Title
Organic Cation Transporters in Drug Disposition and Response

Permalink
https://escholarship.org/uc/item/06q0x5zt

Author
Morrissey, Kari M.

Publication Date
2013

Peer reviewed|Thesis/dissertation

Organic Cation Transporters in Drug Disposition and Response

by

Kari M. Morrissey

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Pharmaceutical Sciences and Pharmacogenomics

in the

GRADUATE DIVISION

ACKNOWLEDGEMENTS

Achieving this doctoral degree would not have been possible without the many friends and colleagues that have supported me throughout the process. I am pleased to acknowledge those who have helped me through this academic journey.

First, I thank my advisor, Dr. Kathleen Giacomini, for her support, encouragement and scientific expertise. It has been an absolute privilege to be mentored by someone so passionate about science, and this dissertation would not have been possible without her support and advice. For me personally, Kathy is an excellent example of a woman in science who has successfully combined career, family life and adventure without compromising her personal goals and I am inspired to attempt to do the same. I also want to thank Drs. Leslie Benet and Deanna Kroetz, members of my thesis committee, for their mentorship and critique of my research projects and dissertation. I would also like to acknowledge additional faculty members who contributed to my scientific and personal development at UCSF, including Dr. Nadav Ahituv, Dr. Lynda Frassetto and Dr. Rada Savic.

The Giacomini laboratory has been a wonderful place to work and I am thankful for the past and present members of the Giacomini laboratory who have helped me survive graduate school. In particular, I would like to thank Sook Wah Yee, Sophie Stocker, Eugene Chen, Richard Castro, Chris Wen, Lu Xu, Matthias Whittwer, Xiaomin Liang, Michael Lin, Ethan Geier, Sirj Goswami, Lawrence Lin, James Shima, Lian Li, Jen

ABSTRACT

ORGANIC CATION TRANSPORTERS IN DRUG DISPOSITION AND RESPONSE

Kari M. Morrissey

Transporters in the kidney play an important role in the tissue distribution and excretion of various prescription drugs and endogenous metabolic waste products. For organic cations that are dependent upon renal elimination, variability in the activities or expression levels of renal organic cation transporters are major sources of intra- and inter-individual variation in secretory clearance. The overall goal of this dissertation research was to enhance our knowledge of the clinical impact of renal organic cation transporters on variation in drug disposition and response. This research primarily focuses on the role of efflux transporters at the apical membrane of the renal proximal tubule: Multidrug and toxin extrusion proteins, MATE1 and MATE2K.

The studies described in this dissertation aimed to understand the importance of two potential sources of variability in renal drug handling: extrinsic (*i.e*., a drug-drug interaction mediated by MATE2K) and intrinsic (*i.e*., expression variants of MATE1 and MATE2K). Our *in vitro* and clincal data suggest that both MATE1 and MATE2K play important roles in the exposure, tissue distribution and response of the antidiabetic drug, metformin. Furthermore, these studies also challenge currently recommended methods and criteria for the conduct and use of *in vitro* experiments to inform the decision to

perform a clinical investigation of a transporter-mediated drug-drug interaction. This dissertation research also demonstrates that expression variants of MATE1 and MATE2K have a clinical impact on the disposition and pharmacologic effects of metformin in healthy volunteers and type II diabetic subjects. Overall, the studies described in this dissertation add to our understanding of the role of MATE1 and MATE2K in renal drug handling and peripheral effects of drugs.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

RENAL TRANSPORTERS IN DRUG DEVELOPMENT[*]

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 co-transport 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 inter-individual 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 >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 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) has recently published a series of decision trees to guide
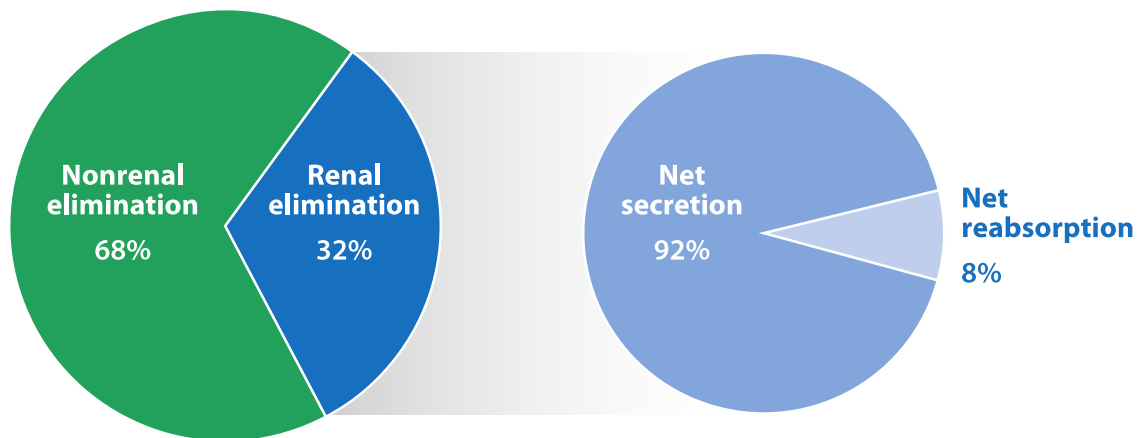
Figure 1. The contribution of the kidney to the elimination of the top 200 prescribed drugs in the United States in 2010 (3). Drugs are considered renally eliminated when ≥25% of their absorbed dose is excreted unchanged in the urine. As certain drugs may appear multiple times on the top 200 list, only unique chemical entities are included (*n* = 114). Net secretion is designated for drugs whose renal clearances exceed their filtration clearances.

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 (*i*) highlight renal transporters that are important in drug elimination and summarize recent data on their expression levels, substrates, inhibitors and associated DDIs, *(ii)* describe how the recent FDA guidelines can be applied to the development of NMEs and (*iii*) 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:

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

where C is the concentration of drug in plasma;

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

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

$$CL_R = \text{fe} \bullet CL_T, \quad (eq.\ 3)$$

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 $\bullet$ 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:

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

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

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

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 (*eqs. 1,4*) with the rate of filtration (*eq. 5*), or in simpler terms CL_R to fu $\bullet$ GFR. If $CL_R >$ fu $\bullet$ GFR, then net secretion is assumed; if $CL_R <$ fu $\bullet$ GFR, then net reabsorption is assumed. In either case, both the processes of secretion and reabsorption

may take place but cannot be differentiated in the net values. Interestingly, drugs that are eliminated by renal mechanisms are more likely to undergo net secretion rather 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.
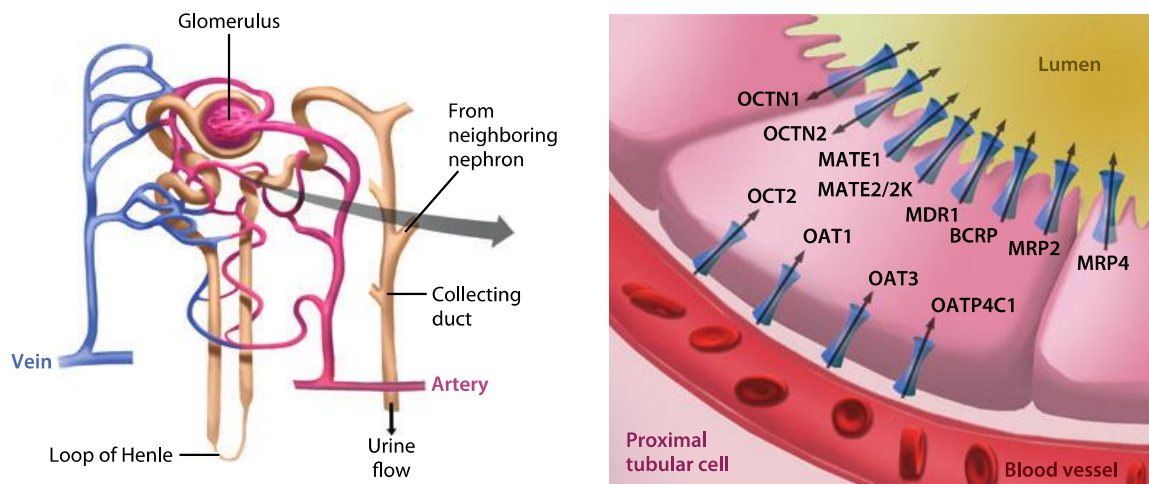
Figure 2. Drug transporters in the nephron of the kidney. Illustration of the nephron (left) and secretory transporters in the proximal tubular cell that facilitate the renal secretory elimination of diverse medications (right).

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.

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 class, 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 understudied transporters. Drugs that are eliminated in the kidney with a net tubular secretion are particularly susceptible to DDIs when co-administered 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

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

Basolateral Transporter	Substrate	Km (μM)	Reference
SLC22A2 (OCT2)	amantadine	27[b]	(1)
	amiloride	95[b]	(3)
	ASP⁺ (4-(4-dimethylamino)styryl-N-methylpyridinium)	24[b]	(3)
	cimetidine	72.6[b]	(6)
	dopamine	1400[b]	(8)
	epinephrine	420[b]	(8)
	famotidine	56.1[b]	(6)
	histamine	940[b]	(8)
	lamivudine	46.3[a]	(13)
	metformin*	680[b], 990[b], 1072[b], 3171[b], 3356[b]	(15-19)
	memantine	34[b]	(1)
	MPP⁺ (N-methylpyridinium)	16[b], 19[a], 19.5[b]	(1; 20; 19)
	norepinephrine	1500[b]	(8)
	prostaglandin E2	0.0289[b]	(22)
	prostaglandin F2α	0.344[b]	(22)
	ranitidine	65.2[b]	(6)
	serotonin	290[b]	(8)
	tetraethylammonium	33.8[a], 76[a]	(24; 20)
	varenicline*	370[b]	(28)
	YM155	2.67[b]	(29)
SLC22A6 (OAT1)	6-carboxyfluorescein	3.93[b]	(31)
	acyclovir*	342[b]	(33)
	adefovir	30[a], 23.8[b]	(34; 35)
	cidofovir	30[b], 58[b], 46[a]	(37; 35; 34)
	dimesna	636[b]	(39)
	edaravone sulfate	10.8[b]	(40)
	ganciclovir	896[b]	(33)
	glutarate	10.7[b]	(31)
	methotrexate*	554[b], 724[a]	(43; 44)
	ochratoxin A	0.42[b]	(46)
	olmesartan	0.0683[b]	(30)
	PAH (para-aminohippurate)	15.4[b], 20.1[b], 28[b], 9.3[a], 5[b], 4[a], 3.9[a]	(35; 49; 50; 34; 51; 53)
	perfluoroheptanoate	50.5[b]	(54)
	perflurooctanoate	43.2[b]	(54)
	probenecid	26[b]	(39)
	prostaglandin E2	0.97[b]	(22)
	prostaglandin F2α	0.575[b]	(22)
	tenofovir*	33.8[b]	(56)
	uric acid	197.6[b]	(58)
	zidovudine*	45.9[b]	(33)
SLC22A8 (OAT3)	1-BSA (1-butanesulfonic acid)	5098[b]	(60)
	adipate	136[b]	(62)

Apical Transporter	Substrate	Km (μM)	Reference
ABCB1 (MDR1, P-gp)	biotin	13[b]	(2)
	colchicine	1640[d]	(4)
	dexamethasone	826[c]	(5)
	digoxin*	73[d], 177[d], 181[c]	(7; 4; 5)
	etoposide	255[b], 461[d]	(9; 4)
	fexofenadine	150[d]	(10)
	indinavir	0.47[c]	(11)
	irinotecan	45.5[b], 116.1[d]	(12)
	loperamide*	11.4[c]	(14)
	nicardipine	2.6[c]	(5)
	paclitaxel	1.4[c], 65[d]	(5; 7)
	rhodamine 123	21[c]	(5)
	ritonavir	0.8[b]	(21)
	saquinavir	14.5[b], 15.4[d]	(21; 7)
	topotecan	78.3[d], 102[b]	(23)
	valinomycin	2.5[c]	(5)
	verapamil	4.1[c]	(5)
	vinblastine	1.7[c], 19[d], 89.2[d], 146[a], 253[b]	(5; 25; 26; 27; 26)
	vincristine	3.7[c]	(5)
ABCC2 (MRP2, cMOAT)	DHEAS (dehydroepiandrosterone sulfate)	14.9[e]	(30)
	estradiol-17β-glucuronide	7.2[e]	(32)
	etoposide	617[b]	(9)
	irinotecan	48.9[e], 90.8[b]	(36; 12)
	methotrexate	480[e]	(38)
	olmesartan	14.9[e]	(30)
	PAH (para-aminohippurate)	880[e], 2100[e], 5000[e]	(41; 42)
	SN-38	180[e]	(32)
	SN-38 glucuronide	5.7[e]	(32)
	valsartan	30.4[e]	(45)
	vinblastine	137.3[b]	(47)
ABCC4 (MRP4)	chenodeoxycholylglycine	5.9[e]	(48)
	chenodeoxycholyltaurine	3.6[e]	(48)
	cholate	14.8[e]	(48)
	cholyltaurine	7.7[e]	(48)
	cyclic AMP	44.5[e]	(55)
	cyclic GMP	9.69[e]	(55)
	deoxycholylglycine	6.7[e]	(48)
	DHEAS (dehydroepiandrosterone sulfate)	1.9[e], 26.2[e]	(57; 30)
	estradiol-17β-glucuronide	30.3[e]	(55)
	folic acid	170[e]	(59)
	methotrexate	220[e], 220[e], 1300[e]	(38; 59; 61)

(continued)

Table 1 *(continued)*

Basolateral Transporter	Substrate	Km (µM)	Reference
SLC22A8 (OAT3), *continued*	α-ketoglutarate	92.8[b]	(62)
	bumetanide*	7.8[a], 1586[b]	(63; 64)
	cimetidine	113[b], 174[b], 57.4[a]	(37; 66; 67)
	cortisol	2.4[a]	(68)
	dimesna	390[b]	(39)
	DMPS (2,3-dimercapto-1-propanesulfonic acid)	40[b]	(60)
	edaravone sulfate	15.1[b]	(40)
	estrone 3-sulfate	2.18[b], 2.21[b], 3.1[a], 6.3[b], 7.5[b]	(49; 67; 72; 50; 73)
	fexofenadine	70.2[b]	(66)
	methotrexate	10.9[a], 17.2[a], 21.1[b]	(44; 67; 43)
	MPS (3-mercapto-1-propanesulfonic acid)	2139[a]	(60)
	ochratoxin A	0.75[b]	(46)
	olmesartan	0.12[b]	(30)
	PAH (para-aminohippurate)	87.2[a]	(67)
	perfluoroheptanoate	65.7[b]	(54)
	perfluorooctanoate	174.5[b]	(54)
	pimelate	634[b]	(62)
	pitavastatin	3.3[a]	(84)
	PNU-288034	44[b]	(85)
	pravastatin	27.2[b]	(87)
	probenecid	32[b]	(39)
	prostaglandin E2	0.345[b]	(22)
	prostaglandin F2α	1.092[b]	(22)
	rosuvastatin	7.4[a]	(93)
	sitagliptin	162[b]	(37)
	suberate	232[b]	(62)
	sulfasalazine	3[a]	(63)
	tetracycline	566.2[b]	(97)
	uric acid	380.3[b]	(58)
	zidovudine	145[b]	(33)
SLCO4C1 (OATP4C1)	digoxin	7.8[b]	(98)
	estrone 3-sulfate	26.6[b]	(99)
	ouabain	0.38[b]	(98)
	T3	5.9[b]	(98)

Apical Transporter	Substrate	Km (µM)	Reference
ABCC4 (MRP4), *continued*	olmesartan	26.2[e]	(30)
	PAH (para-aminohippurate)	160[e]	(42)
	prostaglandin E1	2.1[e]	(65)
	prostaglandin E2	3.4[e]	(65)
	topotecan	1.66[b]	(69)
ABCG2 (BCRP, MXR)	4-MUS (4-methylumbelliferone sulfate)	12.9[e]	(70)
	daunorubicin	2.5[e]	(71)
	doxorubicin	5[e]	(71)
	estradiol-17β-glucuronide	44.2[e]	(74)
	estrone 3-sulfate	6.8[e], 16.6[e]	(75; 70)
	hematoporphyrin	17.8[e]	(76)
	methotrexate	681[e], 1340[e], 1410[e]	(77; 74; 78)
	mitoxantrone	7[e]	(71)
	pitavastatin*	5.73[e]	(79)
	rosuvastatin*	2.02[e], 10.1[e]	(80; 81)
	SN-38	4[e]	(82)
	SN-38 glucuronide	26[e]	(82)
	sulfasalazine	0.7[e]	(83)
	topotecan	213[b]	(23)
SLC22A4 (OCTN1)	ergothioneine	21[b]	(86)
	ipratropium	444[b]	(88)
	tetraethylammonium	195[a], 1800[b]	(89; 90)
SLC22A5 (OCTN2)	acetyl-L-carnitine	8.5[b]	(91)
	D-carnitine	10.9[b], 98.3[b]	(91; 92)
	ipratropium	53[b]	(88)
	L-carnitine	3.5[b], 4.3[b], 4.8[a]	(94; 91; 92)
SLC47A1 (MATE1)	acyclovir	2640[b]	(95)
	cephalexin	5900[b]	(96)
	cimetidine	170[b]	(95)
	estrone 3-sulfate	470[b]	(95)
	ganciclovir	5120[b]	(95)
	guanidine	2100[b]	(95)
	metformin	202[b], 227[b], 780[b]	(15; 100; 95)
	MPP+ (N-methylpyridinium)	16[b], 100[b]	(101; 95)
	paraquat	169[b]	(100)
	PNU-288034	340[b]	(85)
	procainamide	1230[b]	(95)
	tetraethylammonium	220[b], 380[b]	(101; 95)
	topotecan	70[b]	(95)
SLC47A2 (MATE2K)	acyclovir	4320[b]	(95)
	cimetidine	120[b], 370[b]	(95; 102)
	estrone 3-sulfate	850[b]	(95)
	ganciclovir	4280[b]	(95)
	guanidine	4200[b]	(95)
	metformin	1050[b], 1980[b]	(102; 95)
	MPP+ (N-methylpyridinium)	93.5[b], 110[b]	(102; 95)
	procainamide	1580[b], 4100[b]	(95; 102)
	tetraethylammonium	760[b], 830[b]	(95; 102)
	topotecan	60[b]	(95)

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 (9). References are included in the Supplementary References section.

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	Inhibitor	IC$_{50}$, $\underline{K_i}$ (µM)	Reference
SLC22A2 (OCT2)	amantadine	45.9[b], $\underline{28.4}$[b]	(8; 29)
	amitriptyline	14[b]	(19)
	atropine	39[b]	(106)
	Bmim-Cl (1-butyl-3-methylimidazolium chloride)	1.5[b]	(107)
	BmPy-Cl (N-butyl-N-methylpyrrolidinium chloride)	0.48[b]	(107)
	butylscopolamine	764[b]	(106)
	carvedilol	63[b]	(19)
	chloroquine	1096[b]	(19)
	chlorpromazine	14[b]	(19)
	cimetidine*	120[b], 110[b], 373[a]	(19; 29; 24)
	clonidine	16[b], 23[b]	(19; 106)
	cocaine	$\underline{113}$[b]	(8)
	corticosterone	5.35[b]	(29)
	creatinine	580[b]	(115)
	D-Amphetamine	$\underline{10.5}$[b]	(8)
	decynium-22	$\underline{0.1}$[a], 13.8[a]	(20; 24)
	denfluramine	10[b]	(19)
	desipramine	16[a]	(20)
	desloraratidine	60[b]	(19)
	diphenhydramine	15[b], 21[b]	(106; 19)
	disopyramide	324[b]	(19)
	doxepin	13[b]	(19)
	DX-619	$\underline{0.94}$[b]	(117)
	etilefrine	4009[b]	(106)
	EtPy-Cl (1-ethylpyridinium chloride)	36.7[b]	(107)
	famotidine	114[a]	(119)
	flecainide	191[b]	(19)
	flurazepam	60[b]	(19)
	furamidine	182[b]	(122
	grepafloxacin	10.4[b]	(117
	imipramine	6[b]	(19)
	ipratropiumbromide	15[b]	(19)
	ketamine	$\underline{22.7}$[b]	(8)
	levomethadone	60[b]	(19)
	MDMA (3,4-methylenedioxymetamphetamine)	$\underline{1.63}$[b]	(8)
	mefloquine	204[b]	(19)
	memantine	$\underline{7.3}$[b]	(8)
	mepiperphenidol	$\underline{4.8}$[a]	(20)
	metformin	398[b], 521[a], $\underline{289}$[b], $\underline{1380}$[b]	(19; 119; 126)
	mexiletine	55[b]	(19)
	MK-801	$\underline{21.5}$[b]	(8)
	MPP$^+$ (N-methylpyridinium)	4.42[b], $\underline{2.4}$[a]	(29; 20)
	NBuPy-Cl (N-butylpyridinium chloride)	2.29[b]	(107)
	pentamidine	10.6[b]	(122)
	phencyclidine	$\underline{24.9}$[b]	(8)
	phenformin	$\underline{54}$[b], $\underline{111}$[b]	(126; 126)
	prazosin	80.4[b]	(29)

Apical Transporter	Inhibitor	IC$_{50}$, $\underline{K_i}$ (µM)	Reference
ABCB1 (MDR1, P-gp)	amiodarone	5.48[b], 22.5[b], 45.6[b]	(103; 104)
	astemizole	2.73[b]	(105)
	azelastine	16[b], 30[b]	(104
	azithromycin	21.8[d]	(108
	clarithromycin	4.1[d]	(108
	cyclosporine*	1.36[b], 1.4[b], 1.6[b], 6.18[b], $\underline{0.46}$[d], $\underline{2.18}$[b]	(109; 110; 26)
	desethylamiodarone	1.27[b], 11.8[b], 15.4[b], 25.2[b], 41.8[b]	(103; 104)
	dipyridamole	40[b]	(103)
	elacridar	0.027[b], 0.043[b], 0.055[b], 0.18[b], $\underline{0.39}$[d], $\underline{0.44}$[b]	(109; 105; 26)
	erlotinib	2[e]	(111)
	erythromycin	10[d], 22.7[d], 119[d]	(112; 108; 113)
	itraconazole	0.95[b], 2[d]	(105; 114)
	ketoconazole	1.34[b], 3.07[b], 5.49[b], 5.6[b], 6.34[b]	(105; 109; 110)
	paclitaxel	54[b]	(110
	quinidine*	9.4[b], 9.52[b], 14.9[b], 22.9[b], 51.7[b], $\underline{3.23}$[d], $\underline{8.59}$[b]	(105; 103; 109; 26)
	quinine	22.4[b]	(105)
	reserpine	$\underline{1.38}$[d], $\underline{11.5}$[b]	(26)
	ritonavir	3.8[d], 5[d], 28.2[b]	(116; 114; 105)
	roxithromycin	15.4[d]	(108)
	tamoxifen	7.1[b]	(110
	telithromycin	1.8[d]	(108)
	valsopodar	0.11[d]	(116
	verapmil	0.2[e], 4.2[b], 8.44[d], 10.7[b], 17.3[b], 33.5[b], $\underline{8.11}$[d], $\underline{15.1}$[b], $\underline{29}$[b]	(111; 110; 10; 109; 26; 12)
	vinblastine	17.8[b], 18[b], 30.1[b], 89.7[b]	(109; 110)
	zosuquidar	0.024[d], 0.07[b]	(116; 118)
ABCC2 (MRP2, cMOAT)	curcumin	5[e]	(120)
	cyclosporine	10[b], $\underline{4.7}$[e], $\underline{8.11}$[b]	(120; 121; 47)
	daunorubicin	$\underline{49.4}$[b]	(47)
	etoposide	$\underline{756}$[b]	(47)
	gemifloxacin	16[b]	(123
	indomethacin	0.06[e]	(38)
	ketoprofen	1.4[e]	(38)
	MK-571	4[e], 50[d], $\underline{13.1}$[e], $\underline{26.4}$[b]	(124; 12; 121; 47)
	PAK-104P	$\underline{3.7}$[e]	(121)
	reserpine	$\underline{295}$[b]	(47)
	valsopodar	$\underline{28.9}$[e]	(121)
	vincristine	$\underline{802}$[b]	(47)
ABCC4 (MRP4)	benzbromarone	150[e]	(125
	candesartan	16[e]	(58)
	celecoxib	35[e]	(38)
	diclofenac	0.006[e]	(38)

(continued)

13

Table 2 *(continued)*

Basolateral Transporter	Inhibitor	IC$_{50}$, $\underline{K_i}$ (µM)	Reference
SLC22A2 (OCT2), *continued*	procainamide	91.9[b], 50[a]	(29; 20)
	propafenone	25[b]	(19)
	propranolol	229[b]	(19)
	pyridine hydrochloride	790[b]	(107)
	quinidine	8.7[a], 11[b], 13.3[b], 87[b]	(119; 29; 122; 19)
	quinine	23[b], 3.4[a]	(106; 20)
	ranitidine	76[a], 1617[b], 30.5[b], 79[b]	(119; 106; 6)
	sibutramine	29[b]	(19)
	tamoxifen	87[b]	(19)
	tetraethylammonium	189.2[b], 222[a]	(122; 119)
	tetrapentylammonium	1.5[a]	(20)
	trimethoprim	1318[b]	(19)
	verapamil	13.4[b], 85[b]	(29; 19)
	YM155	15.9	(29)
SLC22A6 (OAT1)	1-BSA (1-butanesulfonic acid)	514[b]	(60)
	1-hexylpyridinium chloride	0.35[b]	(107)
	acetazolamide	75[b]	(64)
	acetominophen	639[b]	(128)
	acetylsalicylate	769[b]	(128)
	adefovir	0.9[b], 1.5[b], 1.8[b]	(130)
	adipate	6.2[b]	(62)
	α-ketoglutarate	4.7[b]	(62)
	betamipron	6[b], 16.2[b], 23.6[b]	(130; 35; 49)
	bumetanide	7.6[b]	(64)
	candesartan	17[b]	(58)
	cefadroxil	6140[b]	(131)
	cefamandole	30[b]	(131)
	cefazolin	180[b]	(131)
	cefoperazone	210[b]	(131)
	cefotaxime	3130[b]	(131)
	ceftriaxone	230[b]	(131)
	cephaloridine	1250[b], 740[b]	(31; 131)
	cephalothin	220[b]	(131)
	cephradine	1600[b]	(31
	chlorothiazide	3.78[b]	(64
	cidofovir	60[b]	(31
	cilastatin	1470[b]	(49
	citrinin	3080[b]	(46
	cyclothiazide	84.3[b]	(64
	diclofenac	4[b], 4.46[b]	(130; 128)
	diflunisal	0.85[b]	(130
	DMPS (2,3-Dimercapto-1-propane sulfonate)	19[a], 83[b]	(53; 60)
	ethacrynic acid	29.6[b]	(64)
	etodolac	50[b]	(130)
	flurbiprofen	1.5[b]	(130)
	fluvastatin	26.3[b]	(133)
	fumarate	1733[b]	(62)
	eurosemide	18[b]	(64)
	glutarate	4.9[b], 3.3[b]	(31; 62)
	hippuric acid	20[b]	(136)
	hydrochlorothiazide	67.3[b]	(64)
	ibuprofen	8[b], 55.6[b]	(130; 128)
	indoleacetic acid	83[b]	(136)
	indomethacin	3[b], 3.83[b]	(130; 128)

Apical Transporter	Inhibitor	IC$_{50}$, $\underline{K_i}$ (µM)	Reference
ABCC4 (MRP4), *continued*	dilazep	20[e]	(125)
	dipyridamole	2[e]	(125)
	indomethacin	6.1[e]	(38)
	ketoprofen	11.9[e]	(38)
	losartan	1.5[e]	(58)
	MK-571	10[e]	(125)
	nitrobenzylmercaptopurine riboside	75[e]	(125)
	probenecid	2300[e]	(125)
	sildenafil	20[e]	(125)
	sulfinpyrazone	420[e]	(125)
	sulindac	2.11[e]	(38)
	telmisartan	11[e]	(58)
	trequinsin	10[e]	(125)
	zaprinast	250[e]	(125)
ABCG2 (BCRP, MXR)	17β-estradiol-3-sulfate	14[e]	(70)
	abacavir	385[b]	(127)
	amprenavir	181[b]	(127)
	atazanavir	69.1[b]	(127)
	atorvastatin	14.3[e]	(79)
	AZD9056	32[a], 92[a]	(63)
	cerivastatin	18.1[e]	(79)
	daunomycin	59[e]	(70)
	delaviridine	18.7[b]	(127)
	DHEAS (dehydroepiandrosterone sulfate)	55[e]	(70
	efavirenz	20.6[b]	(127)
	elacrindar	0.31[b]	(129
	erlotinib	0.15[e]	(111)
	fluvastatin	5.43[e]	(79)
	fumitremorgin C	0.47[b], 0.55[e]	(127; 111)
	Ko143	0.01[b]	(127)
	lopinavir	7.66[b]	(127)
	nelfinavir	13.5[b]	(127)
	nilotinib	0.69[e]	(78)
	pitavastatin	2.92[e]	(79)
	rosuvastatin	15.4[e]	(79)
	saquinavir	27.4[b]	(127)
	simvastatin	18[e]	(79)
	SN-38	1.6[e]	(70)
	sulfasalazine	0.73[a]	(63)
SLC22A4 (OCTN1)	disprocynium 24	14.6[b]	(40)
	hercynine	1450[b]	(40)
	L-ergothioneine	9[b]	(90)
	methimazole	7520[b]	(40)
	pyrilamide	182[b]	(40)
	thioperamide	254[b]	(40)
	verapamil	10.8[b]	(40)
SLC22A5 (OCTN2)	cefepime	1700[b]	(94)
	cefoselis	6400[b]	(94)
	cephaloridine	230[b]	(94)
	emetine	4.2[a]	(92)
SLC47A1 (MATE1)	amantadine	111.8[b]	(132)
	cetirizine	371.2[b]	(132)
	chloroquine	2.5[b]	(134)
	chlorpheniramine	87.6[b]	(132)
	cimetidine	1.1[b], 3.8[b]	(132; 135)
	desipramine	55.7[b]	(132)
	diltiazem	12.5[b]	(132)

(continued)

Table 2 *(continued)*

Basolateral Transporter	Inhibitor	IC$_{50}$, $\underline{K}_i$ (µM)	Reference
SLC22A6 (OAT1), *continued*	indoxyl sulfate	83[b]	(136)
	JBP485	226[b], 197[b]	(137)
	ketoconazole	319[a]	(138)
	ketoprofen	1.3[b], 1.4[b], 4.34[b]	(130; 31; 128)
	KW-3902 (Noradamantan-3-yl)-1,3-dipropylxanthine)	7.82[b]	(49)
	losartan	12[b]	(58)
	mefenamic acid	0.83[b]	(128)
	methazolamide	438[b]	(64)
	MPS (3-mercapto-1-propanesulfpnic acid)	204[b]	(60)
	naproxen	5.67[b], 5.8[b]	(128; 130)
	novobiocin	14.9[b]	(72)
	octanoate	5.41[b]	(46)
	olmesartan	0.28[b]	(58)
	ortho-hydroxyhippuric acid	27[b]	(136)
	PAH (para-aminohippurate)	8.8[b], 6.02[b], 92[b]	(31; 46; 137)
	para-hydroxyhippuric acid	25[b]	(136)
	phenacetin	200[b], 275[b]	(130; 128)
	pimelate	18.6[b]	(62)
	piroxicam	20.5[b], 62.8[b], 19.8[b]	(130; 128; 46)
	pratosartan	1.5[b]	(58)
	pravastatin	408[b]	(133)
	probenecid*	3.9[b], 6.3[b], 6.5[b], 7.4[b], 4.29[b], 12.1[b]	(37; 31; 35; 130; 46; 49)
	1-propanesulfonic acid (PSA)	2036[b]	(60)
	rifampin	79.1[a], 62.2[a]	(138)
	salicylate	280[b], 325[b]	(31; 128)
	simvastatin	41.5[a], 73.6[b]	(93; 133)
	suberate	19.3[b]	(62)
	succinate	4825[b]	(62)
	sulfasalazine	4.6[a]	(63)
	sulindac	36.2[b]	(128)
	telmisartan	0.46[b]	(58)
	trichloromethiazide	19.2[b]	(64)
	valsartan	16[b]	(58)
SLC22A8 (OAT3)	acetazolamide	816[b]	(64)
	betamipron	48.3[b]	(49)
	bumetanide	0.75[b]	(64)
	candesartan	0.3[b]	(58)
	cefadroxil	8620[b]	(131)
	cefamandole	50[b]	(131)
	cefazolin	550[b]	(131)
	cefoperazone	1890[b]	(131)
	cefotaxime	290[b]	(131)
	ceftriaxone	4390[b]	(131)
	cephaloridine	2460[b]	(131)
	cephalothin	40[b]	(131)
	chlorothiazide	65.3[b]	(64)
	cilastatin	231[b]	(49)
	cimetidine	79[b]	(37)

Apical Transporter	Inhibitor	IC$_{50}$, $\underline{K}_i$ (µM)	Reference
SLC47A1 (MATE1), *continued*	diphenhydramine	87[b]	(132)
	disopyramide	83.8[b]	(132)
	DX-619	0.82[b]	(117)
	famotidine	0.6[b]	(132)
	imipramine	42[b]	(132)
	metformin	666.9[b]	(132)
	mitoxantrone	4.4[b], 5.2[b]	(15)
	NbuPy-Cl (N-butylpyridinium chloride)	8.5[b]	(107)
	pramipexole	141.4[b]	(132)
	procainamide	217[b]	(132)
	quinidine	29.2[b]	(132)
	ranitidine	17.5[b], 18.9[b], 25.4[b]	(15; 132)
	rapamycin	3.27[b], 3.51[b]	(15)
	ritonavir	13.9[b], 15.4[b]	(15)
	talipexole	66[b]	(132)
	trimethoprim	6.2[b]	(134)
	verapamil	27.5[b]	(132)
SLC47A2 (MATE2K)	amantadine	1167[b]	(132)
	cetirizine	817.6[b]	(132)
	chlorpheniramine	191.2[b]	(132)
	cimetidine	7.3[b], 2.1[b]	(132)
	desipramine	283[b]	(132)
	diltiazem	117[b]	(132)
	diphenhydramine	266.5[b]	(132)
	disopyramide	291.6[b]	(132)
	DX-619	0.1[b]	(117)
	famotidine	9.7[b]	(132)
	imipramine	182.9[b]	(132)
	metformin	6515.7[b]	(132)
	NbuPy-Cl (N-butylpyridinium chloride)	1.6[b]	(107)
	pramipexole	24.1[b]	(132)
	procainamide	178.1[b]	(132)
	quinidine	23.1[b]	(132)
	ranitidine	25[b]	(132)
	talipexole	119.5[b]	(132)
	verapamil	32.1[b]	(132)

(continued)

Table 2 *(continued)*

Basolateral Transporter	Inhibitor	IC$_{50}$, $\underline{K_i}$ (µM)	Reference
SLC22A8 (OAT3), continued	citrinin	$\underline{15.4}$[b]	(46)
	cyclothiazide	27.9[b]	(64)
	diclofenac	7.78[b]	(128)
	ethacrynic acid	0.58[b]	(64)
	fenofibric acid	2.2[b]	(37)
	fluvastatin	5.79[b]	(133)
	fumarate	21100[b]	(62)
	furosemide	1.7[b], 7.31[b]	(37; 64)
	gemfibrozil	6.8	(87)
	glutarate	78.5[b]	(62)
	hydrochlorothiazide	942	(64)
	ibuprofen	3.7[b], 6[b]	(37; 128)
	indapamide	11[b]	(37)
	indomethacin	0.61[b]	(128)
	JBP485	185[b], $\underline{160}$[b]	(137)
	ketoprofen	5.98[b]	(128)
	KW-3902 (Noradamantan-3-yl)-1,3-dipropylxanthine)	$\underline{3.7}$[b]	(49)
	losartan	1.6[b]	(58)
	mefenamic acid	0.78[b]	(128)
	methazolamide	97.5[b]	(64)
	naproxen	4.67[b]	(128
	novobiocin	$\underline{4.77}$[b]	(72)
	octanoate	$\underline{8.6}$[b]	(46)
	olmesartan	0.027[b]	(58)
	para-aminohippurate	$\underline{19.6}$[b]	(46)
	penicillin G	102[b], $\underline{88}$[b]	(137)
	phenacetin	19.4[b]	(128)
	piroxicam	2.52[b], $\underline{4.88}$[b]	(128; 46)
	pratosartan	0.095[b]	(58)
	pravastatin	13.7[b]	(133)
	probenecid*	3.1[b], 5.6[b], $\underline{1.3}$[b], $\underline{4.41}$[b], 9[b]	(37; 66; 46; 49)
	quinapril	6.2[b]	(37)
	simvastatin	32.3[b]	(133)
	sitagliptin	160[b]	(37)
	sulindac	3.62[b]	(128
	telmisartan	1.6[b]	(58)
	trichloromethiazide	71.2[b]	(64)
	valsartan	0.2[b]	(58)
SLCO4C1 (OATP4C1)	3,5,3'-triiodo-L-thyronine	1.3[b]	(98)
	digitoxin	0.12[b]	(98)
	digoxigenin	0.49[b]	(98)
	digoxin	540[b], 119[b]	(98; 99)
	ouabain	0.36[b]	(98)
	thyroxine	8.0[b]	(98)

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 (9). Underlined values represent the *in vitro* inhibition constant, K$_i$. References are included in the Supplementary References section.

