−113], and thus influences intracellular drug accumulation and chemosensitivity. In addition, a number of Phase 2 drug-metabolizing enzymes such as the UGTs are regulated by miRNAs at the posttranscriptional level[114−119]. Findings on miRNA-controlled regulation of DMPK provide new insights into mechanisms behind inter-individual variations in pharmacotherapy.

Recent studies on miRNA regulation in DMPK also led to the development of novel research approaches and technologies. For example, while the luciferase reporter assay, gene mutagenesis and correlation analysis are helpful methods for the assessment of the interactions between miRNAs and target transcripts, a more direct approach has been established which is based on the change of RNA mobility after binding to miRNA, namely RNA electrophoretic mobility shift assay (EMSA)[120,121]. Using this RNA EMSA and other methods, a number of CYP genes (*e.g.*, *CYP2C19*, *CYP2E1* and *CYP2D6*) and regulators have been shown to be regulated post-transcriptionally by particular miRNAs[121−125]. It is also noteworthy that miRNA research is limited to the use of miRNA-expressing plasmids or viruses, or chemically-synthesized or chemo-engineered miRNA mimics[126−128]. To better capture the properties of biologic RNA molecules and cellular miRNA machinery, a novel RNA bioengineering technology has been established for the production of biologic miRNA agents in living cells[109,129−133]. With such novel bioengineered miRNA agents produced cost effectively and on a large scale, extensive functional studies have been conducted and the results showed rather a modest change in the PK of major CYP probe drugs in mouse models[134]. Further studies have demonstrated the utility of miRNAs as therapeutics or sensitizing agents for the treatment of human diseases in various animal models[133,135−139].

There is also growing evidence that lncRNAs may regulate the expression of drug-metabolizing enzymes and transporters. For example, expression of HNF1α antisense RNA 1 (HNF1α-AS1) and HNF4α antisense RNA 1 (HNF4α-AS1) shows a significant influence on the basal and drug-induced expression of drug-metabolizing enzymes in human cells[140]. H19, an lncRNA highly expressed in liver tissues, induces the expression of efflux transporter P-gp/MDR1/ABCB1 in drug-resistant HepG2 cells[141]. The lncRNA MRUL confers the overexpression of ABCB1 in drug-resistant gastric cancer cells[142]. Furthermore, some studies have demonstrated that a lncRNA modulates drug sensitivity through its action on miRNA-transporter axis[143,144]. In addition, as RNA editing and posttranscriptional modifications are critical

for RNA stability and biological function, very recent studies have also demonstrated the alteration of DMPK gene expression following RNA editing[145−147]. Future studies in these areas will undoubtedly advance our understanding of RNA-based regulation in DMPK.

In summary, research on miRNA-controlled regulation of DMPK provides new insights into understanding the post-transcriptional regulatory mechanisms behind inter-individual variations. Novel technologies and research approaches are also established during the investigation of ncRNA regulation of DMPK gene expression, which should have broad impact on biomedical research. Evidence is accumulating that some lncRNAs may be involved in the regulation of DMPK, which represents a new area of research.

3. Drug−drug interactions

3.1. Current status of research on drug−drug interactions

DDIs may result in favorable or toxic effects. Patients frequently use more than one medication at a time. Depending on the clinical settings and the number of drugs prescribed, the incidence of potential DDIs ranges between 15% and 80%[148]. DDIs can be classified mechanistically into 3 major types: physio-chemical incompatibility, PK interactions, and pharmacodynamic interactions[149]. Physio-chemical interactions usually occur when positively and negatively charged compounds are mixed before they are administrated or absorbed. Pharmacokinetics-based DDIs, characterized by altered concentration of unbound drugs that exert pharmacological effects, can be caused by several mechanisms, including: 1) alteration of drug metabolizing enzymes (*e.g.*, CYPs)[150], 2) alteration of transporters involved in the absorption, distribution and excretion of drugs (*e.g.*, MDR1, OAT, OCT, etc)[150], 3) influence on plasma protein binding affinity[149], and 4) changes in the function of organs (*e.g.*, gut motility or stomach content pH)[149]. Pharmacodynamics-based DDIs are characterized by a shift of the unbound drug concentration *versus* response curve[149]. New responses that are not present when either of the drugs is given alone may also be observed when drugs are used in combination.

In vitro, *in vivo* and clinical studies are usually conducted to identify any potential DDIs. The *in vitro* studies are usually simple systems that can be used for high throughput screening and provide mechanistic information for potential DDIs. *In vivo* animal studies are often conducted using clinically relevant dosages and pharmacodynamic endpoints to confirm the *in vitro* observations. If evidence obtained from *in vitro* and *in vivo* animal models suggests strong DDIs potential further clinical trials are recommended[150,151]. Recently, mathematical modeling, particularly physiologically-based pharmacokinetic (PBPK) modeling has also been applied to investigate potential pharmacokinetic-based DDIs. A recent review by Min et al.[152] depicted how pharmacokinetic modeling improves and simplifies the investigation on DDIs. In addition, systematic reviews and databases summarize all the experimental and predicted data on DDIs, which are useful for providing warning and proper advice to patients in clinical practice[153].

Although DDIs between small molecule drugs have been well investigated and documented, knowledges on interactions between drugs and herbs, interactions between therapeutic biologics, and interactions mediated by the gut microbiome are currently not well understood. The cutting-edge investigations on these aspects are briefly introduced in the following sections.

3.2. Current status of research on herb−drug interactions

Herbal plants and herbal products are commonly used as remedies and dietary supplements. When herbs are concurrently administered with drugs unrecognized herb−drug interactions (HDIs) may lead to side effects and toxicity. HDIs basically share the same mechanisms as DDIs. To avoid physio-chemical interactions between herbal components and drugs, it is usually recommended that herbs should be taken at two hours before or after the drugs. Moreover, herbs may sometimes alter the PK and/or pharmacodynamics of the concurrently administered drugs. PK and pharmacodynamic interactions have been reported between herbs and drugs with narrow therapeutic indexes, especially drugs for CNS and cardiovascular diseases[154]. For example, St John's wort (*Hypericum perforatum*) was reported to decrease warfarin plasma concentrations *via* inducing the activity of CYPs, leading to the loss of anticoagulant activity[155]. A traditional Chinese herb Danshen (*Salvia miltiorrhiza*) was reported to interact with warfarin on both its PK profiles and pharmacodynamic effects, resulting in over-anticoagulation and increased risk of bleeding[155].

Investigation of HDIs is often more complicated than those of DDIs, due to the complex herbal components and the batch-to-batch variation of herbal products. As demonstrated in Table 4, compared with DDIs, research on HDIs is still insufficient. *In vitro* screening assays, which are efficient ways for detecting potential DDIs, may not be applicable for testing crude herbs or herb extracts, due to the fact that some of the herbal components may not be bioavailable, and adding such herbal components to the *in vitro* cell/microsome systems may alter results. By using LC−MS/MS, several multi-compound pharmacokinetic studies allowed the simultaneous detection of the plasma/tissue concentrations of multiple components after ingestion of the studied herb, facilitating the discovery of the bioavailable active components and subsequent *in vitro and in vivo* mechanistic studies on potential HDIs[156,157]. Most of the reported HDIs are based on *in vitro* and *in vivo* animal models, providing evidence with low clinical relevance. Moreover, many clinical studies were conducted among healthy populations, where the impact of the herbs on the pharmacodynamics effects of the concurrent drug may not be determined. On the other hand, the wide variation between different batches of herbal products also leads to poor reproducibility of the tests. Although not true in all countries, herbal products in China are generally regulated and used as medicine with standardization of the content of the major active components, and the herbal products are sometimes investigated not only as the effector but also as the affected agent of HDIs. In addition to experimental approaches based on the pre-clinical and clinical data, mathematical models have been established to predict HDIs, demonstrating the feasibility of using PBPK modeling for the prediction of HDIs[152]. For example, PBPK modeling of two major active components from Wuzhi capsule (*Schisandra sphenanthera* extract) predicts its interaction with tacrolimus metabolism by CYP3A4 inhibition[158]. However, the application of modeling and simulation on the investigation of HDIs is still restricted by the limited human pharmacokinetic data of herbal components[152]. More sophisticated designs of clinical studies are warranted to evaluate the safety and efficacy of the concomitant use of herbs and drugs.

3.3. *Trends in drug–drug interactions of therapeutic biologics*

Therapeutic biologics include therapeutic proteins, monoclonal antibodies (mAbs), vaccines, and peptide and nucleic acid derivatives that are manufactured for pharmaceutical uses[159]. Development of therapeutic biologics is growing fast, and in clinical practice the risk of DDIs with biologics is increasing.

3.3.1. *PK of therapeutic biologics*

The PK of biologics is different from those of small molecules. Since most therapeutic biologics undergo rapid degradation in the gastrointestinal tract after oral administration, alternative routes, such as intravenous, intramuscular, and subcutaneous injection are often used for drug delivery[159–161]. The distribution of therapeutic biologics is mainly mediated by interstitial penetration, lymphatic drainage, transcytosis, and receptor-mediated cell uptake[159–161]. Therapeutic proteins usually have a limited volume of distribution and do not bind to plasma proteins, and their biliary and renal excretion is generally negligible[162]. Catabolism *via* proteolytic degradation is the predominant clearance pathway for most therapeutic proteins[159–161], while target antigen-mediated disposition also plays a role[161]. Moreover, fragment crystallizable receptor (FcR)-mediated antibody recycling by monocytes, macrophages, and dendritic cells is a salvage pathway that prolongs the half-lives of many mAbs[159–161]. Immune responses participate in both the catabolism and the antibody recycling process, and therefore immunogenicity can significantly influence the clearance of therapeutic proteins[159]. A recent review by Ferri et al.[162] has summarized the pharmacokinetic DDIs of therapeutic antibodies. Unlike therapeutic proteins, nucleic acid and peptide drugs[160] are rapidly eliminated by peptidases and nucleases[159,163], and may also undergo slow renal excretion[161]. Plasma binding of these oligomers can sometimes be very high and has been reported to affect their distribution and clearance[160].

