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Renal Transporters in Drug Development

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Keywords

renal clearance, SLC transporters, ABC transporters, drug metabolism, nephrotoxicity, pharmacokinetics

Abstract

The kidney plays a vital role in the body's defense against potentially toxic xenobiotics and metabolic waste products through elimination pathways. In particular, secretory transporters in the proximal tubule are major determinants of the disposition of xenobiotics, including many prescription drugs. In the past decade, considerable progress has been made in understanding the impact of renal transporters on the disposition of many clinically used drugs. In addition, renal transporters have been implicated as sites for numerous clinically important drug-drug interactions. This review begins with a description of renal drug handling and presents relevant equations for the calculation of renal clearance, including filtration and secretory clearance. In addition, data on the localization, expression, substrates, and inhibitors of renal drug transporters are tabulated. The recent US Food and Drug Administration drug-drug interaction draft guidance as it pertains to the study of renal drug transporters is presented. Renal drug elimination in special populations and transporter splicing variants are also described.

INTRODUCTION

The kidney plays a vital role in maintaining total body homeostasis by conserving essential nutrients and eliminating potentially toxic xenobiotics, xenobiotic metabolites, and metabolic wastes. The conservation and elimination functions are performed in the physiologic units of the kidney, the nephrons, which number approximately 1 million per kidney in a healthy young adult. The functional components of the nephron include the glomerulus and the renal tubules, the latter of which consist of a monolayer of epithelial cells that is divided into general segments (the proximal tubule, the loop of Henle, and the distal tubule). A major function of the epithelial cells of the renal tubule is to sense and maintain solute balance in the body by reabsorbing glucose, amino acids, and other nutrients and to secrete environmental toxins and high concentrations of endogenous compounds, which could be potentially toxic. The reabsorptive and secretory functions of the renal tubule are performed by a variety of membrane transporters located in the basolateral and luminal membranes of the tubular epithelium.

More than 400 membrane transporter genes in two distinct classes, the solute carrier (SLC) superfamily and the ATP-binding cassette (ABC) superfamily (1), are encoded in the human genome. Typically, SLC transporters are integrated into the membrane and function to move solutes into or out of cells either by facilitated transport along the electrochemical gradient or by cotransport against an electrochemical gradient by utilizing the concentration gradient of another solute. Likewise, ABC transporters are multimembrane-spanning proteins, but they drive the transport of solutes against an electrochemical gradient, utilizing energy from ATP hydrolysis. Similarity maps designed using substrate type, mechanism of transport, evolutionary conservation, and tissue specificity show that transporters that interact with similar chemicals generally cluster together, suggesting that they work in concert, despite their weak sequence similarities (2). Furthermore, in the kidney, evidence from structural, genetic, and functional studies indicate that together SLC and ABC transporters are involved in the renal elimination of a wide array of nutrients, toxins, xenobiotics, and metabolites.

During drug development, renal transporters must be evaluated to understand the pharmacokinetic profiles of new molecular entities (NMEs) and potential sources of interindividual variation in drug disposition, toxicity, and response. For many years, drug developers concentrated on studies of drug metabolism pathways for NMEs as a basis for understanding pharmacokinetic mechanisms and sources of interindividual variation in pharmacokinetics and pharmacodynamics. Recently, it has become clear that transporters play a major role in pharmacokinetics, and that they, together with drug-metabolizing enzymes, are the major determinants of both hepatic and renal drug elimination. Although fecal elimination occurs for some drugs, most drugs or their metabolic end products are ultimately eliminated in the urine. In fact, 32% of the top 200 prescribed drugs in 2010 (3) are cleared by renal mechanisms; drugs are considered renally eliminated when $\geq$25% of the absorbed dose is excreted unchanged in urine (**Figure 1**). Therefore, to understand the mechanisms of elimination of a NME, the transporters involved in the renal clearance of the drug and its active metabolites need to be identified. Variation in the expression levels and activities of renal transporters may be a source of variation in the pharmacokinetics and pharmacodynamics of drugs. Transporters, like drug-metabolizing enzymes, may be targets for drug-drug interactions (DDIs). For example, one drug may inhibit the tubular secretion of a second drug through competitive inhibition mechanisms at a renal transporter. In fact, the US Food and Drug Administration (FDA) recently published a series of decision trees to guide clinical DDI studies of renally cleared drugs (4).

This review focuses on renal drug transporters and their impact on drug elimination, DDIs, and drug development. The goals are to (*a*) highlight renal transporters that are important in

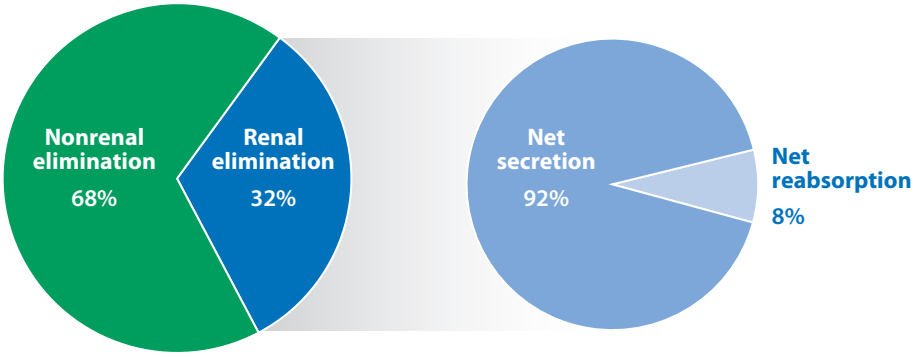

Figure 1

The contribution of the kidney to the elimination of the top 200 prescribed drugs in the United States in 2010 (3). Drugs are considered renally eliminated when ≥25% of their absorbed dose is excreted unchanged in the urine. As certain drugs may appear multiple times on the top 200 list, only unique chemical entities are included ($n = 114$). Net secretion is designated for drugs whose renal clearances exceed their filtration clearances.

drug elimination and summarize recent data on their expression levels, substrates, inhibitors, and associated DDIs, (*b*) describe how the recent FDA guidelines can be applied to the development of NMEs, and (*c*) review differences in renal clearance in special populations. Although reabsorptive transporters are also involved in the renal handling of drugs, this review concentrates largely on secretory transporters, most of which are expressed in the proximal tubule.

ESTIMATION OF RENAL CLEARANCE AND THE CONTRIBUTION OF RENAL SECRETORY TRANSPORTERS

To understand the pharmacokinetic profile of a drug, identifying the routes of its elimination from the body is important. In a typical pharmacokinetic study, total clearance (CL_T) and renal clearance (CL_R) of a drug are determined directly from measurements of drug concentrations in plasma and urine, respectively (5). The difference between total and renal clearance represents nonrenal clearance, which is often attributed to metabolic clearance in the liver. Renal clearance, which reflects the volume of plasma from which a drug is completely removed by the kidney per unit time, can be calculated by several equations:

$$CL_R = \text{rate of urinary excretion}/C, \quad 1.$$

where C is the concentration of drug in plasma;

$$CL_R = \text{total amount excreted unchanged in urine}/AUC, \quad 2.$$

where AUC is the area under the plasma drug concentration-time curve from the time of drug administration extrapolated to infinite time; and

$$CL_R = fe \bullet CL_T, \quad 3.$$

where fe is the fraction of an intravenous dose excreted as unchanged drug in the urine and CL_T is the total body clearance. The term fe may also represent the fraction of the absorbed dose (F • D, where F is the bioavailability of the drug and D is the dose) that is excreted unchanged in the urine after oral administration.

The amount of drug that is excreted in urine is the net result of glomerular filtration, tubular secretion, and tubular reabsorption. The rate at which drugs are excreted in the urine is:

$$\text{Rate of urinary excretion} = (1 - F_R)[\text{rate of filtration} + \text{rate of tubular secretion}], \quad 4.$$

where F_R is the fraction of drug that is reabsorbed from the lumen of the kidney. The rate of filtration is:

$$\text{Rate of filtration} = \text{fu} \bullet \text{GFR} \bullet \text{C}, \quad 5.$$

where fu is the fraction of unbound drug in the plasma and GFR is the glomerular filtration rate.

To determine whether tubular secretion occurs, typically one compares the rate of urinary excretion (Equations 1 and 4) with the rate of filtration (Equation 5), or in simpler terms CL_R to fu • GFR. If $CL_R >$ fu • GFR, then net secretion is assumed; if $CL_R <$ fu • GFR, then net reabsorption is assumed. In either case, both the processes of secretion and reabsorption may take place but are not reflected in the net values. Interestingly, drugs that are eliminated by renal mechanisms are more likely to undergo net secretion than net reabsorption (**Figure 1**). If net secretion is estimated, understanding and predicting potential DDIs or effects of environmental and genetic factors on renal drug elimination require that the transporters responsible for the drug's tubular secretion be identified.

TRANSPORTERS INVOLVED IN RENAL DRUG SECRETION

Transporters expressed on basolateral and apical membranes of the renal tubule epithelium are generally found in the proximal tubule and work in systems to mediate renal drug elimination (**Figure 2**). For a small molecule to be actively secreted into the tubule lumen, at least two distinct transporters are required: one at the basolateral membrane of the tubule cell to accept molecules from the blood and one at the apical membrane to mediate the exit of the molecule to the tubule fluid. Carrier-mediated transport systems at both apical and basolateral membranes have a tendency to be charge selective with distinct systems for anionic and cationic drugs. However, recent studies suggest that there is some overlap (6–8). The systems of transporters that are largely involved in the secretion of cationic drugs include the organic cation transporter OCT2 on the basolateral membrane and the multidrug and toxin extrusion proteins MATE1 and MATE2/2K on the apical membrane. Transporter systems for weakly acidic drugs include the organic anion transporters OAT1 and OAT3 on the basolateral membrane and the multidrug resistance-associated proteins MRP2 and MRP4 on the apical membrane.