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.
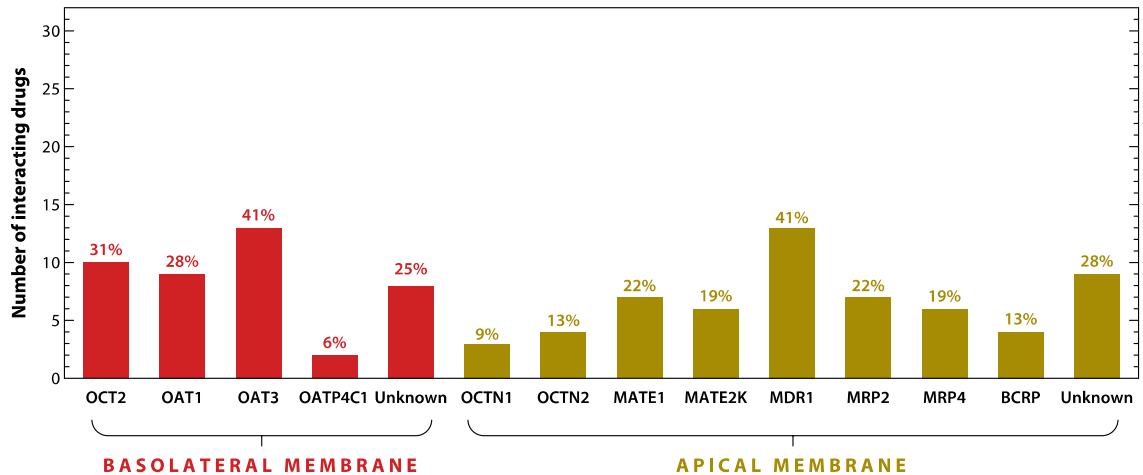
Figure 3. **Interaction of renal secretory transporters with the top 200 prescribed renally secreted medications (i.e., ≥25% of the absorbed dose is excreted unchanged in urine).** The figure includes only drugs predicted to undergo net tubular secretion ($n = 32$). Data are presented as the number of 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.

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.

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 Pharmacological Basis of Therapeutics* (10) and *Clarke's Analysis of Drugs and Poisons* (11)]. 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 concentrations of metformin (12), which increases 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 (13). Nowadays, the coadministration of probenecid with cidofovir, an anti-viral drug, is required by the FDA (14) to protect against cidofovir-mediated nephrotoxicity by inhibiting cidofovir uptake at the basolateral membrane (15; 16). If a DDI were to occur at the apical membrane, the intracellular kidney

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

Implicated Transporter	Interacting Drug	Affected Drug	Clinical Pharmacokinetic Impact on Affected Drug (presented as fold change)					Reference
			AUC	Cmax	CL_R	CL/F	$t_{1/2}$	
OATs	furosemide	lomefloxacin	1.1	NS	0.7	0.9	NS	(139)
OATs	probenecid	cefaclor	2.1	1.5	–	–	1.6	(140)
OATs	probenecid	cephradine	2.4	1.9	–	–	1.5	(140)
OATs	probenecid	famotidine	1.8	1.5	0.4	0.1	NS	(141)
OATs	probenecid	ceftriaxone	0.7	–	–	1.3	0.8	(142)
OATs	probenecid	acyclovir	1.4	–	0.7	NS	–	(143)
OATs	probenecid	cefonicid	2.1	1.2	0.3	–	1.5	(144)
OATs	probenecid	cefoxitin	2.4	–	0.4	–	2	(145)
OATs	probenecid	cidofovir	–	–	0.5	0.6	–	(146)
OATs	probenecid	dicloxacillin	1.9	1.8	0.3	0.5	–	(147)
OATs/MRPs	probenecid	ciprofloxacin	1.7	NS	0.4	0.6	1.5	(148)
OATs/MRPs	probenecid	furosemide	2.7	1.5	0.3	0.4	1.7	(149)
OATs/OCTs	cotrimoxazole (trimethoprim/ sulfamethoxazole)	zidovudine	NS	–	0.4	NS	NS	(150)
OATs/OCTs	cotrimoxazole (trimethoprim/ sulfamethoxazole)	apricitabine	1.7	1.3	0.6	0.6	1.4	(151)
OCTs	trimethoprim	zidovudine	NS	–	0.5	NS	NS	(150)
OCTs/MATEs	cetirizine	pilsicainide	1.4	NS	–	–	–	(152)
OCTs/MATEs[a]	cimetidine	pindolol (S-enantiomer)	1.4	1.3	0.7	–	NS	(153)
OCTs/MATEs[a]	cimetidine	metformin	1.5	1.7	0.7	–	–	(154)
OCTs/MATEs[a]	cimetidine	cephalexin	NS	NS	0.8	0.8	NS	(155)
OCTs/MATEs[a]	cimetidine	ranitidine	1.3	NS	0.7	–	1.3	(155)
OCTs/MATEs[a]	cimetidine	procainamide	1.4	NS	0.6	–	1.3	(156)
OCTs/MATEs[a]	cimetidine	pilsicainide	1.3	NS	0.7	0.7	1.2	(157)
OCTs/MATEs[a]	cimetidine	varenicline	1.3	–	0.8	0.8	–	(28)
OCTs/MATEs[a]	cimetidine	dofetilide[b]	1.5	1.3	0.7	0.7	1.3	(158)
MATEs	pyrimethamine	metformin	1.4	1.4	0.6	–	–	(159)

[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 (17).

[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 are included in the Supplementary References section.

−, not determined; AUC, area under the plasma drug concentration-time curve; CL_R, renal clearance; CL/F, apparent total 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.

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 (18). The publication includes a summary of *in vitro* methods to study transporter-mediated DDIs along with decision trees on the data required to support 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
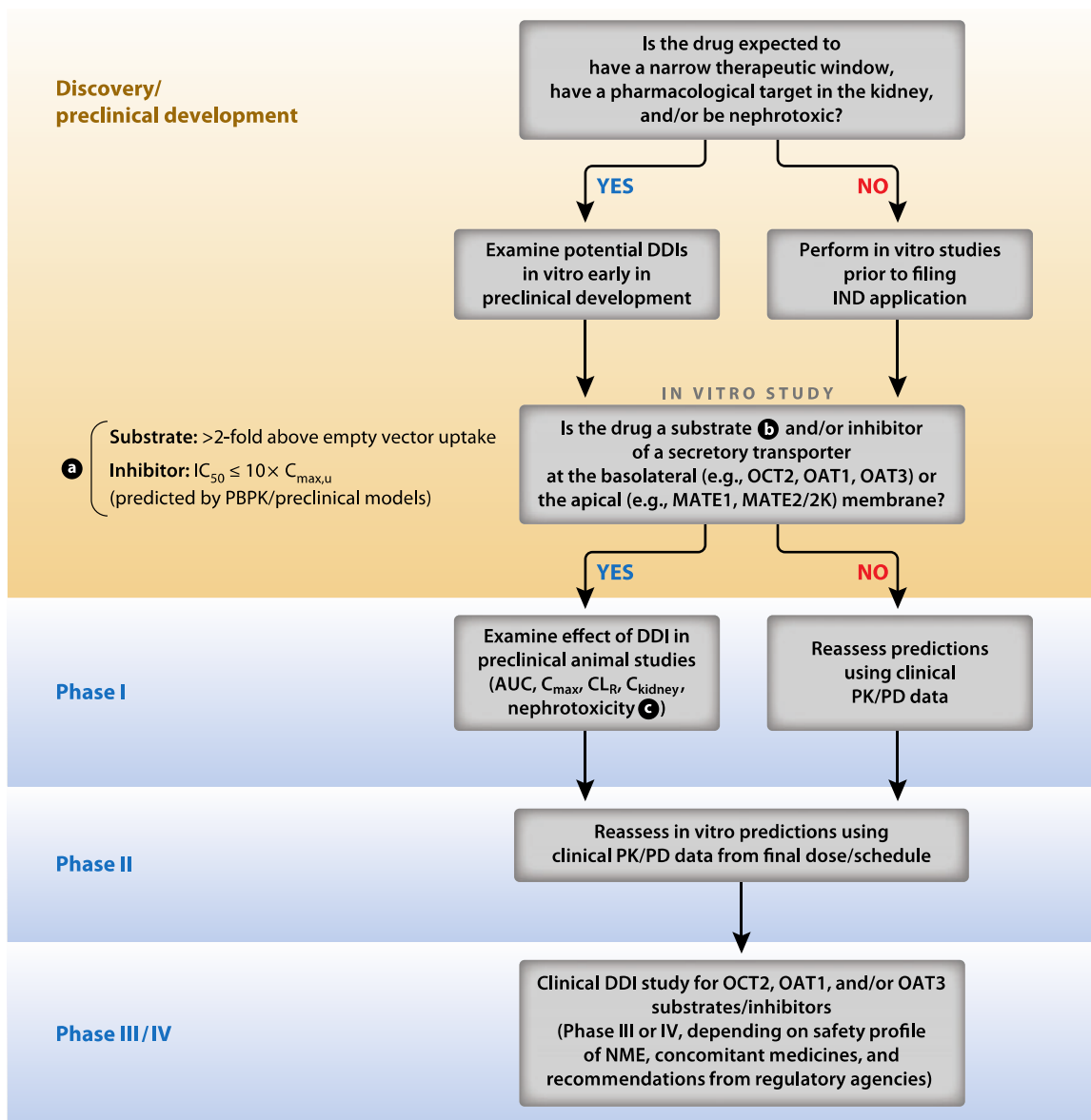
Figure 4. Incorporation of US Food and Drug Administration (FDA) guidelines into the discovery and development of NMEs. Letters a–c refer to items in the figure that are accompanied by the corresponding white letters in black circles. (a) Cutoff values are derived from the FDA DDI draft guidance (4). (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 • GFR)/CL$_T$ ≥ 0.25] or unknown mechanisms. (c) Evaluate NMEs that are inhibitors of apical secretory transporters for

23

nephrotoxicity. NMEs that are inhibitors of basolateral secretory transporters may be protective of potential proximal tubule toxicity.

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.

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 of 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 draft DDI 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 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 post-marketing 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 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 Table 4. 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 (Table 5).

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 (19; 20). 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 and K.M. Giacomini, unpublished data). Variation in transcript levels among individuals may be due to differences in the transcription or

Table 4. The equations to calculate renal clearance and plasma clearance and the creatinine and cystatin C GFR predicative equations for adults and children.

Equation		Comments
$CL_R = \dfrac{U \bullet V}{P}$		Used to calculate urinary clearance, which is the most direct method for measurement of GFR
$CL_R = fe \bullet \dfrac{Dose \bullet F}{AUC}$		Used to calculate urinary clearance
$CL_R = \dfrac{Ae_{0-t}}{AUC_{0-t}}$		Used to calculate urinary clearance
$CL_S = CL_R - fu \bullet GFR$		Used to estimate net secretion for compounds that are eliminated by tubular secretion, primarily by active transport
$CL_{EX} = I \bullet \dfrac{R}{S_{EX}}$		Used to calculate plasma clearance of an exogenous filtration marker; calculated after an intravenous infusion of an exogenous filtration marker to steady-state
Adults		
Cockcroft Gault (CG)	$CL_{CR} = \dfrac{(140 - age) \bullet weight}{72 \bullet S_{CR}}$ $x\ [0.85\ if\ female]$	Most commonly used equation; generally overestimates GFR (due to tubular secretion of creatinine); less accurate in children (<12 years), older, obese, diabetic, pregnant and severely renally impaired individuals (189-191); more accurate than MDRD when plasma creatinine is within the reference interval (192)
MDRD	$eGFR = 186 \bullet S_{CR}^{-1.154} \bullet age^{-0.203}$ $x\ [0.742\ if\ female]\ x\ [1.212\ if\ African\ American]$	More accurate determination of GFR in subjects with reduced renal function; less accurate in obese, pregnant, normal renal function and elderly; takes into account differences in creatinine production rates between individuals of African and European ancestries; generally, the MDRD has been shown to outperform the CG formula, with less bias and higher correlation with the gold standard, inulin (189-191)
CKD-EPI	$eGFR = 144 \bullet \left[\dfrac{S_{CR}}{0.7}\right]^{-0.329(or-1.2098^{a})} \bullet 0.993^{age}$ (for females) $eGFR = 144 \bullet \left[\dfrac{S_{CR}}{0.9}\right]^{-0.411(or-1.2098^{b})} \bullet 0.993^{age}$ (for males)	Formula matches the accuracy of the MDRD at lower GFR values and has an improved accuracy at higher or normal GFR (193; 194); less accurate in pregnant women
Pre-eclampsia pregnancy		
PGFR	$eGFR = 5.14 - 1.22 \bullet S_{CR} + 0.0029 \bullet weight(lbs)$ $+ [0.069\ if\ African\ American] + [-0.0566\ if\ East\ Asian] + [-0.1164\ if\ Hispanic]$	Formula is more accurate than Cockcroft-Gault, MDRD and CKD-EPI (195); validity of this formula in normal pregnancy remains unclear

(continued)

Table 4 *(continued)*

Equation		Comments
Children		
Schwartz	$eGFR = \dfrac{factor^c \cdot height(cm)}{S_{CR}}$	Most commonly used to estimated GFR in children (196-198); less accurate with reduced renal function (199; 200)
Counahan-Barrat	$eGFR = \dfrac{0.43 \cdot height(cm)}{S_{CR}}$	Most commonly used to estimated GFR in children (201); less bias and higher correlation with inulin (202)
Grubb	$eGFR = 84.69 \cdot S_{cystatinC}^{''1.68} \cdot 1.384$	Cystatin C, rather than creatinine, has been advocated for use in determining pediatric GFR (203)

[a]if $S_{CR} > 0.7$ mg dL^{-1}; [b] if $S_{CR} > 0.9$ mg dL^{-1}; [c]Factor: premature to 1 year: 0.33; boys (1-13 years) and girls (1-18 years): 0.55; adolescent boys (13-18 years): 0.70. References are included in the Supplementary References section.

CL_R, renal clearance (mL min^{-1}); U, urine concentration (mg mL^{-1}); V, urine flow (mL min^{-1}); P, average plasma concentration (mg mL^{-1}); fe, fraction of the absorbed dose that is excreted unchanged in the urine after oral administration; Dose, dose of compound (mg); F, bioavailability; AUC, area under the concentration curve (mg mL^{-1} min^{-1}); A_e, amount excreted unchanged in urine (mg); CL_S, net secretory clearance (mL min^{-1}); fu, fraction of unbound drug in plasma; GFR, glomerular filtration rate (mL min^{-1}); CL_{EX}, plasma clearance of exogenous marker (mL min^{-1}); I, infusion concentration of exogenous marker; R, infusion rate (mL min^{-1}); S_{EX}, serum concentration of exogenous marker (mg L^{-1}); eGFR, estimated glomerular filtration rate (mL min^{-1} 1.73m$^{2-1}$); CL_{CR}, creatinine clearance (mL min^{-1}); S_{CR}, serum creatinine (mg dL^{-1}); age (years); weight (kg); $S_{cystatin\ C}$, serum cystatin C (mg L^{-1})

Table 5. Overview of methods and markers to determine GFR.

	Advantage	Disadvantage	Comparison with other methods/markers
METHODS			
Urinary clearance of marker	Can measure GFR accurately in patients with any GFR including pregnant individuals (204)	Timed urine collection required; catheterization of subject is best practice to avoid incomplete collections; caution required if collecting a timed urine < 24 hours using creatinine as a marker because of the circadian rhythm of creatinine clearance (low at night); underestimates GFR in the elderly (205)	Measurement of creatinine clearance using timed urine collections is the gold standard for measuring creatinine clearance; the method is generally similar to predictive equations to estimate GFR from serum creatinine; exceptions to this are people with severe CKD, acute changes in renal function, abnormal muscle mass, extremes of body weight, nutritional status (e.g. vegetarians, excessive creatinine intake) where measurement of clearance using timed urinary collections is preferable
Plasma clearance of marker	Easily measured; no urine samples needed	Overestimates GFR in patients with expanded body space; longer examination periods (~5 hours) for subjects with low GFR; no consensus on formulas used to correct for missing AUC of two-sample method; repeated blood samples are required	Urinary and plasma clearance of exogenous markers in general show high agreement and accuracy; plasma clearance slightly overestimates urinary clearance at lower levels of GFR (206-208)
Nuclear imaging (over kidneys & bladder) with simultaneous counting of radioactive exogenous marker	No urine collection or repeat blood sampling needed; useful for determination of split renal function	Radiation exposure; Inaccuracy compared to renal or plasma clearance	Less accurate than predictive equations; poor correlation with simultaneous urinary or plasma clearance (209-211)

(continued)

Table 5 *(continued)*

	Advantage	Disadvantage	Comparison with other methods/markers
MARKERS			
Endogenous[a]			
Creatinine level in serum or urine	Easily measured Routine laboratory test	Discrepancies in analysis methods between institutions; levels alter with gender, age, races & differences in muscle mass (212); some drugs inhibit creatinine secretion (e.g. cimetidine) thereby increasing serum creatinine without affecting the GFR; not suitable during rapid changes in GFR or certain disease states (e.g. hypoalbuminemia) or at low levels of GFR (213; 214); if only collecting serum creatinine it must be used with a formula such as CG to estimate GFR; each formula has advantages and disadvantages (see Table 4)	Overestimates GFR by <10% (probably reflecting tubular secretion of creatinine) in normal renal function, overestimation increases (max. 30%) as GFR declines
Cystatin C level in serum or plasma	Level independent of muscle mass and sex	Levels affected by age, sex, diabetes, smoking, liver disease, glucocorticoid therapy and thyroid disease (215-217); analysis is 20-30 times more expensive than creatinine; must be used with a formula to estimate GFR; further validation of formulas are required before implementing into clinical practice	Similar or better performance of cystatin C based equations in estimating GFR in children, the elderly, people with mild or moderate renal impairment and renal transplant recipients compared to serum creatinine based equations (218-223)
Exogenous non-radioactive[b]			
Inulin	Gold standard when measuring urinary clearance	Requires constant infusion - difficult to dissolve; expensive	Plasma clearance of inulin exceeds urinary clearance by 5-10 mL/min (206-208)
Iothalamate	Inexpensive	Levels measured using HPLC-expensive; contraindicated in subjects with iodine allergies; possible tubular secretion	Similar (224) or small positive bias of urinary clearance of iothalamate compared to inulin (225; 226) or similar to inulin
Iohexol	Wide availability; stable in biological fluids; adverse reactions are rare	Expensive; difficult to measure concentrations in biological fluid (forms isomers); possible tubular reabsorption or protein binding; contraindicated in subjects with iodine allergies	Iohexol clearance is similar to iothalamate clearance but slightly underestimates inulin clearance (227-229)

(continued)

Table 5 *(continued)*

	Advantage	**Disadvantage**	**Comparison with other methods/markers**
Exogenous radioactive[b]			
^{99m}Tc-DTPA	Easily measured	Dissociation of ^{99m}Tc from DTPA & binding to plasma proteins-underestimates GFR; discrepancies in analysis methods; requirements for storage, administration, disposal or radioactive substance	Poor correlation between ^{99m}Tc-DTPA dynamic renal imaging, urinary or plasma clearance (230; 231); urinary clearance similar to ^{125}I-Iothalamate (230)
[51]Cr-EDTA	Easily measured	Only commercially available in Europe; possible tubular reabsorption; requirements for storage, administration, disposal or radioactive substance	Plasma clearance exceeds urinary clearance but similar to ^{99m}Tc-DTPA (231); urinary clearance underestimates inulin clearance (232)
[125]I-Iothalamate	Easily measured	Contraindicated in subjects with iodine allergies; requirements for storage, administration, disposal or radioactive substance	Plasma clearance higher than with ^{51}Cr-EDTA (225); urinary clearance exceeds inulin clearance (233)

[a]Endogenous biomarkers determined in serum alone must be used with a formula to estimate GFR (eGFR). The urinary clearance of endogenous biomarkers is measured by collection of urine and plasma samples. [b]Nonradioactive and radioactive biomarkers are generally administered intravenously and the urinary clearance or plasma clearance is measured by collection of urine and plasma samples. References are included in the Supplementary References section.

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 transporters 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 the transcription factors, resulting in changes in rates of transcription of the transporter genes. Studies (e.g., the NIH Common Fund's Genotype-Tissue Expression [GTEx]; see http://commonfund.nih.gov/GTEx/) to identify *cis*- and *trans*-eQTLs in the kidney that 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 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 (21-23) may lead to a better
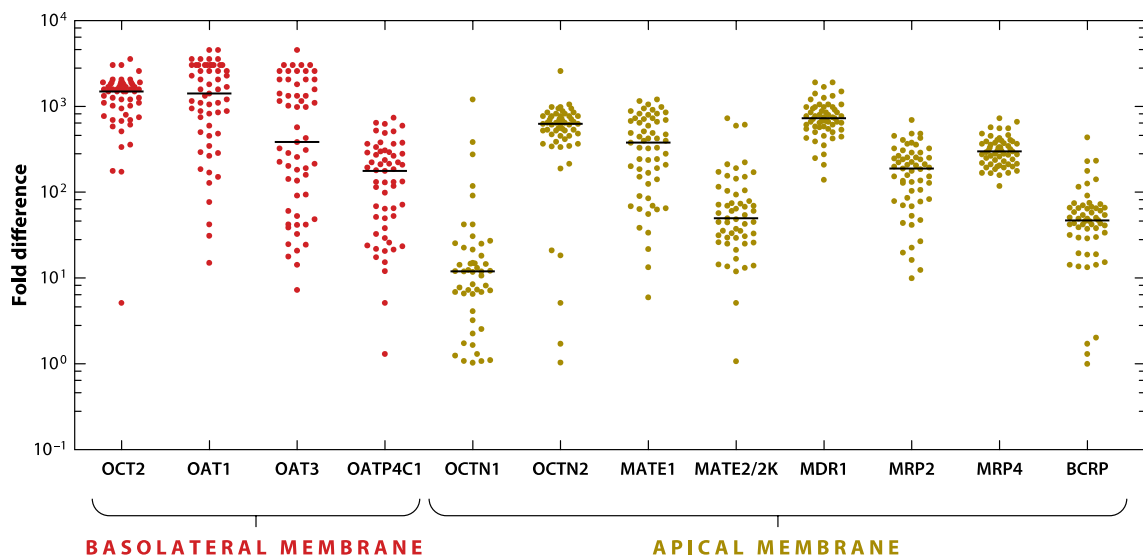
Figure 5. Expression of secretory drug transporters in the kidney of human subjects. Quantitative RT-PCR (reverse-transcription polymerase chain reaction) was performed on RNA obtained from the renal cortex of human donors ($n = 57$) using a custom SYBR® green-based OpenArray® system (Life Technologies, 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/ (24).

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.

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 (25). Furthermore, nonsynonymous coding SNPs, including the OCTN1-L503F (26) and OCT2-A270S (27) 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 (https://pharmacogenetics.ucsf.edu/) and recent literature reviews (28-30).

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, age, pregnancy, sex 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 that might hamper drug excretion (31). 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 6). 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 (31; 32). In fact, the FDA recommends that pharmacokinetic studies be conducted for all drugs, irrespective of their route of elimination, in patients with CKD (33).

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 (34) to levels commonly seen in healthy newborns and premature babies (35). GFR increases postnatally for both term-born and premature infants (36; 37). In term-born infants, this increase is faster than in premature

Table 6.   Comparison of the mRNA and protein expression levels of renal transporters in various special populations.

Transporter	Renal impairment / Chronic kidney disease	Gender	Age	
			Children/ Adolescents	Elderly
OCT2	↓ R (160-163)	F ↑ R (163), M ↑ R (164-166)	↑ R (163; 166), ↓ R (167)	
OAT1	↓ R (163;167), ↑ R (168)	F ↑ R (165), M ↑ R (170), ↔ H (169)	↑ R (165; 171-173), ↓ R (170)	
OAT3	↓ R (163; 167; 174-176), ↑ R (168)	F ↑ R (165), ↔ R (170)	↑ R (165; 173), ↓ R (170)	
OATP4C1	↓ R (98; 176)	F ↓ R (177)	↓ R (177)	
OCTN1		↔ R (178)	↓ R (164; 178)	
OCTN2		↔ R (178)	↓ R (164; 178), ↑ R (179)	
MATE1	↓ R (163; 166)	F ↓ R (180)	↑ R (180)	
MRP2	↑ R (176; 181)	↔ R (165; 182)	↑ R (165), ↓ R (182; 183)	↓ R (183)
MRP4	↑ R (176)	F ↑ R (182)	↓ R (182)	
MDR1	↑ (ARF) R (184), ↔ (CRF) R (181)	M ↑ H (185), ↔ H (186)	↔ H (187), ↑ R (165), ↓ R (183)	↔ R (183)
BCRP	↓ R (188)	↔ R (165; 177)	↓ R (177), ↑ R (165)	

Observations in changes of mRNA or protein levels in renal impairment/chronic kidney disease models were often also reflected in altered excretion processes. The majority of observations are from rodents. Future studies are necessary to determine if similar trends occur in human subjects. 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: ↔ equal, ↑ higher, ↓ lower; changes observed in humans (H) and rodents (R). References are included in the supplementary references section.

ARF, acute renal failure; CRF, chronic renal failure; F, female; M, male.

infants (36-39).

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 (40-43). In children, certain differences in kidney function, e.g., the glomerular filtration of inulin (44) and the excretion of antibiotics (45), 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 solely be explained by these anatomical changes (46-48). 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 (49), the ontogeny of human renal transporters has not been studied extensively and current data are predominately from rodent models (Table 6). 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 (50). 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 (46). Interestingly, cephalosporin-related nephrotoxicity occurs more frequently in adults than children (51; 52). 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 (53-55). 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, a 24-h urine creatinine clearance – rather than the use of predicative equations – remains standard (56). In the setting of preeclampsia, however, renal hyperfiltration is even more pronounced (57) and a new formula for estimating GFR has been developed (58) (Table 4). 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 (59-62).

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 (63). However, no discernable differences in Mdr1 protein expression were observed between normal and pregnant mice (64). With respect to human patients, increases in the renal secretory clearances of metformin (65), amoxicillin (60) and digoxin (66) have been observed in pregnant females. The mechanism(s) for the increase in renal clearance are 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 (67).

Sex

Using creatinine based predictive equations, significant sex 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 compared to men (68; 69). However, measured GFR (using inulin) is also lower in healthy women than men (70), suggesting physiological differences within the kidney may also contribute to sex differences in GFR. The importance of sex is also reflected in all of the adult predictive equations (Table 4).

The influence of sex on renal secretory transporter expression and function is largely unknown. Sex 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 sexes (see Table 6). In human kidneys, there is limited published data comparing transporter expression between sexes. Schuetz et al. (71) detected elevated MDR1 expression in men, but a subsequent study by Wolbold et al. (72) detected no sex differences in MDR1 expression. In a sub analysis of the human kidney expression data (Figure 5), no significant sex differences were observed in the transcript levels of the secretory renal transporters shown in Figure 2 (S.W. Yee, A. Chhibber, C.C. Wen, D.L. Kroetz and K.M. Giacomini, unpublished data). In addition, it is not known whether there are sex differences in the protein levels of renal

secretory transporters. Nonetheless, previous reports suggest an impact of sex on the renal clearance of drugs eliminated by the kidney. For example, the renal clearances of methotrexate and amantadine show distinct differences between sexes, with men having greater renal clearances (73; 74). A systematic study of sex differences in renal clearances and net secretory clearances needs to be conducted for model compounds. If substantial differences are observed, mechanistic studies focused on the expression levels of transporters in the kidney should be performed. These studies are essential to understanding the effect of sex on renal clearance. Indeed, regulatory authorities and the National Institutes of Health have released several publications highlighting the importance of understanding sex differences in pharmacokinetics (75-77).

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 (78-80). 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 (81). In contrast morphine has a higher renal

clearance in Chinese individuals than it does in Caucasian individuals (82). 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 (83). Bioinformatic data analysis based on expressed sequence tags (ESTs) supports this finding, indicating that 35-60% of human gene products are alternatively spliced (84-87). Furthermore, mechanistic studies have demonstrated the importance of alternative splicing on protein localization, regulation and function (88). Renal transporters are no exception and various splicing variants have been described (89).

The most prominent example of splicing variants of renal secretory transporters is MATE2K, a splice variant of MATE2. In comparison to MATE2, MATE2K lacks one exon and is expressed exclusively in the kidney and at a greater abundance (90). *In vitro* experiments demonstrate similar transport activity between MATE2 and MATE2K, suggesting that both MATE2 and MATE2K are involved in the renal elimination of

organic cations (90). Research thus far has largely focused 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 (91; 92). 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 (93). Furthermore, splicing variants of OCTN2 with reduced activity have been identified (94; 95). A splice variant of OCT2 has also been observed (OCT2-A) and consists of only 9 transmembrane domains instead of 12 (96). Nonetheless, data suggest that this splice variant is functional and 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 (96). 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. Investigation 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 intra-individual 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, sex 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.

SUMMARY OF DISSERTATION CHAPTERS

The studies in this dissertation aim to enhance our knowledge of the clinical impact of renal transporters for basic drugs. A brief description of each of the chapters in this dissertation is presented below.

Chapter 2. The UCSF-FDA TransPortal: A Public Drug Transporter Database

In this chapter, the development of the UCSF-FDA TransPortal is described. This database, which was developed in collaboration with the Food and Drug Administration's Office of Clinical Pharmacology, highlights 31 transporters from the ABC and SLC superfamilies. This database summarizes information from ~300 primary literature sources and drug labels on drug transporter expression, tissue distribution, direction of transport, substrate and inhibitor *in vitro* kinetics and clinical drug-drug interactions.

Chapter 3. Identification of Selective and Potent Inhibitors of Renal Organic Cation Transport

To enhance our understanding of transport mechanisms that contribute to renal clearance and drug-drug interactions (DDIs), it is important to discover model inhibitors of renal organic cation transporters that are selective and have a clinical impact on the renal clearance of concomitantly dosed organic cation xenobiotics. In this chapter, a strategic screen was performed to identify clinically significant and selective inhibitors of renal organic cation transport. From this screen, ondansetron, moxifloxacin and norfloxacin were identified as selective inhibitors of the apical kidney transporters, MATE1 and MATE2K, with the potential to inhibit transport at clinically relevant concentrations. Nizatidine was found to be a clinically potent and selective inhibitor of MATE2K that may be useful to understand the clinical significance of MATE2K-mediated drug elimination. This study explores mechanisms of transporter mediated DDIs including allosteric effects.

Chapter 4. The Effect of Nizatidine, a MATE2K Selective Inhibitor, on the Pharmacokinetics and Pharmacodynamics of Metformin in Healthy Volunteers

In this chapter, a healthy volunteer open-label, randomized, two-phase crossover DDI study was conducted to determine the effect of MATE2K-selective inhibition by nizatidine on the exposure and response of metformin. When co-administered, nizatidine increases V/F and $t_{1/2}$ of metformin in healthy volunteers. However, there was no significant difference in metformin's renal clearance (CL_R) or net secretory clearance (CL_{SR}) between treatment arms. In terms of alterations in metformin response, in healthy volunteers, the hypoglycemic activity is enhanced above pre-metformin values only when nizatidine is co-administrated. This study is consistent with the idea that inhibitors of renal efflux transporters may affect the volume of distribution, tissue levels and peripheral effects of drugs. In addition, this study challenges current guidelines that rely on *in vitro* predictions to inform the decision to conduct transporter-mediated clinical DDI studies.

Chapter 5. The Effect of Novel Promoter Variants in MATE1 and MATE2 on the Pharmacokinetics and Pharmacodynamics of Metformin

Clinical studies focused on the effects of transporter promoter variants on drug disposition and response are limited. In this study, we sought to investigate whether common MATE1 and MATE2/2K promoter variants have an effect on the disposition and response of metformin in healthy and type II diabetic subjects. Carriers of MATE1 g.-66T>C, a promoter variant that decreases MATE1 expression, exhibit no difference in

pharmacokinetics, but have an increased effect of metformin. In contrast, carriers of MATE2/2K g.-130G>A have a greater renal clearance of metformin and poorer hypoglycemic response. These studies suggest that MATE2/2K but not MATE1 is important in renal clearance of metformin whereas both MATE1 and MATE2/2K are important determinants of response to the drug. Because of the primary localization of MATE2/2K to the kidney, these studies suggest that the kidney may be an important site for the glycemic action of metformin.

Chapter 6. Development and Characterization of a Humanized MATE2K Mouse

Rodents do not have an ortholog of human MATE2K and it is impossible to directly measure alterations in kidney levels of drugs in human subjects. The development of a humanized MATE2K could help to identify the role of MATE2K in renal elimination. In this chapter, we describe an unsuccessful attempt to develop a humanized MATE2K mouse.

Chapter 7. Conclusions and Perspectives

In this chapter, we summarize the results of the studies in this dissertation, highlighting major findings. In addition, future studies necessary to further understand the role of the organic cation transporters at the apical membrane are discussed.