3.3.2. *Pharmacokinetics-based interactions of therapeutic biologics*

Direct competition between therapeutic biologics and small molecules in PK is not common due to their distinct pharmacokinetic pathways[163]. However, certain indirect pharmacokinetic DDI may occur. Immunosuppressive agents may decrease the immunogenicity of the therapeutic protein so as to hinder its clearance[163]. For example, concomitant treatment with the immunosuppressant methotrexate can decrease the clearance of mAbs including golimumab[164], adalimumab[162], and infliximab[165]. Another indirect pharmacokinetic DDI mechanism is cytokine–CYP modulation. Several biologics with immunomodulatory effects may alter CYP activities *via* modulating the cytokine levels leading to the altered PK of co-administered small molecules that are substrates of the affected CYPs[159,163,166]. For instance, tocilizumab, which can induce CYP3A4 activity by decreasing interleukin 6 levels, was found to reduce simvastatin systemic exposure[167]. Similarly, by triggering inflammation, influenza vaccination has been reported to decrease CYP activity and thus influence the systemic exposure of CYP substrates such as clozapine[168]. PBPK modeling is a powerful tool for the investigation of pharmacokinetic-based interactions between therapeutic biologics

Table 4 Comparison between investigations on DDIs and HDIs[151,153].

Type of investigation	DDIs	HDIs
In vitro studies	• Commonly used for the screening of potential DDIs. • Provide mechanistic information.	• Single component/artificial mixture of major components used in a test. • Does not account for bioavailability. • Provide mechanistic information for certain components.
In vivo animal studies	• Drugs tested in clinically relevant doses. • Provide pharmacokinetics and pharmacodynamics information for clinical trials.	• Crude herbs or herb extracts tested in clinically relevant doses. • Address bioavailability of the herbal components.
Clinical studies	• Retrospective evaluation may not provide sufficient precision to assess DDIs. • Clinical trials on healthy volunteers for pharmacokinetics-based DDIs. • Pharmacodynamics-based DDIs and potential toxicity studies on intended patient populations.	• Most of HDIs evaluation are retrospective and are based on cases reports. • Limited clinical trials and often carried out on healthy volunteers. • Lack of monitoring of pharmacokinetic profiles of the herbal components. • Lack of pharmacodynamics and potential toxicity in patient populations.
Simulation and modeling	• PBPK modeling has been extensively applied to pharmacokinetic-based DDIs with complex mechanisms. • Modeling and simulation are recommended by regulatory agencies[5].	• Only a few herbal products have been predicted of HDIs by PBPK modeling. • Limited human pharmacokinetic data and lack of herbal standardization restrict the application of modeling and simulation on prediction of HDIs.
Systematic reviews and databases	• A number of databases on DDIs analysis have been developed based on solid clinical evidence.	• Few databases on HDIs have been established. • No sufficient clinical data to support the effectiveness and safety of the combination.

and small molecules, and has been successfully applied to quantitatively predict DDIs of CYP-modulating protein drugs (such as blinatumomab and sirukumab) and small molecule CYP substrates in patients[169,170]. On the other hand, pharmacokinetic interaction between two therapeutic biologics has seldom been reported. However, such pharmacokinetic DDIs may occur due to specific binding between two biologics. For example, palifermin is a truncated form of the endogenous fibroblast growth factor which contains the heparin-binding domains. Co-administration of palifermin with heparin was found to increase the systemic exposure to palifermin up to 5-fold[171].

3.3.3. *Pharmacodynamics-based interactions of therapeutic biologics*

Comparing to the pharmacokinetics-based DDIs of therapeutics biologics, their pharmacodynamics-based DDIs are more commonly reported. A large volume of cases has demonstrated pharmacodynamic interactions among various hormones owing to their complex signaling networks[159]. For instance, insulin can interact with numerous drugs including hormones, antidiabetics, antibiotics, antipsychotics, etc[172]. Recombinant growth hormones interact with small molecule hormones such as glucocorticoids, estrogens, thyroxin, etc.[159]. Although co-administration of biologics indicated for the same disease usually results in additive or synergistic efficacy, co-administration may also induce toxicity. Both anakinra and etanercept are approved for the treatment of rheumatoid arthritis. However, combined use of the two biologics led to severe adverse effects including increased risk of infection and increased neutropenia without significant improvement in therapeutic efficacy[173].

3.3.4. *Risk assessment for DDIs of therapeutic biologics*

Due to the distinct pharmacokinetic and pharmacodynamic properties of therapeutic biologics, the classic approach for DDIs prediction for small molecules may not applicable for therapeutic biologics. With the increase in therapeutic biologics in the market, it is critical to call for building strategies and regulations on the potential DDIs involving biologics. Based on the current findings on the major mechanisms for the pharmacokinetic-based DDIs of therapeutic biologics, assessment of the modulation of CYP activities and immunogenicity are recommended. In terms of pharmacodynamics-based DDIs, identification and monitoring of clinical endpoints relevant to both the efficacy as well the adverse effects of therapeutic biologics is highly recommended.

3.4. *Trends in microbiota mediated drug−drug interactions*

Recent studies have indicated that the microbiota is a vital drug target in many disease treatments. Many therapeutics have great effects on altering the composition of the microbiota. As indicated in Fig. 2A, changes in microbiota in the gastrointestinal tract may influence the metabolism of co-administered drugs, leading to altered pharmacokinetics. Findings have shown that gut microbiota can mediate drug metabolism including reduction[174], oxidation[175], dehydroxylation, decarboxylation[176], etc. DDIs between antibiotics and drugs that are metabolized by gut microbiota are commonly reported. Many antibiotics can disturb the PK of a co-administered drug by affecting the enzymatic activities and composition of gut microbiota[177], leading to an altered therapeutic effect. For example, the coagulant drug sulfinpyrazone can be metabolized to sulfinpyrazone sulfide in the gut contents. It was found that the plasma pharmacokinetic profile of sulfinpyrazone and sulfinpyrazone sulfide was changed in patients treated with the antibiotic metronidazole[178]. After reduction *via* azoreductases in gut microbiota, prontosil was metabolized to sulfanilamide, which exhibits potent antibacterial activities. In addition, it was noted in rats that the conversion of prontosil to sulfanilamide can be suppressed by antibiotics, leading to the reduced antibacterial effects[174,179]. Most recently, gnotobiotic mouse models and PBPK models have been established to untangle host and microbial contributions to the pharmacokinetic profile[180]. These novel experimental and computational strategies can be incorporated in future investigations on microbiota-mediated DDIs.

In addition to effects on pharmacokinetics, altered microbiota composition may also lead to pharmacodynamics changes in the concomitant drugs (Fig. 2B). It was noted that the presence of a certain type of bacteria may have an impact on chemotherapy and immunotherapy[181,182]. Clinical trials are currently conducted on microbiota interventions, such as probiotics and fecal microbiota transplant (FMT), to explore their influence on the efficacy and toxicity of co-administrated chemotherapeutic agents, immunotherapeutic agents and anti-inflammatory drugs[183]. The potential benefits of probiotics and FMT to increase the efficacy of pembrolizumab in the treatment of PD-1 resistance patients[184] and to reduce the adverse effects of aspirin[185] and irinotecan[186] are currently under clinical investigation.

Besides well-known influences on the microbiota from antibiotics and probiotics, influences from other types of drugs or natural products are very limited. Although evidence of gut microbiota-mediated DDIs remain limited, the growing interest in microbiota will definitely provide a better understanding on their influence on the PK and pharmacodynamics of drugs. Nevertheless, the impact of herbal medicine on the gut microbiome is unavoidable, and such research is expected to provide more in-depth understanding on herb−drug interactions. In summary, in addition to consideration of classical PK and pharmacodynamic interactions, microbiota-mediated drug−drug/herb−drug interactions are expected to bring additional insight into their therapeutic effects.

3.5. *Summary*

Investigation of herb−drug interactions (HDIs) is often more complicated than that on DDIs, due to the complex herbal components and the batch-to-batch variation of herbal products. More pharmacokinetic and pharmacodynamic data on the bioavailable herbal components from clinical studies using standardized herbal products are warranted for better understanding of HDIs. With the increasing number of therapeutic biologics in the market, it is critical to build strategies and regulations on the potential DDIs involved biologics. Based on the current findings on pharmacokinetic- and pharmacodynamic-based DDIs of therapeutic biologics, assessments on the modulation of CYP activity and immunogenicity, and identification and monitoring of clinical endpoints of the therapeutic biologics is recommended. In addition to consideration of classical PK and pharmacodynamics interactions, microbiota-mediated HDIs/DDIs are expected to bring additional insight into their interactions. Novel experimental and computational strategies, such as gnotobiotic animal models and physiologically-based pharmacokinetic modeling can be incorporated in future investigations on microbiota-mediated HDIs/DDIs.

In summary, the incidence of interactions between various therapeutics is high in patients taking multiple drugs and dietary supplements. Although DDIs between small molecule drugs are

relatively well-characterized, other potential interactions are not fully explored. It is essential to develop efficient strategies for the investigation of the interactions between drugs and herbs, and between therapeutic biologics. Furthermore, the growing knowledge on the microbiota as therapeutic targets and as a site of drug metabolism leads us to pay more attention to microbiota-mediated interactions when examining potential DDIs and HDIs.

4. Disease−drug interactions

Understanding disease−drug interactions is clinically important due to the risk of treatment failure and the incidence of adverse reactions. An accumulation of strong research evidence indicates that disease−drug and drug−disease interactions can have a profound effect on the response to a medication, yet most of the existing results are only from animal models. Moreover, there are differences between animal disease models and human diseases[187]. Differences between different species should be also taken into account. In recent years PBPK modeling has gradually been applied to the prediction of disease−drug interactions[188,57]. However, further clinical study or real-life experience is needed to justify results from PBPK modeling. Additionally, the potential mechanism of disease−drug interactions remains poorly characterized. Therefore, further studies are also needed to reveal the in-depth and comprehensive mechanism involved in disease−drug interactions.