During the drug development process, investigators should determine which transporters are likely to play a role in the renal secretion of a NME by performing in vitro studies to obtain kinetic parameters of drugs with various renal transporters. In the past decade, numerous studies have been performed to identify endogenous compounds, toxins, xenobiotics, and metabolites as substrates and inhibitors of renal secretory transporters (**Tables 1** and **2**). Net tubular secretion is predicted to play an important role in the overall elimination of many commonly prescribed drugs (**Figure 1**). These drugs are diverse in molecular weight, charge, and therapeutic classes and include antibacterials (ciprofloxacin, cephalexin, levofloxacin), antihistamines (famotidine, ranitidine), diuretics (furosemide, trimethoprim), antidiabetics (metformin), and antihyperlipidemics (rosuvastatin, pravastatin). The transporters that play an important role in the renal elimination of these drugs have been predicted by in vitro studies (**Figure 3**). The newly identified transporters (e.g., OATP4C1, MATE1, MATE2K) are less well-characterized than multidrug resistance protein 1 (MDR1), MRPs, OCT2, and OATs, which have been studied for more than a decade. Therefore, the drugs that are secreted by unknown mechanisms may interact with these

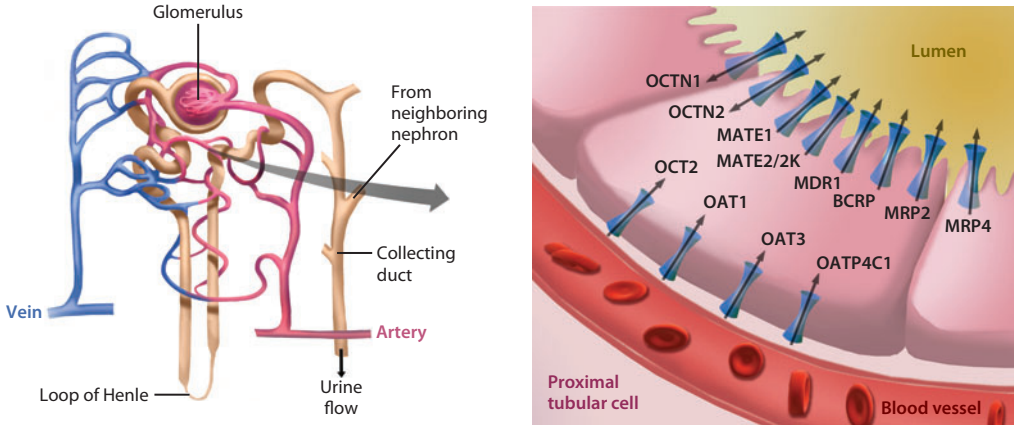

Figure 2

Drug transporters in the nephron of the kidney. Illustration of the nephron (*left*) and secretory transporters in the proximal tubular cell that facilitate the renal secretory elimination of diverse medications (*right*). Abbreviations: BCRP, breast cancer resistance protein; MATE, multidrug and toxin extrusion protein; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter.

understudied transporters. Drugs that are eliminated in the kidney with a net tubular secretion are particularly susceptible to DDIs when coadministered with another medication that interacts with the same transporters.

CLINICAL DRUG-DRUG INTERACTIONS MEDIATED BY RENAL SECRETORY TRANSPORTERS

Historically, DDIs were thought to be mediated primarily by interactions with drug-metabolizing enzymes, but current evidence suggests that they may also be mediated by drug transporters (**Table 3**). Although a particular drug may strongly interact with a specific transporter in vitro, the prediction of a clinical DDI must also consider the plasma concentration, and particularly unbound plasma concentration, of the drug at therapeutic doses [information is available in resources such as *Goodman & Gilman's* (9) and *Clarke's Analysis of Drugs and Poisons* (10)]. The kidney is an important site for transporter-mediated DDIs, and over the years, many clinically important transporter-mediated DDIs in the kidney have been described (**Table 3**). In general, DDIs in the kidney result in higher plasma concentrations of the victim drug when it is a substrate of a renal secretory transporter. For example, cimetidine, an H2-receptor antagonist that is used in the treatment of ulcers and gastric acidity, inhibits the renal clearance of metformin, an antidiabetic agent used to treat type 2 diabetes. This, in turn, results in higher plasma concentrations of metformin (11), which increase its risk of toxicity.

Renal transporter-mediated DDIs have also been exploited to enhance drug concentrations or to protect the kidney. For example, the coadministration of probenecid and penicillin was popularized during World War II as a means of rationing the limited penicillin supplies because it allowed for single-dose administration of penicillin. In brief, probenecid inhibited the renal secretory clearance of penicillin and therefore prolonged its half-life (12). Nowadays, the coadministration of probenecid with cidofovir, an antiviral drug, is required by the FDA (13) to protect against cidofovir-mediated nephrotoxicity by inhibiting cidofovir uptake at the basolateral membrane (14, 15). If a DDI were to occur at the apical membrane, the intracellular kidney

Table 1 Kinetic characteristics of substrates of transporters involved in renal elimination

Basolateral transporter	Substrate	Km (μM)
SLC22A2 (OCT2)	Amantadine	27[b]
	Amiloride	95[b]
	ASP+	24[b]
	Cimetidine	72.6[b]
	Dopamine	1,400[b]
	Epinephrine	420[b]
	Famotidine	56.1[b]
	Histamine	940[b]
	Lamivudine	46.3[a]
	Metformin[f]	680[b], 990[b], 1,072[b], 3,171[b], 3,356[b]
	Memantine	34[b]
	MPP^+	16[b], 19[a], 19.5[b], 19.5[b]
	Norepinephrine	1,500[b]
	Prostaglandin E2	0.0289[b]
	Prostaglandin F2α	0.344[b]
	Ranitidine	65.2[b]
	Serotonin	290[b]
	Tetraethylammonium	33.8[a], 76[a]
	Varenicline[f]	370[b]
	YM155	2.67[b]
SLC22A6 (OAT1)	6-carboxyfluorescein	3.93[b]
	Acyclovir[f]	342[b]
	Adefovir	30[a], 23.8[b]
	Cidofovir	30[b], 58[b], 46[a]
	Dimesna	636[b]
	Edaravone sulfate	10.8[b]
	Ganciclovir	896[b]
	Glutarate	10.7[b]
	Methotrexate[f]	554[b], 724[a]
	Ochratoxin A	0.42[b]
	Olmesartan	0.0683[b]
	PAH	15.4[b], 20.1[b], 28[b], 9.3[a], 5[b], 4[a], 3.9[a]
	Perfluoroheptanoate	50.5[b]
	Perfluorooctanoate	43.2[b]
	Probenecid	26[b]
	Prostaglandin E2	0.97[b]
	Prostaglandin F2α	0.575[b]
	Tenofovir[f]	33.8[b]
	Uric acid	197.6[b]
	Zidovudine[f]	45.9[b]

Apical transporter	Substrate	Km (μM)
ABCB1 (MDR1, P-gp)	Biotin	13[b]
	Colchicine	1,640[d]
	Dexamethasone	826[c]
	Digoxin[f]	73[d], 177[d], 181[c]
	Etoposide	255[b], 461[d]
	Fexofenadine	150[d]
	Indinavir	0.47[c]
	Irinotecan	45.5[b], 116.1[d]
	Loperamide[f]	11.4[c]
	Nicardipine	2.6[c]
	Paclitaxel	1.4[c], 65[d]
	Rhodamine 123	21[c]
	Ritonavir	0.8[b]
	Saquinavir	14.5[b], 15.4[d]
	Topotecan	78.3[d], 102[b]
	Valinomycin	2.5[c]
	Verapamil	4.1[c]
	Vinblastine	1.7[c], 19[d], 89.2[d], 146[a], 253[b]
	Vincristine	3.7[c]
ABCC2 (MRP2, cMOAT)	Dehydroepiandrosterone sulfate	14.9[e]
	Estradiol-17β-glucuronide	7.2[e]
	Etoposide	617[b]
	Irinotecan	48.9[e], 90.8[b]
	Methotrexate	480[e]
	Olmesartan	14.9[e]
	PAH	880[e], 2,100[e], 5,000[e]
	SN-38	180[e]
	SN-38 glucuronide	5.7[e]
	Valsartan	30.4[e]
	Vinblastine	137.3[b]
ABCC4 (MRP4)	Chenodeoxycholylglycine	5.9[e]
	Chenodeoxycholyltaurine	3.6[e]
	Cholate	14.8[e]
	Cholyltaurine	7.7[e]
	Cyclic AMP	44.5[e]
	Dehydroepiandrosterone sulfate	1.9[e], 26.2[e]
	Deoxycholylglycine	6.7[e]
	Estradiol-17β-glucuronide	30.3[e]

(*Continued*)

Table 1 (*Continued*)