REFERENCES

1.   Povey S, Lovering R, Bruford E, Wright M, Lush M, Wain H. 2001. The HUGO Gene Nomenclature Committee (HGNC). *Hum Genet* 109:678-80

2. Schlessinger A, Matsson P, Shima JE, Pieper U, Yee SW, et al. 2010. Comparison of human solute carriers. *Protein Sci* 19:412-28

3. *Top 200 drugs in the US market by dispensed prescriptions*. <http://www.pharmacytimes.com/media/pdf/26babf64401fed80734dbf8dce471e26.pdf> Accessed May 2013

4. *Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm> Accessed May 2013

5. Rowland M, Tozer TN. 2010. *Clinical pharmacokinetics and pharmacodynamics: concepts and applications*. Wolters Kluwer Health/Lippincott William & Wilkins: Philadelphia

6. Hsyu PH, Gisclon LG, Hui AC, Giacomini KM. 1988. Interactions of organic anions with the organic cation transporter in renal BBMV. *Am J Physiol* 254:F56-61

7. Takeda M, Khamdang S, Narikawa S, Kimura H, Kobayashi Y, et al. 2002. Human organic anion transporters and human organic cation transporters mediate renal antiviral transport. *J Pharmacol Exp Ther* 300:918-24

8. Ahn SY, Eraly SA, Tsigelny I, Nigam SK. 2009. Interaction of organic cations with organic anion transporters. *J Biol Chem* 284:31422-30

9. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, et al. 2010. Membrane transporters in drug development. *Nat Rev Drug Discov* 9:215-36

10. Goodman LS, Brunton LL, Chabner B, Knollmann BC. 2011. *Goodman & Gilman's pharmacological basis of therapeutics*. New York: McGraw-Hill

11. Moffat AC. 2004. *Clarke's analysis of drugs and poisons : in pharmaceuticals, body fluids and postmortem material*. London ; Chicago: Pharmaceutical Press

12. Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. 1987. Reduction of metformin renal tubular secretion by cimetidine in man. *Br J Clin Pharmacol* 23:545-51

13. Gibaldi M, Schwartz MA. 1968. Apparent effect of probenecid on the distribution of penicillins in man. *Clin Pharmacol Ther* 9:345-9

14. *Highlights of Prescribing Information: Vistide (Cidofovir)*. <http://www.accessdata.fda.gov/drugsatfda_docs/label/1999/020638s003lbl.pdf> Accessed May 2013

15. Cundy KC, Petty BG, Flaherty J, Fisher PE, Polis MA, et al. 1995. Clinical pharmacokinetics of cidofovir in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 39:1247-52

16. Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, Sweet DH. 1999. The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol Pharmacol* 56:570-80

17. Tsuda M, Terada T, Ueba M, Sato T, Masuda S, et al. 2009. Involvement of human multidrug and toxin extrusion 1 in the drug interaction between cimetidine and metformin in renal epithelial cells. *J Pharmacol Exp Ther* 329:185-91

18.   Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, et al. 2007. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *The Journal of clinical investigation* 117:1422-31

19.   Leabman MK, Giacomini KM. 2003. Estimating the contribution of genes and environment to variation in renal drug clearance. *Pharmacogenetics* 13:581-4

20.   Yin OQ, Tomlinson B, Chow MS. 2006. Variability in renal clearance of substrates for renal transporters in chinese subjects. *J Clin Pharmacol* 46:157-63

21.   Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, et al. 2011. Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *J Neurochem* 117:333-45

22.   Shawahna R, Uchida Y, Decleves X, Ohtsuki S, Yousif S, et al. 2011. Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. *Mol Pharm* 8:1332-41

23.   Ohtsuki S, Schaefer O, Kawakami H, Inoue T, Liehner S, et al. 2012. Simultaneous absolute protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases as a novel approach for the characterization of individual human liver: comparison with mRNA levels and activities. *Drug Metab Dispos* 40:83-92

24.   Morrissey KM, Wen CC, Johns SJ, Zhang L, Huang SM, Giacomini KM. 2012. The UCSF-FDA TransPortal: a public drug transporter database. *Clin Pharmacol Ther* 92:545-6

25.   Choi JH, Yee SW, Ramirez AH, Morrissey KM, Jang GH, et al. 2011. A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. *Clin Pharmacol Ther* 90:674-84

26.   Urban TJ, Brown C, Castro RA, Shah N, Mercer R, et al. 2008. Effects of genetic variation in the novel organic cation transporter, OCTN1, on the renal clearance of gabapentin. *Clin Pharmacol Ther* 83:416-21

27.   Chen Y, Li S, Brown C, Cheatham S, Castro RA, et al. 2009. Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. *Pharmacogenet Genomics* 19:497-504

28.   Urban TJ. 2010. Race, ethnicity, ancestry, and pharmacogenetics. *Mt Sinai J Med* 77:133-9

29.   Yee SW, Chen L, Giacomini KM. 2010. Pharmacogenomics of membrane transporters: past, present and future. *Pharmacogenomics* 11:475-9

30.   DeGorter MK, Xia CQ, Yang JJ, Kim RB. 2012. Drug transporters in drug efficacy and toxicity. *Annu Rev Pharmacol Toxicol* 52:249-73

31.   Sun H, Frassetto L, Benet LZ. 2006. Effects of renal failure on drug transport and metabolism. *Pharmacol Ther* 109:1-11

32.   Naud J, Nolin TD, Leblond FA, Pichette V. 2012. Current understanding of drug disposition in kidney disease. *J Clin Pharmacol* 52:10S-22S

33.   Zhang L, Xu N, Xiao S, Arya V, Zhao P, et al. 2012. Regulatory perspectives on designing pharmacokinetic studies and optimizing labeling recommendations for patients with chronic kidney disease. *J Clin Pharmacol* 52:79S-90S

34. Musso CG, Michelangelo H, Vilas M, Reynaldi J, Martinez B, et al. 2009. Creatinine reabsorption by the aged kidney. *Int Urol Nephrol* 41:727-31

35. Coulthard MG, Hey EN, Ruddock V. 1985. Creatinine and urea clearances compared to inulin clearance in preterm and mature babies. *Early Hum Dev* 11:11-9

36. Gordjani N, Burghard R, Leititis JU, Brandis M. 1988. Serum creatinine and creatinine clearance in healthy neonates and prematures during the first 10 days of life. *Eur J Pediatr* 148:143-5

37. Sonntag J, Prankel B, Waltz S. 1996. Serum creatinine concentration, urinary creatinine excretion and creatinine clearance during the first 9 weeks in preterm infants with a birth weight below 1500 g. *Eur J Pediatr* 155:815-9

38. Aperia A, Broberger O, Elinder G, Herin P, Zetterstrom R. 1981. Postnatal development of renal function in pre-term and full-term infants. *Acta Paediatr Scand* 70:183-7

39. Mannan MA, Shahidulla M, Salam F, Alam MS, Hossain MA, Hossain M. 2012. Postnatal development of renal function in preterm and term neonates. *Mymensingh Med J* 21:103-8

40. Kearns GL. 2000. Impact of developmental pharmacology on pediatric study design: overcoming the challenges. *J Allergy Clin Immunol* 106:S128-38

41. Lichtman SM, Skirvin JA, Vemulapalli S. 2003. Pharmacology of antineoplastic agents in older cancer patients. *Crit Rev Oncol Hematol* 46:101-14

42. Anderson GD, Lynn AM. 2009. Optimizing pediatric dosing: a developmental pharmacologic approach. *Pharmacotherapy* 29:680-90

43. Trifiro G, Spina E. 2011. Age-related changes in pharmacodynamics: focus on drugs acting on central nervous and cardiovascular systems. *Curr Drug Metab* 12:611-20

44. Guignard JP, Torrado A, Da Cunha O, Gautier E. 1975. Glomerular filtration rate in the first three weeks of life. *J Pediatr* 87:268-72

45. Yaffe SJ. 1976. Developmental factors influencing interactions of drugs. *Ann N Y Acad Sci* 281:90-7

46. Hook JB, Hewitt WR. 1977. Development of mechanisms for drug excretion. *Am J Med* 62:497-506

47. Litovitz TL, Flagler SL, Manoguerra AS, Veltri JC, Wright L. 1989. Recurrent poisonings among paediatric poisoning victims. *Med Toxicol Adverse Drug Exp* 4:381-6

48. Chen N, Aleksa K, Woodland C, Rieder M, Koren G. 2006. Ontogeny of drug elimination by the human kidney. *Pediatr Nephrol* 21:160-8

49. Inui KI, Masuda S, Saito H. 2000. Cellular and molecular aspects of drug transport in the kidney. *Kidney Int* 58:944-58

50. van Kalken CK, Giaccone G, van der Valk P, Kuiper CM, Hadisaputro MM, et al. 1992. Multidrug resistance gene (P-glycoprotein) expression in the human fetus. *Am J Pathol* 141:1063-72

51. Tune BM. 1975. Relationship between the transport and toxicity of cephalosporins in the kidney. *J Infect Dis* 132:189-94

52. Jacobs RF, Darville T, Parks JA, Enderlin G. 1992. Safety profile and efficacy of cefotaxime for the treatment of hospitalized children. *Clin Infect Dis* 14:56-65

53. Lindheimer MD, Davison JM, Katz AI. 2001. The kidney and hypertension in pregnancy: twenty exciting years. *Semin Nephrol* 21:173-89

54. Jeyabalan A, Conrad KP. 2007. Renal function during normal pregnancy and preeclampsia. *Front Biosci* 12:2425-37

55. Koetje PM, Spaan JJ, Kooman JP, Spaanderman ME, Peeters LL. 2011. Pregnancy reduces the accuracy of the estimated glomerular filtration rate based on Cockroft-Gault and MDRD formulas. *Reprod Sci* 18:456-62

56. Quadri KH, Bernardini J, Greenberg A, Laifer S, Syed A, Holley JL. 1994. Assessment of renal function during pregnancy using a random urine protein to creatinine ratio and Cockcroft-Gault formula. *Am J Kidney Dis* 24:416-20

57. Alper AB, Yi Y, Rahman M, Webber LS, Magee L, et al. 2011. Performance of estimated glomerular filtration rate prediction equations in preeclamptic patients. *Am J Perinatol* 28:425-30

58. Alper AB, Yi Y, Webber LS, Pridjian G, Mumuney AA, et al. 2007. Estimation of glomerular filtration rate in preeclamptic patients. *Am J Perinatol* 24:569-74

59. Schou M, Amdisen A, Steenstrup OR. 1973. Lithium and pregnancy. II. Hazards to women given lithium during pregnancy and delivery. *Br Med J* 2:137-8

60. Andrew MA, Easterling TR, Carr DB, Shen D, Buchanan ML, et al. 2007. Amoxicillin pharmacokinetics in pregnant women: modeling and simulations of dosage strategies. *Clin Pharmacol Ther* 81:547-56

61. Assael BM, Como ML, Miraglia M, Pardi G, Sereni F. 1979. Ampicillin kinetics in pregnancy. *Br J Clin Pharmacol* 8:286-8

62. Heikkila A, Erkkola R. 1991. Pharmacokinetics of piperacillin during pregnancy. *J Antimicrob Chemother* 28:419-23

63. Wang H, Wu X, Hudkins K, Mikheev A, Zhang H, et al. 2006. Expression of the breast cancer resistance protein (Bcrp1/Abcg2) in tissues from pregnant mice: effects of pregnancy and correlations with nuclear receptors. *Am J Physiol Endocrinol Metab* 291:E1295-304

64. Zhang H, Wu X, Wang H, Mikheev AM, Mao Q, Unadkat JD. 2008. Effect of pregnancy on cytochrome P450 3a and P-glycoprotein expression and activity in the mouse: mechanisms, tissue specificity, and time course. *Mol Pharmacol* 74:714-23

65. Eyal S, Easterling TR, Carr D, Umans JG, Miodovnik M, et al. 2010. Pharmacokinetics of metformin during pregnancy. *Drug Metab Dispos* 38:833-40

66. Hebert MF, Easterling TR, Kirby B, Carr DB, Buchanan ML, et al. 2008. Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study. *Clin Pharmacol Ther* 84:248-53

67. Anderson GD. 2005. Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clin Pharmacokinet* 44:989-1008

68. Fesler P, Mimran A. 2011. Estimation of glomerular filtration rate: what are the pitfalls? *Curr Hypertens Rep* 13:116-21

69. Horio M, Imai E, Yasuda Y, Watanabe T, Matsuo S. 2011. Performance of serum cystatin C versus serum creatinine as a marker of glomerular filtration rate as measured by inulin renal clearance. *Clin Exp Nephrol* 15:868-76

70. Rahn KH, Heidenreich S, Bruckner D. 1999. How to assess glomerular function and damage in humans. *J Hypertens* 17:309-17

71. Schuetz EG, Furuya KN, Schuetz JD. 1995. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J Pharmacol Exp Ther* 275:1011-8

72. Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, et al. 2003. Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* 38:978-88

73. Wong LT, Sitar DS, Aoki FY. 1995. Chronic tobacco smoking and gender as variables affecting amantadine disposition in healthy subjects. *Br J Clin Pharmacol* 39:81-4

74. Godfrey C, Sweeney K, Miller K, Hamilton R, Kremer J. 1998. The population pharmacokinetics of long-term methotrexate in rheumatoid arthritis. *Br J Clin Pharmacol* 46:369-76

75. Mattison D, Zajicek A. 2006. Gaps in knowledge in treating pregnant women. *Gend Med* 3:169-82

76. Uhl K, Parekh A, Kweder S. 2007. Females in clinical studies: where are we going? *Clin Pharmacol Ther* 81:600-2

77. Yang Y, Carlin AS, Faustino PJ, Motta MI, Hamad ML, et al. 2009. Participation of women in clinical trials for new drugs approved by the Food and Drug Administration in 2000-2002. *J Womens Health (Larchmt)* 18:303-10

78. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, et al. 2006. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol* 17:2937-44

79. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, et al. 2007. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem* 53:766-72

80. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, et al. 2009. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 53:982-92

81. Hu OY, Ding PY, Huang CS, Hwang GM, Chu KM. 1997. Pharmacokinetics of fosinoprilat in Chinese and whites after intravenous administration. *J Clin Pharmacol* 37:834-40

82. Zhou HH, Sheller JR, Nu H, Wood M, Wood AJ. 1993. Ethnic differences in response to morphine. *Clin Pharmacol Ther* 54:507-13

83. Johnson JM, Castle J, Garrett-Engele P, Kan Z, Loerch PM, et al. 2003. Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science* 302:2141-4

84. Mironov AA, Fickett JW, Gelfand MS. 1999. Frequent alternative splicing of human genes. *Genome Res* 9:1288-93

85. Brett D, Hanke J, Lehmann G, Haase S, Delbruck S, et al. 2000. EST comparison indicates 38% of human mRNAs contain possible alternative splice forms. *FEBS Lett* 474:83-6

86. Kan Z, Rouchka EC, Gish WR, States DJ. 2001. Gene structure prediction and alternative splicing analysis using genomically aligned ESTs. *Genome Res* 11:889-900

87. Modrek B, Resch A, Grasso C, Lee C. 2001. Genome-wide detection of alternative splicing in expressed sequences of human genes. *Nucleic Acids Res* 29:2850-9

88. Stamm S, Ben-Ari S, Rafalska I, Tang Y, Zhang Z, et al. 2005. Function of alternative splicing. *Gene* 344:1-20

89. Gamba G. 2001. Alternative splicing and diversity of renal transporters. *Am J Physiol Renal Physiol* 281:F781-94

90. Masuda S, Terada T, Yonezawa A, Tanihara Y, Kishimoto K, et al. 2006. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol* 17:2127-35

91. Hosoyamada M, Sekine T, Kanai Y, Endou H. 1999. Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am J Physiol* 276:F122-8

92. Bahn A, Prawitt D, Buttler D, Reid G, Enklaar T, et al. 2000. Genomic structure and in vivo expression of the human organic anion transporter 1 (hOAT1) gene. *Biochem Biophys Res Commun* 275:623-30

93. Robertson EE, Rankin GO. 2006. Human renal organic anion transporters: characteristics and contributions to drug and drug metabolite excretion. *Pharmacol Ther* 109:399-412

94. Lamhonwah AM, Tein I. 1998. Carnitine uptake defect: frameshift mutations in the human plasmalemmal carnitine transporter gene. *Biochem Biophys Res Commun* 252:396-401

95.   Wang Y, Ye J, Ganapathy V, Longo N. 1999. Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. *Proc Natl Acad Sci U S A* 96:2356-60

96.   Urakami Y, Akazawa M, Saito H, Okuda M, Inui K. 2002. cDNA cloning, functional characterization, and tissue distribution of an alternatively spliced variant of organic cation transporter hOCT2 predominantly expressed in the human kidney. *J Am Soc Nephrol* 13:1703-10

SUPPLEMENTARY REFERENCES

1.   Busch AE, Karbach U, Miska D, Gorboulev V, Akhoundova A, et al. 1998. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol Pharmacol* 54:342-52

2.   Luo S, Kansara VS, Zhu X, Mandava NK, Pal D, Mitra AK. 2006. Functional characterization of sodium-dependent multivitamin transporter in MDCK-MDR1 cells and its utilization as a target for drug delivery. *Mol Pharm* 3:329-39

3.   Biermann J, Lang D, Gorboulev V, Koepsell H, Sindic A, et al. 2006. Characterization of regulatory mechanisms and states of human organic cation transporter 2. *Am J Physiol Cell Physiol* 290:C1521-31

4.   Troutman MD, Thakker DR. 2003. Efflux ratio cannot assess P-glycoprotein-mediated attenuation of absorptive transport: asymmetric effect of P-glycoprotein on absorptive and secretory transport across Caco-2 cell monolayers. *Pharm Res* 20:1200-9

5. Kimura Y, Kioka N, Kato H, Matsuo M, Ueda K. 2007. Modulation of drug-stimulated ATPase activity of human MDR1/P-glycoprotein by cholesterol. *Biochem J* 401:597-605

6. Tahara H, Kusuhara H, Endou H, Koepsell H, Imaoka T, et al. 2005. A species difference in the transport activities of H2 receptor antagonists by rat and human renal organic anion and cation transporters. *J Pharmacol Exp Ther* 315:337-45

7. Collett A, Tanianis-Hughes J, Hallifax D, Warhurst G. 2004. Predicting P-glycoprotein effects on oral absorption: correlation of transport in Caco-2 with drug pharmacokinetics in wild-type and mdr1a(-/-) mice in vivo. *Pharm Res* 21:819-26

8. Amphoux A, Vialou V, Drescher E, Bruss M, Mannoury La Cour C, et al. 2006. Differential pharmacological in vitro properties of organic cation transporters and regional distribution in rat brain. *Neuropharmacology* 50:941-52

9. Guo A, Marinaro W, Hu P, Sinko PJ. 2002. Delineating the contribution of secretory transporters in the efflux of etoposide using Madin-Darby canine kidney (MDCK) cells overexpressing P-glycoprotein (Pgp), multidrug resistance-associated protein (MRP1), and canalicular multispecific organic anion transporter (cMOAT). *Drug Metab Dispos* 30:457-63

10. Petri N, Tannergren C, Rungstad D, Lennernas H. 2004. Transport characteristics of fexofenadine in the Caco-2 cell model. *Pharm Res* 21:1398-404

11. Lee CG, Gottesman MM, Cardarelli CO, Ramachandra M, Jeang KT, et al. 1998. HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry* 37:3594-601

12. Luo FR, Paranjpe PV, Guo A, Rubin E, Sinko P. 2002. Intestinal transport of irinotecan in Caco-2 cells and MDCK II cells overexpressing efflux transporters Pgp, cMOAT, and MRP1. *Drug Metab Dispos* 30:763-70

13. Choi MK, Song IS. 2012. Genetic variants of organic cation transporter 1 (OCT1) and OCT2 significantly reduce lamivudine uptake. *Biopharm Drug Dispos* 33:170-8

14. Feng B, Mills JB, Davidson RE, Mireles RJ, Janiszewski JS, et al. 2008. In vitro P-glycoprotein assays to predict the in vivo interactions of P-glycoprotein with drugs in the central nervous system. *Drug Metab Dispos* 36:268-75

15. Meyer zu Schwabedissen HE, Verstuyft C, Kroemer HK, Becquemont L, Kim RB. 2010. Human multidrug and toxin extrusion 1 (MATE1/SLC47A1) transporter: functional characterization, interaction with OCT2 (SLC22A2), and single nucleotide polymorphisms. *Am J Physiol Renal Physiol* 298:F997-1005

16. Kimura N, Masuda S, Tanihara Y, Ueo H, Okuda M, et al. 2005. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. *Drug Metab Pharmacokinet* 20:379-86

17. Choi MK, Jin QR, Jin HE, Shim CK, Cho DY, et al. 2007. Effects of tetraalkylammonium compounds with different affinities for organic cation transporters on the pharmacokinetics of metformin. *Biopharm Drug Dispos* 28:501-10

18. Zolk O, Solbach TF, Konig J, Fromm MF. 2009. Functional characterization of the human organic cation transporter 2 variant p.270Ala>Ser. *Drug Metab Dispos* 37:1312-8

19. Zolk O, Solbach TF, Konig J, Fromm MF. 2009. Structural determinants of inhibitor interaction with the human organic cation transporter OCT2 (SLC22A2). *Naunyn Schmiedebergs Arch Pharmacol* 379:337-48

20. Gorboulev V, Ulzheimer JC, Akhoundova A, Ulzheimer-Teuber I, Karbach U, et al. 1997. Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol* 16:871-81

21. Woodahl EL, Yang Z, Bui T, Shen DD, Ho RJ. 2005. MDR1 G1199A polymorphism alters permeability of HIV protease inhibitors across P-glycoprotein-expressing epithelial cells. *AIDS* 19:1617-25

22. Kimura H, Takeda M, Narikawa S, Enomoto A, Ichida K, Endou H. 2002. Human organic anion transporters and human organic cation transporters mediate renal transport of prostaglandins. *J Pharmacol Exp Ther* 301:293-8

23. Li H, Jin HE, Kim W, Han YH, Kim DD, et al. 2008. Involvement of P-glycoprotein, multidrug resistance protein 2 and breast cancer resistance protein in the transport of belotecan and topotecan in Caco-2 and MDCKII cells. *Pharm Res* 25:2601-12

24. Okuda M, Urakami Y, Saito H, Inui K. 1999. Molecular mechanisms of organic cation transport in OCT2-expressing Xenopus oocytes. *Biochim Biophys Acta* 1417:224-31

25. Hunter J, Jepson MA, Tsuruo T, Simmons NL, Hirst BH. 1993. Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 cells. Kinetics of vinblastine secretion and interaction with modulators. *J Biol Chem* 268:14991-7

26. Tang F, Horie K, Borchardt RT. 2002. Are MDCK cells transfected with the human MDR1 gene a good model of the human intestinal mucosa? *Pharm Res* 19:765-72

27. Jutabha P, Wempe MF, Anzai N, Otomo J, Kadota T, Endou H. 2010. Xenopus laevis oocytes expressing human P-glycoprotein: probing trans- and cis-inhibitory effects on [3H]vinblastine and [3H]digoxin efflux. *Pharmacol Res* 61:76-84

28. Feng B, Obach RS, Burstein AH, Clark DJ, de Morais SM, Faessel HM. 2008. Effect of human renal cationic transporter inhibition on the pharmacokinetics of varenicline, a new therapy for smoking cessation: an in vitro-in vivo study. *Clin Pharmacol Ther* 83:567-76

29. Minematsu T, Iwai M, Umehara K, Usui T, Kamimura H. 2010. Characterization of human organic cation transporter 1 (OCT1/SLC22A1)- and OCT2 (SLC22A2)- mediated transport of 1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)- 4,9-dihydro-1H-naphtho[2,3-d]imidazolium bromide (YM155 monobromide), a novel small molecule survivin suppressant. *Drug Metab Dispos* 38:1-4

30. Yamada A, Maeda K, Kamiyama E, Sugiyama D, Kondo T, et al. 2007. Multiple human isoforms of drug transporters contribute to the hepatic and renal transport of olmesartan, a selective antagonist of the angiotensin II AT1-receptor. *Drug Metab Dispos* 35:2166-76

31. Cihlar T, Ho ES. 2000. Fluorescence-based assay for the interaction of small molecules with the human renal organic anion transporter 1. *Anal Biochem* 283:49-55

32. Cui Y, Konig J, Buchholz JK, Spring H, Leier I, Keppler D. 1999. Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. *Mol Pharmacol* 55:929-37

33. Takeda M, Khamdang S, Narikawa S, Kimura H, Kobayashi Y, et al. 2002. Human organic anion transporters and human organic cation transporters mediate renal antiviral transport. *J Pharmacol Exp Ther* 300:918-24

34. Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, Sweet DH. 1999. The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol Pharmacol* 56:570-80

35. Ho ES, Lin DC, Mendel DB, Cihlar T. 2000. Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. *J Am Soc Nephrol* 11:383-93

36. Chu XY, Kato Y, Ueda K, Suzuki H, Niinuma K, et al. 1998. Biliary excretion mechanism of CPT-11 and its metabolites in humans: involvement of primary active transporters. *Cancer Res* 58:5137-43

37. Chu XY, Bleasby K, Yabut J, Cai X, Chan GH, et al. 2007. Transport of the dipeptidyl peptidase-4 inhibitor sitagliptin by human organic anion transporter 3, organic anion transporting polypeptide 4C1, and multidrug resistance P-glycoprotein. *J Pharmacol Exp Ther* 321:673-83

38. El-Sheikh AA, van den Heuvel JJ, Koenderink JB, Russel FG. 2007. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP)

2/ABCC2- and MRP4/ABCC4-mediated methotrexate transport. *J Pharmacol Exp Ther* 320:229-35

39. Cutler MJ, Urquhart BL, Velenosi TJ, Meyer Zu Schwabedissen HE, Dresser GK, et al. 2012. In vitro and in vivo assessment of renal drug transporters in the disposition of mesna and dimesna. *J Clin Pharmacol* 52:530-42

40. Mizuno N, Takahashi T, Iwase Y, Kusuhara H, Niwa T, Sugiyama Y. 2007. Human organic anion transporters 1 (hOAT1/SLC22A6) and 3 (hOAT3/SLC22A8) transport edaravone (MCI-186; 3-methyl-1-phenyl-2-pyrazolin-5-one) and its sulfate conjugate. *Drug Metab Dispos* 35:1429-34

41. Leier I, Hummel-Eisenbeiss J, Cui Y, Keppler D. 2000. ATP-dependent para-aminohippurate transport by apical multidrug resistance protein MRP2. *Kidney Int* 57:1636-42

42. Smeets PH, van Aubel RA, Wouterse AC, van den Heuvel JJ, Russel FG. 2004. Contribution of multidrug resistance protein 2 (MRP2/ABCC2) to the renal excretion of p-aminohippurate (PAH) and identification of MRP4 (ABCC4) as a novel PAH transporter. *J Am Soc Nephrol* 15:2828-35

43. Takeda M, Khamdang S, Narikawa S, Kimura H, Hosoyamada M, et al. 2002. Characterization of methotrexate transport and its drug interactions with human organic anion transporters. *J Pharmacol Exp Ther* 302:666-71

44. Uwai Y, Taniguchi R, Motohashi H, Saito H, Okuda M, Inui K. 2004. Methotrexate-loxoprofen interaction: involvement of human organic anion transporters hOAT1 and hOAT3. *Drug Metab Pharmacokinet* 19:369-74

45. Yamashiro W, Maeda K, Hirouchi M, Adachi Y, Hu Z, Sugiyama Y. 2006. Involvement of transporters in the hepatic uptake and biliary excretion of valsartan, a selective antagonist of the angiotensin II AT1-receptor, in humans. *Drug Metab Dispos* 34:1247-54

46. Jung KY, Takeda M, Kim DK, Tojo A, Narikawa S, et al. 2001. Characterization of ochratoxin A transport by human organic anion transporters. *Life Sci* 69:2123-35

47. Tang F, Horie K, Borchardt RT. 2002. Are MDCK cells transfected with the human MRP2 gene a good model of the human intestinal mucosa? *Pharm Res* 19:773-9

48. Rius M, Hummel-Eisenbeiss J, Hofmann AF, Keppler D. 2006. Substrate specificity of human ABCC4 (MRP4)-mediated cotransport of bile acids and reduced glutathione. *Am J Physiol Gastrointest Liver Physiol* 290:G640-9

49. Takeda M, Narikawa S, Hosoyamada M, Cha SH, Sekine T, Endou H. 2001. Characterization of organic anion transport inhibitors using cells stably expressing human organic anion transporters. *Eur J Pharmacol* 419:113-20

50. Ueo H, Motohashi H, Katsura T, Inui K. 2005. Human organic anion transporter hOAT3 is a potent transporter of cephalosporin antibiotics, in comparison with hOAT1. *Biochem Pharmacol* 70:1104-13

51. Hosoyamada M, Sekine T, Kanai Y, Endou H. 1999. Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am J Physiol* 276:F122-8

52. Lu R, Chan BS, Schuster VL. 1999. Cloning of the human kidney PAH transporter: narrow substrate specificity and regulation by protein kinase C. *Am J Physiol* 276:F295-303

53. Islinger F, Gekle M, Wright SH. 2001. Interaction of 2,3-dimercapto-1-propane sulfonate with the human organic anion transporter hOAT1. *J Pharmacol Exp Ther* 299:741-7

54. Weaver YM, Ehresman DJ, Butenhoff JL, Hagenbuch B. 2010. Roles of rat renal organic anion transporters in transporting perfluorinated carboxylates with different chain lengths. *Toxicol Sci* 113:305-14

55. Chen ZS, Lee K, Kruh GD. 2001. Transport of cyclic nucleotides and estradiol 17-beta-D-glucuronide by multidrug resistance protein 4. Resistance to 6-mercaptopurine and 6-thioguanine. *J Biol Chem* 276:33747-54

56. Cihlar T, Ho ES, Lin DC, Mulato AS. 2001. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. *Nucleosides Nucleotides Nucleic Acids* 20:641-8

57. Zelcer N, Reid G, Wielinga P, Kuil A, van der Heijden I, et al. 2003. Steroid and bile acid conjugates are substrates of human multidrug-resistance protein (MRP) 4 (ATP-binding cassette C4). *Biochem J* 371:361-7

58. Sato M, Iwanaga T, Mamada H, Ogihara T, Yabuuchi H, et al. 2008. Involvement of uric acid transporters in alteration of serum uric acid level by angiotensin II receptor blockers. *Pharm Res* 25:639-46

59. Chen ZS, Lee K, Walther S, Raftogianis RB, Kuwano M, et al. 2002. Analysis of methotrexate and folate transport by multidrug resistance protein 4 (ABCC4): MRP4 is a component of the methotrexate efflux system. *Cancer Res* 62:3144-50

60. Astorga B, Wunz TM, Morales M, Wright SH, Pelis RM. 2011. Differences in the substrate binding regions of renal organic anion transporters 1 (OAT1) and 3 (OAT3). *Am J Physiol Renal Physiol* 301:F378-86

61. van Aubel RA, Smeets PH, Peters JG, Bindels RJ, Russel FG. 2002. The MRP4/ABCC4 gene encodes a novel apical organic anion transporter in human kidney proximal tubules: putative efflux pump for urinary cAMP and cGMP. *J Am Soc Nephrol* 13:595-603

62. Kaufhold M, Schulz K, Breljak D, Gupta S, Henjakovic M, et al. 2011. Differential interaction of dicarboxylates with human sodium-dicarboxylate cotransporter 3 and organic anion transporters 1 and 3. *Am J Physiol Renal Physiol* 301:F1026-34

63. Elsby R, Fox L, Stresser D, Layton M, Butters C, et al. 2011. In vitro risk assessment of AZD9056 perpetrating a transporter-mediated drug-drug interaction with methotrexate. *Eur J Pharm Sci* 43:41-9

64. Hasannejad H, Takeda M, Taki K, Shin HJ, Babu E, et al. 2004. Interactions of human organic anion transporters with diuretics. *J Pharmacol Exp Ther* 308:1021-9

65. Reid G, Wielinga P, Zelcer N, van der Heijden I, Kuil A, et al. 2003. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. *Proc Natl Acad Sci USA* 100:9244-9

66. Tahara H, Kusuhara H, Maeda K, Koepsell H, Fuse E, Sugiyama Y. 2006. Inhibition of oat3-mediated renal uptake as a mechanism for drug-drug interaction between fexofenadine and probenecid. *Drug Metab Dispos* 34:743-7

67. Cha SH, Sekine T, Fukushima JI, Kanai Y, Kobayashi Y, et al. 2001. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol Pharmacol* 59:1277-86

68. Asif AR, Steffgen J, Metten M, Grunewald RW, Muller GA, et al. 2005. Presence of organic anion transporters 3 (OAT3) and 4 (OAT4) in human adrenocortical cells. *Pflugers Arch* 450:88-95

69. Tian Q, Zhang J, Chan SY, Tan TM, Duan W, et al. 2006. Topotecan is a substrate for multidrug resistance associated protein 4. *Curr Drug Metab* 7:105-18

70. Suzuki M, Suzuki H, Sugimoto Y, Sugiyama Y. 2003. ABCG2 transports sulfated conjugates of steroids and xenobiotics. *J Biol Chem* 278:22644-9

71. Ozvegy C, Litman T, Szakacs G, Nagy Z, Bates S, et al. 2001. Functional characterization of the human multidrug transporter, ABCG2, expressed in insect cells. *Biochem Biophys Res Commun* 285:111-7

72. Duan P, You G. 2009. Novobiocin is a potent inhibitor for human organic anion transporters. *Drug Metab Dispos* 37:1203-10

73. Takeda M, Hosoyamada M, Cha SH, Sekine T, Endou H. 2000. Hydrogen peroxide downregulates human organic anion transporters in the basolateral membrane of the proximal tubule. *Life Sci* 68:679-87

74. Chen ZS, Robey RW, Belinsky MG, Shchaveleva I, Ren XQ, et al. 2003. Transport of methotrexate, methotrexate polyglutamates, and 17beta-estradiol 17-(beta-D-glucuronide) by ABCG2: effects of acquired mutations at R482 on methotrexate transport. *Cancer Res* 63:4048-54

75. Imai Y, Asada S, Tsukahara S, Ishikawa E, Tsuruo T, Sugimoto Y. 2003. Breast cancer resistance protein exports sulfated estrogens but not free estrogens. *Mol Pharmacol* 64:610-8

76. Tamura A, Watanabe M, Saito H, Nakagawa H, Kamachi T, et al. 2006. Functional validation of the genetic polymorphisms of human ATP-binding cassette (ABC) transporter ABCG2: identification of alleles that are defective in porphyrin transport. *Mol Pharmacol* 70:287-96

77. Volk EL, Schneider E. 2003. Wild-type breast cancer resistance protein (BCRP/ABCG2) is a methotrexate polyglutamate transporter. *Cancer Res* 63:5538-43

78. Tiwari AK, Sodani K, Wang SR, Kuang YH, Ashby CR, Jr., et al. 2009. Nilotinib (AMN107, Tasigna) reverses multidrug resistance by inhibiting the activity of the ABCB1/Pgp and ABCG2/BCRP/MXR transporters. *Biochem Pharmacol* 78:153-61

79. Hirano M, Maeda K, Matsushima S, Nozaki Y, Kusuhara H, Sugiyama Y. 2005. Involvement of BCRP (ABCG2) in the biliary excretion of pitavastatin. *Mol Pharmacol* 68:800-7

80. Kitamura S, Maeda K, Wang Y, Sugiyama Y. 2008. Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. *Drug Metab Dispos* 36:2014-23

81. Deng JW, Shon JH, Shin HJ, Park SJ, Yeo CW, et al. 2008. Effect of silymarin supplement on the pharmacokinetics of rosuvastatin. *Pharm Res* 25:1807-14

82. Nakatomi K, Yoshikawa M, Oka M, Ikegami Y, Hayasaka S, et al. 2001. Transport of 7-ethyl-10-hydroxycamptothecin (SN-38) by breast cancer resistance protein ABCG2 in human lung cancer cells. *Biochem Biophys Res Commun* 288:827-32

83. Jani M, Szabo P, Kis E, Molnar E, Glavinas H, Krajcsi P. 2009. Kinetic characterization of sulfasalazine transport by human ATP-binding cassette G2. *Biol Pharm Bull* 32:497-9

84. Fujino H, Saito T, Ogawa S, Kojima J. 2005. Transporter-mediated influx and efflux mechanisms of pitavastatin, a new inhibitor of HMG-CoA reductase. *J Pharm Pharmacol* 57:1305-11

85. Lai Y, Sampson KE, Balogh LM, Brayman TG, Cox SR, et al. 2010. Preclinical and clinical evidence for the collaborative transport and renal secretion of an oxazolidinone antibiotic by organic anion transporter 3 (OAT3/SLC22A8) and multidrug and toxin extrusion protein 1 (MATE1/SLC47A1). *J Pharmacol Exp Ther* 334:936-44

86. Grundemann D, Harlfinger S, Golz S, Geerts A, Lazar A, et al. 2005. Discovery of the ergothioneine transporter. *Proc Natl Acad Sci U S A* 102:5256-61

87. Nakagomi-Hagihara R, Nakai D, Tokui T. 2007. Inhibition of human organic anion transporter 3 mediated pravastatin transport by gemfibrozil and the metabolites in humans. *Xenobiotica* 37:416-26

88. Nakamura T, Nakanishi T, Haruta T, Shirasaka Y, Keogh JP, Tamai I. 2010. Transport of ipratropium, an anti-chronic obstructive pulmonary disease drug, is mediated by organic cation/carnitine transporters in human bronchial epithelial cells: implications for carrier-mediated pulmonary absorption. *Mol Pharm* 7:187-95

89. Yabuuchi H, Tamai I, Nezu J, Sakamoto K, Oku A, et al. 1999. Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. *J Pharmacol Exp Ther* 289:768-73

90. Urban TJ, Yang C, Lagpacan LL, Brown C, Castro RA, et al. 2007. Functional effects of protein sequence polymorphisms in the organic cation/ergothioneine transporter OCTN1 (SLC22A4). *Pharmacogenet Genomics* 17:773-82

91. Ohashi R, Tamai I, Yabuuchi H, Nezu JI, Oku A, et al. 1999. Na(+)-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. *J Pharmacol Exp Ther* 291:778-84

92. Wagner CA, Lukewille U, Kaltenbach S, Moschen I, Broer A, et al. 2000. Functional and pharmacological characterization of human Na(+)-carnitine cotransporter hOCTN2. *Am J Physiol Renal Physiol* 279:F584-91

93. Windass AS, Lowes S, Wang Y, Brown CD. 2007. The contribution of organic anion transporters OAT1 and OAT3 to the renal uptake of rosuvastatin. *J Pharmacol Exp Ther* 322:1221-7

94. Ganapathy ME, Huang W, Rajan DP, Carter AL, Sugawara M, et al. 2000. beta-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. *J Biol Chem* 275:1699-707

95. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. 2007. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol* 74:359-71

96. Watanabe S, Tsuda M, Terada T, Katsura T, Inui K. 2010. Reduced renal clearance of a zwitterionic substrate cephalexin in MATE1-deficient mice. *J Pharmacol Exp Ther* 334:651-6

97. Babu E, Takeda M, Narikawa S, Kobayashi Y, Yamamoto T, et al. 2002. Human organic anion transporters mediate the transport of tetracycline. *Jpn J Pharmacol* 88:69-76

98. Mikkaichi T, Suzuki T, Onogawa T, Tanemoto M, Mizutamari H, et al. 2004. Isolation and characterization of a digoxin transporter and its rat homologue expressed in the kidney. *Proc Natl Acad Sci USA* 101:3569-74

99. Yamaguchi H, Sugie M, Okada M, Mikkaichi T, Toyohara T, et al. 2010. Transport of estrone 3-sulfate mediated by organic anion transporter OATP4C1: estrone 3-sulfate binds to the different recognition site for digoxin in OATP4C1. *Drug Metab Pharmacokinet* 25:314-7

100. Chen Y, Teranishi K, Li S, Yee SW, Hesselson S, et al. 2009. Genetic variants in multidrug and toxic compound extrusion-1, hMATE1, alter transport function. *Pharmacogenomics J* 9:127-36

101. Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. 2005. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci USA* 102:17923-8

102. Masuda S, Terada T, Yonezawa A, Tanihara Y, Kishimoto K, et al. 2006. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol* 17:2127-35

103. Kakumoto M, Takara K, Sakaeda T, Tanigawara Y, Kita T, Okumura K. 2002. MDR1-mediated interaction of digoxin with antiarrhythmic or antianginal drugs. *Biol Pharm Bull* 25:1604-7

104. Katoh M, Nakajima M, Yamazaki H, Yokoi T. 2001. Inhibitory effects of CYP3A4 substrates and their metabolites on P-glycoprotein-mediated transport. *Eur J Pharm Sci* 12:505-13