In recent years, apart from the DDI, disease−drug interactions have attracted lots of attention due to their potential impact on efficacy and safety of clinical therapy. Disease−drug interactions mainly refer to the disease itself can lead to changes in PK and pharmacodynamics of drugs, and also include the influence of alteration of endogenous substrates related to metabolism on disease status. Both effects of disease on drug metabolism and effects of metabolism regulation on diseases have the potential to increase the risk of treatment failure and the incidence of adverse reactions[189]. Although there have been some reports published on disease−drug interactions, there are still many unknown issues to be characterized. This review provides an update on the research on disease−drug interactions and offers an in-depth perspective on new strategies for the elucidation of disease−drug interactions.

4.1. *Effects of diseases on drug metabolism*

Disease is a vital factor affecting clinical medication. Disease changes the PK of a drug by altering the ADME process; on the other hand, disease can also change the sensitivity of the body to drugs by altering the number of receptors and their function in organs. Clinical practice should take into account the effects of a disease on a drug for the best therapeutic outcome and to avoid serious adverse reactions by adjusting the dose, the interval of administration, and the route of administration, etc. Current progress on disease effects on drug metabolism are listed in Table 5.

4.1.1. *Effects of diabetes on drug metabolism*

Diabetes mellitus, commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Diabetes mellitus is also a well-known risk factor for cardiovascular disease and atherosclerotic complications, especially coronary heart disease[209]. In recent years there have been many reports of the effect of diabetes on drug metabolism. Alterations in function and expression of ABC transporters at the blood−brain barrier in diabetes have been observed[210]; for instance, it was found that the uptake of vincristine by cultured rat brain microvessel endothelial cells incubated in diabetic rat serum were higher than uptake in nondiabetic rat serum, which was related to the impairment of P-gp function and expression at the blood−brain barrier of diabetic rats[190]. Moreover, in brain cortex, STZ-induced diabetes mellitus may induce an impairment of function and expression of BCRP. The uptake of prazosin and cimetidine, two typical substrates of BCRP, was significantly increased in diabetic rats compared to uptake in non-diabetic rats[191]. However, different from the

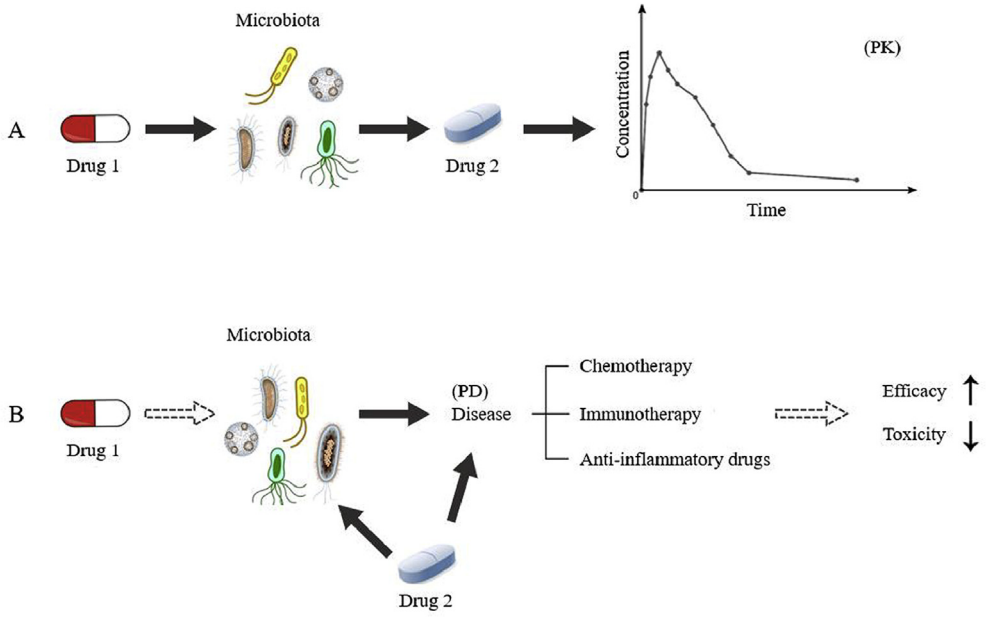

Figure 2 Microbiota-mediated pharmacokinetic and pharmacodynamic interactions between different drugs. (The solid arrow indicates an effect supported by obtained evidence, and the dotted arrow indicates potential effects.)

impairment of function and expression of P-gp and BCRP, diabetes may enhance MRP2 function and expression in liver, kidney and intestine, which then leads to increased excretion of sulfobromophthalein (a substrate of MRP2) *via* the bile, urine and intestinal perfusate[192]. Atorvastatin is a substrate of OATP1B1, an influx transporter expressed on the sinusoidal membrane of hepatocytes. Recent studies found that diabetes mellitus could enhance the hepatotoxicity and decrease exposure to atorvastatin in rats partly through upregulating hepatic *Oatp2*[193,194].

In addition to *Oatp2*, upregulation of hepatic *Cyp3a* also contributes to the decreased exposure to atorvastatin, simvastatin and simvastatin acid in diabetic rats[193−195]. Accumulated evidence shows that diabetes mellitus apparently alters the expression and activity of cytochrome P450 (CYP450) enzymes[196,211]. In diabetic rats, the AUC of theophylline was significantly smaller than that of normal rats because of significantly faster time-averaged total body clearance in diabetic rats, which was attributed to upregulated hepatic CYP1A2 and CYP2E1. Furthermore, diabetes mellitus could significantly increase exposure (area under the curve and peak concentration) to glibenclamide after oral administration. Data with hepatic microsomes suggested the impairment of glibenclamide metabolism and efflux in diabetic rats[197]. Accumulating evidence also has shown that diabetes increased the metabolism of CYP3A4 substrates by upregulating the function and expression of CYP3A4 in hepatic cells[198]. Interestingly, diabetes mellitus showed a tissue-specific effect on CYP3A expression and activity (induced in liver and inhibited in intestine), resulting in opposite pharmacokinetic behavior for verapamil after oral and intravenous administration to diabetic rats[212]. UGTs, the major phase II conjugation enzymes, can also be affected by diabetes mellitus. It was reported that the UGT1 family is adaptively upregulated in the diabetic gastrointestinal tract[199]. Given the essential regulatory role of the gastrointestinal site in drug disposition, such changes in UGTs may have an impact on the metabolism of therapeutic drugs and endogenous substrates.

4.1.2. *Effects of liver disease on drug metabolism*

There is growing evidence to suggest that many hepatic diseases can affect drug metabolism. The effect of liver disease on drug metabolism is mainly due to the alteration of liver hemodynamics and activity of liver microsomal enzymes. Local and systemic liver injuries have a major effect on the expression and activity of DMEs in the liver[213]. For example, compared to control rats, there were significant changes in pharmacokinetic profiles after administrations of rhubarb anthraquinone-extracts in CCl_4-induced liver-injury rats. The plasma concentrations of the four pharmacokinetic markers (Rhein, emodin, aloe-emodin, chrysophanol) of rhubarb anthraquinone extract increased, which indicated that their metabolism and excretion changed after liver injury[200]. Liver failure is often associated with hepatic encephalopathy, due to dyshomeostasis of the central nervous system (CNS). One study showed that the function and expression of P-gp and BCRP decreased, while the function and expression of MRP2 increased in the brain of acute liver failure (ALF) mice[214]. The attenuated function and expression of P-gp at the BBB might enhance phenobarbital distribution in the brain and increase phenobarbital efficacy on the CNS of ALF mice[201]. In addition, ALF could enhance oral plasma exposure of zidovudine in rats by down-regulation of hepatic UGT2B7 and intestinal P-gp[202].

Fatty liver disease, also known as hepatic steatosis, is a condition where excess fat builds up in the liver. Previous research showed that valproic acid with a high-fat diet-induced fatty liver could upregulate UGTs and was accompanied by the increased expression of CAR and PPARα[215]. Further analysis revealed that liver disease in warfarin users was associated with a significant increase in the likelihood of hemorrhage[216].

4.1.3. *Effects of heart failure on drug metabolism*

Heart failure (HF) is considered an epidemic disease in the modern world affecting approximately 1%−2% of the adult population. Many CYP enzymes have been identified in the heart and their levels have been reported to be altered during HF. There is a great deal of discrepancy between various reports on CYP alterations during HF, likely due to differences in disease severity, the species in question and other underlying conditions. A recent review by Aspromonte et al.[217] has summarized a comprehensive modulation of cardiac CYP in patients with HF. In general, cardiac *CYP1B* and *CYP2A*, *CYP2B*, *CYP2J*, *CYP4A* and *CYP11* mRNA levels and related enzyme activities are usually increased in HF[217,218]. On the other hand, HF plays an important role in the down-regulation of hepatic CYP involved in drug metabolism through several mechanisms which include hepatocellular damage, hypoxia, elevated levels of pro-inflammatory cytokines, and increased production of heme oxygenase-1[219]. For example, the plasma concentrations of caffeine (CYP1A2 probe), mephenytoin (CYP2C19 probe), dextromethorphan (CYP2D6 probe) and chlorzoxazone (CYP2E1 probe) were significantly elevated in patients with congestive HF[203]. It was suggested that the doses of these CYP enzymes substrates should be decreased when used in patients with congestive HF.