Basolateral transporter	Substrate	Km (μM)
SLC22A8 (OAT3)	1-BSA	5,098[b]
	Adipate	136[b]
	α-ketoglutarate	92.8[b]
	Bumetanide[f]	7.8[a], 1,586[b]
	Cimetidine	57.4[a], 113[b], 174[b]
	Cortisol	2.4[a]
	Dimesna	390[b]
	DMPS	40[b]
	Edaravone sulfate	15.1[b]
	Estrone 3-sulfate	2.18[b], 2.21[b], 3.1[a], 6.3[b], 7.5[b]
	Fexofenadine	70.2[b]
	Methotrexate	10.9[a], 17.2[a], 21.1[b]
	MPS	2,139[a]
	Ochratoxin A	0.75[b]
	Olmesartan	0.12[b]
	PAH	87.2[a]
	Perfluoroheptanoate	65.7[b]
	Perfluorooctanoate	174.5[b]
	Pimelate	634[b]
	Pitavastatin	3.3[a]
	PNU-288034	44[b]
	Pravastatin	27.2[b]
	Probenecid	32[b]
	Prostaglandin E2	0.345[b]
	Prostaglandin F2α	1.092[b]
	Rosuvastatin	7.4[a]
	Sitagliptin	162[b]
	Suberate	232[b]
	Sulfasalazine	3[a]
	Tetracycline	566.2[b]
	Uric acid	380.3[b]
	Zidovudine	145[b]
SLCO4C1 (OATP4C1)	Digoxin	7.8[b]
	Estrone 3-sulfate	26.6[b]
	Ouabain	0.38[b]
	T3	5.9[b]

Apical transporter	Substrate	Km (μM)
ABCC4 (MRP4), continued	Folic acid	170[e]
	Methotrexate	220[e], 220[e], 1,300[e]
	Olmesartan	26.2[e]
	PAH	160[e]
	Prostaglandin E1	2.1[e]
	Prostaglandin E2	3.4[e]
	Topotecan	1.66[b]
ABCG2 (BCRP, MXR)	4-MUS	12.9[e]
	Daunorubicin	2.5[e]
	Doxorubicin	5[e]
	Estradiol-17β-glucuronide	44.2[e]
	Estrone 3-sulfate	6.8[e], 16.6[e]
	Hematoporphyrin	17.8[e]
	Methotrexate	681[e], 1,340[e], 1,410[e]
	Mitoxantrone	7[e]
	Pitavastatin[f]	5.73[e]
	Rosuvastatin[f]	2.02[e], 10.1[b]
	SN-38	4[e]
	SN-38 glucuronide	26[e]
	Sulfasalazine	0.7[e]
	Topotecan	213[b]
SLC22A4 (OCTN1)	Ergothioneine	21[b]
	Ipratropium	444[b]
	Tetraethylammonium	195[a], 1,800[b]
SLC22A5 (OCTN2)	Acetyl-L-carnitine	8.5[b]
	D-carnitine	10.9[b], 98.3[a]
	Ipratropium	53[b]
	L-carnitine	3.5[b], 4.3[b], 4.8[a]
SLC47A1 (MATE1)	Acyclovir	2,640[b]
	Cephalexin	5,900[b]
	Cimetidine	170[b]
	Estrone 3-sulfate	470[b]
	Ganciclovir	5,120[b]
	Guanidine	2,100[b]
	Metformin	202[b], 227[b], 780[b]
	N-methylpyridinium	16[b], 100[b]
	Paraquat	169[b]
	PNU-288034	340[b]
	Procainamide	1,230[b]
	Tetraethylammonium	220[b], 380[b]
	Topotecan	70[b]
SLC47A2 (MATE2K)	Acyclovir	4,320[b]
	Cimetidine	120[b], 370[b]
	Estrone 3-sulfate	850[b]
	Ganciclovir	4,280[b]

(*Continued*)

Table 1 (*Continued*)

Basolateral transporter	Substrate	Km (μM)	Apical transporter	Substrate	Km (μM)
			SLC47A2 (MATE2K), continued	Guanidine	4,200[b]
				Metformin	1,050[b], 1,980[b]
				N-methylpyridinium	93.5[b], 110[b]
				Procainamide	1580[b], 4,100[b]
				Tetraethylammonium	760[b], 830[b]
				Topotecan	60[b]

In vitro methods: [a]oocytes, [b]transfected S2/HEK293/HeLa/CHO/COS/MDCK/HepG2/HRPE/LLC-PK1 cells, [c]ATPase assay, [d]Caco-2, [e]Sf9/V79/LLC-PK1/HEK293/bile canalicular membrane vesicles.

[f]Denotes drugs that can potentially be used for in vivo (clinical) studies (16).

References can be found in the **Supplemental Material** (follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**).

Abbreviations: AMP, adenosine monophosphate; BCRP, breast cancer resistance protein; cMOAT, canalicular multispecific organic anion transporter; DMPS, 2,3-dimercapto-1-propanesulfonic acid; MATE, multidrug and toxin extrusion protein; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; MUS, methylumbelliferone sulfate; MXR, multixenobiotic resistance protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PAH, *para*-aminohippurate; P-gp, P-glycoprotein.

Table 2 Kinetic characteristics of inhibitors of transporters involved in renal elimination

Basolateral transporter	Substrate	IC_{50}, K_i (μM)	Apical transporter	Substrate	IC_{50}, K_i (μM)
SLC22A2 (OCT2)	Amantadine	45.9[b], 28.4[b]	*ABCB1* (MDR1, P-gp)	Amiodarone	5.48[b], 22.5[b], 45.6[b]
	Amitriptyline	14[b]		Astemizole	2.73[b]
	Atropine	39[b]		Azelastine	16[b], 30[b]
	Bmim-Cl	1.5[b]		Azithromycin	21.8[d]
	BmPy-Cl	0.48[b]		Clarithromycin	4.1[d]
	Butylscopolamine	764[b]		Cyclosporine[f]	1.36[b], 1.4[b], 1.6[b], 6.18[b], 0.46[d], 2.18[b]
	Carvedilol	63[b]			
	Chloroquine	1,096[b]		Desethylamiodarone	1.27[b], 15.4[b], 25.2[b], 11.8[b], 41.8[b]
	Chlorpromazine	14[b]			
	Cimetidine[f]	120[b], 110[b], 373[a]		Dipyridamole	40[b]
				Elacridar	0.027[b], 0.043[b], 0.055[b], 0.18[b], 0.39[d], 0.44[b]
	Clonidine	16[b], 23[b]			
	Cocaine	113[b]		Erlotinib	2[e]
	Corticosterone	5.35[b]		Erythromycin	10[d], 22.7[d], 119[d]
	Creatinine	580[b]		Itraconazole	0.95[b], 2[d]
	D-amphetamine	10.5[b]		Ketoconazole	1.34[b], 3.07[b], 5.49[b], 5.6[b], 6.34[b]
	Decynium-22	0.1[a], 13.8[a]			
	Denfluramine	10[b]		Paclitaxel	54[b]
	Desipramine	16[a]		Quinidine[f]	9.4[b], 9.52[b], 14.9[b], 22.9[b], 51.7[b], 3.23[d], 8.59[b]
	Desloratadine	60[b]			
	Diphenhydramine	15[b], 21[b]		Quinine	22.4[b]
	Disopyramide	324[b]		Reserpine	1.38[d], 11.5[b]
	Doxepin	13[b]		Ritonavir	3.8[d], 5[d], 28.2[b]
	DX-619	0.94[b]		Roxithromycin	15.4[d]
	Etilefrine	4,009[b]		Tamoxifen	7.1[b]
	EtPy-Cl	36.7[b]		Telithromycin	1.8[d]

(*Continued*)

Supplemental Material

Table 2 (*Continued*)

Basolateral transporter	Substrate	IC_{50}, K_i (μM)
SLC22A2 (OCT2), continued	Famotidine	114[a]
	Flecainide	191[b]
	Flurazepam	60[b]
	Furamidine	182[b]
	Grepafloxacin	10.4[b]
	Imipramine	6[b]
	Ipratropium bromide	15[b]
	Ketamine	22.7[b]
	KW-3902	7.82[b]
	Levomethadone	60[b]
	MDMA	1.63[b]
	Mefloquine	204[b]
	Memantine	7.3[b]
	Mepiperphenidol	4.8[a]
	Metformin	398[b], 521[a], 289[b], 1,380[b]
	Mexiletine	55[b]
	MK-801	21.5[b]
	MPP^+	4.42[b], 2.4[a]
	NBuPy-Cl	2.29[b]
	Pentamidine	10.6[b]
	Phencyclidine	24.9[b]
	Phenformin	54[b], 111[b]
	Phenoxybenzamine	4.9[b]
	Prazosin	80.4[b]
	Procainamide	91.9[b], 50[a]
	Propafenone	25[b]
	Propranolol	229[b]
	Pyridine	790[b]
	Quinidine	8.7[a], 11[b], 13.3[b], 87[b]
	Quinine	23[b], 3.4[a]
	Ranitidine	76[a], 1617[b], 30.5[b], 79[b]
	Sibutramine	29[b]
	Tamoxifen	87[b]
	Tetraethylammonium	189.2[b], 222[a]
	Tetrapentylammonium	1.5[a]
	Trimethoprim	1318[b]
	Verapamil	13.4[b], 85[b]
	YM155	15.9