105. Keogh JP, Kunta JR. 2006. Development, validation and utility of an in vitro technique for assessment of potential clinical drug-drug interactions involving P-glycoprotein. *Eur J Pharm Sci* 27:543-54

106. Muller J, Lips KS, Metzner L, Neubert RH, Koepsell H, Brandsch M. 2005. Drug specificity and intestinal membrane localization of human organic cation transporters (OCT). *Biochem Pharmacol* 70:1851-60

107. Cheng Y, Martinez-Guerrero LJ, Wright SH, Kuester RK, Hooth MJ, Sipes IG. 2011. Characterization of the inhibitory effects of N-butylpyridinium chloride and structurally related ionic liquids on organic cation transporters 1/2 and human toxic extrusion transporters 1/2-k in vitro and in vivo. *Drug Metab Dispos* 39:1755-61

108. Eberl S, Renner B, Neubert A, Reisig M, Bachmakov I, et al. 2007. Role of p-glycoprotein inhibition for drug interactions: evidence from in vitro and pharmacoepidemiological studies. *Clin Pharmacokinet* 46:1039-49

109. Rautio J, Humphreys JE, Webster LO, Balakrishnan A, Keogh JP, et al. 2006. In vitro p-glycoprotein inhibition assays for assessment of clinical drug interaction potential of new drug candidates: a recommendation for probe substrates. *Drug Metab Dispos* 34:786-92

110. Wang E, Casciano CN, Clement RP, Johnson WW. 2001. The farnesyl protein transferase inhibitor SCH66336 is a potent inhibitor of MDR1 product P-glycoprotein. *Cancer Res* 61:7525-9

111. Noguchi K, Kawahara H, Kaji A, Katayama K, Mitsuhashi J, Sugimoto Y. 2009. Substrate-dependent bidirectional modulation of P-glycoprotein-mediated drug resistance by erlotinib. *Cancer Sci* 100:1701-7

112. Ekins S, Kim RB, Leake BF, Dantzig AH, Schuetz EG, et al. 2002. Three-dimensional quantitative structure-activity relationships of inhibitors of P-glycoprotein. *Mol Pharmacol* 61:964-73

113. Eriksson UG, Dorani H, Karlsson J, Fritsch H, Hoffmann KJ, et al. 2006. Influence of erythromycin on the pharmacokinetics of ximelagatran may involve inhibition of P-glycoprotein-mediated excretion. *Drug Metab Dispos* 34:775-82

114. Cook JA, Feng B, Fenner KS, Kempshall S, Liu R, et al. 2010. Refining the in vitro and in vivo critical parameters for P-glycoprotein, [I]/IC50 and [I2]/IC50, that allow for the exclusion of drug candidates from clinical digoxin interaction studies. *Mol Pharm* 7:398-411

115. Ciarimboli G, Lancaster CS, Schlatter E, Franke RM, Sprowl JA, et al. 2012. Proximal tubular secretion of creatinine by organic cation transporter OCT2 in cancer patients. *Clin Cancer Res* 18:1101-8

116. Choo EF, Leake B, Wandel C, Imamura H, Wood AJ, et al. 2000. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug Metab Dispos* 28:655-60

117. Imamura Y, Murayama N, Okudaira N, Kurihara A, Okazaki O, et al. 2011. Prediction of fluoroquinolone-induced elevation in serum creatinine levels: a case of drug-endogenous substance interaction involving the inhibition of renal secretion. *Clin Pharmacol Ther* 89:81-8

118. Shaik N, Giri N, Pan G, Elmquist WF. 2007. P-glycoprotein-mediated active efflux of the anti-HIV1 nucleoside abacavir limits cellular accumulation and brain distribution. *Drug Metab Dispos* 35:2076-85

119. Bourdet DL, Pritchard JB, Thakker DR. 2005. Differential substrate and inhibitory activities of ranitidine and famotidine toward human organic cation transporter 1 (hOCT1; SLC22A1), hOCT2 (SLC22A2), and hOCT3 (SLC22A3). *J Pharmacol Exp Ther* 315:1288-97

120. Wortelboer HM, Usta M, van der Velde AE, Boersma MG, Spenkelink B, et al. 2003. Interplay between MRP inhibition and metabolism of MRP inhibitors: the case of curcumin. *Chem Res Toxicol* 16:1642-51

121. Chen ZS, Kawabe T, Ono M, Aoki S, Sumizawa T, et al. 1999. Effect of multidrug resistance-reversing agents on transporting activity of human canalicular multispecific organic anion transporter. *Mol Pharmacol* 56:1219-28

122. Ming X, Ju W, Wu H, Tidwell RR, Hall JE, Thakker DR. 2009. Transport of dicationic drugs pentamidine and furamidine by human organic cation transporters. *Drug Metab Dispos* 37:424-30

123. Vadlapatla RK, Vadlapudi AD, Kwatra D, Pal D, Mitra AK. 2011. Differential effect of P-gp and MRP2 on cellular translocation of gemifloxacin. *Int J Pharm* 420:26-33

124. Yamazaki M, Li B, Louie SW, Pudvah NT, Stocco R, et al. 2005. Effects of fibrates on human organic anion-transporting polypeptide 1B1-, multidrug resistance protein 2- and P-glycoprotein-mediated transport. *Xenobiotica* 35:737-53

125. Reid G, Wielinga P, Zelcer N, De Haas M, Van Deemter L, et al. 2003. Characterization of the transport of nucleoside analog drugs by the human multidrug resistance proteins MRP4 and MRP5. *Mol Pharmacol* 63:1094-103

126. Umehara KI, Iwatsubo T, Noguchi K, Kamimura H. 2007. Comparison of the kinetic characteristics of inhibitory effects exerted by biguanides and H2-blockers on human and rat organic cation transporter-mediated transport: Insight into the development of drug candidates. *Xenobiotica* 37:618-34

127. Weiss J, Rose J, Storch CH, Ketabi-Kiyanvash N, Sauer A, et al. 2007. Modulation of human BCRP (ABCG2) activity by anti-HIV drugs. *J Antimicrob Chemother* 59:238-45

128. Khamdang S, Takeda M, Noshiro R, Narikawa S, Enomoto A, et al. 2002. Interactions of human organic anion transporters and human organic cation transporters with nonsteroidal anti-inflammatory drugs. *J Pharmacol Exp Ther* 303:534-9

129. Ahmed-Belkacem A, Pozza A, Munoz-Martinez F, Bates SE, Castanys S, et al. 2005. Flavonoid structure-activity studies identify 6-prenylchrysin and tectochrysin as potent and specific inhibitors of breast cancer resistance protein ABCG2. *Cancer Res* 65:4852-60

130. Mulato AS, Ho ES, Cihlar T. 2000. Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. *J Pharmacol Exp Ther* 295:10-5

131. Khamdang S, Takeda M, Babu E, Noshiro R, Onozato ML, et al. 2003. Interaction of human and rat organic anion transporter 2 with various cephalosporin antibiotics. *Eur J Pharmacol* 465:1-7

132. Tsuda M, Terada T, Ueba M, Sato T, Masuda S, et al. 2009. Involvement of human multidrug and toxin extrusion 1 in the drug interaction between cimetidine and metformin in renal epithelial cells. *J Pharmacol Exp Ther* 329:185-91

133. Takeda M, Noshiro R, Onozato ML, Tojo A, Hasannejad H, et al. 2004. Evidence for a role of human organic anion transporters in the muscular side effects of HMG-CoA reductase inhibitors. *Eur J Pharmacol* 483:133-8

134. Muller F, Konig J, Glaeser H, Schmidt I, Zolk O, et al. 2011. Molecular mechanism of renal tubular secretion of the antimalarial drug chloroquine. *Antimicrob Agents Chemother* 55:3091-8

135. Ito S, Kusuhara H, Yokochi M, Toyoshima J, Inoue K, et al. 2012. Competitive inhibition of the luminal efflux by multidrug and toxin extrusions, but not basolateral uptake by organic cation transporter 2, is the likely mechanism underlying the pharmacokinetic drug-drug interactions caused by cimetidine in the kidney. *J Pharmacol Exp Ther* 340:393-403

136. Motojima M, Hosokawa A, Yamato H, Muraki T, Yoshioka T. 2002. Uraemic toxins induce proximal tubular injury via organic anion transporter 1-mediated uptake. *Br J Pharmacol* 135:555-63

137. Zhu Y, Meng Q, Wang C, Liu Q, Sun H, et al. 2012. Organic anion transporters involved in the excretion of bestatin in the kidney. *Peptides* 33:265-71

138. Choi MK, Jin QR, Choi YL, Ahn SH, Bae MA, Song IS. 2011. Inhibitory effects of ketoconazole and rifampin on OAT1 and OATP1B1 transport activities: considerations on drug-drug interactions. *Biopharm Drug Dispos* 32:175-84

139. Sudoh T, Fujimura A, Shiga T, Sasaki M, Harada K, et al. 1994. Renal clearance of lomefloxacin is decreased by furosemide. *Eur J Clin Pharmacol* 46:267-9

140. Welling PG, Dean S, Selen A, Kendall MJ, Wise R. 1979. Probenecid: an unexplained effect on cephalosporin pharmacology. *Br J Clin Pharmacol* 8:491-5

141. Inotsume N, Nishimura M, Nakano M, Fujiyama S, Sato T. 1990. The inhibitory effect of probenecid on renal excretion of famotidine in young, healthy volunteers. *J Clin Pharmacol* 30:50-6

142. Stoeckel K, Trueb V, Dubach UC, McNamara PJ. 1988. Effect of probenecid on the elimination and protein binding of ceftriaxone. *Eur J Clin Pharmacol* 34:151-6

143. Laskin OL, de Miranda P, King DH, Page DA, Longstreth JA, et al. 1982. Effects of probenecid on the pharmacokinetics and elimination of acyclovir in humans. *Antimicrob Agents Chemother* 21:804-7

144. Pitkin D, Dubb J, Actor P, Alexander F, Ehrlich S, et al. 1981. Kinetics and renal handling of cefonicid. *Clin Pharmacol Ther* 30:587-93

145. Vlasses PH, Holbrook AM, Schrogie JJ, Rogers JD, Ferguson RK, Abrams WB. 1980. Effect of orally administered probenecid on the pharmacokinetics of cefoxitin. *Antimicrob Agents Chemother* 17:847-55

146. Cundy KC, Petty BG, Flaherty J, Fisher PE, Polis MA, et al. 1995. Clinical pharmacokinetics of cidofovir in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 39:1247-52

147. Beringer PM, Kriengkauykiat J, Zhang X, Hidayat L, Liu S, et al. 2008. Lack of effect of P-glycoprotein inhibition on renal clearance of dicloxacillin in patients with cystic fibrosis. *Pharmacotherapy* 28:883-94

148. Jaehde U, Sorgel F, Reiter A, Sigl G, Naber KG, Schunack W. 1995. Effect of probenecid on the distribution and elimination of ciprofloxacin in humans. *Clin Pharmacol Ther* 58:532-41

149. Vree TB, van den Biggelaar-Martea M, Verwey-van Wissen CP. 1995. Probenecid inhibits the renal clearance of furosemide and its acyl glucuronide. *Br J Clin Pharmacol* 39:692-5

150. Chatton JY, Munafo A, Chave JP, Steinhauslin F, Roch-Ramel F, et al. 1992. Trimethoprim, alone or in combination with sulphamethoxazole, decreases the renal excretion of zidovudine and its glucuronide. *Br J Clin Pharmacol* 34:551-4

151. Shiveley L, Struthers-Semple C, Cox S, Sawyer J. 2008. Pharmacokinetics of apricitabine, a novel nucleoside reverse transcriptase inhibitor, in healthy volunteers treated with trimethoprim-sulphamethoxazole. *J Clin Pharm Ther* 33:45-54

152. Tsuruoka S, Ioka T, Wakaumi M, Sakamoto K, Ookami H, Fujimura A. 2006. Severe arrhythmia as a result of the interaction of cetirizine and pilsicainide in a patient with renal insufficiency: first case presentation showing competition for excretion via renal multidrug resistance protein 1 and organic cation transporter 2. *Clin Pharmacol Ther* 79:389-96

153. Somogyi AA, Bochner F, Sallustio BC. 1992. Stereoselective inhibition of pindolol renal clearance by cimetidine in humans. *Clin Pharmacol Ther* 51:379-87

154. Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. 1987. Reduction of metformin renal tubular secretion by cimetidine in man. *Br J Clin Pharmacol* 23:545-51

155. van Crugten J, Bochner F, Keal J, Somogyi A. 1986. Selectivity of the cimetidine-induced alterations in the renal handling of organic substrates in humans. Studies with anionic, cationic and zwitterionic drugs. *J Pharmacol Exp Ther* 236:481-7

156. Somogyi A, McLean A, Heinzow B. 1983. Cimetidine-procainamide pharmacokinetic interaction in man: evidence of competition for tubular secretion of basic drugs. *Eur J Clin Pharmacol* 25:339-45

157. Shiga T, Hashiguchi M, Urae A, Kasanuki H, Rikihisa T. 2000. Effect of cimetidine and probenecid on pilsicainide renal clearance in humans. *Clin Pharmacol Ther* 67:222-8

158. Abel S, Nichols DJ, Brearley CJ, Eve MD. 2000. Effect of cimetidine and ranitidine on pharmacokinetics and pharmacodynamics of a single dose of dofetilide. *Br J Clin Pharmacol* 49:64-71

159. Kusuhara H, Ito S, Kumagai Y, Jiang M, Shiroshita T, et al. 2011. Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at oral microdose and at therapeutic dose in healthy subjects. *Clin Pharmacol Ther* 89:837-44

160. Habu Y, Yano I, Okuda M, Fukatsu A, Inui K. 2005. Restored expression and activity of organic ion transporters rOAT1, rOAT3 and rOCT2 after hyperuricemia in the rat kidney. *Biochemical Pharmacology* 69:993-9

161. Habu Y, Yano I, Takeuchi A, Saito H, Okuda M, et al. 2003. Decreased activity of basolateral organic ion transports in hyperuricemic rat kidney: roles of organic ion transporters, rOAT1 rOAT3 and rOCT2. *Biochemical Pharmacology* 66:1107-14

162. Ji L, Masuda S, Saito H, Inui K. 2002. Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy. *Kidney International* 62:514-24

163. Matsuzaki T, Morisaki T, Sugimoto W, Yokoo K, Sato D, et al. 2008. Altered pharmacokinetics of cationic drugs caused by down-regulation of renal rat organic cation transporter 2 (Slc22a2) and rat multidrug and toxin extrusion 1 (Slc47a1) in ischemia/reperfusion-induced acute kidney injury. *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 36:649-54

164. Alnouti Y, Petrick JS, Klaassen CD. 2006. Tissue distribution and ontogeny of organic cation transporters in mice. *Drug Metabolism and Disposition* 34:477-82

165. de Zwart L, Scholten M, Monbaliu JG, Annaert PP, Van Houdt JM, et al. 2008. The ontogeny of drug metabolizing enzymes and transporters in the rat. *Reproductive Toxicology* 26:220-30

166. Nishihara K, Masuda S, Ji L, Katsura T, Inui K-I. 2007. Pharmacokinetic significance of luminal multidrug and toxin extrusion 1 in chronic renal failure rats. *Biochemical Pharmacology* 73:1482-90

167. Deguchi T, Takemoto M, Uehara N, Lindup WE, Suenaga A, Otagiri M. 2005. Renal Clearance of Endogenous Hippurate Correlates with Expression Levels of Renal Organic Anion Transporters in Uremic Rats. *Journal of Pharmacology and Experimental Therapeutics* 314:932-8

168. Zhang R, Yang X, Li J, Wu J, Peng W-X, et al. 2008. Upregulation of rat renal cortical organic anion transporter (OAT1 and OAT3) expression in response to ischemia/reperfusion injury. *American Journal of Nephrology* 28:772-83

169. Burckhardt BC, Burckhardt G. 2003. Transport of organic anions across the basolateral membrane of proximal tubule cells. *Reviews of Physiology, Biochemistry and Pharmacology, Vol 146* 146:95-158

170. Buist SCN, Cherrington NJ, Choudhuri S, Hartley DP, Klaassen CD. 2002. Gender-specific and developmental influences on the expression of rat organic anion transporters. *Journal of Pharmacology and Experimental Therapeutics* 301:145-51

171. Nakajima N, Sekine T, Cha SH, Tojo A, Hosoyamada M, et al. 2000. Developmental changes in multispecific organic anion transporter 1 expression in the rat kidney. *Kidney International* 57:1608-16

172. Sekine T, Endou H. 2009. Children's toxicology from bench to bed - Drug-induced Renal Injury (3): Drug transporters and toxic nephropathy in childhood. *Journal of Toxicological Sciences* 34:SP259-65

173. Sweet DH, Bush KT, Nigam SK. 2001. The organic anion transporter family: from physiology to ontogeny and the clinic. *American Journal of Physiology-Renal Physiology* 281:F197-205

174. Aoyama I, Enomoto A, Niwa T. 2003. Effects of oral adsorbent on gene expression profile in uremic rat kidney: cDNA array analysis. *American Journal of Kidney Diseases* 41:S8-S14-S8-S

175. Di Giusto G, Anzai N, Endou H, Torres AM. 2008. Elimination of organic anions in response to an early stage of renal ischemia-reperfusion in the rat: role of

basolateral plasma membrane transporters and cortical renal blood flow. *Pharmacology* 81:127-36

176. Naud J, Michaud J, Beauchemin S, Hebert MJ, Roger M, et al. 2011. Effects of chronic renal failure on kidney drug transporters and cytochrome P450 in rats. *Drug Metab Dispos* 39:1363-9

177. Cheng XG, Klaassen CD. 2009. Tissue Distribution, Ontogeny, and Hormonal Regulation of Xenobiotic Transporters in Mouse Kidneys. *Drug Metabolism and Disposition* 37:2178-85

178. Slitt AL, Cherrington NJ, Hartley DP, Leazer TM, Klaassen CD. 2002. Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. *Drug Metabolism and Disposition* 30:212-9

179. Garcia-Delgado M, Peral MJ, Duran JM, Garcia-Miranda P, Calonge ML, Ilundain AA. 2009. Ontogeny of Na+/l-carnitine transporter and of gamma-trimethylaminobutyraldehyde dehydrogenase and gamma-butyrobetaine hydroxylase genes expression in rat kidney. *Mechanisms of Ageing and Development* 130:227-33

180. Lickteig AJ, Cheng X, Augustine LM, Klaassen CD, Cherrington NJ. 2008. Tissue distribution, ontogeny and induction of the transporters Multidrug and toxin extrusion (MATE) 1 and MATE2 mRNA expression levels in mice. *Life Sciences* 83:59-64

181. Laouari D, Yang R, Veau C, Blanke I, Friedlander G. 2001. Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure. *Am J Physiol Renal Physiol.* 280:F636-45

182. Maher JM, Slitt AL, Cherrington NJ, Cheng XG, Klaassen CD. 2005. Tissue distribution and hepatic and renal ontogeny of the multidrug resistance-associated protein (MRP) family in mice. *Drug Metabolism and Disposition* 33:947-55

183. Rosati A, Maniori S, Decorti G, Candussio L, Giraldi T, Bartoli F. 2003. Physiological regulation of P-glycoprotein, MRP1, MRP2 and cytochrome P450 3A2 during rat ontogeny. *Development Growth & Differentiation* 45:377-87

184. Huang ZH, Murakami T, Okochi A, Yumoto R, Nagai J, Takano M. 2000. Expression and function of P-glycoprotein in rats with glycerol-induced acute renal failure. *European Journal of Pharmacology* 406:453-60

185. Schuetz EG, Furuya KN, Schuetz JD. 1995. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *The Journal of Pharmacology and Experimental Therapeutics* 275:1011-8

186. Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, et al. 2003. Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* 38:978-88

187. van Kalken CK, Giaccone G, van der Valk P, Kuiper CM, Hadisaputro MM, et al. 1992. Multidrug resistance gene (P-glycoprotein) expression in the human fetus. *Am J Pathol* 141:1063-72

188. Lu H, Klaassen C. 2008. Gender Differences in mRNA Expression of ATP-Binding Cassette Efflux and Bile Acid Transporters in Kidney, Liver, and Intestine of 5/6 Nephrectomized Rats. *Drug Metabolism and Disposition* 36:16-23

189. Froissart M, Rossert J, Jacquot C, Paillard M, Houillier P. 2005. Predictive performance of the modification of diet in renal disease and Cockcroft-Gault equations for estimating renal function. *J Am Soc Nephrol* 16:763-73

190. Fehrman-Ekholm I, Skeppholm L. 2004. Renal function in the elderly (>70 years old) measured by means of iohexol clearance, serum creatinine, serum urea and estimated clearance. *Scand J Urol Nephrol* 38:73-7

191. Cirillo M, Anastasio P, De Santo NG. 2005. Relationship of gender, age, and body mass index to errors in predicted kidney function. *Nephrol Dial Transplant* 20:1791-8

192. Bostom AG, Kronenberg F, Ritz E. 2002. Predictive performance of renal function equations for patients with chronic kidney disease and normal serum creatinine levels. *J Am Soc Nephrol* 13:2140-4

193. Murata K, Baumann NA, Saenger AK, Larson TS, Rule AD, Lieske JC. 2011. Relative performance of the MDRD and CKD-EPI equations for estimating glomerular filtration rate among patients with varied clinical presentations. *Clin J Am Soc Nephrol* 6:1963-72

194. Michels WM, Grootendorst DC, Verduijn M, Elliott EG, Dekker FW, Krediet RT. 2010. Performance of the Cockcroft-Gault, MDRD, and new CKD-EPI formulas in relation to GFR, age, and body size. *Clin J Am Soc Nephrol* 5:1003-9

195. Alper AB, Yi Y, Rahman M, Webber LS, Magee L, et al. 2011. Performance of estimated glomerular filtration rate prediction equations in preeclamptic patients. *Am J Perinatol* 28:425-30

196. Brion LP, Fleischman AR, McCarton C, Schwartz GJ. 1986. A simple estimate of glomerular filtration rate in low birth weight infants during the first year of life: noninvasive assessment of body composition and growth. *J Pediatr* 109:698-707

197. Schwartz GJ, Feld LG, Langford DJ. 1984. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. *J Pediatr* 104:849-54

198. Schwartz GJ, Haycock GB, Edelmann CM, Jr., Spitzer A. 1976. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics* 58:259-63

199. Martini S, Prevot A, Mosig D, Werner D, van Melle G, Guignard JP. 2003. Glomerular filtration rate: measure creatinine and height rather than cystatin C! *Acta Paediatr* 92:1052-7

200. Seikaly MG, Browne R, Bajaj G, Arant BS, Jr. 1996. Limitations to body length/serum creatinine ratio as an estimate of glomerular filtration in children. *Pediatr Nephrol* 10:709-11

201. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM. 1976. Estimation of glomerular filtration rate from plasma creatinine concentration in children. *Arch Dis Child* 51:875-8

202. van Rossum LK, Mathot RA, Cransberg K, Zietse R, Vulto AG. 2005. Estimation of the glomerular filtration rate in children: which algorithm should be used? *Pediatr Nephrol* 20:1769-75

203. Grubb A, Nyman U, Bjork J, Lindstrom V, Rippe B, et al. 2005. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the modification of diet in renal disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. *Clin Chem* 51:1420-31

204. Quadri KH, Bernardini J, Greenberg A, Laifer S, Syed A, Holley JL. 1994. Assessment of renal function during pregnancy using a random urine protein to creatinine ratio and Cockcroft-Gault formula. *Am J Kidney Dis* 24:416-20

205. Musso CG, Michelangelo H, Vilas M, Reynaldi J, Martinez B, et al. 2009. Creatinine reabsorption by the aged kidney. *Int Urol Nephrol* 41:727-31

206. Van Acker BA, Koomen GC, Arisz L. 1995. Drawbacks of the constant-infusion technique for measurement of renal function. *Am J Physiol* 268:F543-52

207. Florijn KW, Barendregt JN, Lentjes EG, van Dam W, Prodjosudjadi W, et al. 1994. Glomerular filtration rate measurement by "single-shot" injection of inulin. *Kidney Int* 46:252-9

208. Hellerstein S, Berenbom M, Alon U, Warady BA. 1993. The renal clearance and infusion clearance of inulin are similar, but not identical. *Kidney Int* 44:1058-61

209. Bhatt MK, Bartlett ML, Mallitt KA, McTaggart S, Ravi Kumar AS. 2011. Correlation of various published radionuclide glomerular filtration rate estimation techniques and proposed paediatric normative data. *Nucl Med Commun* 32:1088-94

210. Ma YC, Zuo L, Zhang CL, Wang M, Wang RF, Wang HY. 2007. Comparison of 99mTc-DTPA renal dynamic imaging with modified MDRD equation for glomerular filtration rate estimation in Chinese patients in different stages of chronic kidney disease. *Nephrol Dial Transplant* 22:417-23

211. Rodby RA, Ali A, Rohde RD, Lewis EJ. 1992. Renal scanning 99mTc diethylene-triamine pentaacetic acid glomerular filtration rate (GFR) determination compared with iothalamate clearance GFR in diabetics. The Collaborative Study Group for

The study of Angiotensin-Converting Enzyme Inhibition in Diabetic Nephropathy. *Am J Kidney Dis* 20:569-73

212. Heymsfield SB, Arteaga C, McManus C, Smith J, Moffitt S. 1983. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 37:478-94

213. Stevens LA, Levey AS. 2005. Measurement of kidney function. *Med Clin North Am* 89:457-73

214. Shemesh O, Golbetz H, Kriss JP, Myers BD. 1985. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int* 28:830-8

215. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, et al. 2004. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 65:1416-21

216. Wiesli P, Schwegler B, Spinas GA, Schmid C. 2003. Serum cystatin C is sensitive to small changes in thyroid function. *Clin Chim Acta* 338:87-90

217. Risch L, Herklotz R, Blumberg A, Huber AR. 2001. Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. *Clin Chem* 47:2055-9

218. Zahran A, Qureshi M, Shoker A. 2007. Comparison between creatinine and cystatin C-based GFR equations in renal transplantation. *Nephrol Dial Transplant* 22:2659-68

219. Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec L. 2006. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. *Nephrol Dial Transplant* 21:1855-62

220. White C, Akbari A, Hussain N, Dinh L, Filler G, et al. 2005. Estimating glomerular filtration rate in kidney transplantation: a comparison between serum creatinine and cystatin C-based methods. *J Am Soc Nephrol* 16:3763-70

221. Van Den Noortgate NJ, Janssens WH, Delanghe JR, Afschrift MB, Lameire NH. 2002. Serum cystatin C concentration compared with other markers of glomerular filtration rate in the old old. *J Am Geriatr Soc* 50:1278-82

222. Filler G, Priem F, Lepage N, Sinha P, Vollmer I, et al. 2002. Beta-trace protein, cystatin C, beta(2)-microglobulin, and creatinine compared for detecting impaired glomerular filtration rates in children. *Clin Chem* 48:729-36

223. Finney H, Newman DJ, Thakkar H, Fell JM, Price CP. 2000. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. *Arch Dis Child* 82:71-5

224. Skov PE. 1970. Glomerular filtration rate in patients with severe and very severe renal insufficiency. Determined by simultaneous inulin, creatinine and 125 iothalamate clearance. *Acta Med Scand* 187:419-28

225. Odlind B, Hallgren R, Sohtell M, Lindstrom B. 1985. Is 125I iothalamate an ideal marker for glomerular filtration? *Kidney Int* 27:9-16

226. Tessitore N, Lo Schiavo C, Corgnati A, Previato G, Valvo E, et al. 1979. 125I-iothalamate and creatinine clearances in patients with chronic renal diseases. *Nephron* 24:41-5

227. Erley CM, Bader BD, Berger ED, Vochazer A, Jorzik JJ, et al. 2001. Plasma clearance of iodine contrast media as a measure of glomerular filtration rate in critically ill patients. *Crit Care Med* 29:1544-50

228. Gaspari F, Perico N, Ruggenenti P, Mosconi L, Amuchastegui CS, et al. 1995. Plasma clearance of nonradioactive iohexol as a measure of glomerular filtration rate. *J Am Soc Nephrol* 6:257-63

229. Brown SC, O'Reilly PH. 1991. Iohexol clearance for the determination of glomerular filtration rate in clinical practice: evidence for a new gold standard. *J Urol* 146:675-9

230. LaFrance ND, Drew HH, Walser M. 1988. Radioisotopic measurement of glomerular filtration rate in severe chronic renal failure. *J Nucl Med* 29:1927-30

231. Rehling M, Moller ML, Thamdrup B, Lund JO, Trap-Jensen J. 1984. Simultaneous measurement of renal clearance and plasma clearance of 99mTc-labelled diethylenetriaminepenta-acetate, 51Cr-labelled ethylenediaminetetra-acetate and inulin in man. *Clin Sci (Lond)* 66:613-9

232. Brochner-Mortensen J. 1978. Routine methods and their reliability for assessment of glomerular filtration rate in adults, with special reference to total [51Cr]EDTA plasma clearance. *Dan Med Bull* 25:181-202

233. Perrone RD, Steinman TI, Beck GJ, Skibinski CI, Royal HD, et al. 1990. Utility of radioisotopic filtration markers in chronic renal insufficiency: simultaneous comparison of 125I-iothalamate, 169Yb-DTPA, 99mTc-DTPA, and inulin. The Modification of Diet in Renal Disease Study. *Am J Kidney Dis* 16:224-35

CHAPTER 2

THE UCSF-FDA TRANSPORTAL: A PUBLIC DRUG TRANSPORTER DATABASE [*]

Drug transporters play a key role in the absorption, distribution and elimination of many drugs, and they appear to be important determinants of therapeutic and adverse drug activities. Although a large body of data pertaining to drug transporters is available, there are few databases that inform drug developers, regulatory agencies and academic scientists about transporters that are important in drug action and disposition. In this article, we inform the scientific community about the UCSF-FDA TransPortal, a new and valuable online resource for research and drug development.

To provide a central resource for information about important drug transporters, we have developed a free-of-charge online drug transporter database, University of California, San Francisco – Food and Drug Administration (UCSF-FDA) TransPortal (http://bts.ucsf.edu/fdatransportal). We have highlighted 31 drug transporters from the ATP-binding cassette (ABC) and solute carrier (SLC) transporter superfamilies that play a critical role in drug disposition, toxicity and efficacy—including transporters listed in the 2012 US FDA draft drug interaction guidance (1) and the International Transporter Consortium white paper (2). For each transporter, we have compiled primary literature on its expression levels, subcellular localization and direction of transport in the kidney,

liver, small intestine, placenta and blood–brain barrier (Figure 1). In addition, we have listed known inhibitors and substrates of each transporter and summarized transport kinetic data (K_m, K_i, IC_{50}) from *in vitro* studies. Finally, clinical drug–drug interactions attributed to drug transporters are listed, along with a description of the impact on the affected drug's pharmacokinetics and pharmacodynamics.

Other drug transporter databases include the University of Tokyo's TP-search (http://125.206.112.67/tp-search/login.php), Q. Yan's Human Membrane Transporter Database (http://lab.digibench.net/transporter), M. Müller's ABC-Transporter Database (http://nutrigene.4t.com/translink.htm), C. Yuzong's Drug ADME Associated Protein Database (http://xin.cz3.nus.edu.sg/group/admeap/admeap.asp), UCSF's Pharmacogenomics of Membrane Transporters (http://pharmacogenetics.ucsf.edu), and the University of Washington's Metabolism and Transport Drug Interaction Database (http://www.druginteractioninfo.org). However, several of the databases have not been updated in recent years and none includes data on expression levels of drug transporters across human tissues. Furthermore, in general, the databases provide limited information on the substrates and inhibitors of the 31 transporters that are included in TransPortal.

TransPortal currently contains information from more than 297 primary literature sources and drug labels. From these sources, TransPortal provides messenger RNA expression levels for 31 transporters in five human tissues that play a role in drug–drug interactions. In addition, the database provides information on 482 substrates, 866 inhibitors and 48
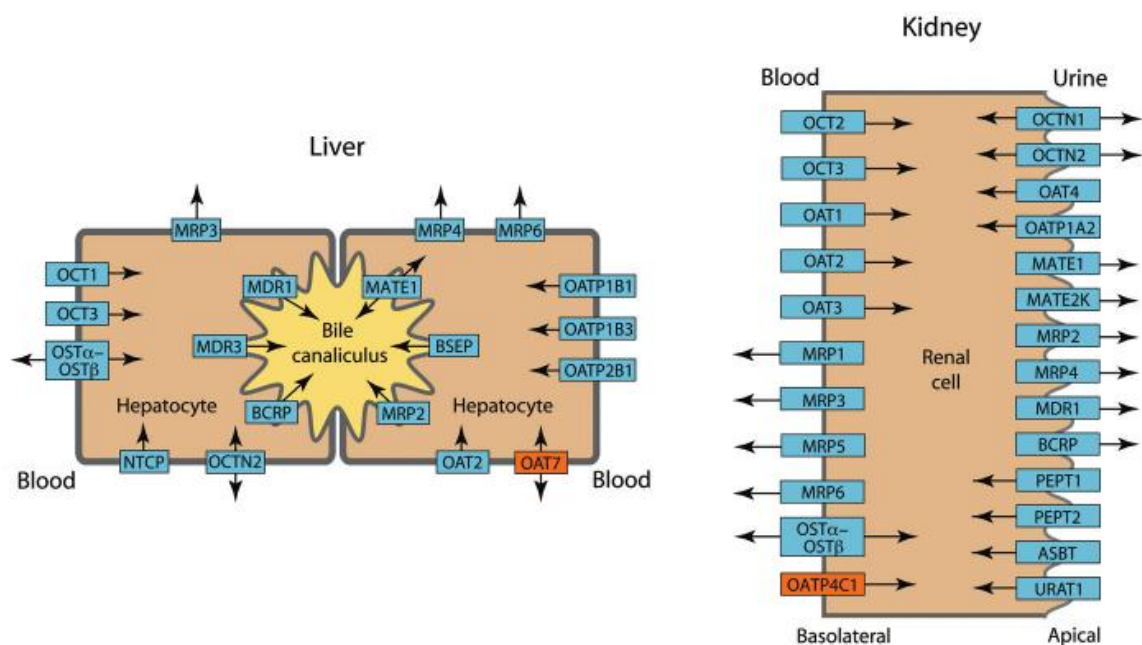
Figure 1. Representative TransPortal screenshot of drug transporters in human liver and kidney. For definitions of the abbreviations, please see the glossary at http://bts.ucsf.edu/fdatransportal. Transporters currently included within TransPortal are designated in blue and transporters that are not currently contained in the database are highlighted in orange.

clinical drug–drug interactions. The database is also text-searchable, user-friendly and in compliance with Section 508 of the Rehabilitation Act, with many links to PubMed, drug labels and websites within the database. The UCSF-FDA TransPortal, supported by the FDA Critical Path Initiative (http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/default.htm), is thus an important tool for research and for enhancing the development of safer medications.

REFERENCES

1. *Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm> Accessed May 2013

2. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, et al. 2010. Membrane transporters in drug development. *Nat Rev Drug Discov* 9:215-36

CHAPTER 3

IDENTIFICATION OF SELECTIVE AND POTENT INHIBITORS OF RENAL ORGANIC CATION TRANSPORT

INTRODUCTION

The body defends itself against potentially toxic xenobiotics, xenobiotic metabolites and metabolic wastes by elimination, in which the kidney plays a critical role. To accomplish this task, the functional units of the kidney, the nephrons, determine the degree of renal elimination through the balance of glomerular filtration, tubular secretion and tubular reabsorption. For renal tubular secretion to occur, a molecule must first pass from the blood into the renal tubular cell, and then into the tubular lumen to the urine. Current data suggest that in the proximal tubule of the kidney, organic cations (including many basic drugs) are transported from the blood to the renal tubule cell by organic cation transporter 2 (OCT2) and are eliminated in the urine by the concerted action of the H^+/organic cation antiporters, multidrug and toxin extrusion 1 (MATE1) and 2K (MATE2K).

Due to the overlapping specificities of drugs for renal secretory transporters, drug-drug interactions (DDIs) occur in clinical situations and impact the pharmacokinetics of drugs that are renally excreted. For example, in healthy volunteers the renal clearance of the anti-diabetic organic cation, metformin, is decreased by the co-administration of cimetidine or pyrimethamine (1; 2). For both drugs, the DDI appears to result from inhibition of MATE1 and MATE2K-mediated, rather than OCT2-mediated metformin

elimination (1; 3; 4). With the exception of cimetidine and pyrimethamine, there are few drugs identified as clinically potent and selective inhibitors of renal organic cation transporters. In addition, the inhibitors that have been identified are either promiscuous and inhibit multiple transporter families (e.g. quinidine) or are more potent inhibitors of organic cation transporter 1 (OCT1), an organic cation transporter that is highly expressed in the liver (e.g. procainamide) (4-6).