4.1.4. *Effects of renal disease on drug metabolism*

Evaluation of drug metabolism in patients with end-stage renal disease is important because these patients use a large number of medications and are at risk of adverse reactions and DDI. Previous studies found that end-stage renal disease patients had a 50% increase in the plasma warfarin *S/R* ratio relative to control subjects. This may be reflective of a selective decrease in hepatic CYP3A and CYP2C9 activity in renal failure[204,205]. Furthermore, results from a “cocktail” approach showed that the enzyme activities of CYP3A4 and CYP2C9 of patients with renal failure were selectively inhibited[220]. Therefore, if CYP34A and CYP2C9 substrates are used in patients with renal failure, the dose needs to be lowered. Although chronic renal failure (CRF) has been found to be associated with a decrease in liver CYP, the mechanism remains poorly understood. The *N*-demethylation of erythromycin was decreased by more than 35% ($P < 0.001$) in hepatocytes incubated with serum from rats with CRF[221]. It is speculated that the mediator(s) of uremic serum may down-regulate the CYP of normal hepatocytes. In addition, a recent study investigated the effects of adenine-induced chronic kidney disease (CKD) in rats on the activities of some XMEs in liver and kidneys. It was found that the plasma theophylline concentration was significantly increased in rats with CKD[206]. Moreover, a reduced metabolism of midazolam could be observed in rats with acute kidney injury (AKI)[207].

4.1.5. *Effects of sepsis on drug metabolism*

Sepsis is the systemic inflammatory response syndrome caused by infection, which is a common complication following surgery, especially abdominal surgery, with higher mortality. It has been well documented that hepatocellular dysfunction occurs early in sepsis and contributes to multiple organ failure and ultimately death[222]. Among them, the effects of polymicrobial sepsis on the

Table 5 Summary of the effects of specific diseases on drug metabolism.

Type of diseases	Affected drugs	Related mechanisms
Diabetes mellitus	• Vincristine and other P-gp substrates: increased uptake[190]. • Prazosin, cimetidine and other BCRP substrates: increased uptake[191]. • Sulfobromophthalein and other MRP2 substrates: enhanced excretion[192]. • Atorvastatin, simvastatin: decreased exposure[193−195]. • Theophylline: increased metabolism[196] • Glibenclamide: inhibited metabolism and decreased the efflux[197]. • CYP3A4 substrates: increased metabolism[198]. • UGT1 substrates: increased metabolism[199].	• Impairment of P-gp function and expression. • Impairment of BCRP function and expression. • Induction of MRP2. • Upregulated OATP2, CYP3A. • Induction of CYP1A2 and CYP2E1. • Inhibition of CYP2C11 and BCRP. • Upregulated CYP3A4. • Upregulated UGT1.
Liver disease	• Rhein, emodin, aloe-emodin, chrysophanol: inhibited metabolism[200]. • Phenobarbital: enhance distribution[201]. • Zidovudine: inhibited metabolism and decreased the efflux[202]. • MRP2 substrates: enhanced efflux[201].	• Inhibition of CYP and UGT metabolism. • Inhibition of P-gp and BCRP (brain). • Down-regulation of UGT2B7 and P-gp. • Induction of MRP2 (brain).
Heart failure	• Caffeine and other CYP1A2 substrates: inhibited metabolism[203]. • Mephenytoin and other CYP2C19 substrates: inhibited metabolism[203]. • Dextromethorphan and other CYP2D6 substrates: inhibited metabolism[203]. • Chlorzoxazone and other CYP2E1 substrates: inhibited metabolism[203].	• Down-regulation of CYP1A2. • Down-regulation of CYP2C19. • Down-regulation of CYP2D6. • Down-regulation of CYP2E1
Renal disease	• Erythromycin and other CYP3A substrates: inhibited metabolism[204]. • Warfarin and other CYP2C9 substrates: inhibited metabolism[205]. • Theophylline and other CYP1A1 substrates: inhibited metabolism[206]. • Midazolam and other CYP3A11 substrates: inhibited metabolism[207].	• Inhibition of CYP3A. • Inhibition of CYP2C9. • Inhibition of CYP1A1. • Inhibition of CYP3A11.
Sepsis	• CYP1A1 substrates: inhibited metabolism[208]. • CYP1A2 substrates: inhibited metabolism[208]. • CYP2E1 substrates: inhibited metabolism[208].	• Down-regulation of CYP1A1. • Down-regulation of CYP1A2. • Down-regulation of CYP2E1.

activity and gene expression of hepatic microsomal CYP450 have attracted considerable attention due to their potential disease−drug interactions in clinical therapy. It has been reported that the major hepatic CYP isoforms CYP1A1, 1A2, 2B1, 2E1 were down-regulated during polymicrobial sepsis[208,203−224]. Moreover, results from mechanistic studies show that nitric oxide (NO) and the AhR play key potential roles in down-regulation of hepatic CYP during sepsis[225,226]. Therefore, treatment with pharmaceutical agents that regulate or are metabolized by CYP enzymes might be approached cautiously in the septic patient.

On the other hand, early and appropriate antimicrobial treatment is the predominant intervening measure to decrease patient mortality[227]. However, the pathophysiologic changes during sepsis such as systemic capillary leak syndrome, altered shift of body fluid and hypoalbuminemia can lead to changes in pharmacokinetics/pharmacodynamics (PK/PD) parameters such as apparent volume of distribution (V_d)[228] and clearance (CL)[229] that affect the achievement of PK targets and increase the risk of treatment failure with routine dosing. In addition, it is likely to cause low blood protein symptoms in sepsis due to the increased capillary permeability, decreased hepatic albumin synthesis and a large number of infusions[230]. Therefore, the effect of hypoalbuminemia on antibiotic PK also cannot be ignored. It is crucial to reduce patient mortality by adjusting antimicrobial doses and improving drug infusion to optimize antimicrobial therapy according to the characteristics of PK/PD during sepsis.

4.2. Effects of endogenous metabolism mediated by nuclear receptors on diseases

In recent years the regulation of endogenous metabolism mediated by nuclear receptors on diseases has received increasing attention with improvements in bioanalytical technology, especially the intervention of the various “omics”. Among them, PXR and CAR are two closely related and liver-enriched nuclear hormone receptors originally defined as xenobiotic receptors. However, an

increasing body of evidence suggests that PXR and CAR also have endobiotic functions that impact glucose and lipid metabolism, as well as the pathogenesis of metabolic diseases. PXR and CAR not only regulate the transcription of drug-metabolizing enzymes and transporters, but also orchestrate energy metabolism and immune responses[231]. The cutting-edge investigations on these aspects are briefly shown in Table 6.

A recent study revealed that PXR ablation inhibited high-fat diet-induced obesity, hepatic steatosis, and insulin resistance[232]. These results may help to establish PXR as a novel therapeutic target, and PXR antagonists may be used for the prevention and treatment of obesity and type 2 diabetes. PXR was also reported to play a vital role in maintaining biliary bile acid homeostasis by regulating the biosynthesis and transport of bile salts[233]. Activation of the PXR pathway was associated with decreased lithocholic acid-induced cholestasis in mice[241]. PXR may be developed as a therapeutic target for cholesterol gallstone disease. Interestingly, study has revealed a function of PXR in enlarging liver size and changing liver cell fate by activation of the yes-associated protein (YAP) signaling pathway. This has implications for understanding the physiological functions of PXR[242]. In addition, PXR plays an important endobiotic role in adrenal steroid homeostasis. Activation of PXR markedly increased plasma concentrations of corticosterone and aldosterone[234]. These results suggest that PXR is a potential endocrine disrupting factor that may have broad implications in steroid homeostasis and drug−hormone interactions.

CAR has also been increasingly appreciated for its endobiotic functions in influencing glucose and lipid metabolism, with dysregulation implicated in two of the most prevalent metabolic disorders, obesity and type 2 diabetes[243]. Further study found that CAR suppresses hepatic gluconeogenesis by facilitating the ubiquitination and degradation of PGC1α[244]. Given the metabolic benefits of CAR activation, CAR may represent an attractive therapeutic target to manage obesity and type 2 diabetes.

Nonalcoholic steatohepatitis (NASH) is common and medically significant because it is closely related to metabolic syndrome and has the potential to progress into the more harmful cirrhosis. Emerging evidence points to an important function of AhR in the uptake of fatty acids in the liver and the pathogenesis of fatty liver disease[236]. Activation of the AhR sensitizes mice to NASH by deactivating mitochondrial sirtuin deacetylase Sirt3[245]. These results suggest that the use of AhR antagonists might be a viable approach to prevent and treat NASH.

LXRs are known as sterol sensors that impact cholesterol and lipid homeostasis, as well as inflammation. The hepatic functions of LXRs are well documented and the pathophysiological role of LXRs was uncovered progressively in recent years. Activation of LXR prevents lipopolysaccharide-induced lung injury by regulating antioxidant enzymes and the implication of this regulation is pulmonary tissue protection[237]. Moreover, a recent study demonstrated that activation of LXR attenuates OA-induced acute respiratory distress syndrome by attenuating the inflammatory response and enhancing antioxidant capacity[238].

FXR, a nuclear receptor mainly expressed in enterohepatic tissues, is a master regulator for bile acid, lipid and glucose homeostasis[246]. Emerging evidence indicates that restoration of FXR protein levels may represent a new strategy for enterohepatic and metabolic diseases. Hepatitis B virus X protein (HBx) is a hepatitis B virus protein that has multiple cellular functions, but its role in the pathogenesis of hepatocellular carcinoma (HCC) has been controversial. It was reported that transactivation of FXR by full-length HBx may represent a protective mechanism to inhibit HCC[247]. Additionally, FXR antagonism was also reported to be pivotal in attenuating obstructive cholestasis in bile duct-ligated mice[235]. These results suggest that FXR may be developed as a therapeutic target for cholesterol gallstone disease.

The tumor suppressor p53 is traditionally recognized as a surveillance molecule to preserve genome integrity. Recent studies have demonstrated that it contributes to metabolic diseases. It was found that the activation of p53 participated in promoting bile acid disposition and alleviating cholestatic syndrome by up-regulating the expression of *Cyp2b10*, *Sult2a1* and *Abcc2/3/4*, which provides a potential therapeutic target for cholestasis[235]. In addition, p53 could attenuate acetaminophen-induced hepatotoxicity by regulating the CYPs, SULTs and MRPs, which provides a potential new therapeutic target for APAP-induced liver injury[248].