Apical transporter	Substrate	IC_{50}, K_i (μM)
ABCB1 (MDR1, P-gp), continued	Valsopodar	0.11[d]
	Verapamil	0.2[e], 4.2[b], 8.44[d], 10.7[b], 17.3[b], 33.5[b], 8.11[d], 15.1[b], 29[b]
	Vinblastine	17.8[b], 18[b], 30.1[b], 89.7[b]
	Zosuquidar	0.024[d], 0.07[b]
ABCC2 (MRP2, cMOAT)	Curcumin	5[e]
	Cyclosporine	10[b], 4.7[e], 8.11[b]
	Daunorubicin	49.4[b]
	Etoposide	756[b]
	Gemifloxacin	16[b]
	Indomethacin	0.06[e]
	Ketoprofen	1.4[e]
	MK-571	4[e], 50[d], 13.1[e], 26.4[b]
	PAK-104P	3.7[e]
	Reserpine	295[b]
	Valsopodar	28.9[e]
	Vincristine	802[b]
ABCC4 (MRP4)	Benzbromarone	150[e]
	Candesartan	16[e]
	Celecoxib	35[e]
	Diclofenac	0.006[e]
	Dilazep	20[e]
	Dipyridamole	2[e]
	Indomethacin	6.1[e]
	Ketoprofen	11.9[e]
	Losartan	1.5[e]
	MK-571	10[e]
	Nitrobenzylmercaptopurine riboside	75[e]
	Probenecid	2,300[e]
	Sildenafil	20[e]
	Sulfinpyrazone	420[e]
	Sulindac	2.11[e]
	Telmisartan	11[e]
	Trequinsin	10[e]
	Zaprinast	250[e]
ABCG2 (BCRP, MXR)	17β-estradiol-3-sulfate	14[e]
	Abacavir	385[b]
	Amprenavir	181[b]
	Atazanavir	69.1[b]
	Atorvastatin	14.3[e]
	AZD9056	32[a], 92[a]

(*Continued*)

Table 2 (*Continued*)

Basolateral transporter	Substrate	IC_{50}, K_i (μM)
SLC22A6 (OAT1)	1-BSA	514[b]
	1-hexylpyridinium chloride	0.35[b]
	Acetazolamide	75[b]
	Acetaminophen	639[b]
	Acetylsalicylate	769[b]
	Adefovir	0.9[b], 1.5[b], 1.8[b]
	Adipate	6.2[b]
	α-ketoglutarate	4.7[b]
	Betamipron	6[b], 16.2[b], 23.6[b]
	Bumetanide	7.6[b]
	Candesartan	17[b]
	Cefamandole	30[b]
	Cefazolin	180[b]
	Cefoperazone	210[b]
	Cefotaxime	3,130[b]
	Ceftriaxone	230[b]
	Cephaloridine	1,250[b], 740[b]
	Cephalothin	220[b]
	Cephradine	1,600[b]
	Chlorothiazide	3.78[b]
	Cidofovir	60[b]
	Cilastatin	1,470[b]
	Citrinin	3,080[b]
	Cyclothiazide	84.3[b]
	Diclofenac	4[b], 4.46[b]
	Diflunisal	0.85[b]
	DMPS	19[a], 83[b]
	Ethacrynic acid	29.6[b]
	Etodolac	50[b]
	Flurbiprofen	1.5[b]
	Fluvastatin	26.3[b]
	Fumarate	1,733[b]
	Furosemide	18[b]
	Glutarate	4.9[b], 3.3[b]
	Hippuric acid	20[b]
	Hydrochlorothiazide	67.3[b]
	Ibuprofen	8[b], 55.6[b]
	Indoleacetic acid	83[b]
	Indomethacin	3[b], 3.83[b]
	Indoxyl sulfate	83[b]

Apical transporter	Substrate	IC_{50}, K_i (μM)
ABCG2 (BCRP, MXR), continued	Cerivastatin	18.1[e]
	Daunomycin	59[e]
	Dehydroepiandrosterone sulfate	55[e]
	Delavirdine	18.7[b]
	Efavirenz	20.6[b]
	Elacridar	0.31[b]
	Erlotinib	0.15[e]
	Fluvastatin	5.43[e]
	Fumitremorgin C	0.47[b], 0.55[e]
	Ko143	0.01[b]
	Lopinavir	7.66[b]
	Nelfinavir	13.5[b]
	Nilotinib	0.69[e]
	Pitavastatin	2.92[e]
	Rosuvastatin	15.4[e]
	Saquinavir	27.4[b]
	Simvastatin	18[e]
	SN-38	1.6[e]
	Sulfasalazine	0.73[a]
SLC22A4 (OCTN1)	Disprocynium 24	14.6[b]
	Hercynine	1,450[b]
	L-ergothioneine	9[b]
	Methimazole	7,520[b]
	Pyrilamide	182[b]
	Thioperamide	254[b]
	Verapamil	10.8[b]
SLC22A5 (OCTN2)	Cefepime	1,700[b]
	Cefoselis	6,400[b]
	Cephaloridine	230[b]
	Emetine	4.2[a]
SLC47A1 (MATE1)	Amantadine	111.8[b]
	Cetirizine	371.2[b]
	Chloroquine	2.5[b]
	Chlorpheniramine	87.6[b]
	Cimetidine	1.1[b], 3.8[b]
	Desipramine	55.7[b]
	Diltiazem	12.5[b]
	Diphenhydramine	87[b]
	Disopyramide	83.8[b]
	DX-619	0.82[b]
	Famotidine	0.6[b]
	Imipramine	42[b]

(*Continued*)

Table 2 (*Continued*)

Basolateral transporter	Substrate	IC_{50}, K_i (μM)
SLC22A6 (OAT1), continued	JBP485	226[b], 197[b]
	Ketoconazole	319[a]
	Ketoprofen	1.3[b], 1.4[b], 4.34[b]
	KW-3902	3.7[b]
	Losartan	12[b]
	Mefenamic acid	0.83[b]
	Methazolamide	438[b]
	MPS	204[b]
	Naproxen	5.67[b], 5.8[b]
	Novobiocin	14.9[b]
	Octanoate	5.41[b]
	Olmesartan	0.28[b]
	Ortho-hydroxyhippuric acid	27[b]
	Para-aminohippurate	8.8[b], 6.02[b], 106[b], 92[b]
	Para-hydroxyhippuric acid	25[b]
	Phenacetin	200[b], 275[b]
	Pimelate	18.6[b]
	Piroxicam	20.5[b], 62.8[b], 19.8[b]
	Pratosartan	1.5[b]
	Pravastatin	408[b]
	Probenecid[f]	3.9[b], 6.3[b], 6.5[b], 7.4[b], 4.29[b], 12.1[b]
	Rifampin	79.1[a], 62.2[a]
	Salicylate	280[b], 325[b]
	Simvastatin	41.5[a], 73.6[b]
	Suberate	19.3[b]
	Sulfasalazine	4.6[a]
	Sulindac	36.2[b]
	Telmisartan	0.46[b]
	Trichloromethiazide	19.2[b]
	Valsartan	16[b]
SLC22A8 (OAT3)	Acetazolamide	816[b]
	Betamipron	48.3[b]
	Bumetanide	0.75[b]
	Candesartan	0.3[b]
	Cefamandole	50[b]
	Cefazolin	550[b]

Apical transporter	Substrate	IC_{50}, K_i (μM)
SLC47A1 (MATE1), continued	Metformin	666.9[b]
	Mitoxantrone	4.4[b], 5.2[b]
	NBuPy-Cl	8.5[b]
	Pramipexole	141.4[b]
	Procainamide	217[b]
	Quinidine	29.2[b]
	Ranitidine	17.5[b], 18.9[b], 25.4[b]
	Rapamycin	3.27[b], 3.51[b]
	Ritonavir	13.9[b], 15.4[b]
	Talipexole	66[b]
	Trimethoprim	6.2[b]
	Verapamil	27.5[b]
SLC47A2 (MATE2K)	Amantadine	1,167[b]
	Cetirizine	817.6[b]
	Chlorpheniramine	191.2[b]
	Cimetidine	7.3[b], 2.1[b]
	Desipramine	283[b]
	Diltiazem	117[b]
	Diphenhydramine	266.5[b]
	Disopyramide	291.6[b]
	DX-619	0.1[b]
	Famotidine	9.7[b]
	Imipramine	182.9[b]
	Metformin	6,515.7[b]
	NBuPy-Cl	1.6[b]
	Pramipexole	24.1[b]
	Procainamide	178.1[b]
	Quinidine	23.1[b]
	Ranitidine	25[b]
	Talipexole	119.5[b]
	Verapamil	32.1[b]

(*Continued*)

Table 2 (*Continued*)

Basolateral transporter	Substrate	IC_{50}, K_i (μM)
SLC22A8 (OAT3), continued	Cefoperazone	1,890[b]
	Cefotaxime	290[b]
	Cephaloridine	2,460[b]
	Cephalothin	40[b]
	Chlorothiazide	65.3[b]
	Cilastatin	231[b]
	Cimetidine	79[b]
	Citrinin	15.4[b]
	Cyclothiazide	27.9[b]
	Diclofenac	7.78[b]
	Ethacrynic acid	0.58[b]
	Fenofibric acid	2.2[b]
	Fluvastatin	5.79[b]
	Furosemide	1.7[b], 7.31[b]
	Gemfibrozil	6.8
	Glutarate	78.5[b]
	Hydrochlorothiazide	942
	Ibuprofen	3.7[b], 6[b]
	Indapamide	11[b]
	Indomethacin	0.61[b]
	JBP485	185[b], 160[b]
	Ketoprofen	5.98[b]
	Losartan	1.6[b]
	Mefenamic acid	0.78[b]
	Methazolamide	97.5[b]
	Naproxen	4.67[b]
	Novobiocin	4.77[b]
	Octanoate	8.6[b]
	Olmesartan	0.027[b]
	Para-aminohippurate	19.6[b]
	Penicillin G	102[b], 88[b]
	Phenacetin	19.4[b]
	Piroxicam	2.52[b], 4.88[b]
	Pratosartan	0.095[b]
	Pravastatin	13.7[b]
	Probenecid[f]	3.1[b], 5.6[b], 1.3[b], 4.41[b], 9[b]
	Quinapril	6.2[b]
	Simvastatin	32.3[b]
	Sitagliptin	160[b]
	Sulindac	3.62[b]
	Telmisartan	1.6[b]
	Trichloromethiazide	71.2[b]
	Valsartan	0.2[b]

(*Continued*)

Table 2 (*Continued*)

Basolateral transporter	Substrate	IC_{50}, K_i (μM)
SLCO4C1 (OATP4Cl)	Digitoxin	0.12[b]
	Digoxigenin	0.49[b]
	Digoxin	540[b], 119[b]
	Ouabain	0.36[b]
	Thyroxine	8.0[b]

Supplemental Material

In vitro methods: [a]oocytes, [b]transfected S2/HEK293/HeLa/CHO/COS/MDCK/HepG2/HRPE/LLC-PK1 cells, [c]ATPase assay, [d]Caco-2, [e]Sf9/V79/LLC-PK1/HEK293/bile canalicular membrane vesicles.