In vitro methods for predicting transporter-mediated DDIs are evolving. Typically, one compares the *in vivo* $C_{max,u}$ (maximum plasma concentration that is not bound to plasma proteins) of a potential inhibitor to its *in vitro* IC_{50} (concentration at half the maximum inhibition of active transport). Recently, the International Transporter Consortium and the US FDA have proposed that if the $C_{max,u}/IC_{50}$ value is ≥ 0.1 a clinical investigation of a transporter-mediated DDI should be considered (7-9).

Applying these recommendations to the current study, an initial *in vitro* screen of 73 over-the-counter and prescription drugs was conducted at a single concentration of each drug: 10x the drug's C_{max} observed in human subjects multiplied by the drug's fraction unbound, if known. Only drugs that exceed 50% transport inhibition of metformin uptake were further characterized for potency and selectivity against the other non-renal transporters known to transport organic cations: OCT1, OCT3 and plasma membrane monoamine transporter (PMAT). This study provides a novel approach to identify selective probe inhibitors of renal organic cation transporters at clinically relevant concentrations and to predict their transporter-mediated DDI liabilities.

MATERIALS AND METHODS

Drugs and Reagents

Gabapentin, pregabalin and topiramate were purchased from Cayman Chemical Company (Ann Arbor, MI). Neomycin, carmofur and mycophenolate mofetil were purchased from Enzo Life Sciences International (Farmingdale, NY), LKT laboratories (St Paul, MN) and United States Pharmacopeia (Rockville, MD), respectively. Chlorphenesin, diphenidol, doxepin, ethambutol, pyrazinamide and thalidomide were purchased from MP Biomedicals (Solon, OH). Abacavir, atazanavir, cefpodoxime, darunavir, emtricitabine, famiciclovir, lacosamide, moxifloxacin, nevirapine, oseltamavir, rabeprazole, raltegravir, telebivudine and zonisamide were purchased from Toronto Research Chemicals (North York, Ontario, Canada). [^{14}C]-labeled metformin was purchased from Moravek Biochemicals and Radiochemicals (Brea, CA). All other chemicals were purchased from Sigma (St. Louis, MO). Cell culture reagents were purchased from Univerisity of California, San Francisco's Cell Culture Facility.

Cell Lines

Flp-In human embryonic kidney (HEK293-Flp-In) cells stably expressing human OCT1 (HEK-OCT1), OCT2 (HEK-OCT2), OCT3 (HEK-OCT3), MATE1 (HEK-MATE1), MATE2K (HEK-MATE2K) and the pcDNA5/FRT empty vector (HEK-EV) were previously established in our laboratory (10-17). Madin−Darby canine kidney type II (MDCK-II) cells stably expressing human PMAT (MDCK-PMAT) and the pcDNA3.1(+) vector (MDCK-EV) were also established previously (18).

Cell Culture

Stably transfected cells were maintained in Dulbecco's modified eagle medium (DMEM) H-21 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 U/mL streptomycin, 2 mM glutamine and 200 μg/mL hygromycin B (HEK cell lines) or 800 μg/mL geneticin (MDCK cell lines). All cell lines were grown at 37°C in a humidified atmosphere with 5% CO_2.

Metformin Cellular Uptake Study

Cells were suspended in cell culture medium, seeded on poly-D-lysine-coated 48-well plates (Greiner Bio-One, Monroe, NC) and grown to ~90% confluency (~48 hours post seeding). Immediately prior to uptake, HEK-EV, HEK-OCT1, HEK-OCT2 and HEK-OCT3 cells were preincubated for 20 min with Hank's balanced salt solution (HBSS, 5.4 mM KCl, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 137 mM NaCl, 0.34 mM Na_2HPO_4, 5.6 mM D-glucose, 1.3 mM $CaCl_2$, 0.49 mM $MgCl_2$, 0.41 mM $MgSO_4$, pH 7.4). HEK-EV, HEK-MATE1 and HEK-MATE2K cells were preincubated with HBSS plus 30 mM NH_4Cl for 20 mins. MDCK-EV and MDCK-PMAT cells were preincubated with Krebs-Ringer-Henseleit Buffer (KRH, 5.6 mM glucose, 125 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM HEPES, pH 6.6) for 20 min. Preincubation media was removed and uptake was initiated with the addition of uptake buffer (HBSS [HEK cell lines] or KRH [MDCK cell lines] containing 10 μmol/L unlabeled and [^{14}C]-labeled metformin or [^{14}C]-labeled metformin with a test inhibitor at its 10x $C_{max,u}$) at 37°C for a period of time for which linear uptake was observed (2-5

min). At the end of the uptake, cells were washed twice with ice-cold buffer (HBSS [HEK cell lines] or KRH [MDCK cell lines]) and lysed with 0.1 N NaOH/0.1% SDS. Intracellular radioactivity was determined by liquid scintillation counting and normalized per well of protein content as measured by bicinchoninic acid protein assay (Thermo Scientific, Rockford, IL). Each test condition was conducted in triplicate.

Compounds that were selective inhibitors of the renal organic cation transporters at concentrations that were 10x $C_{max,u}$ were subjected to experimental IC_{50} determination. Studies were conducted exactly as described above using increasing concentrations of the inhibitor (ranging from 0-40x $C_{max,u}$ of the inhibitor for a total of 8 concentrations in triplicate).

Data Analysis

Determination of IC_{50} Values

After adjusting for protein quantity and subtracting non-specific transport of metformin (measured from empty vector cells, HEK-EV and MDCK-EV), residual values were normalized to the rate of uptake in the absence of the inhibitor (set at 100%). Dose response curves and IC_{50} values were obtained using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Briefly, inhibitor concentrations were transformed to log scale and dose-response inhibition curves were fitted with the following equation:

$$\% \text{ metformin uptake} = \text{bottom} + (100-\text{bottom})/(1+10^{\log IC50-\log[\text{inhibitor}]*\text{hill coefficient}}),$$

where bottom is the plateau of maximum inhibition observed.

Absolute IC_{50} values were calculated by interpolation of the fitted curve. Since the concentration of metformin used for uptake experiments (10 μM) is much lower than its K_m (Michaelis-Menten constant) for each transporter (~200-3000 μM) (Chapter 1), the calculated IC_{50} values would be identical to the inhibitory constant, K_i (assuming a competitive mechanism of inhibition).

Physicochemical properties and clinical concentration of test inhibitors

Predicted charge at pH 7.4 was calculated using MarvinView Version 5.3.6 (ChemAxon, http://www.chemaxon.com). Maximum plasma concentrations (C_{max}) of test inhibitors in human subjects and percent protein binding in human plasma were obtained from literature sources (Table 1; (19; 20)). Maximum plasma concentrations not bound to plasma protein ($C_{max,u}$) were calculated by $C_{max,u} = C_{max} \bullet f_u$, where f_u is the unbound fraction in human plasma.

RESULTS

Identification of Drugs That Inhibit Renal Organic Cation Transporters at Clinically Relevant Concentrations

A medium throughput screen to identify inhibitors of the renal organic cation transporters, OCT2, MATE1 and MATE2K was performed by measuring alterations in [14C]-labeled metformin uptake. FDA-approved medications (n=73) were selected to be screened for inhibitory potential if they met one or more of the following critera: (*i*) C_{max} in human subjects was greater than 0.1 μM, (*ii*) unbound fraction in human plasma (f_u) was greater than 0.10 and (iii) predicted to have a positive or neutral charge at physiological pH (pH

Table 1. Summary of physicochemical properties and clinical concentrations of test inhibitors (n=73).

Drugs	Physicochemical Properties		Clinical Concentrations				
	Predicted charge at pH 7.4	Molecular Weight (g/mol)	Dose	Frequency, Route	C_{max} (μM)	% Protein Binding	Calculated $C_{max,u}$ (μM)
abacavir	0.05	286.3	1200 mg	single, oral	33.53	50	16.77
allopurinol	-0.07	136.1	300 mg	single, oral	19.10	5	18.15
amantidine	1	151.3	150 mg	single, oral	3.70	67	1.22
amoxicillin	-0.49	365.4	1000 mg	single, oral	38.59	20	30.87
ampicillin	-0.48	349.4	500 mg	single, oral	11.45	20	9.16
atazanavir	0	704.9	400 mg	multiple, oral	7.66	86	1.07
auranofin	0.91	679.5	6 mg	multiple, oral	1.03	60	0.41
azithromycin	1.96	749.0	1000 mg	single, oral	1.09	50	0.55
bretylium tosylate	0	414.4	5 mg/kg	single, oral	0.34	5	0.32
carmofur	-0.72	257.3	100 mg	single, oral	10.49	46	5.62
cefaclor	-0.48	385.8	500 mg	single, oral	33.70	25	25.27
cefadroxil	-0.49	363.4	500 mg	single, oral	44.03	20	35.22
cefpodoxime	-1	427.5	800 mg	single, oral	15.46	29	10.98
cephalexin	-0.48	365.4	500 mg	single, oral	9.36	14	8.05
chlorphenesin	0	245.7	800 mg	single, oral	69.19	0	69.19
cimetidine	0.12	252.4	200 mg	single, oral	7.53	26	5.57
clofazimine	0.01	473.4	400 mg	single, oral	0.34	0	0.34
clofibrate	0	242.7	1000 mg	single, oral	325.50	98	6.51
cycloserine	0.01	102.1	750 mg	multiple, oral	333.01	20	266.41
darunavir	0	593.7	600 mg	multiple, oral	9.20	95	0.46
diazoxide	1	230.7	300 mg	single, oral	86.69	90	8.67
diphenhydramine	0.97	255.4	100 mg	single, oral	0.63	80	0.13
diphenidol	0.99	309.4	50 mg	single, oral	0.65	90	0.06
disopyramide	1	339.5	200 mg	single, oral	14.14	80	2.83
doxepin	1	279.4	250 mg	multiple, oral	0.47	80	0.09
emtricitabine	0	247.2	200 mg	multiple, oral	7.30	4	7.01
eplerenone	0	414.5	50 mg	multiple, oral	2.41	60	0.97
ethambutol	1.15	204.3	15 mg/kg	single, oral	27.41	40	16.45
famiciclovir	0.01	321.3	500 mg	single, oral	10.40	20	8.32
furosemide	-1	330.7	80 mg	single, oral	14.82	99	0.15
gabapentin	0	171.2	2400 mg	single, oral	44.68	3	43.34
ganciclovir	0.79	255.2	1000 mg	single, oral	4.70	2	4.61
indapamide	-0.03	365.8	5 mg	single, oral	0.71	80	0.14
lacosamide	0	250.3	1000 mg	multiple, oral	87.10	15	74.03
lamotrigine	0.03	256.1	200 mg	single, oral	9.76	56	4.30
levetiracetam	0	170.2	5.4 mg/kg	multiple, oral	36.96	10	33.26
moxifloxacin	0.03	401.4	400 mg	single, oral	6.23	39	3.77
mycophenolate mofetil	0.05	433.5	2000 mg	single, oral	54.90	97	1.65
naloxone	0.73	327.4	16 mg	single, oral	18.63	40	11.18
neomycin	5.28	614.6	6000 mg	multiple, oral	130.17	30	91.12
nevirapine	0	266.3	400 mg	single, oral	11.52	60	4.61
niacin	-0.99	123.1	2000 mg	single, oral	125.90	20	100.72

Drugs	Physicochemical Properties		Clinical Concentrations				
	Predicted charge at pH 7.4	Molecular Weight (g/mol)	Dose	Frequency, Route	C_{max} (μM)	% Protein Binding	Calculated $C_{max,u}$ (μM)
nizatidine	0.83	331.5	150 mg	single, oral	4.40	35	2.86
norfloxacin	-0.01	319.3	400 mg	multiple, oral	4.70	15	3.99
omeprazole	-0.01	345.4	80 mg	single, oral	7.99	95	0.40
ondansetron	0.47	293.4	8 mg	single, oral	0.13	75	0.03
orphenadrine	0.97	269.4	100 mg	single, oral	0.78	20	0.62
oseltamavir	0.99	312.4	400 mg	multiple, oral	3.52	3	3.42
oxcarbazepine	1	252.3	300 mg	single, oral	20.49	40	12.29
pergolide	0.99	314.5	0.138 mg	single, oral	5.72	90	0.57
praziquantel	0	312.4	50 mg/kg	single, oral	20.17	85	3.02
pregabalin	0	159.2	300 mg	single, oral	56.46	0	56.46
procainamide	0.98	235.3	1000 mg	single, oral	22.52	15	19.15
procyclidine	0.99	287.4	10 mg	single, oral	0.42	95	0.02
pyrazinamide	0	123.1	3000 mg	single, oral	1088.55	50	544.27
quinine	0.98	378.5	600 mg	single, oral	7.61	70	2.28
rabeprazole	-0.01	359.4	20 mg	single, oral	1.13	96.3	0.04
raltegravir	-0.48	444.4	800 mg	multiple, oral	2.71	83	0.46
ranitidine	0.83	314.4	1600 mg	single, oral	6.31	15	5.36
rufinamide	0	238.2	1600 mg	multiple, oral	28.76	34	18.98
salsalate	-1	258.2	1000 mg	single, oral	81.33	18	66.69
stavudine	0	224.2	40 mg	single, oral	5.42	0	5.42
streptomycin	2.8	581.6	250 mg	single, oral	3.95	65	1.38
telebivudine	0	242.2	120 mg	multiple, oral	15.27	3	14.82
thalidomide	0	258.2	200 mg	single, oral	7.75	66	2.63
topiramate	0	339.4	400 mg	single, oral	22.69	17	18.83
tramadol	0.99	263.4	200 mg	single, oral	2.45	20	1.96
trimethoprim	0.36	290.3	300 mg	single, oral	32.72	70	9.82
valacyclovir	0.54	324.3	1000 mg	single, oral	17.45	18	14.33
valproate	-0.99	144.2	500 mg	single, oral	511.79	90	51.18
vigabatrin	0	129.2	1000 mg	single, oral	236.07	0	236.07
voriconazole	0	349.3	6 mg/kg	multiple, oral	6.74	58	2.83
zonisamide	0	212.2	800 mg	single, oral	10.84	40	6.50

Maximum plasma concentrations (C_{max}) of test inhibitors in human subjects and percent

protein binding in human plasma were obtained from literature sources (19; 20).

7.4).   These criteria were used to maximize the identification of a clinically potent inhibitor of renal organic cation transporters.   The selected drugs span across various therapeutic classes (e.g., antibiotic, antiulcer, antiarrhythmic, antiemetic) and are also variable in terms of the fraction of a dose that is eliminated as unchanged drug in the urine.

Of these 73 drugs, 4 (naloxone, quinine, procainamide and praziquantel) were inhibitors of OCT2, 10 (trimethoprim, cimetidine, ranitidine, moxifloxacin, chlorphenesin, quinine, clofazimine, abacavir, norfloxacin and ondansetron) were inhibitors of MATE1 and 11 (trimethoprim, chlorphenesin, cimetidine, ranitidine, moxifloxacin, norfloxacin, procainamide, ondansetron, famiciclovir, nizatidine and quinine) were inhibitors of MATE2K, with $\geq$ 50% inhibition of $[^{14}C]$-metformin uptake at 10x $C_{max,u}$ (Figure 1a-c). These compounds (n=15) were then assessed for their potential to inhibit other organic cation transporters, OCT1 and OCT3, that are not thought to play a significant role in renal drug elimination.

Interestingly, metformin uptake increased ($\geq$125%) in the presence of some drugs, particularly in both MATE1 and MATE2K-transfected cells for amantidine, ampicillin, auranofin, diphenhydramine, diphenidol, doxepin, indapamide, vigabatrin and in all three OCT2, MATE1 and MATE2K-transfected cells for ganciclovir, procyclidine, streptomycin. Similarity analysis of the physicochemical properties of the drugs that enhanced metformin uptake and chemical structures did not reveal any common characteristics of the compounds that enhanced metformin uptake.

Figure 1a
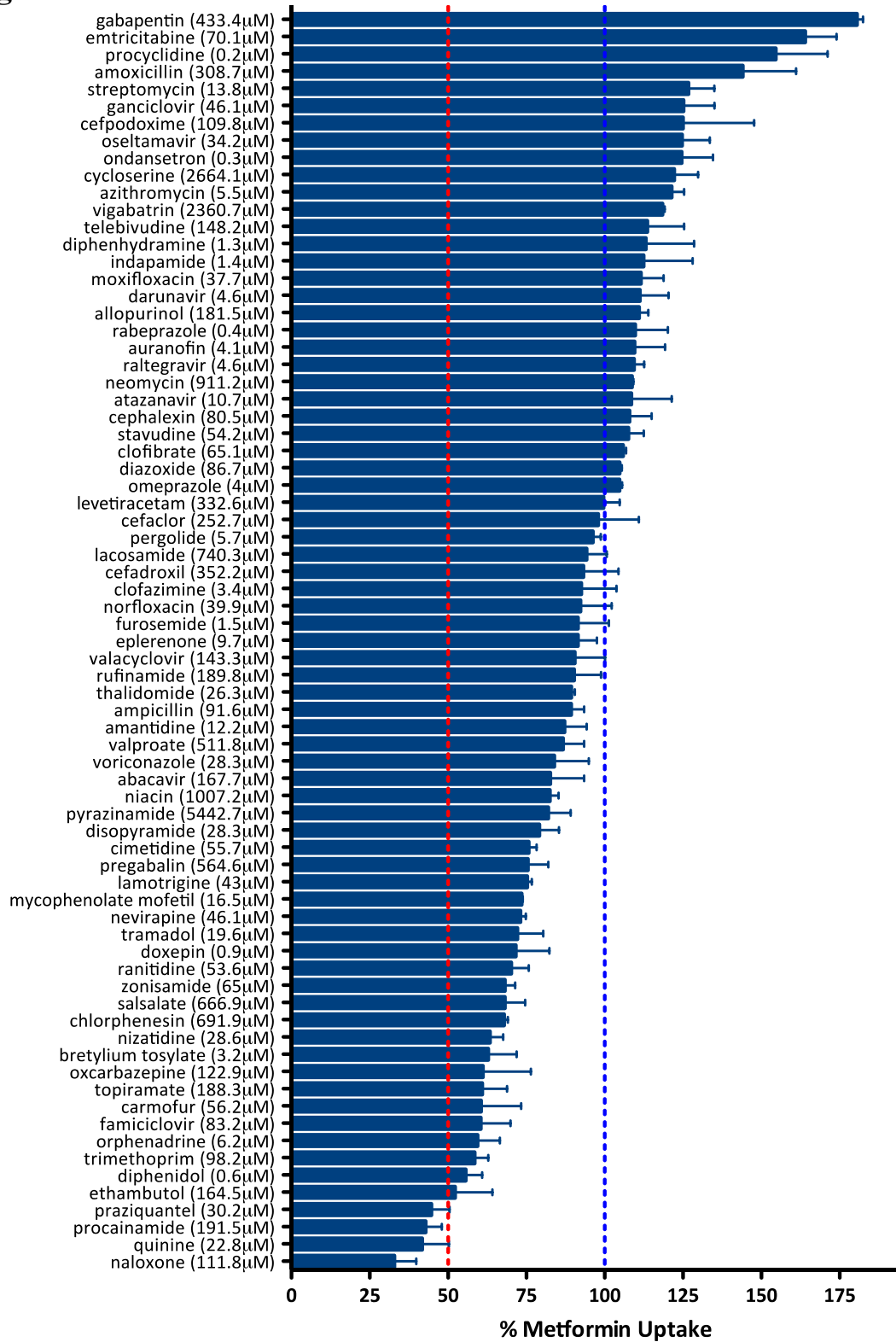
Figure 1b
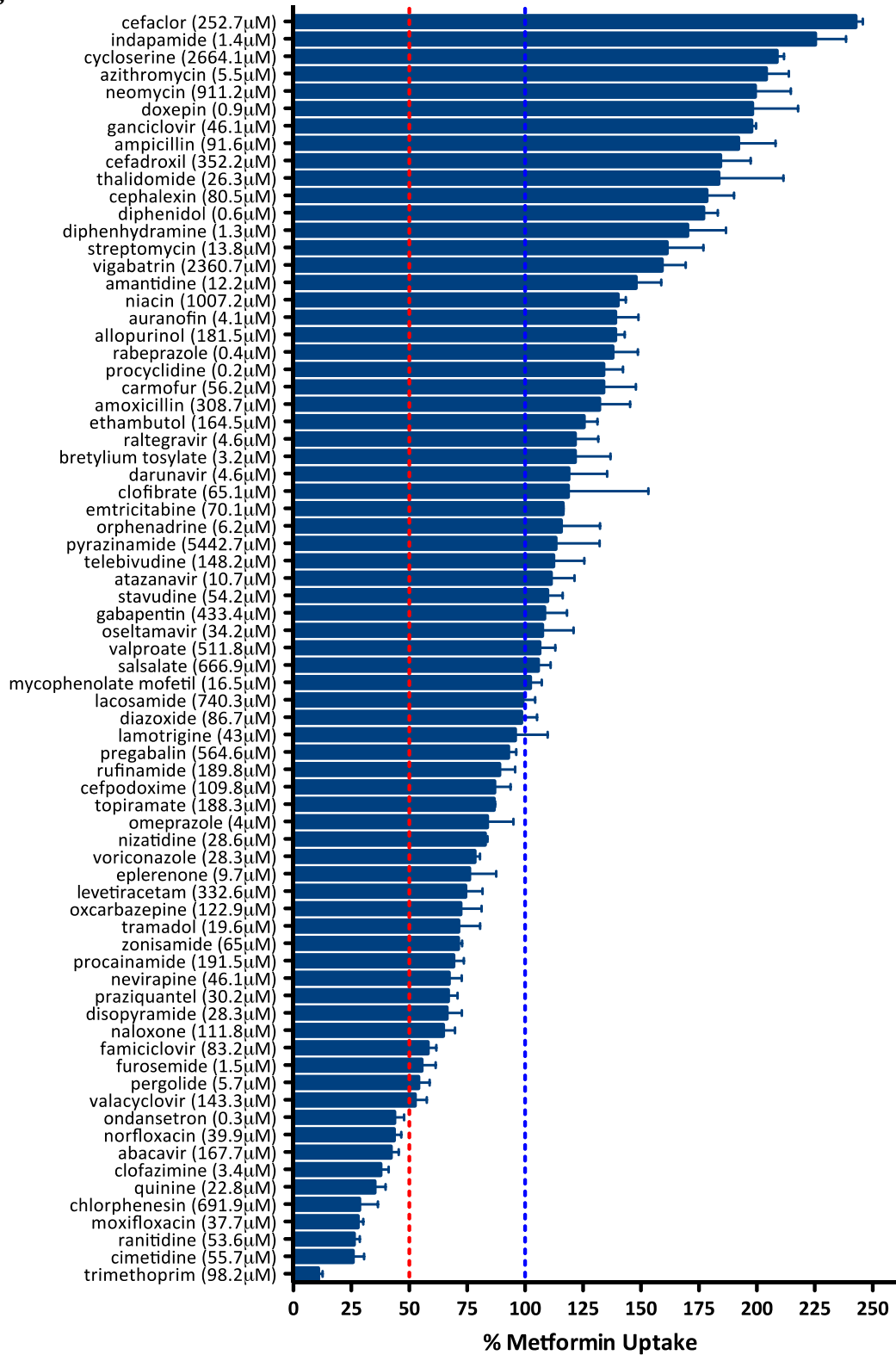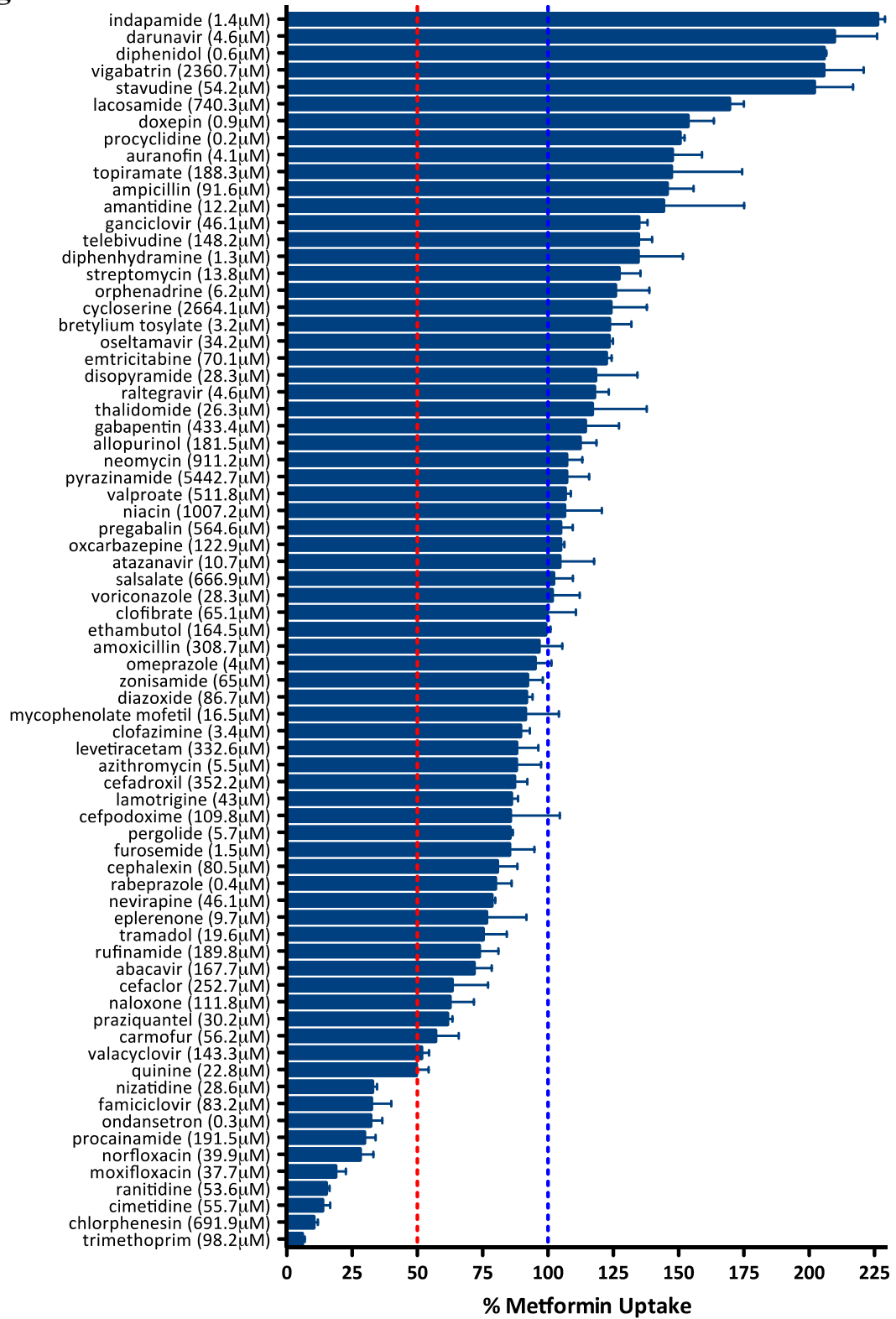
Figure 1c

Figure 1. Potency of test inhibitors of renal organic cation transporters at clinically relevant concentrations. Results of single-concentration cell-based inhibition screen at the 10x candidate inhibitor's own $C_{max,u}$ (tested concentration in parentheses) against cells stably expressing (a) OCT2, (b) MATE1 and (c) MATE2K. The blue and red lines are drawn at 100% (no inhibitor) and 50% [^{14}C]-metformin uptake, respectively. Drugs that display $\leq$ 50% of metformin uptake at 10x their $C_{max,u}$ were classified as clinically potent inhibitors and were further characterized for selectivity. Data are presented as mean $\pm$ SD (samples in triplicate from one experiment).

Selectivity of Potent Renal Organic Cation Transporter Inhibitors Against Non-Renal Organic Cation Transporters

Based on the OCT2, MATE1 and MATE2K screening results, inhibitors were grouped as follows: (*i*) OCT2-selective (naloxone and praziquantel), (*ii*) MATE1-selective (abacavir and clofazimine), (*iii*) MATE2K-selective (famiciclovir and nizatidine), (*iv*) MATE1/MATE2K dual inhibitors (chlorphenesin, cimetidine, moxifloxacin, norfloxacin, ondansetron, ranitidine and trimethoprim) and (*v*) OCT2/apical inhibitors (procainamide and quinine). These compounds were then tested for their ability to inhibit the non-renal organic cation transporters, OCT1 and OCT3. Of these compounds, moxifloxacin, norfloxacin, ondansetron, ranitidine and nizatidine did not inhibit OCT1- or OCT3-mediated metformin uptake at their 10x $C_{max,u}$ (metformin uptake was >50%) and were therefore designated as selective inhibitors of the renal organic cation transporters (Figure 2a-e). These drugs were then subjected to follow-up IC_{50} determinations against six known metformin transporters (OCT1, OCT2, OCT3, MATE1, MATE2K and PMAT).

The inhibition curves and calculated IC_{50} values of moxifloxacin, norfloxacin, ondansetron, ranitidine and nizatidine are shown in Figure 3a-e and Table 2, respectively. Of note, moxifloxacin, norfloxacin and ondansetron, were identified as potential clinical inhibitors of both MATE1 ($C_{max,u}/IC_{50}$ is 0.74, 0.20 and 0.62, respectively) and MATE2K ($C_{max,u}/IC_{50}$ is 3.16, 0.84 and 0.39, respectively), but not of any of the other metformin transporters. In addition, nizatidine was identified as a potent and selective inhibitor of MATE2K ($C_{max,u}/IC_{50}$=0.37), but not of any of the other metformin transporters. Although ranitidine was a potent inhibitor of MATE1 and MATE2K-mediated transport,

Figure 2a
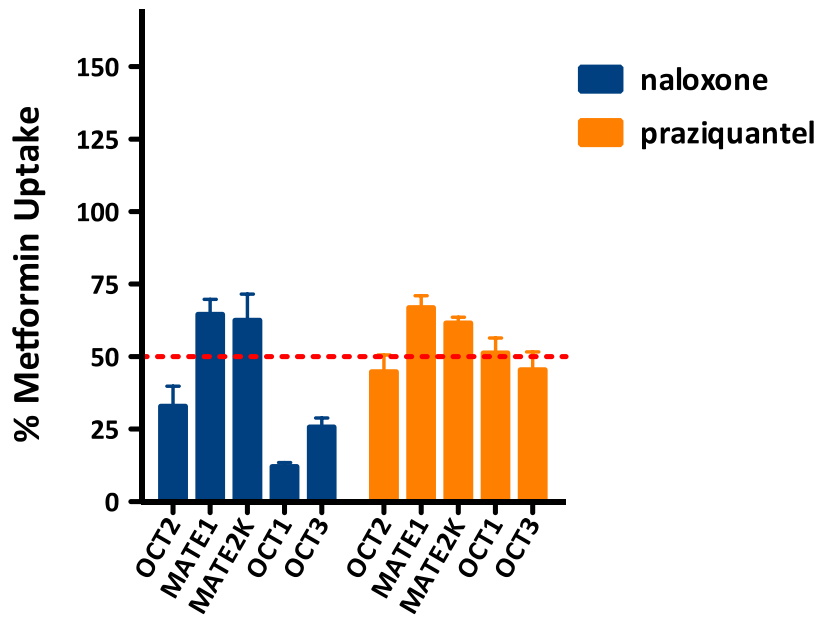
Figure 2b
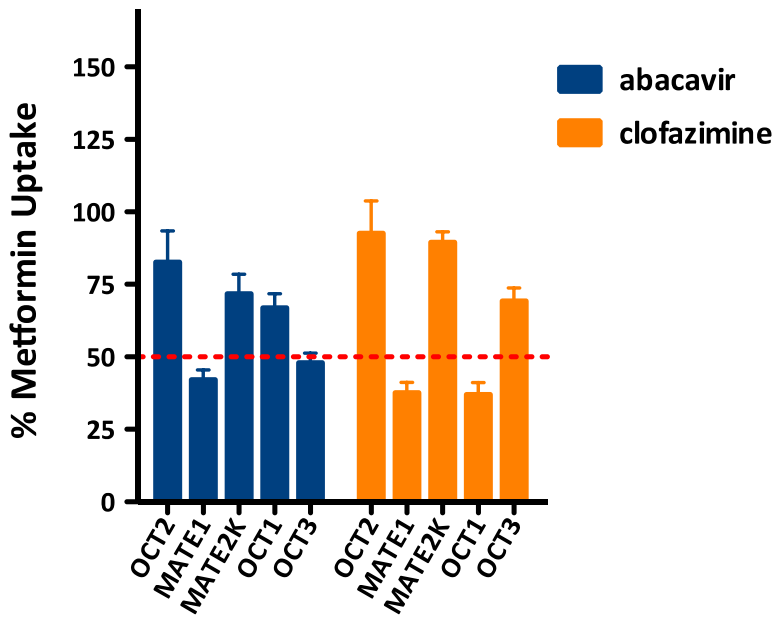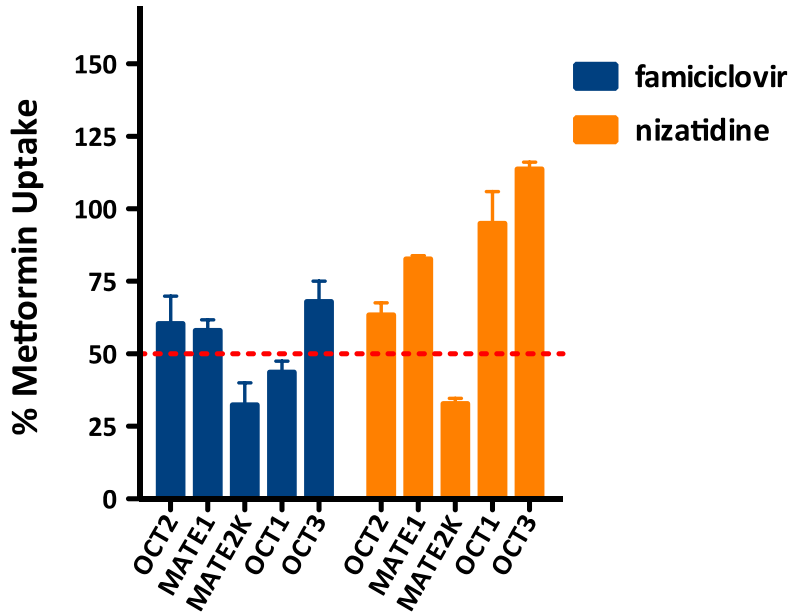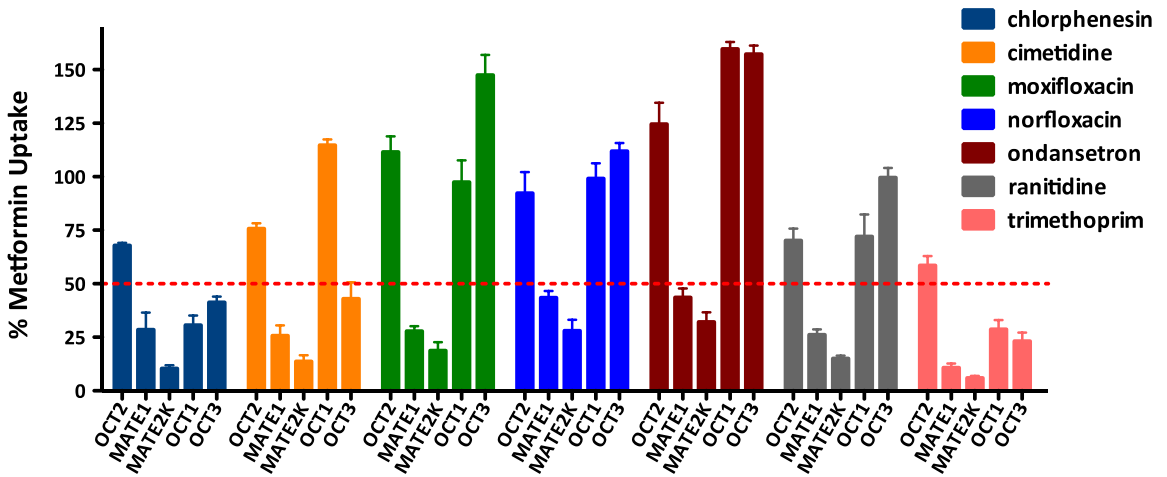
Figure 2c
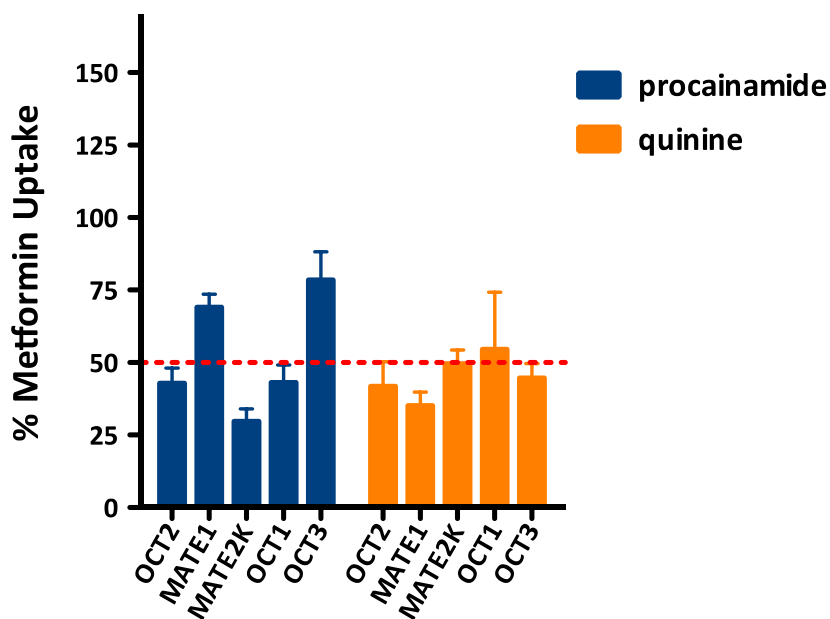
Figure 2d

Figure 2e

Figure 2. Selectivity of top OCT2, MATE1 and MATE2K inhibitors against non-renal organic cation transporters at clinically relevant concentrations. (a) OCT2-selective, (b) MATE1-selective, (c) MATE2K-selective, (d) MATE1/MATE2K-dual inhibitors and (e) OCT2/apical inhibitors from Figure 1 were tested for their ability to inhibit OCT1 and OCT3 at a concentration of 10x their $C_{max,u}$. The red line is drawn at 50% of $[^{14}C]$-metformin uptake. Drugs that display $\geq$ 50% inhibition of metformin uptake at 10x their $C_{max,u}$ in OCT1 and OCT3-expressing cells were classified as selective and clinically potent inhibitors of renal organic cation transporters. Data are presented as mean $\pm$ SD (samples in triplicate from one experiment).