Metabolism regulation mediated by downstream targets of the above transcriptional factors may also play an important role in diseases. For example, NAD(P)H: quinone oxidoreductase 1 (NQO1) has been reported to be a prognostic biomarker and a promising therapeutic target for patients with NSCLC due to its frequent overexpression and significantly increased activity in NSCLC. It was found that depleting tumor-NQO1 potentiates anoikis and inhibits the growth of NSCLC[239]. Furthermore, recent results from a metabolomics analysis have revealed that inhibition of cell proliferation upon NQO1 depletion was accompanied by suppressed glycometabolism in NQO1 high-expression human NSCLC A549 cells. Also, NQO1 depletion significantly decreased the gene expression of hexokinase II[240].

4.3. *Summary*

Understanding disease−drug interactions is clinically important due to the risk of treatment failure and the incidence of adverse reactions. An accumulation of strong research evidence indicates that disease−drug and drug−disease interactions can have a profound effect on the response to a medication, but most of the existing results are only from animal models. In recent years, PBPK modeling has also gradually been applied to the prediction of disease−drug interactions[57,188]. However, further clinical study or real-life experience is certainly needed to justify the results from PBPK modeling. Additionally, the potential mechanisms of disease−drug interactions are not well-characterized. Therefore, further studies are needed to reveal the in-depth and comprehensive mechanism involved in disease−drug interactions.

5. Mathematical modeling

The application of mathematical modeling to problems in PK has a rich history in the form of pharmacokinetic modeling to explore how simulation can be used to improve our understanding of common issues not readily addressed in human pharmacology[249]. Animal models are mainly used in experimental physiology, experimental pathology and experimental therapeutics, especially in the study of new drugs. In the earliest stage of drug discovery/development, various cell-based models and animal models were used for the prediction of human PK and toxicokinetics[250]. In this section, the current status and future challenges on PBPK modeling and animal models are summarized.

5.1. *The current status and challenges of PBPK modeling*

As early as 1937[251] physiological parameters were introduced into pharmacokinetic parameter estimation. The term PBPK model appeared in 1977[252]. Although the PBPK method was proposed a long time ago, it was applied to support new drug development in the last decade since the mechanism of drug metabolism and transport gained clarity. Two known milestones of extensive application for industry are: 1) A PBPK review team was set up in the office of clinical pharmacology (CDER/FDA, US) because of increasing numbers of PBPK submissions in 2013[253]; 2) PBPK guidance was released by FDA[254] and EMA[255] respectively during 2016−2018. A total of 217 PBPK submissions were reviewed by the FDA in 2016[256]. As one of the four major pharmacometric research methods[257], the strategy of waiving clinical trials through PBPK study has been extensively accepted in western society and is gradually being accepted in China.

5.1.1. *Basic concepts of PBPK*

PBPK can be utilized to mechanistically understand and predict *in vivo* pharmacokinetic characteristics from a whole body perspective by integrating system-specific parameters (such as physiological parameters), drug-specific parameters (such as physical−chemical and mechanistic pharmacokinetic data), and specific PBPK model structure[258]. It can quantitatively describe drug concentration kinetics in the blood and each tissue through a series of mathematical differential equations, which allows it to accurately predict target tissue drug concentration as well as to understand drug absorption, distribution, metabolism, elimination, and transportation (ADMET) processes. Because it incorporates system-specific parameters into equations of each tissue, it can also be used to predict drug concentration in tissues under different scenarios, such as co-administration of enzyme inhibitor or in a specific population (hepatic- or renal-impaired patients, pediatrics, or elders), which could support new drug development strategy, clinical trial design, and improved clinical development efficiency.

5.1.2. *PBPK in drug development*

5.1.2.1. PK drug−drug interaction study. As of August 2016, 60% of PBPK study cases submitted to FDA were related to drug−drug interactions (DDI)[256]. Among the three predominant DDI mechanisms, enzyme-[259], transporter-[260], and disease-mediated DDI[261,262], enzyme-mediated DDI cases showed the best predictive performance in PBPK. Hsueh et al.[259] summarized 104 publications with DDI predictions, a total of 126 and 360 cases were reported for drug as metabolic "victim" and "perpetrator" respectively. The predictive performance of CYP3A- and CYP2D6-mediated DDI was found to be the best for new drugs as victim using the PBPK method. Two enzymes are involved in metabolism of large proportion of marketed drugs and well-established probe perpetrators are available[256,263]. The predictive performance was poorer for new drugs as perpetrator[259]. In order to accurately predict the quantitative effects of an enzyme inhibitor[264] or inducer[265] on a substrate, the FDA suggested the following study strategy[256,266]: a) Develop an initial PBPK model of enzyme−substrate based on *in vitro* data followed by verification using human single-dose PK data; b) develop a PBPK model of inducer or inhibitor and validate its enzyme modulation effect using *in vivo* (or literature data) data; c) predict the effect of inhibitor/inducer on substrate PK characteristics in humans using the PBPK model, which will support DDI study strategy or clinical trial design, especially for the dose selection; d) if a dedicated DDI was required and conducted, then the initial PBPK model will be verified and modified based on observed DDI data; e) predict other untested scenarios and validate dose selection. Following this strategy, predictive performance was summarized in report published by Hsueh et al.[259]. As stated in submitted cases to the FDA, AUCR or C_{max}R (ratio of AUC or C_{max}) was estimated within the range of (0.80, 1.25) and (0.50, 2.00) for higher than 73% and 77% cases respectively. Although overall DDI of CYP-mediated interactions could be estimated well, prediction of time-dependent DDI and intestinal enzyme-mediated DDI was still challenging[256].

Because tissue concentration can indicate efficacy or safety better than plasma concentration, it is more important to be predicted, especially for the drugs with a "disconnected" concentration in tissues compared to plasma concentration, which may be caused by significantly different distribution through transporters[267]. Unfortunately, the best prediction method theoretically, the PBPK method, showed worse predictive performance for transporter-mediated DDI compared to that of enzyme-mediated DDI, which was due to ubiquitous tissue distribution, unique cellular localization, and competing active and passive processes[268]. Furthermore, the lack of knowledge pertaining to disease- or population-specific factors makes PBPK more challenging for transporter-mediated DDI prediction. In order to accurately predict unbound and intra/subcellular drug concentrations while considering the role of a transporter, selecting appropriate *in vitro* (such as imaging) and *in vivo* experimental methods to determine tissue concentration followed by verification of PBPK model in animals may be helpful[267]. Recently,

Table 6 Summary of endogenous substances related to diseases and nuclear receptors.

Endogenous substance	Diseases involved	Related nuclear receptor or protein
Glucose and lipid	• Obesity and type 2 diabetes[232]	• PXR, CAR
Biliary bile acid	• Cholesterol gallstone[233,234]	• PXR, FXR
	• Cholestatic syndrome[235]	• P53
Corticosterone and aldosterone	• Steroid dyshomeostasis[234]	• PXR
Fatty acids	• Nonalcoholic steatohepatitis[236]	• AhR
Sterol	• Lung injury[237]	• LXR
Oleic acid	• Acute Respiratory Distress Syndrome[238]	• LXR
Hexokinase II	• Non-small cell lung cancer[239,240]	• NQO1

disease-mediated DDI received greater attention, especially for renal impairment affecting liver enzymes[262]. However, research on disease-mediated DDI are limited, and few PBPK cases to predict this kind of DDI have been reported[269,270], and so further research to uncover the rationales behind of disease and physiological parameters is needed.

5.1.2.2. Specific population study. One of the most known characteristics of the PBPK model is that it can integrate drug-specific and system-specific parameters, which includes age, gender, disease status, and specific physiological status. This characteristic allows us to predict PK exposure changes by mechanistically changing specific parameters according to the different populations, such as pediatric, elderly, and in patients with hepatic or renal impairment. However, accurate prediction for these specific populations is still quite challenging because changes in system-specific parameters generally are not available or quantified accurately[271]. The FDA and other scientists summarized PBPK prediction strategy in patients with renal impairment[272] or hepatic impairment[273], in the elderly[274], pediatric[275], fetal[276], and pregnant patients[277] but, because of the above limits, these predictions could be utilized only to aid in clinical trial design in these specific populations rather than to waive these dedicated clinical trials without any verification in these specific populations.

5.1.2.3. Generic drug development. In comparison to the *in vitro*−*in vivo* correlation (IVIVC) method the PBPK method was advantageous because it could identify the contribution of penetration, intestinal metabolism and transport to the absorption−drug concentration−time curve. Therefore, PBPK analysis can estimate *in vivo* dissolution characteristics more accurately, which will be useful to guide drug development[278,279]. Therefore, the US FDA continuously held modeling and simulation workshops to make PBPK methods more useful in generic drug development[280−282] as well as suggested a research strategy for industry[283,284]. Additionally, physiologically-based oral absorption modeling can be utilized to guide Quality by Design (QbD) and predict food effects, effect of acid-reducing agents, SUPAC activities, and to influence label language. Although it was potentially powerful, its application is still limited because of physiological information missing in PBPK system models. The European OrBiTo (Oral Biopharmaceutical Tool) Project results showed that less than 50% of drugs could receive 2-fold error prediction performance using the PBPK modeling method[285]. Modified *in vitro* experiment data with more similarities to *in vivo* status and accurate physiological parameters affecting the rate-limiting absorption process may be able to improve its predictive performance.

5.1.2.4. Other applications and trends of PBPK modeling methods. In addition to the above applications, the PBPK modeling method also could be used to predict first-in-human PK profiles[286]. It may be helpful for those drugs with nonlinear metabolism characteristics. Recently, a semi-PBPK model (or minimal PBPK model)[287,288] was reported to extensively survey human biologics PK profiles to assess the predominant clearance site and dynamically describe system plasma concentrations and two other virtual compartments, lumped tissues with continuous and fenestrated vascular endothelium. This semi-PBPK model structure could allow investigators quickly estimate PBPK parameters using system drug concentrations considering drug-receptor binding in systems as well as in tissues, as described in two recent reviews[289,290].