[f]Denotes drugs that can potentially be used for in vivo (clinical) studies (16).

References can be found in the **Supplemental Material**.

Abbreviations: BCRP, breast cancer resistance protein; BSA, butanesulfonic acid; cMOAT, canalicular multispecific organic anion transporter; DMPS, 2,3-dimercapto-1-propanesulfonic acid; MATE, multidrug and toxin extrusion protein; MDMA, 3,4-methylenedioxy-*N*-methylamphetamine; MDR, multidrug resistance protein; MPS, 3-mercapto-1-propanesulfonic acid; MRP, multidrug resistance-associated protein; MXR, multixenobiotic resistance protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein.

concentrations would increase, as would the risk for drug-induced nephrotoxicity. Therefore, understanding the site at which the DDI takes place is important.

The study of renal transporter-mediated DDIs in drug development was a focus of a recent publication by the International Transporter Consortium (ITC), a diverse group of experts from academia, industry, and the FDA (16). The publication includes a summary of in vitro methods to study transporter-mediated DDIs along with decision trees on the data required to support

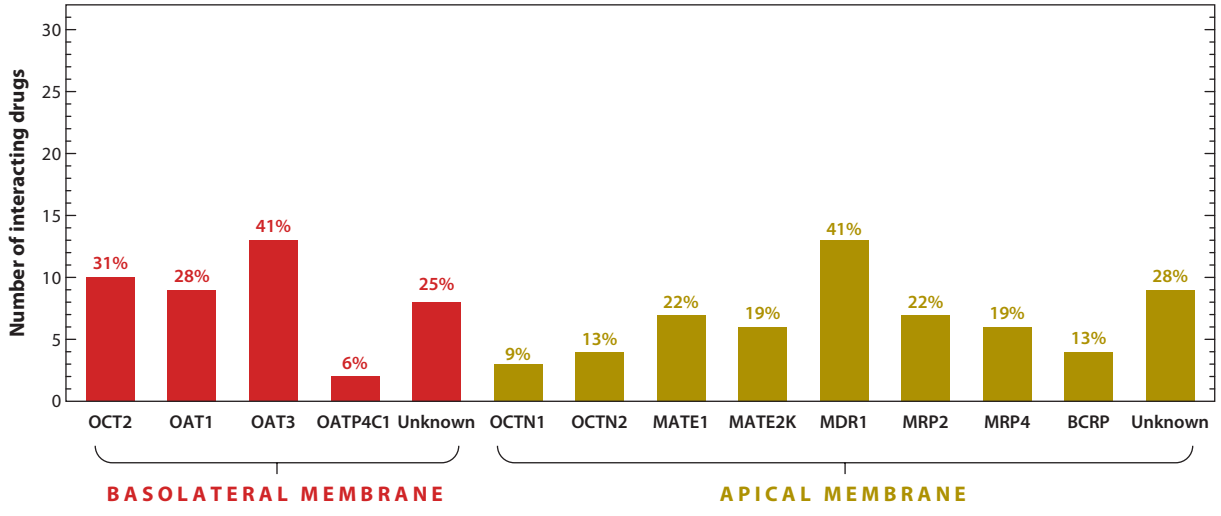

Figure 3

Interaction of renal secretory transporters with the top 200 prescribed renally secreted medications (i.e., ≥25% of the absorbed dose is excreted unchanged in urine). The figure includes only drugs predicted to undergo net tubular secretion ($n = 32$). Data are presented as the number of drugs that interact with a single transporter (or unknown transporter) at either the basolateral or apical membrane. Several drugs are predicted to interact with more than one transporter at either membrane. Abbreviations: BCRP, breast cancer resistance protein; MATE, multidrug and toxin extrusion protein; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter.

go/no-go decisions about initiating clinical transporter-mediated DDI studies, and a list of model drugs that potentially could be used in a clinical investigation of a transporter-mediated DDI (**Tables 1** and **2**).

Recently, the FDA has issued a draft guidance (4) that includes modifications of the ITC recommendations. Regarding the transporters expressed in the kidney, the guidance focuses on three transporters: OCT2, OAT1, and OAT3. However, future updates will likely provide additional guidelines for other renal secretory drug transporters. Briefly, when renal secretion is important [defined as $(CL_R - fu \bullet GFR)/CL_T \geq 0.25$] for a NME's elimination, it is recommended that the NME be evaluated in vitro as a potential substrate of OCT2, OAT1, and OAT3 (**Figure 4**). If a NME is determined to be a substrate of any of these transporters (defined when intracellular accumulation of the NME is twofold above empty vector in overexpressing OCT2, OAT1, and/or OAT3 cells), a clinical DDI study with a prototypic inhibitor is recommended. Because inhibition could occur regardless of the NME's route of elimination, all NMEs must be evaluated as potential inhibitors of renal secretory transporters. In the FDA draft guidance (4), if a NME has an IC_{50} value (concentration associated with half the maximum inhibition in an in vitro assay of OCT2, OAT1, or OAT3 transport) of less than ten times its $C_{max,u}$ (maximum plasma concentration that is not bound to plasma proteins), a clinical DDI study with a sensitive substrate is recommended.

In the current FDA DDI draft guidance, a clinical DDI is defined as a clinically significant change in the victim drug's AUC and/or C_{max}. However, for drugs that are cleared by renal mechanisms, the site of the DDI must be considered—that is, whether the DDI is occurring at the apical or basolateral membrane. For example, for a drug that is targeted to the kidney for pharmacological action (e.g., a diuretic), blocking the uptake into the kidney would potentially reduce its access to its pharmacological target and, therefore, reduce its pharmacological effect. In contrast, if a secretory transporter at the apical membrane is inhibited, drug concentrations within the renal cell are increased, resulting in enhanced pharmacological effects or, in some cases, enhanced renal toxicities. In both cases, the site of the DDI has a direct effect on drug efficacy and toxicity, which may not be reflected in changes in plasma concentrations.

Although the current FDA guidance is in its draft stage, it is important to remember that a clinical DDI at a renal drug transporter may have profound effects on plasma concentrations, renal cell drug levels, drug activity, and/or potential toxicities (**Table 3**). For this reason, these guidelines must be strategically incorporated into the research and development of investigational drugs (**Figure 4**). For all NMEs, it is advantageous to identify potential DDI liabilities and to test them in vitro prior to conducting Phase I clinical trials. In particular, a DDI could easily halt drug development if the victim drug has a narrow therapeutic window, has a pharmacological target in the kidney, or is nephrotoxic. Therefore, for these types of drugs, it is particularly beneficial to identify potential transporter-mediated DDIs in the early stages of preclinical development. Once pharmacokinetic studies are initiated in human subjects, predictions can be reassessed utilizing the clinically relevant concentrations of the NME. Depending on the potency of the interaction at OCT2, OAT1, and OAT3, clinical DDI studies may be requested by the FDA in Phase III or in the postmarketing phase.

DESIGN OF A CLINICAL DRUG-DRUG INTERACTION STUDY

The FDA recommends that in vivo DDI studies be conducted using a crossover design (4). Commonly, DDI studies are performed in healthy volunteers, but sometimes more specific populations are required (e.g., certain genotypes, individuals with renal impairment). Regulatory agencies ask that drug developers provide specific recommendations regarding the clinical significance of any

Table 3 Examples of clinical drug-drug interactions mediated by renal secretory transporters

Implicated transporters	Interacting drug	Affected drug	Clinical pharmacokinetic impact on affected drug (presented as fold change[c])				
			AUC	C_{max}	CL_R	CL/F	$t_{1/2}$
OATs	Furosemide	Lomefloxacin	1.1	NS	0.7	0.9	NS
OATs	Probenecid	Cefaclor	2.1	1.5	–	–	1.6
OATs	Probenecid	Cephradine	2.4	1.9	–	–	1.5
OATs	Probenecid	Famotidine	1.8	1.5	0.4	0.1	NS
OATs	Probenecid	Ceftriaxone	0.7	–	–	1.3	0.8
OATs	Probenecid	Acyclovir	1.4	–	0.7	NS	–
OATs	Probenecid	Cefonicid	2.1	1.2	0.3	–	1.5
OATs	Probenecid	Cefoxitin	2.4	–	0.4	–	2
OATs	Probenecid	Cidofovir	–	–	0.5	0.6	–
OATs	Probenecid	Dicloxacillin	1.9	1.8	0.3	0.5	–
OATs/MRPs	Probenecid	Ciprofloxacin	1.7	NS	0.4	0.6	1.5
OATs/MRPs	Probenecid	Furosemide	2.7	1.5	0.3	0.4	1.7
OATs/OCTs	Cotrimoxazole (trimethoprim/sulfamethoxazole)	Zidovudine	NS	–	0.4	NS	NS
OATs/OCTs	Cotrimoxazole (trimethoprim/sulfamethoxazole)	Apricitabine	1.7	1.3	0.6	0.6	1.4
OCTs	Trimethoprim	Zidovudine	NS	–	0.5	NS	NS
OCTs/MATEs	Cetirizine	Pilsicainide	1.4	NS	–	–	–
OCTs/MATEs[a]	Cimetidine	Pindolol (*S*-enantiomer)	1.4	1.3	0.7	–	NS
OCTs/MATEs[a]	Cimetidine	Metformin	1.5	1.7	0.7	–	–
OCTs/MATEs[a]	Cimetidine	Cephalexin	NS	NS	0.8	0.8	NS
OCTs/MATEs[a]	Cimetidine	Ranitidine	1.3	NS	0.7	–	1.3
OCTs/MATEs[a]	Cimetidine	Procainamide	1.4	NS	0.6	–	1.3
OCTs/MATEs[a]	Cimetidine	Pilsicainide	1.3	NS	0.7	0.7	1.2
OCTs/MATEs[a]	Cimetidine	Varenicline	1.3	–	0.8	0.8	–
OCTs/MATEs[a]	Cimetidine	Dofetilide[b]	1.5	1.3	0.7	0.7	1.3
MATEs	Pyrimethamine	Metformin	1.4	1.4	0.6	–	–