Figure 3a
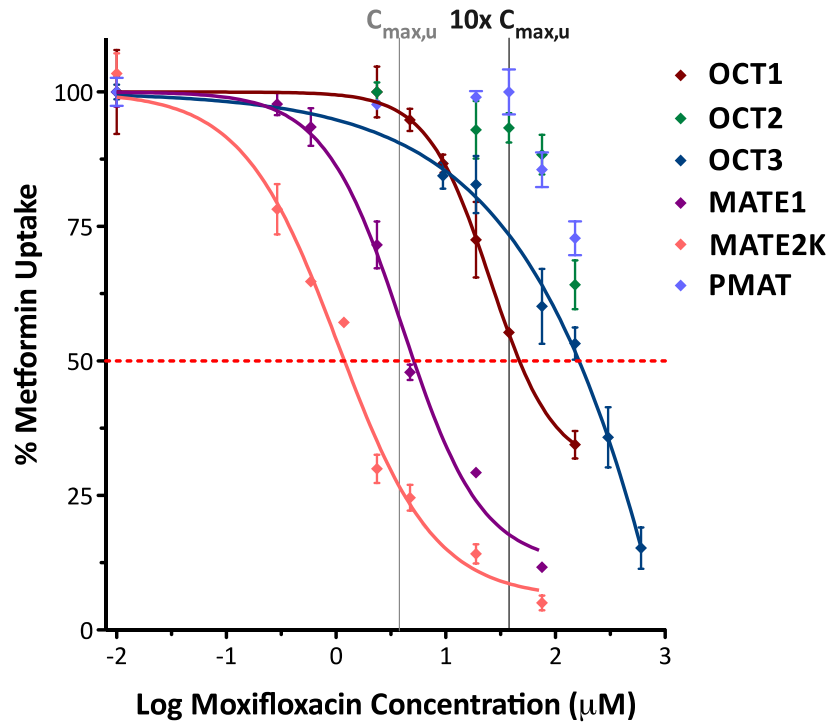
Figure 3b
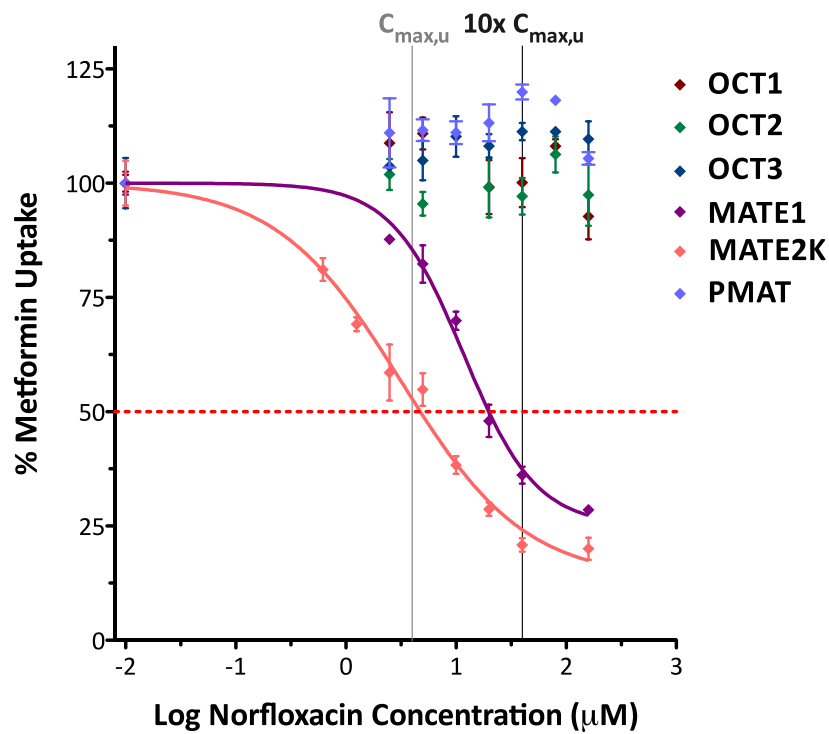
Figure 3c
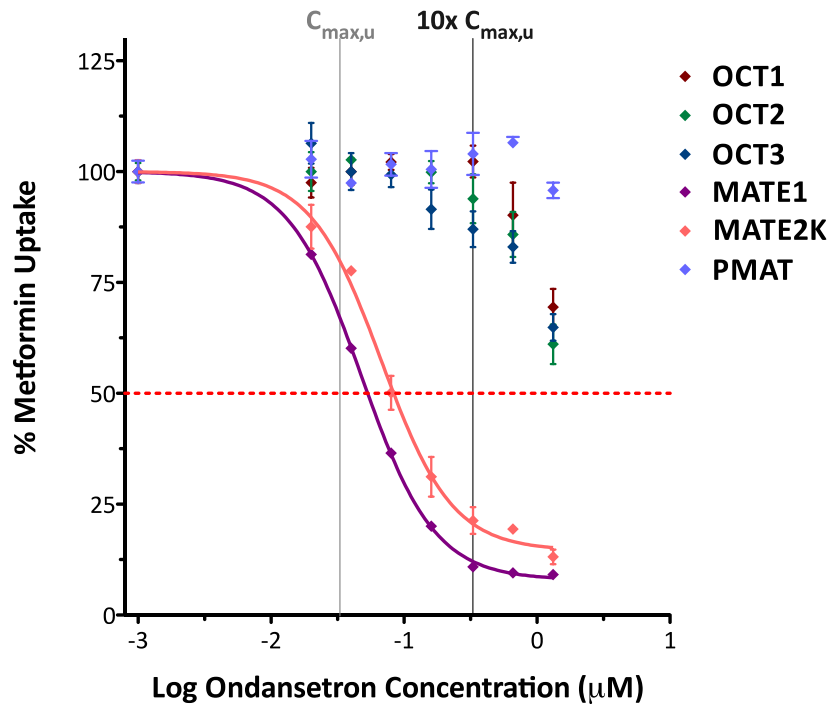
Figure 3d
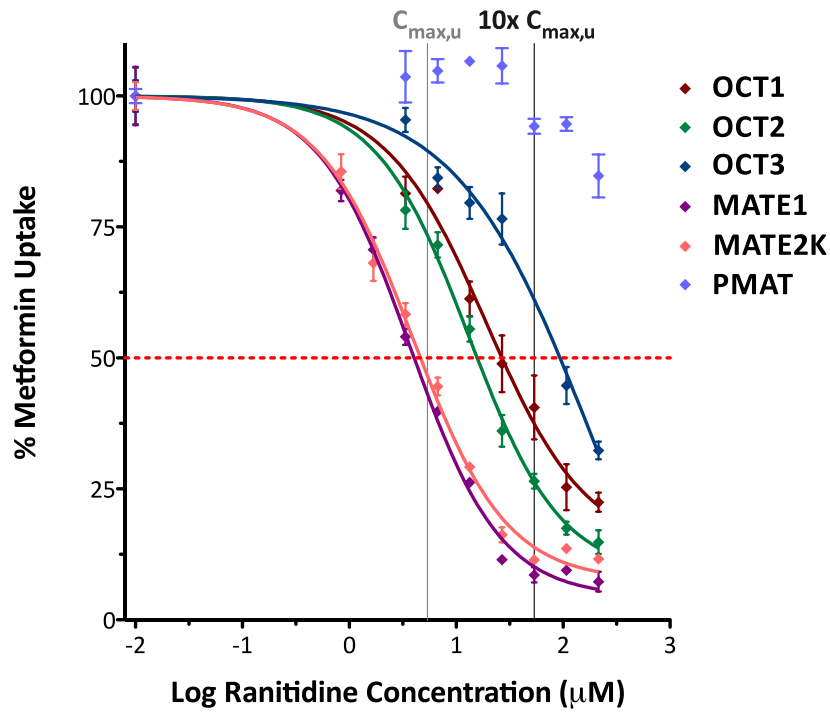
Figure 3e
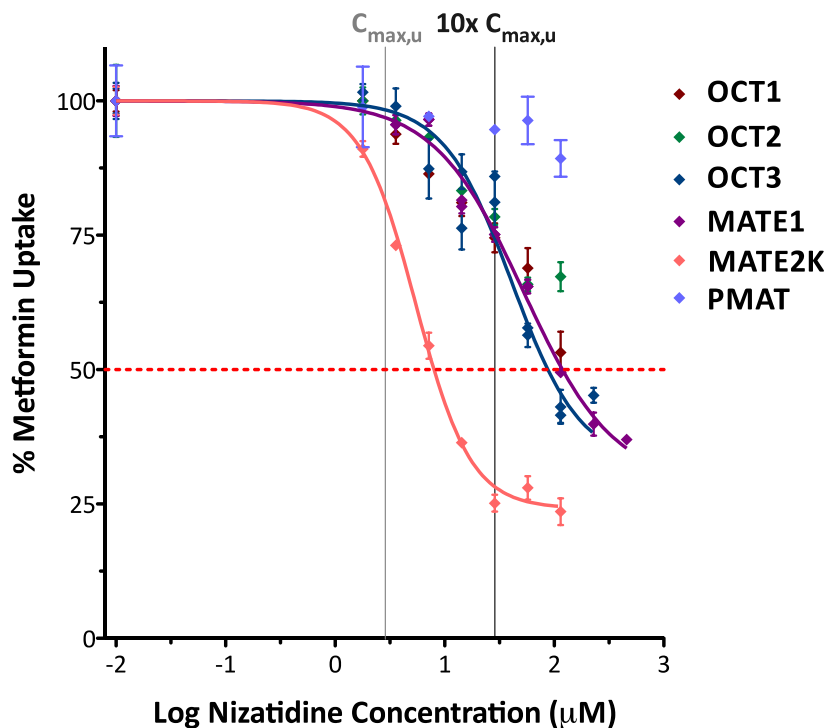
Figure 3. Determination of inhibition potency kinetics of top hits in organic cation transporter cell lines. Increasing concentrations of (a) moxifloxacin, (b) norfloxacin, (c) ondansetron, (d) ranitidine and (e) nizatidine were analyzed for their ability to inhibit metformin uptake in cells stably overexpressing OCT1, OCT2, OCT3, MATE1, MATE2K and PMAT. IC_{50} values were determined for drugs/cell lines that display $\geq$ 50% inhibition of metformin uptake (horizontal red line). The vertical grey and black lines are drawn at 1x and 10x the inhibitor's $C_{max,u}$, respectively. Values are presented as mean $\pm$ SEM (n = 3).

Table 2. *In vitro* **potency of putative clinical inhibitors in cells expressing OCT1, OCT2, OCT3, MATE1, MATE2K and PMAT.**

Drug	Parameter	OCT1	OCT2	OCT3	MATE1	MATE2K	PMAT
moxifloxacin	IC_{50} (µM)	47.0	>151	>151	5.12	1.19	>151
	C_{max}/IC_{50}	**0.13**	<0.04	<0.04	**1.22**	**5.22**	<0.04
	$C_{max,u}/IC_{50}$	0.08	<0.02	<0.02	**0.74**	**3.16**	<0.02
norfloxacin	IC_{50} (µM)	>160	>160	>160	19.9	4.74	>160
	C_{max}/IC_{50}	<0.03	<0.03	<0.03	**0.24**	**0.99**	<0.03
	$C_{max,u}/IC_{50}$	<0.02	<0.02	<0.02	**0.20**	**0.84**	<0.02
ondansetron	IC_{50} (µM)	>1.32	>1.32	>1.32	0.05	0.08	>1.32
	C_{max}/IC_{50}	<0.10	<0.10	<0.10	**2.47**	**1.57**	<0.10
	$C_{max,u}/IC_{50}$	<0.02	<0.02	<0.02	**0.62**	**0.39**	<0.02
ranitidine	IC_{50} (µM)	26.8	15.7	93.5	4.04	4.63	>214
	C_{max}/IC_{50}	**0.24**	**0.40**	0.07	**1.56**	**1.36**	<0.03
	$C_{max,u}/IC_{50}$	**0.20**	**0.34**	0.06	**1.33**	**1.16**	<0.02
nizatidine	IC_{50} (µM)	>115	>115	41.1	52.7	7.81	>115
	C_{max}/IC_{50}	<0.04	<0.04	**0.11**	0.08	**0.56**	<0.04
	$C_{max,u}/IC_{50}$	<0.02	<0.02	0.07	0.05	**0.37**	<0.02

C_{max} and calculated $C_{max,u}$ were obtained from literature values (see Table 1). C_{max}/IC_{50} and $C_{max,u}/IC_{50}$ values that exceed the 0.1 cut-off are bolded.

IC_{50}, concentration at half the maximum inhibition of active transport; C_{max}, maximum plasma concentration; $C_{max,u}$, maximum plasma concentration that is not bound to plasma proteins; OCT, organic cation transporter; MATE, multidrug and toxin extrusion transporter; PMAT, plasma membrane monoamine transporter.

it did not demonstrate selective inhibition of the two MATE transporters. In all cases, the Hill coefficient was greater than one, suggesting positive cooperativity where more than one binding site is involved in the inhibition.

DISCUSSION

Historically, DDIs are thought to be mediated primarily by interactions with drug-metabolizing enzymes. Current evidence suggests a role for drug transporters in mediating clinical DDIs (7; 21), however these types of DDIs are less well characterized. Further, as noted previously, prior to this study, few inhibitors of renal organic cation transporters have been identified and of these inhibitors none has been shown to selectively inhibit metformin uptake or efflux that is mediated by only one of the renal organic cation transporters. In this study, a strategic screen was implemented to quickly identify probe inhibitors of renal organic cation transport that are selective and can inhibit transport at clinically relevant concentrations.

In general, inhibition of renal organic cation transport at either membrane would result in a reduced renal clearance of metformin. However, depending on whether an inhibitor affects the transport of a drug at the basolateral or apical membrane, the drug's toxicity and/or pharmacodynamics may be affected. For example, for a drug with a mechanism of action that is dependent upon its transport into the renal cell, blocking the OCT2-mediated uptake into the kidney would potentially reduce its access to its pharmacological target and therefore, its pharmacological effect. In contrast, if MATE1 or MATE2K is inhibited, there would be increased levels of the drug within the renal cell,

resulting in enhanced pharmacologic effects or, in some cases, enhanced renal toxicities. Therefore, it is important to identify probe inhibitors of each transporter that are selective and inhibit at clinically relevant concentrations.

In this study, 15 of 73 drugs that were screened were identified as inhibitors of one or more renal organic cation transporters, with IC_{50} values $< 10x$ times their $C_{max,u}$, the cutoff that the FDA and the International Transporter Consortium have recommended to follow up with a clinical DDI study (7; 8). Furthermore, we have identified moxifloxacin, norfloxacin and ondansetron as potential clinical inhibitors of both MATE1 (IC_{50} equals 1.4x, 5.0x and 1.6x $C_{max,u}$, respectively) and MATE2K (IC_{50} equals 0.3x, 1.2x and 2.6x $C_{max,u}$, respectively), but not any of the other organic cation transporters. Nizatidine was identified as a clinically potent and selective inhibitor of MATE2K (IC_{50} equals 2.7x $C_{max,u}$) and may be useful to understand the clinical significance of MATE2K-mediated drug elimination.

Surprisingly, some drugs appeared to stimulate metformin uptake in OCT2, MATE1 and MATE2K-transfected cells. However, these effects could not be explained by any physicochemical property (e.g., molecular weight, hydrogen bond donors, predicted charge at physiological pH) or chemical structure (data not reported). This enhancement of *in vitro* activity has also been observed for a variety of solute carrier and ATP-binding cassette transporters including the organic anion transporter, OAT1, multidrug resistance-associated protein, MRP2, organic anion-transporting polypeptide, OATP1B1/1B3, and OCT2 (22-26). Previous reports have observed the dependence of OCT2-mediated

transport on inside-negative membrane potential (27; 28). Interestingly, OCT2-mediated uptake of metformin was increased by ~2-fold in the presence of gabapentin, a gamma-aminobutyric acid (GABA) analog that is known to alter calcium currents. This suggests that alterations in membrane potential by test compounds may explain an enhancement in metformin uptake. In our data set, the enhancement in metformin uptake was more common in MATE-transfected cell lines, which are particularly affected by alterations in pH as they transport organic cations in exchange for a proton (29-31). Therefore, it is possible that the test compounds could have altered the pH gradient, leading to an enhancement in metformin uptake by MATE-transfected cells. Enhancement in OCT2- and MATE-mediated metformin uptake in the presence of test compounds may also be explained by interaction of the test compound with an allosteric binding site. In this mechanism, a test compound could bind to an allosteric site that could in turn alter the transporter's structure, and enhance its affinity for metformin.

In this study, we used a medium-throughput cell based screen with cells that overexpress each organic cation transporter to measure the *in vitro* inhibition kinetics of test compounds. Utilizing this method, we were able to quickly decipher the potential for inhibition of each transporter at clinically relevant concentrations. However, additional studies with cellular systems (e.g. primary proximal tubular cells, OCT2/MATE dual-transfected polarized cells) may help to understand the potential interplay of multiple transport and metabolism processes. In addition, these studies were performed without preincubation of the test compound prior to initiation of uptake, which may not be applicable to clinical situations where the perpertrator drug concentrations are at steady-

state prior to administration of the victim drug and may not identify mechanism-based inhibitors. Therefore, it is possible that the potential for a drug interaction with metformin and the identified inhibitors may have been underestimated by this *in vitro* study design.

Using clinical pharmacokinetic literature and a cell-based screening assay, candidate inhibitors of renal organic cation transport that inhibit at clinically relevant concentrations were identified. This design represented an innovative screening strategy focused on clinically relevant unbound drug concentrations rather than using a single drug concentration for all compounds, which may not be clinically relevant. From this screen, moxifloxacin (antibacterial), norfloxacin (antibacterial), ondansetron (antiemetic) and nizatidine (antiulcer) were identified as selective and potent renal organic cation transport inhibitors and have the potential to cause clinically relevant DDIs. While these drugs are not used to treat typical comorbidities of type II diabetes (e.g. hypertension), they may be co-prescribed with metformin for patients with bacterial infections, nausea and ulcers. In these patients, the disposition and response of metformin may be altered. Clinical studies are necessary to investigate the effect of these selective inhibitors on the renal clearance of metformin and other organic cation xenobiotics in human subjects.

REFERENCES

1.   Kusuhara H, Ito S, Kumagai Y, Jiang M, Shiroshita T, et al. 2011. Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at

oral microdose and at therapeutic dose in healthy subjects. *Clin Pharmacol Ther* 89:837-44

2.   Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. 1987. Reduction of metformin renal tubular secretion by cimetidine in man. *Br J Clin Pharmacol* 23:545-51

3.   Ito S, Kusuhara H, Kuroiwa Y, Wu C, Moriyama Y, et al. 2010. Potent and specific inhibition of mMate1-mediated efflux of type I organic cations in the liver and kidney by pyrimethamine. *J Pharmacol Exp Ther* 333:341-50

4.   Tsuda M, Terada T, Ueba M, Sato T, Masuda S, et al. 2009. Involvement of human multidrug and toxin extrusion 1 in the drug interaction between cimetidine and metformin in renal epithelial cells. *J Pharmacol Exp Ther* 329:185-91

5.   Bourdet DL, Pritchard JB, Thakker DR. 2005. Differential substrate and inhibitory activities of ranitidine and famotidine toward human organic cation transporter 1 (hOCT1; SLC22A1), hOCT2 (SLC22A2), and hOCT3 (SLC22A3). *J Pharmacol Exp Ther* 315:1288-97

6.   Minematsu T, Iwai M, Umehara K, Usui T, Kamimura H. 2010. Characterization of human organic cation transporter 1 (OCT1/SLC22A1)- and OCT2 (SLC22A2)-mediated transport of 1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)- 4,9-dihydro-1H-naphtho[2,3-d]imidazolium bromide (YM155 monobromide), a novel small molecule survivin suppressant. *Drug Metab Dispos* 38:1-4

7.   Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, et al. 2010. Membrane transporters in drug development. *Nat Rev Drug Discov* 9:215-36

8.   *Guidance for Industry (Draft): Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm> Accessed May 2013

9.   Hillgren KM, Keppler D, Zur AA, Giacomini KM, Stieger B, et al. 2013. Emerging transporters of clinical importance: An update from the international transporter consortium. *Clin Pharmacol Ther* e-pub ahead of print

10. Zhang S, Lovejoy KS, Shima JE, Lagpacan LL, Shu Y, et al. 2006. Organic cation transporters are determinants of oxaliplatin cytotoxicity. *Cancer Res* 66:8847-57

11. Chen Y, Zhang S, Sorani M, Giacomini KM. 2007. Transport of paraquat by human organic cation transporters and multidrug and toxic compound extrusion family. *J Pharmacol Exp Ther* 322:695-700

12. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, et al. 2007. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 117:1422-31

13. More SS, Li S, Yee SW, Chen L, Xu Z, et al. 2010. Organic cation transporters modulate the uptake and cytotoxicity of picoplatin, a third-generation platinum analogue. *Mol Cancer Ther* 9:1058-69

14. Choi JH, Yee SW, Ramirez AH, Morrissey KM, Jang GH, et al. 2011. A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. *Clin Pharmacol Ther* 90:674-84

15. Kido Y, Matsson P, Giacomini KM. 2011. Profiling of a prescription drug library for potential renal drug-drug interactions mediated by the organic cation transporter 2. *J Med Chem* 54:4548-58

16. Minematsu T, Giacomini KM. 2011. Interactions of tyrosine kinase inhibitors with organic cation transporters and multidrug and toxic compound extrusion proteins. *Mol Cancer Ther* 10:531-9

17. Wittwer MB, Zur AA, Khuri N, Kido Y, Kosaka A, et al. 2013. Discovery of potent, selective multidrug and toxin extrusion transporter 1 (MATE1, SLC47A1) inhibitors through prescription drug profiling and computational modeling. *J Med Chem* 56:781-95

18. Zhou M, Xia L, Wang J. 2007. Metformin transport by a newly cloned proton-stimulated organic cation transporter (plasma membrane monoamine transporter) expressed in human intestine. *Drug Metab Dispos* 35:1956-62

19. Goodman LS, Brunton LL, Chabner B, Knollmann BC. 2011. *Goodman & Gilman's pharmacological basis of therapeutics*. New York: McGraw-Hill

20. Moffat AC, Osselton MD, Widdop B. 2004. *Clarke's analysis of drugs and poisons : in pharmaceuticals, body fluids and postmortem material*. London ; Chicago: Pharmaceutical Press

21. Lau YY, Huang Y, Frassetto L, Benet LZ. 2007. effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. *Clin Pharmacol Ther* 81:194-204

22. Pedersen JM, Matsson P, Bergstrom CA, Norinder U, Hoogstraate J, Artursson P. 2008. Prediction and identification of drug interactions with the human ATP-

binding cassette transporter multidrug-resistance associated protein 2 (MRP2; ABCC2). *J Med Chem* 51:3275-87

23. Vanwert AL, Srimaroeng C, Sweet DH. 2008. Organic anion transporter 3 (oat3/slc22a8) interacts with carboxyfluoroquinolones, and deletion increases systemic exposure to ciprofloxacin. *Mol Pharmacol* 74:122-31

24. Kindla J, Muller F, Mieth M, Fromm MF, Konig J. 2011. Influence of non-steroidal anti-inflammatory drugs on organic anion transporting polypeptide (OATP) 1B1- and OATP1B3-mediated drug transport. *Drug Metab Dispos* 39:1047-53

25. Karlgren M, Vildhede A, Norinder U, Wisniewski JR, Kimoto E, et al. 2012. Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): influence of protein expression on drug-drug interactions. *J Med Chem* 55:4740-63

26. Mulgaonkar A, Venitz J, Grundemann D, Sweet DH. 2013. Human organic cation transporters 1 (SLC22A1), 2 (SLC22A2), and 3 (SLC22A3) as disposition pathways for fluoroquinolone antimicrobials. *Antimicrob Agents Chemother* 57:2705-11

27. Busch AE, Karbach U, Miska D, Gorboulev V, Akhoundova A, et al. 1998. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol Pharmacol* 54:342-52

28. Dudley AJ, Bleasby K, Brown CD. 2000. The organic cation transporter OCT2 mediates the uptake of beta-adrenoceptor antagonists across the apical membrane of renal LLC-PK1 cell monolayers. *Br J Pharmacol* 131:71-9

29. Masuda S, Terada T, Yonezawa A, Tanihara Y, Kishimoto K, et al. 2006. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol* 17:2127-35

30. Terada T, Masuda S, Asaka J, Tsuda M, Katsura T, Inui K. 2006. Molecular cloning, functional characterization and tissue distribution of rat H+/organic cation antiporter MATE1. *Pharm Res* 23:1696-701

31. Tsuda M, Terada T, Asaka J, Ueba M, Katsura T, Inui K. 2007. Oppositely directed H+ gradient functions as a driving force of rat H+/organic cation antiporter MATE1. *Am J Physiol Renal Physiol* 292:F593-8

CHAPTER 4

THE EFFECT OF NIZATIDINE, A MATE2K SELECTIVE INHIBITOR, ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF METFORMIN IN HEALTHY VOLUNTEERS

INTRODUCTION

In the proximal tubule of the kidney, basic drugs are transported from the renal cells to the tubule lumen through the concerted action of the H^+/organic cation antiporters, multidrug and toxin extrusion 1 (MATE1) and 2K (MATE2K). Dual inhibitors of the MATE transporters (e.g., cimetidine, pyrimethamine) have previously been shown to have a clinical impact on the pharmacokinetics of concomitantly administered organic cations (e.g. metformin, procainamide, ranitidine) (1-4). However, the clinical impact of selective MATE1 or MATE2K inhibition on the pharmacokinetics and pharmacodynamics of basic drugs is unknown.

MATE2K is believed to be an important renal transporter for many drugs. In comparison to MATE1, which is expressed in multiple tissues (e.g., kidney, liver, muscle), MATE2K is predominately expressed in the kidney (5), and at equivalent or higher levels than MATE1 (S.W. Yee, A. Chhibber, D.L. Kroetz and K.M. Giacomini, unpublished data). MATE2K also specifically transports some drugs (e.g., oxaliplatin), which do not appear to be substrates of MATE1 (6; 7). Studies from our laboratory have shown that a common MATE2K promoter variant (g.-130G>A, rs12943590) is associated with poor response to the biguanide, metformin in type II diabetic subjects (8). Taken together,

these data suggest that MATE2K is important for the renal elimination of many basic drugs.

As transporter-mediated drug-drug interactions occur in clinical situations and have an impact on pharmacokinetics and drug safety, regulatory agencies in the United States and the European Union have issued guidances on when to conduct a clinical drug-drug interaction (DDI) study. The US Food and Drug Administration (FDA) recommends that a clinical investigation of a transporter-mediated drug interaction should be conducted when the I_{fu}/IC_{50} ratio (maximum plasma concentration of the inhibitor that is not bound to plasma proteins divided by the concentration associated with half the maximum inhibition in an *in vitro* assay) of the new molecular entity is greater than 0.1 (9). The European Medicines Agency (EMA) guidance is more stringent with a clinical study initiation cut-off of greater than 0.02 (10). Although the current guidances focus primarily on the uptake transporters in the kidney (organic cation transporter 2 and organic anion transporters 1 and 3), the EMA and a recent publication from the International Transporter Consortium (ITC) recommends to extend these guidelines for MATE-mediated drug interactions (11).

Through *in vitro* assays we have identified the histamine 2 antagonist, nizatidine, as a selective inhibitor of MATE2K-mediated transport (Chapter 3). At a 150 mg single oral dose, the I_{fu}/IC_{50} ratio is 0.37 (Chapter 3), above the cut-off for when a clinical DDI investigation is recommended. The goal of this study is to determine the clinical impact of selective MATE2K transport inhibition on the exposure and response of metformin in

healthy volunteers. To maximize the potential for a clinical impact of MATE2K inhibition by nizatidine, a 600 mg oral dose of nizatidine (the maximum recommended daily dose) was administered to healthy volunteers. The hypotheses of this study were (*i*) the coadministration of metformin and nizatidine will reduce the renal clearance (CL_R) of metformin, increase metformin kidney levels and potentially lead to increased plasma concentrations and (*ii*) the interaction will enhance the hypoglycemic activity of metformin.

MATERIALS AND METHODS

Subjects and Study Design

This was an open-label, randomized, two-treatment crossover study conducted in healthy subjects (n=12; n=6, each sex) at the General Clinical Research Center (GCRC) at San Francisco General Hospital (SFGH). The committee on Human Research at the University of California, San Francisco (Institutional Review Board (IRB) 11-06968) approved this study and all subjects were recruited directly from the Study of Pharmacogenetics in Ethnically Diverse Populations (IRB 10-03167). To be eligible for this study, volunteers had to provide written informed consent, be between the ages of 18 and 45 years, not be on any medications other than oral contraceptives and not have any known allergies to iodine. Screening included a comprehensive medical history, physical examination and laboratory studies (complete blood count, electrolytes, BUN and creatinine, albumin, and liver enzymes). Volunteers with elevated liver enzymes, anemia, elevated creatinine concentrations or a positive pregnancy test were excluded.

Once enrolled, volunteers were asked to follow a controlled carbohydrate diet (200-250 g/day) 3 days prior to each of their inpatient visits. Subjects were admitted to the clinical facility the night before the first dose and remained there for the duration of the study (36 hours). After an overnight fast (10 hours), study participants received either (*i*) an 850 mg oral dose of metformin (Glucophage®) or (*ii*) a simultaneous 850 mg oral dose of metformin and a 600 mg oral dose of nizatidine (Axid®). Each study participant received both treatments separated by a minimum of 7 days and the order of treatment was determined by randomization. A 3-hour oral glucose tolerance test (OGTT, 75 g) was conducted 2 hours after metformin dosing (with or without nizatidine). Baseline OGTT's, without metformin, were obtained from the same volunteers in a previous study (12). Standardized meals were provided on both study days after completion of the OGTT. Following the metformin dose, volunteers were asked to drink 8 oz of water every 2 hours to maintain urine flow and pH. At 10 hours after metformin dosing, a 1500 mg dose of iohexol (Omnipaque®) was administered by slow IV push for 5 minutes followed by flushing the IV line with 10 mL of normal saline.

Timed blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, 13, 14, 16, 22 and 24 post metformin dosing for the determination of plasma metformin and nizatidine concentrations. Additional blood samples were collected at 10, 12, 12.5, 13, 13.5 and 14 hours post metformin dosing for the determination of iohexol clearance. For metformin pharmacodynamics, blood samples were collected at 0, 15, 30, 45, 60, 90, 120 and 180 min after glucose administration. An additional blood sample was collected at 12 hours after the second dose of metformin to determine serum creatinine. Urine

samples were collected during the following time intervals: 0-2, 2-4, 4-8, 8-12 and 12-24 hours after metformin dosing for the calculation of metformin and creatinine renal clearances.

Analytical Methods

Metformin concentrations in plasma and urine were assayed by a validated liquid chromatography-tandem mass spectrometry method (13). Nizatidine plasma concentrations were measured in tandem using the transitions m/z 332.29 to 58.08. Both the intra-day and inter-day coefficients of analysis variation were <5%. Iohexol concentrations in plasma were measured by University of Minnesota Physicians Outreach Labs (Minneapolis, MN). Lactate and glucose concentrations in plasma were analyzed by ARUP laboratories (Salt Lake City, UT). Creatinine concentrations in plasma and urine were measured by the clinical laboratories of SFGH.

Data Analysis

Clinical Pharmacokinetics

The concentration-time curves of metformin and nizatidine were plotted using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). The pharmacokinetic parameters of metformin, nizatidine and iohexol were determined by non-compartmental analysis (WinNonlin 4.1, Pharsight Corporation, Mountain View, CA).

Statistical Analysis

Using previously reported metformin pharmacokinetic data in healthy volunteers (14), a sample size of 12 was needed to detect a 30% difference in metformin's renal clearance between the two treatments with >80% power. Data are presented as mean ± SD unless indicated otherwise. Paired nonparametric Student's *t*-tests were used to analyze the differences in metformin pharmacokinetic and pharmacodynamic parameters using GraphPad Prism 4.0. A statistically significant result was defined when $p < 0.05$.

RESULTS

Study Population

Healthy male and female African American (n=6), Asian (n=3) and Caucasian (n=3) subjects were randomized to treatment arms. All 12 subjects completed the study and no adverse events were reported. The subject demographics of the healthy volunteers are shown in Table 1.

Pharmacokinetics of Nizatidine

To verify that nizatidine achieved high enough concentrations predicted to alter MATE2K activity, the pharmacokinetics of nizatidine were determined after a single 600 mg dose (Figure 1, Table 2). Nizatidine reached a C_{max} of 4.2 ± 0.3 µg/mL (12.7 µM) and with 35% protein binding (15), its calculated $C_{max,u}$ was 2.7 ± 0.2 µg/mL (8.2 µM). This value is greater than its *in vitro* MATE2K inhibition potency ($C_{max,u}/IC_{50}=1.1$) and above the recommended cut-off for conducting a clinical DDI study.

Pharmacokinetics of Metformin

Table 1. Demographic characteristics of healthy volunteers (n=12)

Characteristic	Value
Age (years)	28 (22 - 33)
Weight (kg)	74 (49 - 101)
Height (cm)	176 (160 - 192)
BMI (kg/m^2)	23.9 (18.1 - 28.6)
eGFR (mL/min)	114 (89 - 143)

Data presented as mean (range).

BMI, body mass index; eGFR, estimation of glomerular filtration rate as assessed by MDRD (Modification of Diet in Renal Disease; equation is listed in Chapter 1, Table 4) at screening.
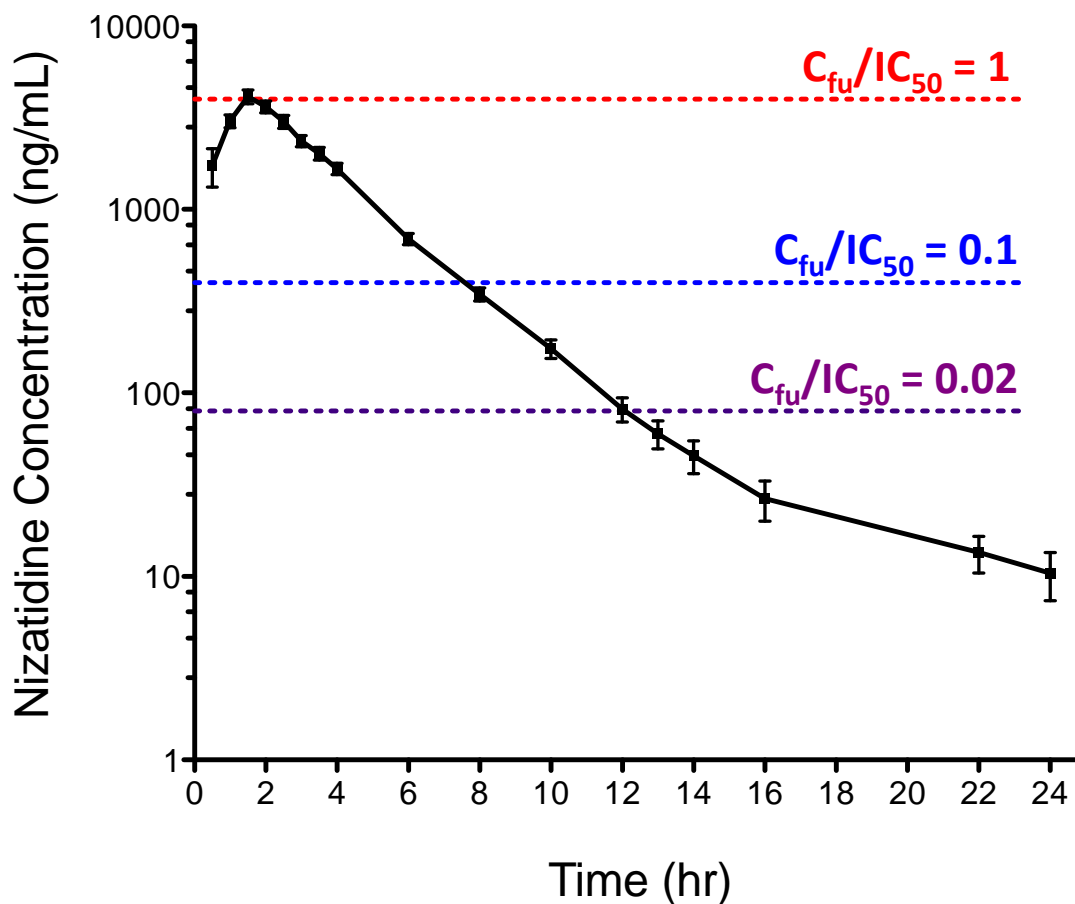
Figure 1. **Mean nizatidine plasma concentrations following administration of a single oral dose to 12 healthy volunteers.** Nizatidine plasma concentrations were determined after a 600 mg single oral dose in combination with metformin. The red, blue and purple horizontal lines are drawn at C_{fu}/IC_{50} ratios of 1, 0.1 (FDA cut-off) and 0.02 (EMA cut-off), respectively. Data represent mean ± SEM.