The PBPK modeling method is not an independent modeling method, and sometimes it is better to be integrated with other modeling methods for better results. In order to understand PK characteristics in mechanism, allometric scaling[291] and *in vitro*−*in vivo* extrapolation methods[292] can also be used to analyze preclinical data and compare the results with human data, which can provide more key information from different angles to develop a PBPK model more accurately indicating the real disposition process in humans[293]. Taking advantage of the PBPK ability to predict drug tissue concentration, a PBPK-PD model could be developed to capture pharmacodynamic characteristics in a more accurate way with more understanding of the mechanism[294,295], which is helpful for those drugs with significantly inconsistent exposure between system and targeted tissues. For a new moiety entity clinical development, verification of an established PBPK model based on human data with the specific ADMET mechanism is required, which may need an additional clinical trial. Recently, global development is going to become routine strategy, and ethnic differences in PK characteristics will be important. Therefore, PBPK could support evaluation of ethnic differences by its unique contribution to the mechanistic understanding[296]. Because population PK (PopPK) is routinely used to identify the key factors affecting PK profiles followed by quantifying these key factors, a PopPK study could verify PBPK simulations under some extreme scenarios, which may allow sponsors to waive some dedicated clinical trials (PBPK-PopPK strategy)[297]. Under many scenarios, we only pay attention to drug concentration in tissues related to PK, PD, or safety characteristics, so we don't need to accurately capture drug kinetics in other tissues. Therefore, in order to increase parameter reliability without a decrease in PBPK power, we could shrink the typical PBPK model integrating each tissue in humans to a semi-PBPK model integrating necessary target tissues and replace other tissues with one or two compartments.

Along with the coadministration of herbal or natural products, the potential herb−drug interaction is gaining increasing attention, and can be predicted using a PBPK modeling method. But accurate prediction of herb−drug interactions is still a challenging mission because of the complex composition and relatively limited knowledge of individual constituents that produce the interactions. A feasible procedure is to firstly identify the major constituents followed by compound−compound interaction prediction as previous introduced[158,298]. The major concern with this procedure is to prove that the interaction of major constituents is similar to that of the whole herb.

5.2. Summary

In summary, PBPK can be utilized to mechanistically understand and predict *a priori in vivo* pharmacokinetic characteristics from a whole body perspective by integrating system-specific and drug-specific parameters. PBPK modeling has been routinely conducted for new entities to illustrate pharmacokinetic characteristics when drug−drug interactions happen or when dosing in specific populations needs optimization. The predictive performance of CYP3A- and CYP2D6-mediated DDI was found to be best for new drugs as victim using PBPK method, which could be applied to waive part of clinical trial. Due to unclear changes in transporter-mediated mechanism and system-specific parameters in specific populations, PBPK modeling power is limited to supporting clinical trial design.

6. Novel animal models for DMPK studies

Animal models are mainly used in experimental physiology, experimental pathology and experimental therapeutics, especially in the study of new drugs. In the earliest stage of drug discovery/development, various cell-based models and animal models were used for the prediction of human PK and toxicokinetics[250]. The common laboratory animals for DMPK include rats, rabbits, dogs, monkeys, etc. However, with the development of gene editing technology, animal models of special ADME genes are needed to better study the mechanisms of DMPK, including the metabolic pathway and its regulatory mechanism.

6.1. Conventional transgenic animal models for DMPK research

Traditional animal models are constructed by homologous recombination in embryonic stem cells. This method implements foreign gene knock-in, but the recombination efficiency is very low, and the recombinant site has certain randomness[299]. In 2009, the discovery and application of nucleic acid engineering enzymes greatly advanced gene knock-in technology[300]. Zinc-finger nucleases (ZFNs) are the first nucleic acid engineering enzymes to be discovered[300]. They cleave DNA at specific sites to form double-strand breaks (DSBs), which are then repaired by cell homology and used as templates by exogenous donor DNA. The repair of DSBs result in knocking out the foreign gene[300]. Another engineering nuclease that was subsequently discovered for gene editing is transcription activator-like effector nucleases (TALENs)[300,301]. Since the 1990s, *Cyp* knockout (KO) mice have been successfully constructed using gene KO techniques, such as *Cyp1a2*, *Cyp2e1*, *Cyp2c9*, *Cyp3a4* and *Cyp2d6*[302–305]. In recent years some of mouse models have been used to study the DMPK of drugs under specific *Cyp* knockout conditions. To overcome the differences in subtype composition, protein expression, catalytic activity and substrate specificity between mouse and human CYP enzymes, scientists have built humanized animal models to better evaluate drug metabolism characteristics of human CYPs. For example, in 2007 humanized *Cyp1a1/2* mice were constructed for a toxicology study[306]. Humanized *Cyp2c19* mice for drug metabolism[307], humanized *Cyp3a4* mice for drug interactions[308], and humanized *Cyp2d6* mice for drug interactions[309] were reported in 2008, 2011 and 2012, respectively. In 2012, *Cyp2c* knockout mice and *Cyp2c9* humanized mice were generated for drug metabolism and drug interaction studies[304]. In 2015, humanized *Cyp2b6* mice were also constructed for drug metabolism[310].

Both *Cyp* gene KO and humanized mouse models have been constructed by traditional knockout techniques, *i.e.* homologous recombination of foreign DNA fragments with genes of the same or similar sequence in the host genome, thus replacing the corresponding gene sequences in the genome of the recipient cells and integrating them into the host. In the cell genome, the key technologies of this method include the acquisition of embryonic stem cells, the design of target, and the screening of embryonic stem cells. Homologous recombination is time-consuming, costly, as well as inefficient in gene editing, and may lead to adverse mutations. As it is difficult to obtain and culture embryonic stem cells in rats, the construction and application of knockout or knock-in rat models have lagged behind the mouse models. Rats are a rodent model animal widely used in DMPK and have many advantages over mice, such as larger size, easy manipulation, high tolerance to blood volume loss and large sample size. Moreover, rats in certain physiological and pathological states such as diabetes and breast cancer, are closer to humans than mice[311,312]. Therefore, it is particularly urgent to construct novel rat models of DMPK-related genes through KO and humanization.

6.2. Novel CRISPR/Cas9-based animal models for DMPK research

The clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated 9 (Cas9) system, as the third generation of artificial nuclease technology, provides a promising tool for genetic engineering. It offers an efficient approach to develop genetically modified animal models and a potential strategy for targeted gene therapies. The CRISPR/Cas9 system allows simultaneous digestion of multiple targets at multiple sites in the same cell, making it possible to knock out or knock in multiple genes. CRISPR/Cas9 as a new gene editing technology has many characteristics and advantages, including high targeting accuracy, simultaneous knockout of multiple sites of target genes, simplicity of operation and no species restriction. In recent years, CRISPR/Cas9 has been applied to the study of drug absorption, disposition, metabolism and excretion, as well as the preparation of ADME animal models.

Today CRISPR/Cas9 technology enables DMPK scientists to develop better and more predictable ADME models *in vitro* and *in vivo*, especially to study ADME genes that have not been fully explored previously. Most published papers of CRISPR/Cas9-mediated ADME describe CYP drug metabolic enzymes and ABC drug transporters. For example, in 2016, the rat *Cyp2d* gene locus (containing *Cyp2d1-5*) was knocked out and replaced with human *CYP2D6* in Wistar rats, but a functional characterization was not reported[313]. In the same year the rat *Cyp2e1* gene was knocked out in Sprague Dawley rats, and the KO rats were physiological normal and lost the expression and function of the CYP2E1 enzyme[314]. In 2017, *Cyp3a1* and *Cyp3a2* double KO rats were generated by CRISPR/Cas9 technology[315], and *Cyp2c* (*Cyp2c6*, *Cyp2c11* and *Cyp2c12*) genes were also knocked out in rats[316]. Finally, *Cyp2c11* gene was knocked out in Sprague Dawley rats[317]. *In vitro* and *in vivo* metabolic studies of the CYP substrates indicated that the target CYP isoform was functionally inactive in all KO rats[314,315]. It should be noted that KO models resulted in the compensatory regulation of other CYP isoforms involved in drug metabolism[314,315]. However, the potential mechanisms of these compensatory changes remain unclear. In addition, these KO models showed some differences, such as changes in serum testosterone concentrations[315] or alkaline phosphatase[314]. Some of these differences can be attributed to the deficiency of CYP functions, such as CYP3A-mediated testosterone metabolism. Therefore, these physiological changes in KO rats should be considered when comparing ADME data from KO models with data from wild-type rats. In addition to the rat KO models, a *Cyp2b9/10/13* KO mouse model was also generated *via* CRISPR/Cas9 technology[318]. It is interesting that there were no significant compensatory changes in other CYP isoforms in *Cyp2b* KO mice, which may be due to low CYP2B hepatic expression, especially in male mice[318]. In 2019, a novel MDR1 (*Mdr1a/b*) double-knockout rat model was generated in Sprague Dawley rats by the CRISPR/Cas9 technology[319]. The loss of MDR1 function significantly increased digoxin uptake in *Mdr1a/b*$^{-/-}$ rats. The MDR1 KO rat model is of great significance to study the function of MDR1 in drug transport, toxicity and drug resistance.

6.3. Summary

In summary, genome editing based on CRISPR/Cas9 has been identified as a breakthrough technology in constructing animal models. Novel animal models are not only conducive to the basic research of human diseases, but also can be used to study the molecular mechanisms of drug pharmacodynamics, toxicity and clinical use. Furthermore, DMPK animal models will promote the study of DMPK mechanisms and strengthen the relationship between drug metabolism and pharmacology/toxicology. For example, the potentials and mechanisms of DDI between erlotinib and docetaxel was studies by using *Cyp3a1/2* KO rats[320]. Docetaxel significantly increased the maximum concentration and systemic exposure of erlotinib in wild type (WT) rats, but the DDI was significantly attenuated in *Cyp3a1/2* KO rats, suggesting that the CYP3A plays the perpetrating role of docetaxel on erlotinib.