[a]In vitro inhibition potency values indicate that cimetidine is a much stronger inhibitor of MATEs than OCTs, suggesting that the MATEs are the predominant sites of the DDIs (94).

[b]The PD of the affected drug was also altered.

[c]Calculation of fold change: fold change in the presence of the interacting drug = value with interacting drug divided by value without interacting drug. Fold change >1: increase in pharmacokinetic value. Fold change <1: decrease in pharmacokinetic value.

References can be found in the **Supplemental Material**.

Abbreviations and symbols: –, not determined; AUC, area under the plasma drug concentration-time curve; CL_R, renal clearance; CL/F, apparent clearance; C_{max}, maximum plasma concentration; DDI, drug-drug interaction; MATE, multidrug and toxin extrusion protein; MRP, multidrug resistance-associated protein; NS, not significant; OAT, organic anion transporter; OCT, organic cation transporter; $t_{1/2}$, half-life.

Supplemental Material

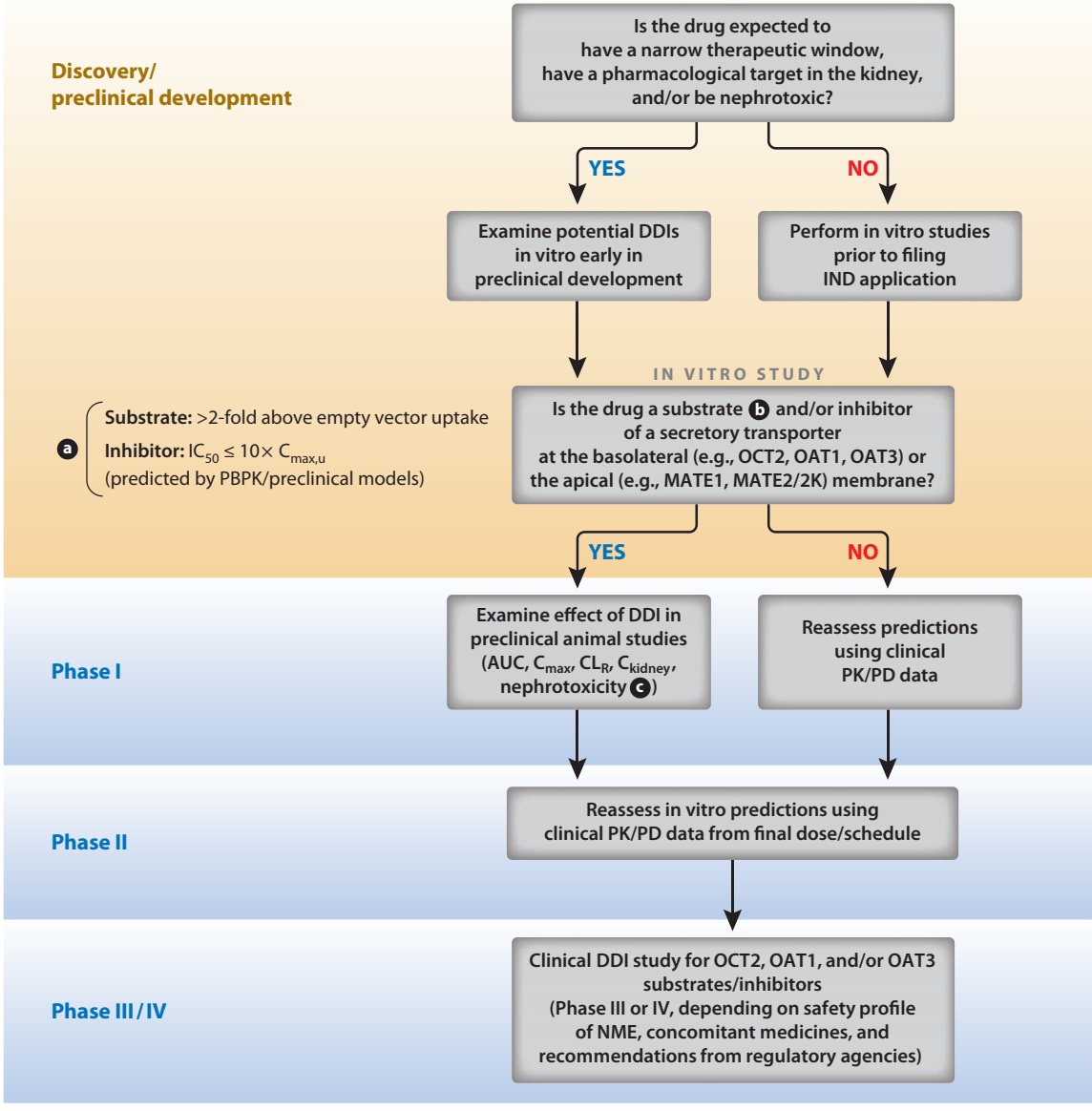

Figure 4

Incorporation of US Food and Drug Administration (FDA) guidelines into the discovery and development of NMEs. Letters *a–c* refer to items in the figure that are accompanied by the corresponding white letters in black circles. (*a*) Cutoff values are derived from the FDA DDI draft guidance. (*b*) In vitro studies to investigate the NME as a substrate are recommended by the FDA DDI draft guidance (4) when the NME is cleared primarily by renal secretion [$(CL_R - fu \bullet GFR)/CL_T \geq 0.25$] or unknown mechanisms. (*c*) Evaluate NMEs that are inhibitors of apical secretory transporters for nephrotoxicity. NMEs that are inhibitors of basolateral secretory transporters may be protective of potential proximal tubule toxicity. Abbreviations: AUC, area under the plasma drug concentration-time curve; CL_R, renal clearance; C_{kidney}, concentration of drug in the kidney; C_{max}, maximum plasma concentration; $C_{max,u}$, maximum plasma concentration that is not bound to plasma proteins; DDI, drug-drug interaction; IC_{50}, concentration associated with half the maximum inhibition in an in vitro assay of OCT2, OAT1, or OAT3 transport; IND, investigational new drug; MATE, multidrug and toxin extrusion protein; NME, new molecular entity; OAT, organic anion transporter; OCT, organic cation transporter; PBPK, physiologically based pharmacokinetics; PK/PD, pharmacokinetics/pharmacodynamics.

reported DDI (primarily focusing on differences in AUC and C_{max}) based on what is known about the dose-response and/or pharmacokinetic/pharmacodynamic relationships of the victim drug (4).

For renally eliminated drugs, an accurate determination of GFR is essential to understanding the contribution of secretory and reabsorptive mechanisms to renal clearance. In human subjects, GFR can be measured directly by calculating the urinary or plasma clearances of endogenous or exogenous filtration markers or indirectly by using predictive equations. The different equations used to calculate renal and plasma clearance and to predict GFR are compiled and assessed in **Supplemental Table 1** (follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**). In clinical practice, GFR is more commonly estimated using predictive equations rather than direct measurement. However, in clinical studies, GFR can be measured by calculating the plasma clearance of exogenous markers (e.g., inulin) or, more commonly, by calculating the clearance of endogenous markers (e.g., creatinine). Notably, each of the methods and markers used to measure GFR has important advantages and disadvantages (**Supplemental Table 2**).

Supplemental Material

RENAL TRANSPORTERS AS SOURCES OF PHARMACOKINETIC VARIATION

For drugs that are eliminated by secretion, interindividual variation in the expression levels or activities of secretory transporters are major sources of variation in secretory clearance. Specifically, genetic or heritable factors have been estimated to account for 64–94% of the interindividual variation in the renal clearances of several medications including metformin, amoxicillin, cephalexin, famotidine, and ampicillin (17, 18). Presumably, environmental factors account for the remainder of the variation.