C_{fu}, unbound concentration in plasma; IC_{50}, concentration at half the maximum inhibition of active transport (determined in Chapter 3); FDA, Food and Drug Administration; EMA, European Medicines Agency

Table 2. Summary of the pharmacokinetic parameters of nizatidine after a single 600 mg oral dose in healthy volunteers (n=12).

Parameter	mean ± SD
t_{max} (h)	1.9 ± 0.5
C_{max} (µg/mL)	4.2 ± 1.0
AUC_{0-24} (µg*h/mL)	14.1 ± 1.8
AUC_{inf} (µg*h/mL)	14.1 ± 1.8
V/F (L)	168 ± 48
CL/F (mL/min)	716 ± 80
$t_{1/2}$ (h)	2.8 ± 0.9

t_{max}, time to the maximal plasma concentration; C_{max}, maximal plasma concentration; AUC_{0-24}, area under the concentration-time curve from 0-24 h time point; AUC_{inf}, area under the concentration-time curve from 0 to infinity; V/F, apparent volume of distribution; CL/F, apparent oral clearance; $t_{1/2}$, plasma terminal elimination half-life

Plasma concentration-time profiles for metformin were similar following administration of metformin alone or with nizatidine (Figure 2). When nizatidine was co-administered, metformin's V/F (apparent volume of distribution) and $t_{1/2}$ (half-life) increased by 38% and 24%, respectively ($p < 0.05$, Table 3). V/F and $t_{1/2}$ values for metformin with and without nizatidine are shown in Figure 3. The co-administration of nizatidine had no significant effect on metformin t_{max} (time to the maximal plasma concentration), C_{max} (maximal plasma concentration), AUC_{0-24} (area under the concentration-time curve from 0-24 h time point), AUC_{inf} (area under the concentration-time curve from 0 to infinity), CL/F (apparent oral clearance), CL_R (renal clearance) and CL_{SR} (renal clearance by secretion) (Figure 2, Table 3).

Evaluation of Alterations in Metformin-Mediated Toxicty With Nizatidine Co-Administration

A rare, but serious adverse effect associated with metformin is lactic acidosis (16), where plasma lactate levels exceed 5 mM. With nizatidine co-administration, there was no difference in plasma lactate levels in comparison to the metformin alone treatment arm (Figure 4). In addition, there was no difference in the C_{max} of plasma lactate between treatment arms (metformin alone, 1.7 ± 0.1 mM; metformin with nizatidine, 1.6 ± 0.1 mM) and the individual subject plasma concentrations were below the 5 mM toxicity threshold.

Hypoglycemic Action of Metformin With Nizatidine Co-Administration
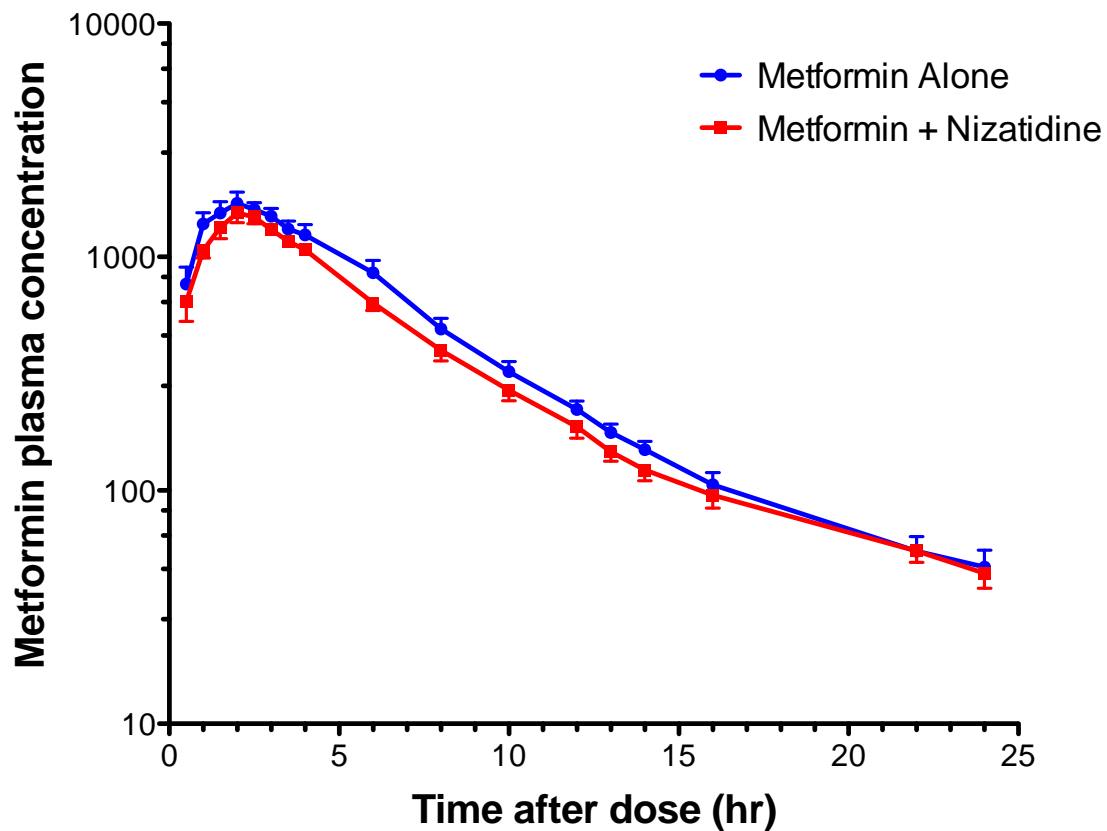
Figure 2. Mean metformin plasma concentration-time curves after administration of metformin alone or with nizatidine to healthy volunteers. Metformin plasma concentrations were determined after a single oral dose (850 mg) alone (blue) or in combination with a single oral dose (600 mg) of nizatidine (red). Data represent mean ± SEM.

Table 3. Summary of the pharmacokinetic parameters of metformin in healthy volunteers with and without nizatidine co-administration.

	Metformin Alone	Metformin + Nizatidine	p-value
t_{max} (h)	2.46±0.66	2.13±0.38	0.09
C_{max} (µg/mL)	1.81±0.60	1.68±0.55	0.43
AUC_{0-24} (µg*h/mL)	11.0±3.1	9.6±2.1	0.20
AUC_{inf} (µg*h/mL)	11.4±3.3	10.0±2.4	0.25
V/F (L)	577±179	799±239	0.01
CL/F (mL/min)	1360±440	1480±330	0.46
$t_{1/2}$ (h)	5.1±1.4	6.3±1.6	0.05
[a]Amount in urine$_{0-24}$ (mg)	437±86	376±128	0.10
[a]CL_R (mL/min)	736±204	670±133	0.18
CL_{IHX} (mL/min)	118±19	110±13	0.09
[a]CL_{CR} (mL/min)	156±40	147±26	0.28
[a]CL_{SR} (mL/min)	615±201	558±134	0.22

[a]Due to incomplete urine collection from one subject, the amount in urine, CL_R, CL_{CR}, CL_{SR} were calculated from 11 subjects. Data is presented as mean ± SD.

t_{max}, time to the maximal plasma concentration; C_{max}, maximal plasma concentration; AUC_{0-24}, area under the concentration-time curve from 0-24 h time point; AUC_{inf}, area

under the concentration-time curve from 0 to infinity; V/F, apparent volume of distribution; CL/F, apparent oral clearance; $t_{1/2}$, plasma terminal elimination half-life; CL_R, renal clearance; CL_{IHX}, iohexol clearance, CL_{CR}, creatinine clearance; CL_{SR}, renal clearance by secretion

Figure 3a
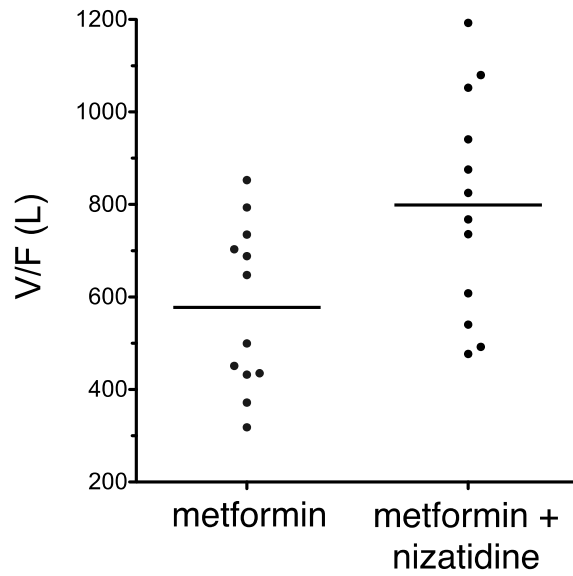
Figure 3b
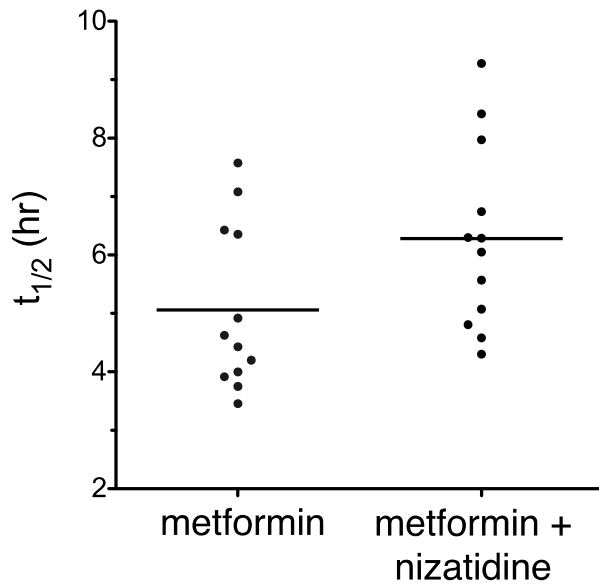
Figure 3. Volume of distribution (a) and half-life (b) alterations between treatment groups. Dots and horizontal lines represent individual data points and mean value of all subjects, respectively.

V/F, apparent volume of distribution; $t_{1/2}$, plasma terminal elimination half-life.
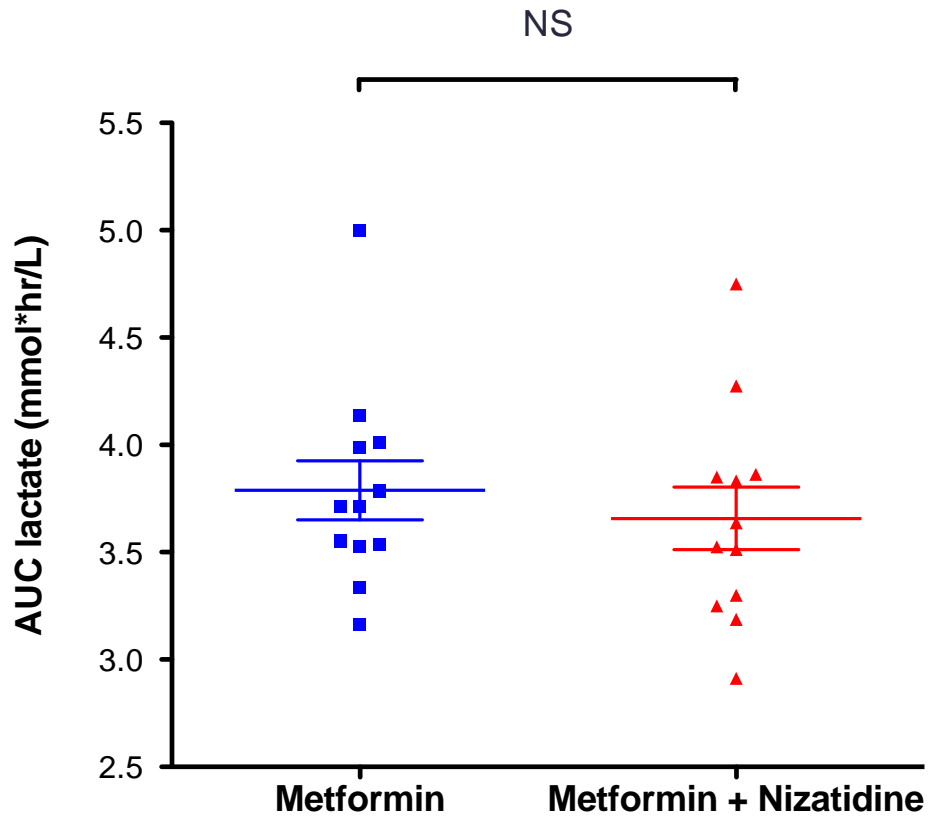
Figure 4. The effect of nizatidine on plasma lactate concentrations after metformin treatment. The area under the plasma lactate concentration-time curve (AUC lactate) was calculated during the oral glucose tolerance test. Symbols, horizontal line and brackets represent individual data points, mean value of all subjects and standard error of the mean, respectively. NS, not significant.

The glucose-lowering effect of metformin was determined in healthy subjects (n=12) by administering a 3-hour oral glucose tolerance test (OGTT). We observed similar areas under the glucose concentration-time curve (AUC) after the OGTT between pre-metformin and metformin alone treatment periods (pre-metformin, 3298 ± 145 mg hr/L; metformin alone, 3100 ± 121 mg hr/L; p>0.1; Figure 5). However, after metformin was co-administered with nizatdine, there was a significantly lower glucose AUC (greater response) compared to pre-metformin values (pre-metformin, 3298 ± 145 mg hr/L; metformin with nizatidine, 3015 ± 74 mg hr/L; p=0.03; Figure 5).

DISCUSSION

Previous clinical investigations of renal organic cation transporter-mediated drug interactions have focused on drugs that are predicted to alter the activity of more than one transporter (1-4; 17-20). Further, in most of the previous studies, alterations in the pharmacologic effects of the victim drugs were not investigated. To our knowledge, this is the first study to investigate the clinical impact of a selective MATE2K inhibitor, nizatidine, on the exposure and response to metformin. Our major findings include: (*i*) Nizatidine co-administration increases the apparent volume of distribution (V/F) and half-life of metformin ($t_{1/2}$) in healthy volunteers. (*ii*) Despite reaching unbound maximum concentrations ($C_{max,u}$) that are greater than its *in vitro* inhibition potency of MATE2K-mediated transport (determined in Chapter 3), nizatidine had no effect on metformin's renal clearance (CL_R) or net secretory clearance (CL_{SR}). (*iii*) In healthy volunteers, the hypoglycemic activity of metformin was enhanced above pre-metformin values only when nizatidine was co-administered.
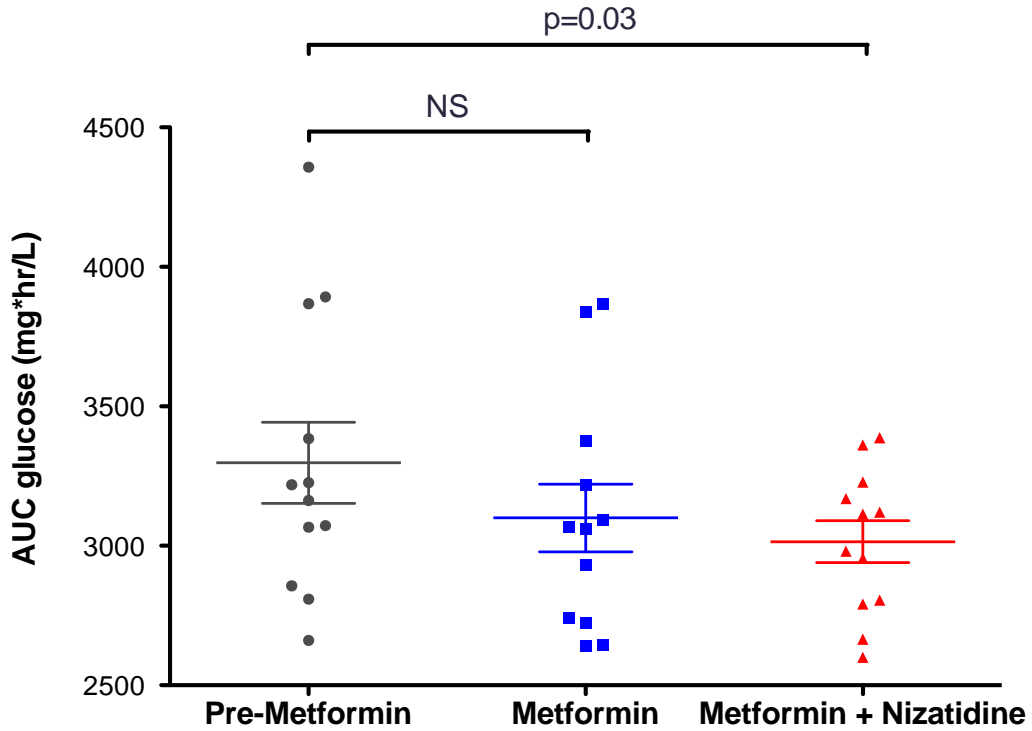
Figure 5. The effect of nizatidine on plasma glucose concentrations after metformin treatment. The area under the plasma glucose concentration-time curve (AUC glucose) was calculated during the oral glucose tolerance test. Symbols, horizontal line and brackets represent individual data points, mean value of all subjects and standard error of the mean, respectively. NS, not significant.

In a previous clinical drug-drug interaction study, there was a significant reduction in the CL_R and CL_{SR} of metformin upon co-administration of a MATE1/MATE2K dual inhibitor, pyrimethamine (2). However, in the current study, we did not observe a significant reduction in the CL_R or CL_{SR} of metformin when co-administered with nizatidine, a MATE2K selective inhibitor. Compared to pyrimethamine, the half-life of nizatidine is much shorter (pyrimethamine $t_{1/2}$, ~4 days (15); nizatidine $t_{1/2}$, 2.8 ± 0.3 hours). It is probable that the duration of inhibition is an important determinant of alterations in CL_R, which is generally estimated over a 24-hour period. However, we also examined the fractional CL_R and CL_{SR} (e.g. 0-2 hour, 2-4 hour, 0-4 hour) of metformin, and found no differences between treatment arms (data not shown). Metformin is likely to be transported by both MATE1 and MATE2K and the fractional contribution of each MATE is not known. Therefore, selective inhibition of MATE2K may be insufficient to have a measurable effect on the CL_R and CL_{SR} of metformin. It is also possible that the *in vitro* methods used in this study predicted an IC_{50} that was too high (Chapter 3) and that a much lower *in vivo* IC_{50} value may occur in the renal proximal tubule. The IC_{50} for nizatidine on MATE2K-mediated transport was determined *in vitro* using a proton-driven influx of metformin. However, *in vivo* MATE2K functions as an efflux pump. A previous study examining MATE1 transport of MPP^+ (1-methyl-4-phenylpyridinium) demonstrated that there are symmetrical interactions of H^+ with inward-facing and outward-facing MATE1 (21). This has not been confirmed for MATE2K-mediated transport of metformin. However, it should be noted that for *in vitro* studies of both pyrimethamine and cimetidine, MATE1/2K broad inhibitors of metformin transport, influx methods were used to predict IC_{50} values (2; 22). Finally, it is possible that

143

systemic concentrations of nizatidine were too low to have an effect on MATE2K-mediated transport, despite the fact that they were greater than *in vitro* concentrations required to inhibit MATE2K-mediated transport of metformin. These results directly challenge the current guidelines provided by the ITC and EMA and suggest that the current cut-off ($I_{fu}/IC_{50} \geq 0.1$ and 0.02, by the ITC and EMA, respectively), is too conservative. An analysis of published *in vitro* and clinical DDI data of MATE1/MATE2K dual inhibitors (e.g. cimetidine, pyrimethamine) revealed that an I_{fu}/IC_{50} ratio > 2 would have predicted a clinical effect on renal clearance (I_{fu}/IC_{50} ranged from 2.1-10.9; data not shown) (1-4; 18-20; 23). In addition to adjustment of the I_{fu}/IC_{50} cut-off, future guidances may also want to consider the half-life of the inhibitor as well as if there are other transporters present at the same membrane that could compensate for any decrease in transporter activity.

In this study, we observed a significant increase in metformin V/F and a related increase in metformin $t_{1/2}$. These studies are consistent with previous reports that inhibitors of renal transporters have a direct effect on the victim drug's volume of distribution (24). As MATE2K is highly expressed in the kidney, an increase in metformin V/F suggests that metformin may be accumulating in the renal cell when its MATE2K-mediated efflux is blocked by nizatidine co-administration.

Interestingly, while we did not see any alterations in systemic levels of metformin, the hypoglycemic activity is enhanced above pre-metformin values only when nizatidine is co-administered. While the kidney is mostly known for its role in the reabsorption of

glucose from the filtrate, it also has a significant impact on glucose uptake from blood (20% of total uptake in the post-absorptive state) (25), gluconeogenesis (20-25% of total glucose release in the post-absorptive state) (26-28) and glucose utilization (post-absorptive, 5-10%; post-prandial, 10-15% of total) (29; 30). In the liver, metformin is known to enhance glucose uptake, decrease glucose production and increase glucose utilization (for review see (31)). From our observations that nizatidine increases metformin V/F and hypoglycemic activity, we hypothesize that nizatidine sequesters metformin in the kidney where it is then able to enhance glucose uptake and utilization by the kidney. In the current study design where there are high levels of glucose and a single dose of metformin, it is unlikely that alterations in gluconeogenesis would play a role.

Although we did see an enhancement of hypoglycemic activity above pre-metformin levels with metformin and nizatidine co-administration, it was a very modest response. This is not surprising given that our subjects are healthy volunteers with normal fasting glucose values (<110 mg/dL). A similar study design with type II diabetic subjects or pre-diabetics would be informative to understand the impact of nizatidine co-administration on glucose tolerance. Furthermore, although there is no evidence to show that nizatidine has an impact on glucose tolerance, it would also be beneficial to include a nizatidine alone arm to confirm that it is truly differences in metformin's pharmacokinetics that enhances the hypoglycemic activity.

A previous report from our laboratory demonstrated that carriers of MATE2K g.-130G>A, a promoter variant that increases MATE2K expression, exhibit a reduced effect of metformin in type II diabetic subjects (8). For drugs that are metabolized by CYP2D6, an individual who is an extensive metabolizer can respond like a poor metabolizer when taking a CYP2D6 inhibitor (e.g., quinidine, paroxetine, fluoxetine) (32-34). The inhibition of transporters by other drugs and carriers of transporter expression variants is an important point of intersection between pharmacogenetics and drug response that has not, to our knowledge, been investigated. It is possible that carriers of MATE2K g.-130G>A, when taking metformin and nizatidine together, will have an improved ability to achieve glycemic control.

Our results suggest that metformin is accumulating in the kidney when nizatidine is co-administered. An increase in kidney levels as a result of a drug interaction could have a significant impact on other basic drugs that are effluxed by MATE2K, particularly those that are nephrotoxic (e.g., platinum-based anticancer agents). Of the platinum-based agents, cisplatin is the most nephrotoxic (35), and it is hypothesized that this nephrotoxicity may be a result of renal cell accumulation as it is a weak substrate of MATE2K (6; 7). Interestingly, oxaliplatin is predicted to be exclusively effluxed by MATE2K (6; 7) does not demonstrate substantial nephrotoxicity (35). It is possible that a drug interaction at MATE2K could enhance the potential for nephrotoxicity of platinum agents, including oxaliplatin, which has limited nephrotoxicity as a single agent. Retrospective studies would be informative to determine whether the concomitant administration of nizatidine and a platinum-based therapeutic enhances kidney toxicities.

146

In summary, selective inhibition of MATE2K-mediated transport by nizatidine increases the apparent volume of distribution and hypoglycemic activity of metformin. However, despite achieving unbound maximum concentrations that are greater than the *in vitro* inhibition potency of MATE2K-mediated transport, nizatidine did not change metformin's renal clearance or net secretory clearance between treatment arms. These studies are consistent with the idea that inhibitors of renal efflux transporters may affect the volume of distribution, tissue levels and peripheral effects of drugs. Furthermore, this study suggests that better *in vitro/in vivo* predictive methods and criteria are required to predict renal DDIs mediated by MATEs.

REFERENCES

1.	Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. 1987. Reduction of metformin renal tubular secretion by cimetidine in man. *Br J Clin Pharmacol* 23:545-51

2.	Kusuhara H, Ito S, Kumagai Y, Jiang M, Shiroshita T, et al. 2011. Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at oral microdose and at therapeutic dose in healthy subjects. *Clin Pharmacol Ther* 89:837-44

3.	Somogyi A, McLean A, Heinzow B. 1983. Cimetidine-procainamide pharmacokinetic interaction in man: evidence of competition for tubular secretion of basic drugs. *Eur J Clin Pharmacol* 25:339-45

4. van Crugten J, Bochner F, Keal J, Somogyi A. 1986. Selectivity of the cimetidine-induced alterations in the renal handling of organic substrates in humans. Studies with anionic, cationic and zwitterionic drugs. *J Pharmacol Exp Ther* 236:481-7

5. Masuda S, Terada T, Yonezawa A, Tanihara Y, Kishimoto K, et al. 2006. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol* 17:2127-35

6. Yokoo S, Yonezawa A, Masuda S, Fukatsu A, Katsura T, Inui K. 2007. Differential contribution of organic cation transporters, OCT2 and MATE1, in platinum agent-induced nephrotoxicity. *Biochem Pharmacol* 74:477-87

7. Yonezawa A, Masuda S, Yokoo S, Katsura T, Inui K. 2006. Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1-3 and multidrug and toxin extrusion family). *J Pharmacol Exp Ther* 319:879-86

8. Choi JH, Yee SW, Ramirez AH, Morrissey KM, Jang GH, et al. 2011. A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. *Clin Pharmacol Ther* 90:674-84

9. *Guidance for Industry (Draft): Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm> Accessed May 2013

10. *European Medicines Agency's Guideline on the Investigation of Drug Interactions*.

<http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf.> Accessed May 2013

11. Hillgren KM, Keppler D, Zur AA, Giacomini KM, Stieger B, et al. 2013. Emerging transporters of clinical importance: An update from the international transporter consortium. *Clin Pharmacol Ther* e-pub ahead of print

12. Stocker SL, Morrissey KM, Yee SW, Castro RA, Xu L, et al. 2013. The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. *Clin Pharmacol Ther* 93:186-94

13. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, et al. 2008. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther* 83:273-80

14. Chen Y, Li S, Brown C, Cheatham S, Castro RA, et al. 2009. Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. *Pharmacogenet Genomics* 19:497-504

15. Moffat AC, Osselton MD, Widdop B. 2004. *Clarke's analysis of drugs and poisons : in pharmaceuticals, body fluids and postmortem material*. London; Chicago: Pharmaceutical Press

16. Howlett HC, Bailey CJ. 1999. A risk-benefit assessment of metformin in type 2 diabetes mellitus. *Drug Saf* 20:489-503

17. Abel S, Nichols DJ, Brearley CJ, Eve MD. 2000. Effect of cimetidine and ranitidine on pharmacokinetics and pharmacodynamics of a single dose of dofetilide. *Br J Clin Pharmacol* 49:64-71

18. Feng B, Obach RS, Burstein AH, Clark DJ, de Morais SM, Faessel HM. 2008. Effect of human renal cationic transporter inhibition on the pharmacokinetics of varenicline, a new therapy for smoking cessation: an in vitro-in vivo study. *Clin Pharmacol Ther* 83:567-76

19. Shiga T, Hashiguchi M, Urae A, Kasanuki H, Rikihisa T. 2000. Effect of cimetidine and probenecid on pilsicainide renal clearance in humans. *Clin Pharmacol Ther* 67:222-8

20. Somogyi AA, Bochner F, Sallustio BC. 1992. Stereoselective inhibition of pindolol renal clearance by cimetidine in humans. *Clin Pharmacol Ther* 51:379-87

21. Dangprapai Y, Wright SH. 2011. Interaction of H+ with the extracellular and intracellular aspects of hMATE1. *Am J Physiol Renal Physiol* 301:F520-8

22. Tsuda M, Terada T, Ueba M, Sato T, Masuda S, et al. 2009. Involvement of human multidrug and toxin extrusion 1 in the drug interaction between cimetidine and metformin in renal epithelial cells. *J Pharmacol Exp Ther* 329:185-91

23. Morrissey KM, Stocker SL, Wittwer MB, Xu L, Giacomini KM. 2013. Renal transporters in drug development. *Annu Rev Pharmacol Toxicol* 53:503-29

24. Grover A, Benet LZ. 2009. Effects of drug transporters on volume of distribution. *AAPS J* 11:250-61

25. Stumvoll M, Chintalapudi U, Perriello G, Welle S, Gutierrez O, Gerich J. 1995. Uptake and release of glucose by the human kidney. Postabsorptive rates and responses to epinephrine. *J Clin Invest* 96:2528-33

26. Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC. 1996. Contributions of gluconeogenesis to glucose production in the fasted state. *J Clin Invest* 98:378-85

27. Stumvoll M, Meyer C, Mitrakou A, Nadkarni V, Gerich JE. 1997. Renal glucose production and utilization: new aspects in humans. *Diabetologia* 40:749-57

28. Gerich JE. 2000. Physiology of glucose homeostasis. *Diabetes Obes Metab* 2:345-50

29. Gerich JE. 1993. Control of glycaemia. *Baillieres Clin Endocrinol Metab* 7:551-86

30. Woerle HJ, Meyer C, Dostou JM, Gosmanov NR, Islam N, et al. 2003. Pathways for glucose disposal after meal ingestion in humans. *Am J Physiol Endocrinol Metab* 284:E716-25

31. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. 2012. Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics* 22:820-7

32. Feifel N, Kucher K, Fuchs L, Jedrychowski M, Schmidt E, et al. 1993. Role of cytochrome P4502D6 in the metabolism of brofaromine. A new selective MAO-A inhibitor. *Eur J Clin Pharmacol* 45:265-9

33. Alfaro CL, Lam YW, Simpson J, Ereshefsky L. 2000. CYP2D6 inhibition by fluoxetine, paroxetine, sertraline, and venlafaxine in a crossover study: intraindividual variability and plasma concentration correlations. *J Clin Pharmacol* 40:58-66

34.   Borges S, Desta Z, Li L, Skaar TC, Ward BA, et al. 2006. Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther* 80:61-74

35.   Rabik CA, Dolan ME. 2007. Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treat Rev* 33:9-23

CHAPTER 5

THE EFFECT OF NOVEL PROMOTER VARIANTS IN MATE1 AND MATE2 ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF METFORMIN[*]

ABSTRACT

Interindividual variation in response to metformin, first-line therapy for type 2 diabetes, is substantial. Given that transporters are determinants of metformin pharmacokinetics, we examined the effects of promoter variants in both multidrug and toxic extrusion protein 1 (MATE1) (g.-66T>C, rs2252281) and MATE2 (g.-130G>A, rs12943590) on variation in metformin disposition and response. The pharmacokinetics and glucose-lowering effects of metformin were assessed in healthy volunteers (n=57) receiving metformin. The renal and secretory clearances of metformin were higher (22% and 26%, respectively) in carriers of variant MATE2 who were also MATE1 reference ($P < 0.05$). Both MATE genotypes were associated with altered post-metformin glucose tolerance, with variant carriers of MATE1 and MATE2 having an enhanced ($P < 0.01$) and reduced ($P < 0.05$) response, respectively. Consistent with these results, patients with diabetes (n=145) carrying the MATE1 variant showed enhanced metformin response. These findings suggest that promoter variants of MATE1 and MATE2 are important determinants of metformin disposition and response in healthy volunteers and diabetic patients.

INTRODUCTION

As the first-line therapy for the treatment of type 2 diabetes, metformin is the most frequently prescribed anti-diabetic drug (1). Although controversial, studies suggest that metformin's pharmacological action is related to its activation of adenosine monophosphate-activated protein kinase, which reduces hepatic glucose production, enhances glucose uptake in hepatic cells and peripheral tissues, decreases absorption of glucose from the gastrointestinal tract and increases insulin sensitivity in peripheral tissues (2; 3).

The pharmacokinetics of metformin have been studied extensively in both healthy volunteers and patients with type 2 diabetes. About 50% of an oral dose is absorbed (4; 5) into the blood and rapidly distributed to various tissues. Metformin is not bound to plasma proteins (6) and is eliminated into urine as unchanged drug (4; 7). The renal clearance of metformin is much greater than glomerular filtration rate, suggesting a significant contribution of tubular secretion to its elimination. Considerable interindividual variability in the renal clearance of metformin has been observed in healthy volunteers (150-700 mL/min) (7), which includes a strong genetic component (8; 9). In addition to pharmacokinetic interindividual variability, the response to metformin varies substantially, with ≥30% of patients receiving metformin monotherapy classified as nonresponders (10).

Metformin relies on facilitated transport for delivery to the liver, kidney and peripheral tissues. Indeed, previous studies demonstrate that membrane transporters contribute to

154

the interindividual variability in the pharmacokinetics and pharmacodynamics of metformin (11-15). Metformin is transported primarily by the organic cation transporters (OCTs), particularly OCT1 and OCT2, and multidrug and toxin extrusion proteins (MATEs), namely MATE1 and MATE2. MATE1 and OCT1 have been implicated as determinants of metformin response primarily due to their tissue distribution at major sites of metformin action. MATE1 is highly expressed in the kidney and liver (Figure 1) with lower expression in skeletal muscle and adipose tissue (16), whereas, OCT1 is predominantly expressed in the liver. Previous reports from our laboratory and others have shown that OCT1 is the major determinant of metformin uptake into hepatocytes and polymorphisms of OCT1/*SLC22A1* are associated with reduced response to metformin in both healthy volunteers (11; 12) and diabetic patients (17; 18). Of note, MATE1 and OCT1 have been shown to mediate transcellular transport of metformin *in vitro* (19; 20) and to affect metformin response in diabetic patients (14). Other studies indicate MATE1/*SLC47A1* polymorphisms alone affect metformin response in diabetic patients (21).