7. Non-classical xenobiotic metabolic pathways

Drug metabolism or drug biotransformation is the process by which xenobiotics are enzymatically modified to make them more readily excretable and eliminate pharmacological activity. Drug metabolism is the prominent process in drug disposition. Understanding the metabolic fate and the corresponding enzymes are important with regard to metabolite toxicity and drug−drug interaction risks. Detailed data from drug metabolism studies aid in the drug clinical practice and drug design and modification. Over the past decades the basic mechanism and rules of drug metabolism, especially mediated by CYP, have been clarified. The strategies and approaches used for drug metabolism investigations have come to maturity and industrialization. Recently, with the rapid development of the separation technology and qualitative techniques, such as IMS-TOF/MS or novel 2D NMR technology, and the considerable amount of attention directed at non-CYP enzymes, several undesirable drug metabolites have been identified, and novel metabolic reactions were discovered. Some outwardly rational reactions are newly described based on the novel understandings of the mechanism underlying common biotransformations. This section briefly reviews a series of cases of novel metabolic reactions and pathways to provide readers new insights into investigations on drug metabolism.

7.1. Oxidative pathways

Oxidative pathways, including sp^3-hybridized *C*-hydroxylation, unsaturated *C*-oxidation, *N*-dealkylation/deamination, *O*-dealkylation, *S*-dealkylation, *N*-oxidation, *S*-oxidation, and oxidative cleavage of esters and amides classified by functional groups are the most common biotransformations. In recent years some unexpected oxidative reactions or pathways have been reported.

Aromatic ring-containing drugs are most common and generally metabolized by P450-mediated π-electron oxidations to form an arene-epoxidized intermediate. The latter undergoes a hydride shift spontaneously to produce stable phenol metabolite(s). However, for some specific structures, unstable epoxides are preferentially attacked by nucleophilic substances, thereby leading to reactive intermediate-related covalent attachments. For example, for cocaine, the covalent adducts of biological thiols are first characterized[321]. *In vitro* investigations revealed that CYP1A2, 2C9, and 2D6 catalyze the formation of a reactive epoxide intermediate from the oxidation of the cocaine phenyl moiety (Fig. 3A). Although an aryl moiety is generally considered a stable functional group, epoxide ring opening is attacked by nucleophilic thiolates, such as *N*-acetylcysteine or glutathione, for cocaine.

Carbon−carbon cleavage and formation reactions are rare in xenobiotic metabolism. Recent studies have focused on the roles of flavin-containing monooxygenases (FMOs) to catalyze unexpected Baeyer−Villiger oxidations, which is a kind of carbon−carbon bond cleavage reaction. E7016, a potential anticancer agent with a 4-hydroxypiperidine moiety was confirmed to be a substrate of FMO5[322]. The generation of the major ring-opened hydroxylcarboxylate metabolite was proposed by a three-step reaction, as follows: dehydrogenation of the secondary alcohol on the parent drug to form piperidine-4-one, followed by insertion of an oxygen atom to form a lactone *via* the Baeyer−Villiger oxidation, and further CEs-mediated hydrolysis. Recently, the 2,3-dihydropyridin-4-one (DHPO) ring in MRX-I (an analog of the antibiotic linezolid) was also reported to undergo a similar carbon−carbon cleavage reaction in humans[323]. However, different from piperidine-4-one, Baeyer−Villiger oxidation of the DHPO ring forms an enol lactone and is further hydrolyzed to an enol, which can be transformed to an aldehyde intermediate by enol−aldehyde tautomerism. The aldehyde intermediate underwent either oxidation catalyzed by short-chain dehydrogenase, aldehyde ketone reductase, and aldehyde dehydrogenase (ALDH) or reduction mediated by ALDH to generate the observed directed DHPO ring-opening metabolites MRX459 or MRX455-1, respectively (Fig. 3B). $H_2^{18}O$ experiments were conducted to elucidate the mechanism underlying the formation of the two metabolites.

7.2. Reductive metabolic pathways

The majority of *in vivo* biotransformations are oxidation, while reductive reactions preferentially occur in anaerobic or low-O conditions. A considerable number of the same enzymes that catalyze oxidative metabolism, such as P450s, aldo-keto reductase, carbonyl reductase, xanthine oxidase, aldehyde oxidase, and quinone oxidoreductase, can also be involved in reductions. Under the catalysis of some specific enzymes or the involvement of reducing agents, some uncommon reductive metabolic pathways are observed.

NADPH-cytochrome P450 reductase (POR) and cytochrome-b5 is crucial for P450 electron transporter chain integrity because they donate electrons to P450s from NADPH. Thus, most marketed recombinant P450 enzymes generally contain cytochrome-b5 and POR to enhance their oxidative efficiencies. In some cases, POR alone can also catalyze one-electron reduction, such as with aristolochic acid[324]. Another reported substrate of POR is an aldehyde intermediate (M-CHO) that is formed during the metabolism of imrecoxib, which is a moderate COX-2 inhibitor[325]. POR expresses dual effects on further M-CHO metabolism, namely oxidation to form carboxylic acid metabolite (M2) and unexpected reduction to form a hydroxymethyl metabolite (M1), by donating electrons to P450s or competitively to the substrate, respectively (Fig. 4A). The two opposite metabolic pathways, especially M-CHO reduction, led to an underestimation of the amount of M2 in static *in vitro* incubations.

7.3. Hydrolysis pathways

Many drugs with specific functional moieties, including esters, amides, thioesters, epoxides, sulfates, and glucuronides can be

metabolized by adding water. Hydrolysis is generally carried out by the corresponding enzymes, such as esterase or amidases. Prodrug design has received increasing interest, thereby leading to considerable attention to the important roles of hydrolytic metabolism. Some novel hydrolytic enzymes also catalyze undesirable reactions.

For example, arylacetamide deacetylase (AADAC) is a serine hydrolase expressed in human liver and intestine that is rarely reported compared with other hydrolytic enzymes (CEs and paraxonase). Only one AADAC isoform is present in humans. AADAC is identified as a lipase that is capable of hydrolyzing endogenous cholesterol ester[326]; however, it has been recently found to be responsible for some clinical drugs, such as prasugrel[327] and vicagrel[328]. Different from clopidogrel, the thiolactone metabolite of vicagrel is formed *via* a rapid hydrolysis before intestinal absorption[329]. The first activation step for vicagrel was initially believed to be mediated only by human intestine CES-2 (CES2) until a recent finding showed that AADAC also contributed to vicagrel hydrolysis (Fig. 4B). The activation of the parent drug before entering the systemic circulation guarantees short onset time and avoidance of "clopidogrel resistance" attributed to CYP2C9 gene polymorphisms.

Another case of hydrolytic enzymes newly identified is dipeptidyl peptidases (DPPs), which can catalyze the hydrolysis of cyanopyrrolidine DDP-4 inhibitors. Generally, a nitrile group in the drug structures prevents metabolism because of its well-known inertness, and as a result, a nitrile moiety is increasing introduced as a block on metabolically labile sites in drug design[330]. However, for vildagliptin, anagliptin, and besigliptin (not saxagliptin), the biotransformation of the nitrile group into carboxylic acid is the major metabolic pathway *in vivo* by the DPP family such as DPP-4, DPP-2, DPP-8, DPP-9, and fibroblast activation protein-α[331]. Among them, DPP-2 has the highest hydrolytic capacity after DPP-4. However, other substrates containing a nitrile group, such as lacosamide and flutamide, cannot be hydrolyzed by DPPs probably because the nitrile moiety in these structures cannot be positioned in the catalytic triad of Asp-His-Ser of DPPs.