There is a large amount of interindividual variation in the expression levels of mRNA transcripts of renal drug transporters. Quantitative RT-PCR (reverse-transcription polymerase chain reaction) data of the kidney cortex from 57 human donors show that there is variable expression of the mRNA transcripts of secretory transporters among kidney tissues (**Figure 5**) that cannot be accounted for by gender or age (S.W. Yee, A. Chhibber, C.C. Wen, D.L. Kroetz & K.M. Giacomini, unpublished data). Variation in transcript levels among individuals may be due to differences in the transcription or degradation rates of mRNA transcripts. Transcription rates are influenced by the binding of transcription factors, which may be repressors or enhancers, to the transporter gene. Single-nucleotide polymorphisms (SNPs) in enhancer or repressor regions of the transporter genes, termed *cis*-eQTLs (expression quantitative trait loci), can alter the binding of the enhancers or repressors, resulting in changes in transcription rates. Furthermore, SNPs in the transcription factor genes themselves (*trans*-eQTLs) may also result in changes in the expression levels or protein structures of transcription factors, resulting in changes in rates of transcription of transporter genes. Studies (e.g., the NIH Common Fund's Genotype-Tissue Expression; see **http://commonfund.nih.gov/GTEx/**) to identify *cis*- and *trans*-eQTLs in the kidney are ongoing and are expected to provide information on the sources of variation in transcript levels of renal drug transporters.

Of the transporters localized to the basolateral membrane and known to play a role in renal drug secretion, OCT2 and OAT1 transcripts are most abundant (median of 57 donors), followed by OAT3 and OATP4C1. Of the secretory transporters expressed on the apical membrane, MDR1, OCTN2, MATE1, MRP4, and MRP2 are expressed at a higher level in comparison with MATE2/2K, breast cancer resistance protein (BCRP), and OCTN1 (**Figure 5**). Because mRNA levels may not reflect transporter protein levels, it is not known whether differences in the transcript levels will translate to differences in transporter protein levels on the plasma membrane

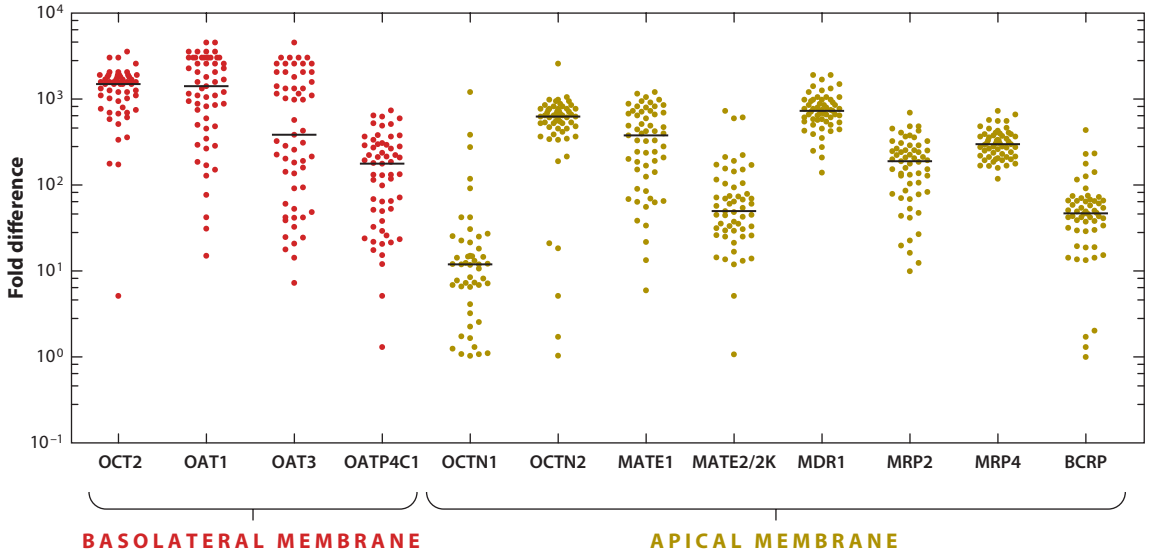

Figure 5

Expression of secretory drug transporters in the kidney of human subjects. Quantitative RT-PCR (reverse-transcription polymerase chain reaction) was performed on RNA obtained from the renal cortex of human donors ($n = 57$) using a custom SYBR® green-based OpenArray® system (Life Technologies, Grand Island, New York). Data are normalized to the mean of three housekeeping genes and are presented as $2^{-\Delta\Delta Ct}$ (black horizontal lines are the median values). For additional information on the expression of other drug transporters in the kidney, refer to the UCSF-FDA TransPortal at **http://bts.ucsf.edu/fdatransportal/** (95). Abbreviations: BCRP, breast cancer resistance protein; MATE, multidrug and toxin extrusion protein; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter.

of the renal tubule among the various transporters. Furthermore, the variation in protein levels of transporters in the kidney is also not known. Advances in proteomic methods (19–21) may lead to a better understanding of the levels of renal transporters in the kidney and interindividual differences in expression levels of transporter proteins.

Recent studies suggest that genetic polymorphisms in renal drug transporters may play an important role in the variability of the pharmacokinetics and pharmacodynamics of certain medications, presumably by causing changes in transporter expression levels and activity. For example, a common promoter variant of MATE2/2K is predicted to increase MATE2/2K expression and is associated with a poorer hypoglycemic response to metformin (22). Furthermore, nonsynonymous coding SNPs, including the OCTN1-L503F (23) and OCT2-A270S (24) polymorphisms, have been associated with altered transporter function and variation in plasma drug concentrations. For a more comprehensive review of genetic variants and their impact on the pharmacokinetics and pharmacodynamics of xenobiotics, see the Pharmacogenetics of Membrane Transporters Database (**http://pharmacogenetics.ucsf.edu/**) and recent literature reviews (25–27).

RENAL CLEARANCE ALTERATIONS IN SPECIAL POPULATIONS

In addition to DDIs and genetics, current information suggests that many other factors contribute to variation in renal drug clearance. This section describes the effects of chronic kidney disease,

Table 4 Comparison of mRNA and protein expression levels of renal transporters in various special populations[a]

Transporter	Renal impairment/chronic kidney disease	Gender	Age	
			Children/adolescents	Elderly
OCT2	$\downarrow^{R}$	F $\uparrow^{R}$; M $\uparrow^{R}$	$\uparrow^{R}$;$\downarrow^{R}$	
OAT1	$\downarrow^{R}$; $\uparrow^{R}$	F $\uparrow^{R}$; M $\uparrow^{R}$; $\leftrightarrow^{H}$	$\uparrow^{R}$; $\downarrow^{R}$	
OAT3	$\downarrow^{R}$; $\uparrow^{R}$	F $\uparrow^{R}$; $\leftrightarrow^{R}$	$\uparrow^{R}$; $\downarrow^{R}$	
OATP4C1	$\downarrow^{R}$	F $\downarrow^{R}$	$\downarrow^{R}$	
OCTN1		$\leftrightarrow^{R}$	$\downarrow^{R}$	
OCTN2		$\leftrightarrow^{R}$	$\downarrow^{R}$; $\uparrow^{R}$	
MATE1	$\downarrow^{R}$	F $\downarrow^{R}$	$\uparrow^{R}$	
MRP2	$\uparrow^{R}$	$\leftrightarrow^{R}$	$\uparrow^{R}$; $\downarrow^{R}$	$\downarrow^{R}$
MRP4	$\uparrow^{R}$	F $\uparrow^{R}$	$\downarrow^{R}$	
MDR1	$\uparrow$ (ARF) ^{R}; $\leftrightarrow$ (CRF) ^{R}	M $\uparrow^{H}$; $\leftrightarrow^{H}$	$\leftrightarrow^{H}$; $\uparrow^{R}$; $\downarrow^{R}$	$\leftrightarrow^{R}$
BCRP	$\downarrow^{R}$	$\leftrightarrow^{R}$	$\downarrow^{R}$; $\uparrow^{R}$	

[a]Observations in changes of mRNA or protein levels in renal impairment/chronic kidney disease models were often also reflected in altered excretion processes. Ontogenic expression levels and gender differences refer to young animals; values for newborn animals may be different. Changes reflect differences in mRNA-expression or transporter protein quantity with the following symbols: $\leftrightarrow$ equal, $\uparrow$ higher, $\downarrow$ lower; changes observed in humans (^{H}) and rodents (^{R}).

References can be found in the **Supplemental Material**.

Abbreviations: ARF, acute renal failure; BCRP, breast cancer resistance protein; CRF, chronic renal failure; F, female; M, male; MATE, multidrug and toxin extrusion protein; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter.

age, pregnancy, gender, and ethnicity on interindividual differences in the renal clearance of drugs. Where available, specific information is presented on transporters in the kidney.

Chronic Kidney Disease

Diseases of the kidney, such as chronic kidney disease (CKD), acute changes in kidney function, or renal impairment, alter the renal clearance of xenobiotics, and in some cases dose modifications are necessary. An accurate determination of GFR is of particular importance when prescribing certain medications to patients with CKD. Reduced GFR in patients with CKD is often accompanied by other aberrations, including diminished drug transporter expression, reduced metabolic enzyme activity, and accumulation of uremic toxins which might hamper drug excretion (28). Numerous studies using rodent models have suggested that CKD is associated with a decrease in the expression levels of Oct2, Oatp4c1, Mate1, and Bcrp and with an increase in the mRNA levels of Mrp2, Mrp4, and Mdr1 (**Table 4**). Currently, there is no information regarding the effect of CKD on the expression levels of drug transporters in human kidneys. In addition, due to alterations in renal drug handling, CKD can also impair hepatic drug metabolism, uptake, and biliary excretion of both renally and nonrenally cleared compounds (28, 29). In fact, the FDA recommends that pharmacokinetic studies be conducted for all drugs, irrespective of their route of elimination, in patients with CKD (30).

Age

In addition to the structural changes in the kidney associated with aging, older adults also exhibit physiological changes such as decreased GFR and altered tubular handling of creatinine.

Creatinine production decreases in healthy older individuals, and net creatinine reabsorption appears to increase (31) to levels commonly seen in healthy newborns and premature babies (32). GFR increases postnatally for both term-born and premature infants (33, 34). In term-born infants, this increase is faster than in premature infants (33–36).