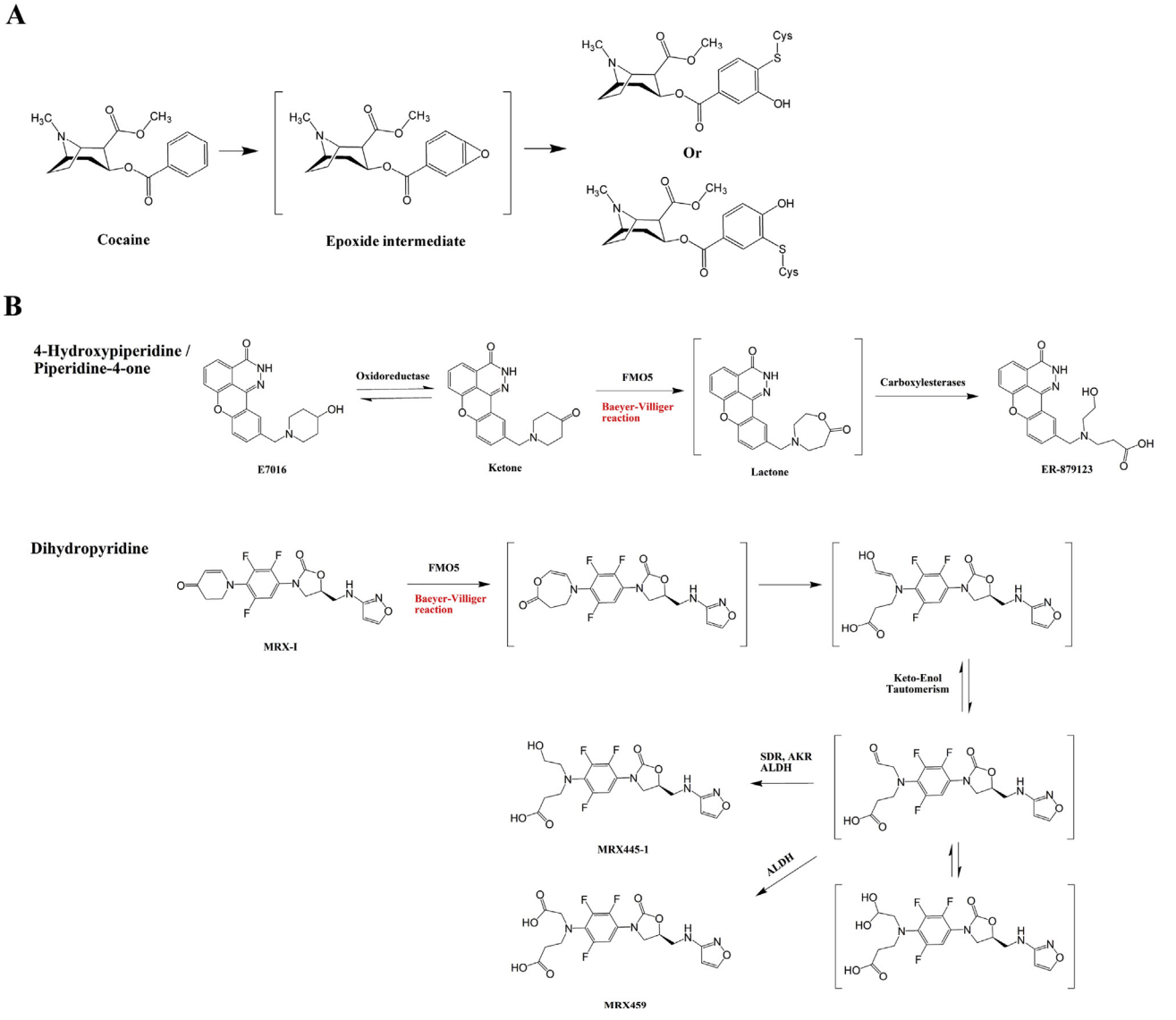

Figure 3 Cases of some unusual metabolic pathways of oxidation, including: (A) proposed mechanism for cocaine metabolism to thiol-related adduction, and (B) Baeyer−Villiger oxidation mediated by FMO5.

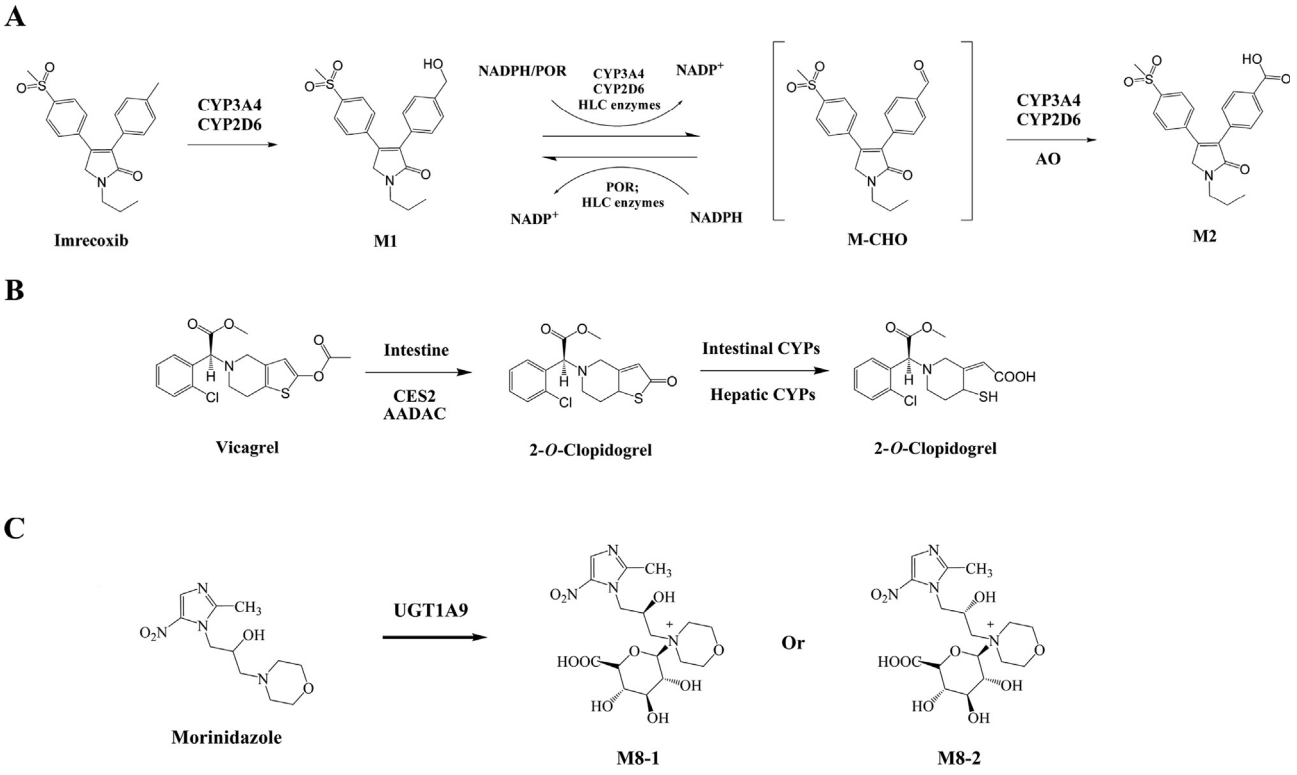

Figure 4 Instances of some unusual metabolic pathways for reduction, hydrolysis, and conjugation, including: (A) formation mechanism for M1 and M2, the major imrecoxib metabolites in humans, (B) hydrolysis pathways of vicagrel in humans, and (C) N^+-glucuronidation of morinidazole.

7.4. Conjugation pathways

Generally, conjugation pathways involve the addition of an endogenous hydrophilic group to a drug or its metabolite(s), including glucuronidation, sulfation, glutathione conjugation, amino acid conjugation, acetylation, and methylation. Conjugation generally introduces polar groups to facilitate drug excretion, except for methylation and acetylation. Although this finding is true for many cases, several unusual conjugative reactions were reported in recent years.

The substrates for glucuronidation generally have an OH (*i.e.*, alcohols, phenol, and carboxylic acids), amino (*i.e.*, aliphatic tertiary amine, aromatic primary amine, and sulfonamide) or thiophenol group. In general, for drugs possessing both tertiary amine and hydroxyl groups, *O*-glucuronidation is always formed preferentially over *N*-glucuronidation. However, a reversible regioselectivity is observed in the conjugative metabolism of morinidazole in humans, where glucuronidation prefers the tertiary nitrogen of the morpholine ring to the aliphatic hydroxyl group at the side chain of morinidazole (Fig. 4C)[332]. Additionally, molecular modeling studies indicated that the regioselectivity for morinidazole glucuronidation is unrelated to steric hindrance. UGT1A4, as well as UGT1A3 and 2B10, are often considered enzymes that play important roles in N^+-glucuronidation[333–335]. However, according to the morinidazole experience, UGT1A9 can be identified as a new UGT isoform specializing in tertiary aliphatic amine *N*-glucuronidation.

In addition, several novel conjugates, including carnitine conjugation to cyclopropanecarboxylic acid[336] creatinine conjugation to andrographolide[337], and phosphoethanolamine conjugation to pimasertib[338], recently have been discovered. The combination or further modification of the common conjugation process has been also reported[339,340].

7.5. Summary

In recent years there has been an increased effort to better understand the role of enzymes beyond P450, UDP-glucuronosyltransferase, and aldehyde oxidase in drug metabolism. Recently, several biological enzymes responsible for endogenous substrate catalysis, such as dipeptidyl peptidases and arylacetamide deacetylase, are newly proven to have additional capabilities in drug transformation. Drugs that rely on these non-P450 enzymes for their *in vivo* clearance, however, usually undergo non-classical metabolic pathways. The basic mechanism and rules of drug metabolism cannot be characterized based on the structures of the drugs alone, because the presence of metabolic intermediates that would allow for the intra-molecular rearrangement are likely factors in unusual metabolite formation. This subtle but potentially significant hypothesis suggests that the electron or radical-mediated modulation of biotransformation characteristics may represent uncommon underlying mechanisms for undesirable metabolic pathways, with relevant toxicological consequences.

Several novel and unusual reactions and pathways have been reviewed. Most of these reactions are attributed to (I) metabolic intermediate formation and rearrangement and (II) the involvement of novel metabolic enzymes, especially non-P450s.

Considering the availability of sophisticated and sensitive analytical instrumentation, as well as the introduction of modern approaches in drug metabolism investigation, new metabolic reactions continue to be discovered. Accurately predicting drug metabolism in an empirical manner and clarifying the metabolism mechanisms responsible for drug adverse reactions and drug−drug interactions will increase in the future. Additionally, valuable inspiration may be provided for rational drug design and modification with the expansion of metabolic enzymes, many of which are recognized as new therapeutic targets.

8. Conclusions and perspectives

DMPK research is essential for understanding the efficacy and safety of medications. Integrated studies on drug-metabolizing enzymes and transporters underlying the ADME processes as well as their transcriptional and posttranscriptional regulation mechanisms provide a comprehensive understanding of interindividual variations in pharmacotherapy. Future studies in these areas will undoubtedly advance our understanding to achieve better prediction of PK properties. Understanding the DDIs and disease−drug interactions is clinically important as such interactions may increase the risk of adverse reactions or lead to treatment failure. Although DDIs between small molecule drugs are relatively well-characterized, other potential interactions are not fully explored, including interactions with herbal biologics and other new forms of therapeutics. Furthermore, more attention should be paid to the microbiota-mediated drug interactions when examining potential DDIs and HDIs. There is emerging evidence indicating that disease−drug interactions can have a profound impact on the therapeutic outcomes. Further studies are needed to reveal the critical mechanisms by which disease−drug interactions are produced. While the benefits of PBPK are obvious for clinical trials, it is better to integrate PBPK with other modeling methods and consult experimental findings to design clinical trials in support of new drug development. Novel animal models such as those created through CRISPR-Cas9-based gene editing techniques should be an invaluable addition to current tools for PK studies. With the application of sensitive and accurate analytical instruments and technologies, many new metabolic reactions and biotransformation pathways have been and will be discovered. Predicting drug metabolism more accurately and clarifying the metabolic mechanisms responsible for adverse drug reactions and DDIs will become possible in the future. Collectively, DMPK research awaits further innovation and mechanistic studies while DMPK remains a critical component in drug development, and is essential for practicing precision medication.

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Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2019.10.001.

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