Given the difference in GFR between adults and children or the elderly, doses of renally cleared drugs need to be adjusted for both pediatric and geriatric patients to reduce side effects and enhance appropriate therapeutic responses (37–40). In children, certain differences in kidney function, e.g., the glomerular filtration of inulin (41) and the excretion of antibiotics (42), can clearly be attributed to kidney maturation on an anatomical level, e.g., length and number of nephrons. However, other differences, e.g., the increased clearance of digoxin in young children, cannot be explained solely by these anatomical changes (43–45). In such cases, transporters are likely to play a crucial role; however, the underlying molecular processes for differences in renal clearance are poorly understood in a developmental context. Even though abundant information exists on renal drug transport in adults (46), the ontogeny of human renal transporters has not been studied extensively, and current data are predominantly from rodent models (**Table 4**). Furthermore, the rodent data often conflict, and further research is necessary to obtain conclusive evidence for ontogenic differences. In humans, MDR1 mRNA is detected in the kidney by 7 weeks of gestation, and its tissue distribution pattern differs from that seen in adult tissues (47). In addition, a disproportional increase in organic anion secretion relative to kidney mass has been reported in human subjects, suggesting a specific maturation of the organic anion transport system during development (43). Interestingly, cephalosporin-related nephrotoxicity occurs more frequently in adults than in children (48, 49). The reasons for this are largely unknown, although differences in transporter expression could, in part, explain these observations.

Pregnancy

During normal pregnancy, GFR and renal blood flow begin to increase in the first trimester and peak in the second trimester at approximately 40–60% and 50–85%, respectively, of prepregnancy values (50–52). Increases in GFR during pregnancy are expected to result in enhanced renal elimination. Therefore, caution and an accurate estimate of GFR are important when administering renally cleared drugs in pregnant individuals. For estimating GFR in normal pregnancy (53), a 24-h urine creatinine clearance—rather than the use of predictive equations—remains standard. In the setting of preeclampsia (54), however, renal hyperfiltration is even more pronounced, and a new formula for estimating GFR has been developed (55) (**Supplemental Table 1**). To achieve therapeutic effects with drugs in which GFR is a major determinant of their total clearance (e.g., lithium, amoxicillin, piperacillin), dose adjustments are recommended in pregnant women (56–59).

There is limited knowledge regarding the effect of pregnancy on transporter expression, and the majority of information stems from rodent models. In mice, pregnancy has been associated with elevated levels of Bcrp protein and mRNA (60). However, no discernible differences in Mdr1 protein expression were observed between normal and pregnant mice (61). With respect to human patients, increases in the renal secretory clearances of metformin (62), amoxicillin (57), and digoxin (63) have been observed in pregnant females. The mechanism(s) for the increase in renal clearance is not known, but possible explanations include enhanced secretory transporter expression/function, decreased tubular reabsorption, and enhanced renal blood flow. For a review of medications that are affected by pregnancy-induced changes in drug pharmacokinetics and the potential impact of drug transporters, see the recent review by Anderson (64).

Supplemental Material

Gender

Using creatinine-based predictive equations, significant gender differences in GFR have been identified. These discrepancies are attributed primarily to differences in creatinine production since the muscle mass of women is approximately 15% smaller than that of men (65, 66). However, measured GFR (using inulin) is also lower in healthy women than in men (67), suggesting that physiological differences within the kidney may also contribute to gender differences in GFR. The importance of gender is also reflected in its inclusion in all of the adult predictive equations (**Supplemental Table 1**).

The influence of gender on renal secretory transporter expression and function is largely unknown. Gender differences in transporter expression have been studied extensively in rodent models, but this field remains controversial since there are several conflicting reports on the direction of expression differences between genders (see **Table 4**). In human kidneys, there is limited published data comparing transporter expression between genders. Schuetz et al. (68) detected elevated MDR1 expression in men, but a subsequent study by Wolbold et al. (69) detected no gender differences in MDR1 expression. In a subanalysis of the human kidney expression data (**Figure 5**), no significant gender differences were observed in the transcript levels of the renal secretory transporters shown in **Figure 2** (S.W. Yee, A. Chhibber, C.C. Wen, D.L. Kroetz & K.M. Giacomini, unpublished results). In addition, it is not known whether there are gender differences in the protein levels in renal secretory transporters. Nonetheless, previous reports suggest an impact of gender on the renal clearance of drugs eliminated by the kidney. For example, the renal clearances of methotrexate and amantadine show distinct differences between genders, with men having greater renal clearances than females (70, 71). A systematic study of gender differences in renal clearances and net secretory clearances needs to be conducted for model compounds. If substantial differences are observed, mechanistic studies that focus on the expression levels of transporters in the kidney should be performed. These studies are essential to understanding the effect of gender on renal clearance. Indeed, regulatory authorities and the National Institutes of Health have released several publications highlighting the importance of understanding gender differences in pharmacokinetics (72, 73, 74).

Ethnicity

The predictive equations used to calculate GFR differ among ethnic groups, and ethnic-specific coefficients have been proposed to improve the calculation of GFR (75–77). It is unclear whether GFR itself varies among ethnic groups or whether these ethnic-specific predictive equations are necessary to reflect differences in the rate of endogenous creatinine production, secretion, or reabsorption or discrepancies in assay methodology between ethnic groups.

Interethnic differences in drug absorption, metabolism, and response have been extensively reported. Ethnic differences in renal clearance, although less common, have been demonstrated. For example, the renal clearance of fosinoprilat is greater in Caucasian subjects than in Chinese subjects (78). In contrast, morphine has a higher renal clearance in Chinese individuals than it does in Caucasian individuals (79). These ethnic differences could be attributed to intrinsic factors (e.g., genetics) and extrinsic factors (e.g., diet). Currently, there is little information about the relative contribution of these factors to the overall difference in drug disposition and response. Future studies are required to learn more about ethnic differences in renal clearance of these drugs and others and about the mechanisms associated with such differences, including allele frequency differences of genetic polymorphisms, which may be associated with variation in the expression level and activity of renal transporters.

ALTERNATIVE SPLICING OF RENAL SECRETORY TRANSPORTERS

Alternative splicing is a mechanism in eukaryotic cells to increase the coding capacity of genes and is predicted to occur in ~74% of all human genes (80). Bioinformatic data analysis based on expressed sequence tags (ESTs) supports this finding, indicating that 35–60% of human gene products are alternatively spliced (81–84). Furthermore, mechanistic studies have demonstrated the importance of alternative splicing on protein localization, regulation, and function (85). Renal transporters are no exception, and various splicing variants have been described (86).

The most prominent example of splicing variants of renal secretory transporters is MATE2K, a splice variant of MATE2. In comparison with MATE2, MATE2K lacks one exon and is expressed predominantly in the kidney (87) and at a greater abundance (87). In vitro experiments demonstrate similar transport activity between MATE2 and MATE2K, suggesting that both are involved in the renal elimination of organic cations (87). Research thus far has focused largely on MATE2K since it was identified several years before the functionality of MATE2 was determined. In addition, variants of OAT1 have been identified (OAT1-1, OAT1-2, OAT1-3, OAT1-4); however, preliminary reports suggest that only OAT1-1 and OAT1-2 are functional (88, 89). Three splice variants of OAT3 have been identified, but whether they are translated into functional proteins is unknown because transporter function has not been evaluated (90). Furthermore, splicing variants of OCTN2 with reduced activity have been identified (91, 92). A splice variant of OCT2 has also been observed (OCT2-A) and consists of only 9 transmembrane domains instead of 12 (93). Nonetheless, data suggest that this splice variant is functional and that it exhibits different kinetics for several compounds compared with OCT2. For example, the uptake of 1-methyl-4-phenylpyridinium (MPP^+) was greater in OCT2-expressing cells than in OCT2-A-expressing cells, and the uptake of tetraethylammonium (TEA) was inhibited by levofloxacin and procainamide in OCT2-A-expressing cells, but not in OCT2-expressing cells (93). Although splice variants have been identified for multiple renal secretory transporters, the clinical impact of these variants on the renal elimination of drugs has not been determined, and future research is necessary to define their clinical significance and the mechanisms by which splicing is regulated.

SUMMARY POINTS

1. Renal drug transporters are important determinants of the total clearance of commonly prescribed drugs.
2. Renal secretory transporters are implicated in numerous clinically significant DDIs, generally leading to increased plasma levels of drugs and potential safety issues. Understanding whether the interaction will potentiate or reduce possible nephrotoxicity requires knowledge of the specific site (apical or basolateral membrane) of the interaction.
3. Identification of transporter-mediated DDI liabilities early in the drug development process is important, particularly if the NME has a narrow therapeutic window, has a pharmacological target in the kidney, or is nephrotoxic.
4. The inter- and intraindividual variation in renal drug clearance arises from multiple factors, including drug interactions, genetics, disease status, ethnicity, and age.

FUTURE ISSUES

1. An understanding of the contribution and interplay of both intrinsic (e.g., genetics) and extrinsic (e.g., environment) factors on renal drug clearance is needed.
2. Examination of transporter ontogeny and age-related events is required to optimize drug therapy in pediatric and geriatric populations.
3. The influence of pregnancy, gender, and ethnicity on renal drug elimination is largely unknown and understudied. Elucidating the underlying mechanisms and their impact on drug dosing requires future studies.
4. Further studies are necessary to understand the clinical impact of splicing variants and genetic polymorphisms of transporters on renal drug elimination.

DISCLOSURE STATEMENT

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Errata

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