In addition to the effects on pharmacodynamics, transporters play a major role in metformin renal elimination (22). In particular, OCT2 mediates the entry of metformin into the renal tubular cells, whereas MATE1 and MATE2 contribute to the efflux of metformin into the urine (19; 20) (Figure 2). Previous studies have shown that a nonsynonymous variant in OCT2/*SLC22A2* (A270S, rs316019) alters the renal clearance of metformin in healthy volunteers (15; 23; 24). In addition, renal clearance and tissue distribution of metformin is altered in Mate1(-/-), but not Mate1(+/-) mice (25; 26).
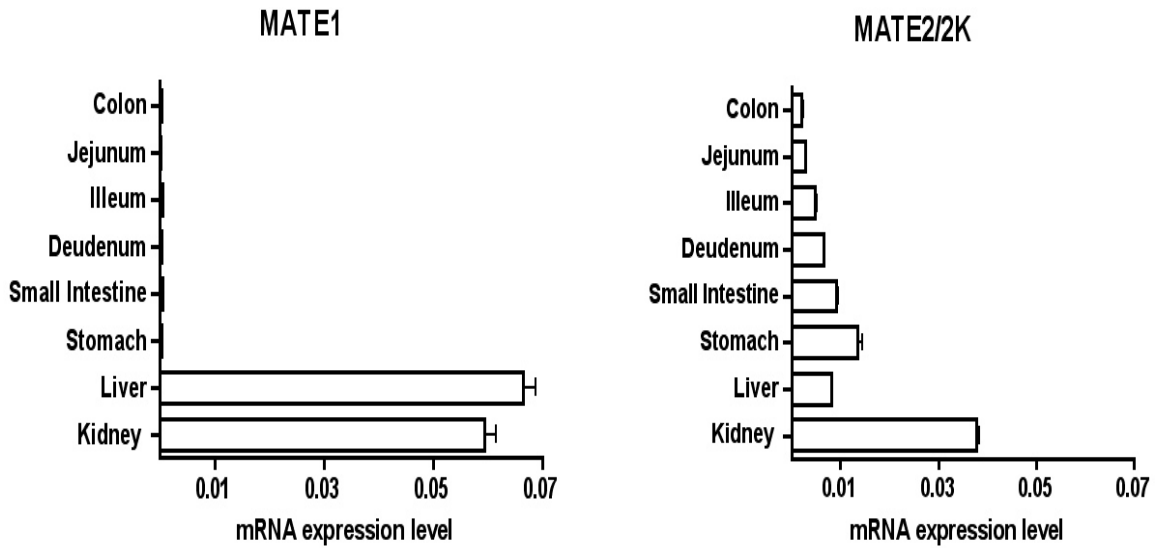
Figure 1. The expression profile of MATE1 and MATE2/2K in various human tissues. Total RNA isolated from various human tissues (BioChain, Hayward, CA) were reverse transcribed and mRNA levels of MATE1 and MATE2 were determined by real-time PCR (27) using commercially available primer-probe sets (Life Technologies, Grand Island, NY). The delta cycle threshold (C_T) values for all the genes in each sample were calculated by subtracting the mean C_T values for three housekeeping genes (GAPDH, β-Actin, and PGK-1) from the C_T for each target gene. The relative quantity of each gene was then determined by calculating the $2^{-\Delta C_T}$ value. Data represent the mean and standard deviation from two experiments.
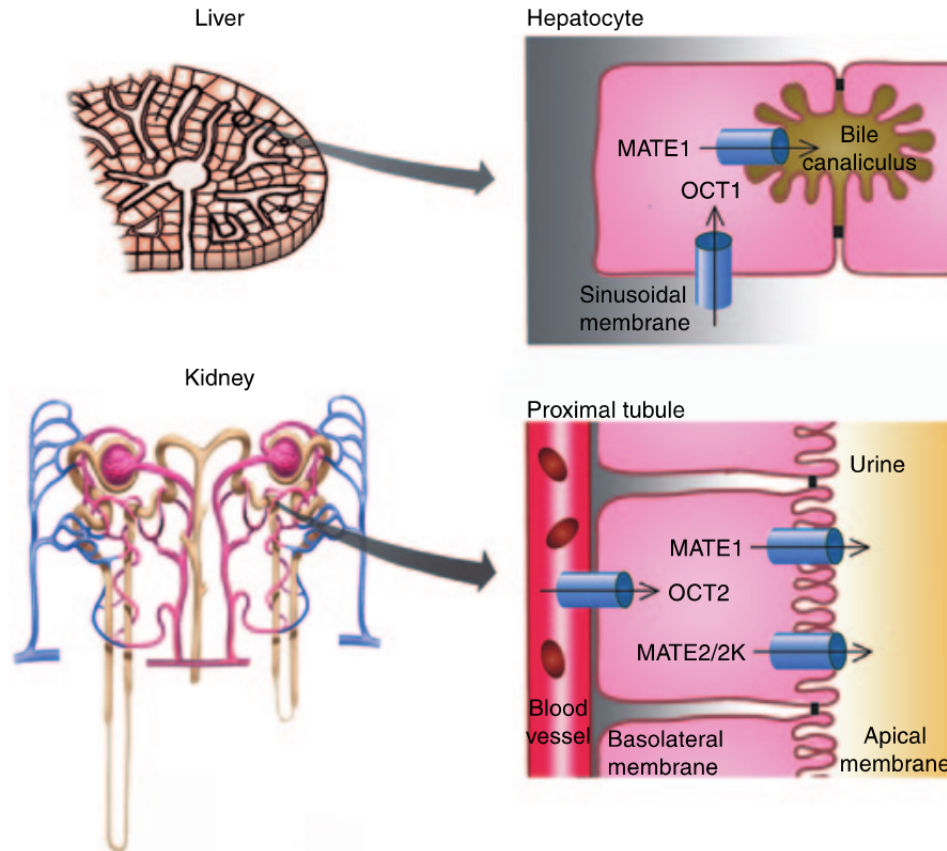
Figure 2. Representative drawing of metformin transporters in the hepatocyte of the liver and nephron of the kidney. This drawing shows the liver (top left) and nephron (bottom left) and the cation transporters in the hepatocyte (top right) and proximal tubule cell (bottom right) that have been identified as important determinants of the pharmacokinetics and response to metformin. Multidrug and toxin extrusion protein 2 (MATE2) in the kidney has two functional isoforms, MATE2 and MATE2-K. OCT, organic cation transporter.

However, the effect of genetic variation in MATE1/*SLC47A1* and MATE2/*SLC47A2* on the pharmacokinetics of metformin in humans remains unclear.

Until recently, clinical studies that focused on the effects of promoter variants on drug disposition and response has been less well studied than coding and intronic region polymorphisms. Recently, our laboratory has shown that a common promoter variant, MATE2 (g.-130G>A, rs12943590), increases luciferase activity *in vitro* and associates with reduced response to metformin in diabetic patients (28). In addition, another common promoter variant, MATE1 (g.-66T>C, rs2252281) exhibited reduced luciferase activity in reporter assays *in vitro* and was shown to associate with reduced expression of MATE1 mRNA transcripts in the kidney (29). In the current study, we hypothesized that these two promoter variants are determinants of metformin renal clearance and antidiabetic response in healthy volunteers and diabetic patients. Because the two variants may have opposing effects, we also considered gene-gene interactions in our association studies. Our data demonstrate that in the absence of the MATE1 promoter variant, the MATE2 promoter variant is associated with an increased renal clearance of metformin. Of note, both variants associate with the glucose-lowering effects of metformin in healthy volunteers and in diabetic patents, but in opposite directions.

MATERIALS AND METHODS

Healthy Human Volunteers

The Committee on Human Research at the University of California, San Francisco (Institutional Review Board (IRB) 10-03087 and 10-02578) approved this study. Healthy

male and female volunteers were recruited directly from the Study of Pharmacogenetics in Ethnically Diverse Populations (IRB 10-03167) and participants were enrolled only after informed consent was provided. To be eligible for this study, volunteers had to be older than 18 years of age and not taking any medications other than vitamins and/or oral contraceptives. Screening included a comprehensive medical history, physical examination and laboratory studies (complete blood count, electrolytes, BUN and creatinine, albumin, and liver enzymes).   Volunteers with elevated liver enzymes, anemia, elevated creatinine concentrations or a positive pregnancy test were excluded.

Genotyping

MATE2 (g.-130G>A, rs12943590), OCT1 coding variants (R61C (rs12208357), G401S (rs34130495), 420del (rs72552763) and G465R (rs34059508)) and OCT2 (A270S, rs316019) were genotyped by a TaqMan assay using standard procedures. MATE1 (g.-66T>C, rs2252281) was genotyped by PCR amplification followed by sequencing of the promoter region. The OCT1 coding variants (R61C, G401S and G465R) were genotyped only in the type 2 diabetic cohort.

Clinical Study Procedures

Once enrolled, volunteers were advised to maintain stable activity levels 7 days before starting the study. After the initial 3-day carbohydrate controlled diet (200-250 g/day), volunteers were admitted to the General Clinical Research Center (GCRC) at San Francisco General Hospital and remained there for the duration of the study (72 h). After an overnight fast (10 h), each subject underwent a 3 hour oral glucose tolerance test

(OGTT, 75 g; day 1). Volunteers were dosed with 1,000 mg metformin (Major Pharmaceuticals, Livonia, MI) in the evening of study day 1 followed by a dose of 850 mg early on the second study day (day 2). A second OGTT was administered 2 h after metformin administration on study day 2. Standardized meals were provided on both study days after completion of the OGTT. Following the metformin dose, volunteers were asked to drink 8 oz of water every 2 h to maintain urine flow and pH.

Timed blood samples were collected after the first (0, 0.5, 1, 2 and 11 h) and second (0, 0.5, 1, 1.5, 2, 2.25, 2.5, 2.75, 3, 3.5, 4, 6, 8, 10, 12 and 24 h) metformin dose, respectively, for the determination of plasma metformin concentrations. For metformin pharmacodynamics (glucose/insulin concentrations) blood samples were collected at 0, 15, 30, 45, 60, 90, 120 and 180 min after glucose administration. An additional blood sample was collected at 12 h after the second dose of metformin to determine serum creatinine. Urine samples were collected during the following time intervals: 0-12 h after the first dose of metformin and 0-2, 2-4, 4-6, 6-8, 8-12 and 12-24 h after the second dose of metformin.

Analytical Methods

Metformin concentrations in plasma and urine were assayed by a validated liquid chromatography-tandem mass spectrometry method (11). The quantification limit was 4 mg/L for plasma and 40 ng/mL for urine. Both the intra-day and inter-day coefficients of analysis variation were <5%. Glucose concentrations in plasma and creatinine concentrations in plasma and urine were determined using standard colorimetric assays.

Insulin concentrations in plasma were determined using an immunoassay (Mercodia, NC) following the manufacturer's instructions.

Clinical Pharmacokinetics

The concentration-time profile of metformin was evaluated by non-compartmental analysis (WinNonlin 4.1, Pharsight Corporation, Mountain View, CA). The pharmacokinetics of metformin from both plasma and urine were calculated after the second dose as described previously (11).

Patients With Type 2 Diabetes

Diabetic patients of Caucasian or African American ethnicity were recruited into a multicenter retrospective study described previously (28). Briefly, all patients were prescribed metformin monotherapy as their initial hypoglycemic medication, had glycosylated hemoglobin (HbA1c) levels measured both before and after commencement of metformin treatment and a medication possession ratio (number of days supply during observation period/number of days in observation period) greater than 0.8. The IRBs of the Marshfield Clinic Research Foundation, Kaiser Permanente South East, Georgia and University of California, San Francisco approved this study and informed consent was obtained. The review process for utilizing and linking DNA stored in a biobank to electronic medical records has been described previously (30).

Statistical Analysis

Data are presented as mean $\pm$ SD unless indicated otherwise. Unpaired and paired nonparametric Student's *t*-tests were used to analyze the differences in metformin pharmacokinetic and pharmacodynamic parameters, respectively, for each genotype using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). A statistically significant result was defined when $P < 0.05$. The 95% confidence intervals for the relative change in HbA1c were calculated by a nonparametric bootstrap method using the R software package (www.r-project.org, Version 2.12.0). Linear regression and multivariate analyses were carried out using the R software.

All analyses were conducted, first, by assessing the effect of each variant alone, and second, after the exclusion of individuals carrying confounding genotypes on the pharmacokinetics and pharmacodynamics of metformin. A recessive genetic model was used, unless the analyses were underpowered, in which case a dominant genetic model was used. In the secondary analyses, we removed individuals who were carrying at least one minor allele for MATE1 (g.-66T>C), MATE2 (g.-130G>A) or a reduced-function OCT1 coding variant (R61C, G401S, 420del and G465R) or OCT2 coding variant (A270S) that could confound the measured parameters. This secondary analysis was conducted because the MATE1 (g.-66T>C) variant opposes the effects of the MATE2 (g.-130G>A) variant (i.e., the MATE1 and MATE2 polymorphisms result in reduced and enhanced promoter activity, respectively, in reporter assays). In addition, coding variants in OCT1 and OCT2 are known to effect metformin pharmacodynamics (11; 12; 17; 18) and pharmacokinetics (15; 23; 24), respectively, and could confound the effects of MATE variants on the disposition and response to metformin.

RESULTS

Healthy male and female Asian (*n*=18), African American, (*n*=33) and Caucasian (*n*=6) volunteers were genotyped for MATE1 (g.-66T>C, rs2252281), MATE2 (g.-130G>A, rs12943590), OCT1 (420del, rs72552763) and OCT2 (A270S, rs316019). All alleles were in Hardy-Weinberg equilibrium. The pharmacokinetics and pharmacodynamics of metformin were evaluated in these volunteers after oral dosing of the drug (1,850 mg in total). The study design and characteristics of the patients with type 2 diabetes have been reported previously (28). Demographic characteristics for the healthy volunteers and a subset of patients are shown in Tables 1 and 2. In this study, we first discuss our analysis of the association of the MATE1 variant with the pharmacokinetics and pharmacodynamics of metformin in healthy volunteers and diabetic patients, followed by an analysis focused on the MATE2 promoter variant. For both promoter variants, we first analyzed the effect of either variant alone, and then adjusted for each of the additional transporter variants (see Methods).

The MATE1 Promoter Variant, g.-66T>C, Has No Effect on the Pharmacokinetics of Metformin in Healthy Volunteers

The pharmacokinetic parameters obtained in the present study are similar to those previously reported in healthy volunteers (11; 15; 31-34). The MATE1 g.-66T>C genotype had no significant effect on the pharmacokinetics of metformin (reference *n*=32, variant *n*=25; Figure 3), even after adjusting for creatinine clearance (CL_{CR}). The pharmacokinetics of metformin remained similar even after exclusion of volunteers carrying the OCT1 or OCT2 polymorphisms (Table 3).

Table 1. Demographic characteristics of the healthy volunteers in the total cohort (n=57).

	Total cohort	MATE1 (-66T>C)		MATE2 (-130G>A)	
		Reference (TT)	Variant (TC & CC)	Reference (GG)	Variant (GA & AA)
N	57	32	25	27	30
Sex, n (%)					
Female	36 (63)	21 (66)	15 (60)	16 (59)	20 (67)
Male	21 (37)	11 (34)	10 (40)	11 (41)	10 (33)
Ethnicity, n (%)					
Asian	18 (32)	13 (41)	5 (20)	3 (11)	15 (50)
African American	33 (58)	14 (44)	19 (76)	21 (78)	12 (40)
Caucasian	6 (11)	5 (16)	1 (4)	3 (11)	3 (10)
Age (years)	28 (18 - 45)	29 (18 - 45)	26 (18 - 39)	28 (21 - 45)	27 (18 - 44)
Weight (kg)	77 (50 - 136)	74 (50 - 124)	80 (53 - 136)	83 (51 - 136)	71* (50 -116)
Height (cm)	172 (149 - 198)	171 (158 - 192)	172 (149 - 198)	173 (158 - 198)	170 (149 - 192)
BMI ($kg/(m^2)$)	25.8 (18.0 - 43.6)	25.1 (18.0 - 39.4)	26.7 (19.8 - 43.6)	27 (19 - 44)	24* (18 - 33)
CL_{CR} (mL/min)	121 (57 - 225)	114 (57 - 208)	130 (67 - 225)	119 (59 - 192)	122 (57 - 225)

Data represented as mean (range) unless stated otherwise. *$P<0.05$ compared to reference genotype.

BMI, body mass index; CL_{CR}, creatinine clearance measured from the urine over 24 h

Table 2.   Baseline characteristics of patients with type 2 diabetes on metformin monotherapy compared across the three clinical sites and based on ethnicity.

	Clinical site			Ethnicity		
	Marshfield Clinic	Kaiser South East	BioVu	Caucasian	African American	Total
N	47	57	41	90	55	145
Sex, n (%)						
Male	22	18	18	44	14	58
Female	25	39	23	46	41	87
Ethnicity, n (%)						
Caucasian	47	11	32			
African-American	0	46	9			
Age (years)	56 ± 14 (55)	58 ± 10 (58)	57 ± 13 (53)	56 ± 13 (54)	59 ± 12 (58)	57 ± 12 (56)
BMI (kg/m^2)	36.8 ± 8.4 (35.7) (n=47)	38.2 ± 8.1 (37.9) (n=23)	33.2 ± 6.0* (30.9) (n=40)	37.0 ± 8.4 (34.4) (n=82)	35.3 ± 5.8 (34.3) (n=28)	35.8 ± 7.8 (34.4) (n=110)
Baseline HbA1c (%)	7.90 ± 1.48 (7.50)	8.29 ± 1.43 (8.10)	7.98 ± 1.50 (7.50)	7.97 ± 1.45 (7.60)	8.25 ± 1.48 (8.10)	8.07 ± 1.46 (7.80)
Average metformin daily dose (mg)	869 ± 271 (978)	913 ± 458 (1000)	951 ± 472 (1000)	936 ± 381 (1000)	866 ± 453 (1000)	909 ± 410 (1000)
eGFR (mL/min/1.73m^2)	81 ± 24 (76) (n=47)	82 ± 16 (82) (n=57)	NA	80 ± 23 (76) (n=58)	83 ± 16 (83) (n=46)	82 ± 20 (79) (n=104)

Data shown represent mean ± SD unless stated otherwise. *$P < 0.05$ compared to Kaiser South East clinical sites.

BMI, body mass index; HbA1c, glycoslyated hemoglobin; NA, not available; eGFR, estimated creatinine clearance from the MDRD equation (Chapter 1, Table 4)
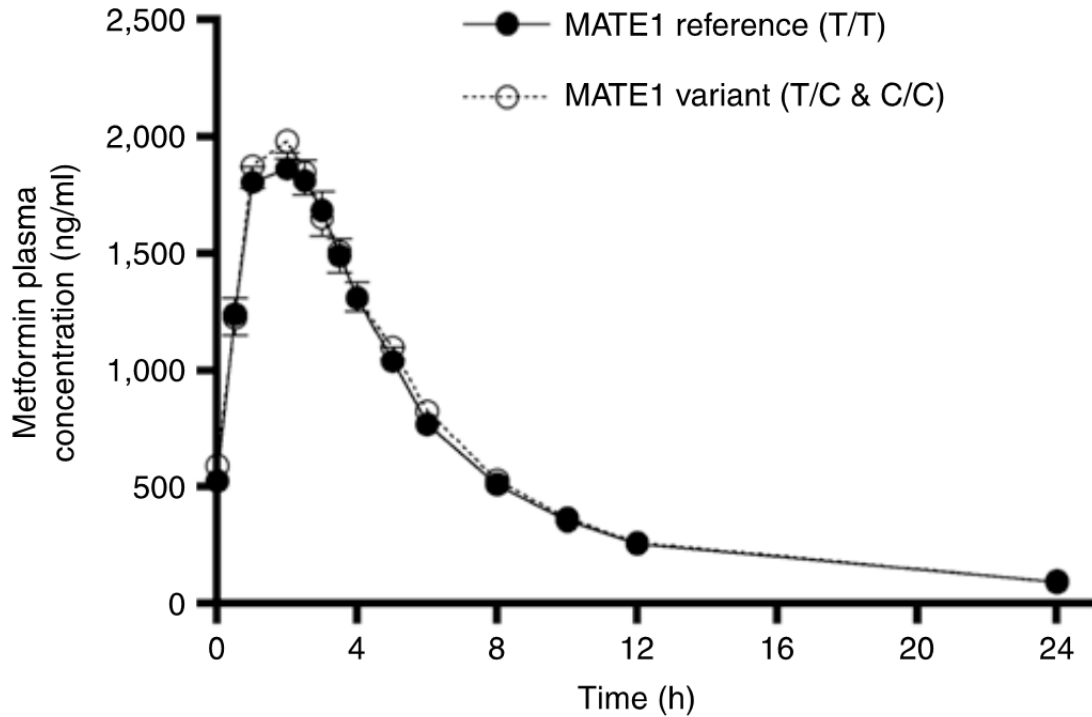
Figure 3. **The effect of multidrug and toxin extrusion protein 1 (MATE1) (g.–66T>C) on the pharmacokinetics of metformin in 57 healthy volunteers.** Shown is the mean plasma concentration–time curve of metformin after oral administration to healthy volunteers who carry at least one MATE1 (g.–66T>C) variant allele ($n = 25$, open circles) or those who are homozygous for the reference MATE1 allele ($n = 32$, filled circles). The volunteers were given two doses of metformin (1,850 mg in total). The plasma metformin concentration–time curves after the second dose are shown. Data represent mean $\pm$ SEM.

Table 3. **Summary of the metformin pharmacokinetic parameters in healthy volunteers with known OCT1 and OCT2 genotype and homozygous for the MATE1 reference allele (-66T/T) and heterozygous or homozygous for the MATE1 variant allele (-66T/C or -66C/C).**

	All subjects		OCT1 reference (AA)		OCT2 reference (CC)	
	MATE1 reference (TT)	MATE1 variant (TC & CC)	MATE1 reference (TT)	MATE1 variant (TC & CC)	MATE1 reference (TT)	MATE1 variant (TC & CC)
N	32	25	29	20	40	5
T_{max} (h)	1.81 ± 0.68	1.71 ± 0.59	1.81 ± 0.68	1.70 ± 0.63	1.74 ± 0.64	1.90 ± 0.52
C_{max} (ng/mL)	2220 ± 570	2000 ± 390	2180 ± 560	2190 ± 540	2177 ± 599	2080 ± 305
AUC_{0-24} (ng.h/mL)	13300 ± 3100	13700 ± 2800	13300 ± 3100	13600 ± 2900	13200 ± 3000	13900 ± 1400
AUC_{inf} (ng.h/mL)	14500 ± 3200	13700 ± 2400	14200 ± 3200	14500 ± 3200	14000 ± 3200	15000 ± 1400
V/F (L)	565 ± 182	596 ± 200	586 ± 197	549 ± 164	586 ± 185	545 ± 107
CL/F (mL/min)	1029 ± 243	1069 ± 216	1049 ± 252	1017 ± 223	1059 ± 251	954 ± 91
$T_{1/2}$ (h)	6.33 ± 1.52	6.40 ± 1.33	6.42 ± 1.65	6.24 ± 1.27	6.41 ± 1.59	6.59 ± 1.10
Amount in $urine_{0-24}$ (mg)	370 ± 96	384 ± 94	367 ± 99	378 ± 94	364 ± 69	454 ± 156
CL_R (mL/min)	530 ± 151	530 ± 154	524 ± 147	536 ± 146	530 ± 132	601 ± 233
CL_{CR} (mL/min)	117 ± 35	145 ± 40	114 ± 31	130 ± 42	122 ± 35	128 ± 26
CL_{SR} (mL/min)	416 ± 150	400 ± 143	410 ± 146	404 ± 134	408 ± 134	472 ± 213

Data were obtained from healthy volunteers given two doses of metformin. The first dose (1000 mg) was given at 1800 h on study day 1 and the second dose (850 mg) was given at 0700 h on study day 2. Blood and urine samples for the pharmacokinetic analysis were collected 0-24 h after the second dose. T_{max}, time to the maximal plasma concentration; C_{max}, maximal plasma concentration; AUC_{0-24}, area under the concentration-time curve from 0-24 h time point; AUC_{inf}, area under the plasma concentration versus time curve from 0 to infinity; V/F, apparent volume of distribution; CL/F, apparent oral clearance; $T_{1/2}$, plasma terminal elimination half-life; CL_R, renal clearance; CL_{CR}, creatinine clearance; CL_{SR}, renal clearance by secretion.

The MATE1 Promoter Variant, g.-66T>C, is Associated With a Greater Response to Metformin in Healthy Volunteers

Before metformin dosing, the area under the curve (AUC) of glucose (mean±SD, reference, 359±56 mg/dL/h; variant 352±77 mg/dL/h) and insulin (reference, 129±83 mU/L/h; variant 141±102 mU/L/h) were similar between MATE1 genotypes. After metformin administration, volunteers who were homozygous for the variant MATE1 allele had significantly lower glucose AUC (greater response) after the oral glucose tolerance test (OGTT) than those volunteers carrying at least one reference allele (reference, 309±39 mg/dL/h; variant, 250±37 mg/dL/h; $P = 0.002$; Figure 4a-b). The association of the MATE1 allele with glucose AUC persisted in subsequent analysis of volunteers who were also homozygous for the reference OCT1 (MATE1 reference, n=43, 308±40 mg/dL/h; MATE1 variant, n=5, 242±41 mg/dL/h; $P = 0.005$) or OCT2 (MATE1 reference, n=41, 306±41 mg/dL/h; MATE1 variant, n=4, 262±12 mg/dL/h; $P = 0.03$) polymorphisms. We were unable to detect a significant effect of the MATE1 variant in healthy volunteers after removal of individuals with the MATE2 g.-130G>A variant because of a reduction in sample size, which resulted in a substantial loss of power. Insulin AUC (reference 124±74 mU/L/h; variant 109±68 mU/L/h) and concentrations 2 h after glucose administration (reference 41±32 mU/L; variant 27±18 mU/L/h) were similar for both MATE1 reference and variant volunteers.

The MATE1 Promoter Variant, g.-66T>C, is Associated With a Greater Response to Metformin in Type II Diabetes Mellitus Patients

The effect of the MATE1 promoter variant on the response to metformin (relative change
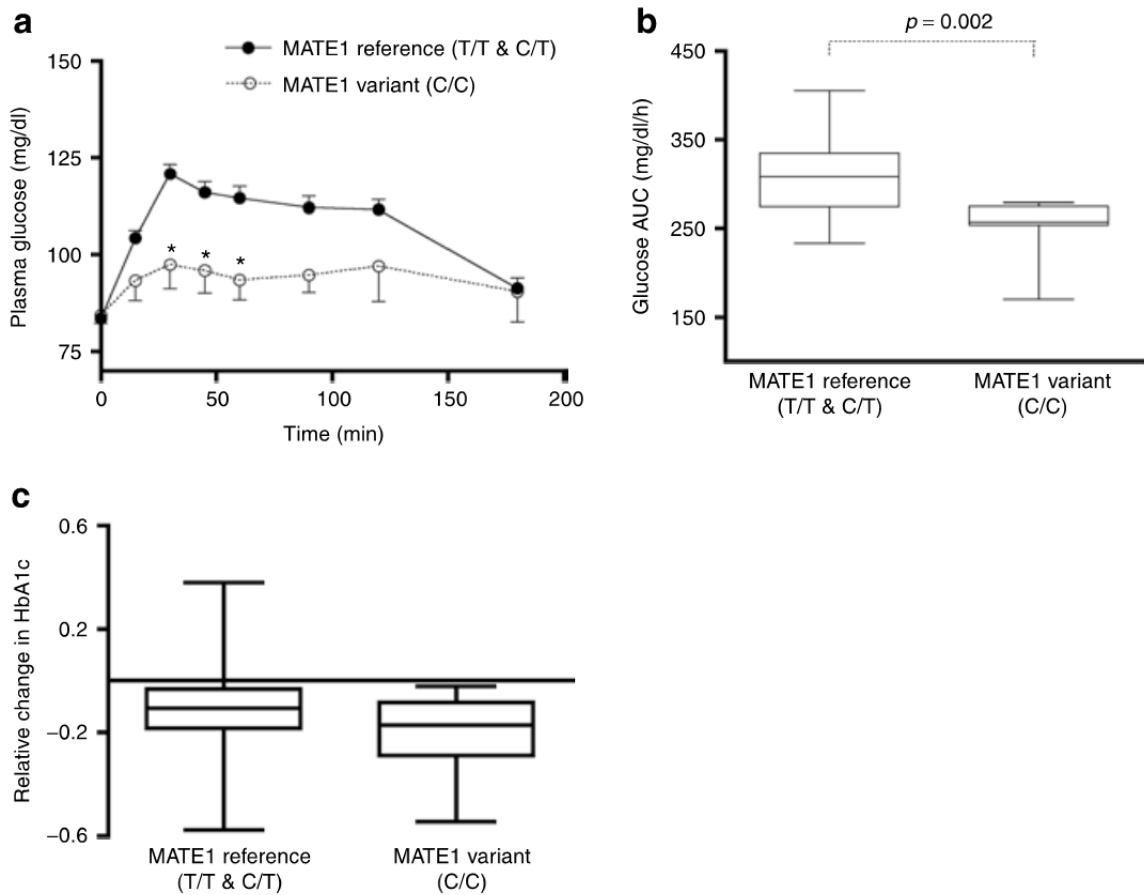
Figure 4. The multidrug and toxin extrusion protein 1 (MATE1) promoter variant (g.–66T>C) is associated with different response to metformin in healthy volunteers and patients with type 2 diabetes. (a) The time course of plasma glucose concentrations after an oral glucose tolerance test (OGTT) during metformin treatment in healthy volunteers carrying at least one reference MATE1 allele ($n = 49$, filled circles) and those carrying two MATE1 variant ($n = 8$, open circles) alleles. The data are expressed as mean ± SEM; $*P < 0.05$ as compared with volunteers with at least one reference allele. (b) The glucose exposure with OGTT (area under the curve, AUC) after metformin treatment in the same healthy volunteers represented in (a). (c) The relative change in glycosylated hemoglobin (HbA1c) in patients with type 2 diabetes ($n = 145$) receiving metformin monotherapy who are homozygous for the major alleles of OCT1 and carrying at least

169

one reference MATE1 allele (n = 122) or homozygous for the MATE1 variant allele (n = 23). Relative change in HbA1c was calculated as follows: (treatment minus baseline HbA1c)/baseline HbA1c. Relative change of −0.15 is interpreted as a decrease in HbA1c level by 15% from baseline. The box plots (b and c) display the median and interquartile range (the 25th–75th percentile). The whiskers display lower and upper values within 1.5 times the interquartile range beyond the 25th and 75th percentile.

in glycosylated hemoglobin, HbA1c) was examined in type 2 diabetic patients from a previously described (28) cohort of Caucasian (n=185) and African American (n=64) patients receiving metformin monotherapy. The MATE1 promoter variant alone was not associated with the relative change in HbA1c ($P > 0.6$). However, in our secondary analysis, in which we examined the effect of the MATE1 promoter variant together with other transporter variants, we obtained the following results: The MATE1 promoter variant was not associated with response to metformin after removal of patients who were carriers of OCT2 A270S or MATE2 g.-130G>A. In contrast, when patients carrying one or more OCT1 reduced-function variants were removed from the analysis, the MATE1 variant allele had a significant effect on response (Figure 4c, Table 4). That is, Caucasian and African American patients homozygous for the MATE1 variant allele had a significantly larger relative change in HbA1c levels (i.e., greater response to metformin) than patients carrying at least one reference MATE1 allele (Figure 4c, Table 4, $P = 0.01$).

The MATE2 Promoter Variant, g.-130G>A, is Associated With Increased Renal Clearance

With the exception of half-life, the pharmacokinetics of metformin were similar for individuals with the MATE2 reference (n=27) and those with the variant allele (n=30, Figure 5a and Table 5), even after adjustment for creatinine clearance (CL_{CR}). The elimination half-life was longer in volunteers carrying at least one MATE2 (g.-130G>A) variant allele as compared to those with the reference MATE2 genotype. This association remained even after adjustment for sex, body mass index (BMI) and MATE1 (g.-66T>C, rs2252281) genotype ($P = 0.02$).

Table 4.   Association analyses of MATE1 g.–66T>C with metformin response (relative change in HbA1c) in patients with type 2 diabetes.

Genotype group g.–66T→C	Number	Mean relative change (95% confidence interval)
(i) Caucasians (*n* = 90)		
TT	37	−0.15 (−0.20, −0.10)
TC	42	−0.088 (−0.12, −0.057)
CC	11	−0.27 (−0.36, −0.19)
TT/TC	79	−0.12 (−0.15, −0.088)
Statistical analyses		
Kruskal–Wallis test		$P = 0.0015$
Mann–Whitney test (TT/TC vs. CC)		$P = 0.0011$
Linear regression model (recessive): relative change = variant allele + dose		Coefficient = −0.14 (−0.23, −0.051); $P = 0.0022$
(ii) Caucasians and African Americans (*n* = 145)		
TT	59	−0.16 (−0.20, −0.12)
TC	63	−0.078 (−0.11, −0.048)
CC	23	−0.21 (−0.27, −0.016)
TT/TC	122	−0.12 (−0.14, −0.09)
Statistical analyses		
Kruskal–Wallis test		$P = 0.0008$
Mann–Whitney test (TT/TC vs. CC)		$P = 0.0064$
Linear regression model (recessive): relative change = variant allele + dose + ethnicity		Coefficient = −0.087 (−0.15 −0.021); $P = 0.011$

Mean relative change of HbA1c levels was calculated for each MATE1 g.–66T>C genotype group in the Caucasians (*n* = 90), or the combined Caucasian and African Americans (*n* = 145). All patients were homozygous for the organic cation transporter 1 major alleles. These major alleles are R61C (rs12208357), G410S (rs34130495), 420Del (rs72552763), and G465R (rs34059508). The 95% confidence intervals were calculated by nonparametric bootstrap estimates of the 95% confidence interval. Relative change =

(treatment HbA1c minus baseline HbA1c)/baseline HbA1c. Note: Relative change of −0.15 is interpreted as a decrease in HbA1c level by 15% from baseline. In the linear regression model, the coefficient represents the decrease (negative value) in relative change in HbA1c for those patients who are homozygous for the variant g.-66T>C allele. HbA1c, glycosylated hemoglobin; MATE1, multidrug and toxin extrusion protein 1.
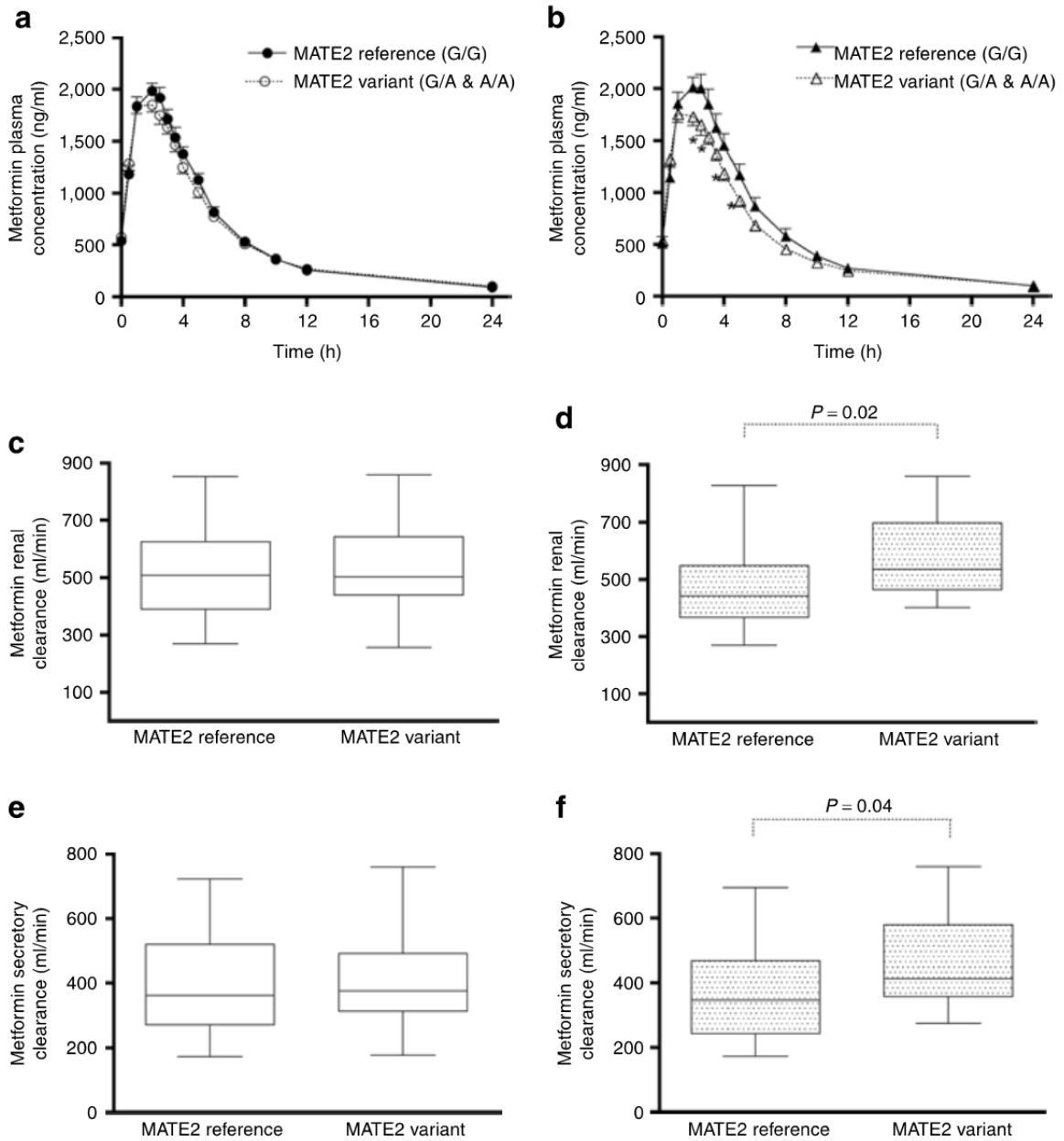
Figure 5. The effect of multidrug and toxin extrusion protein 2 (MATE2) (g.– 130G>A) on the pharmacokinetics of metformin in 57 healthy volunteers. The plasma concentration–time curves of metformin after oral administration to healthy volunteers (a) who carry at least one MATE2 variant allele (*n* = 30, open circles) or those who carry only MATE2 reference alleles (*n* = 27, filled circles) and carry either the reference or variant alleles of MATE1 or (b) who carry at least one MATE2 variant allele

(n = 17, open triangles) or those who carry only MATE2 reference alleles (n = 15, filled triangles) and only carry reference alleles for MATE1. Data represent mean ± SEM; *P < 0.05 as compared with volunteers with at least one reference allele. The (c) renal clearance and (e) net tubular secretion of the same volunteers (open boxes) depicted in (a). The (d) renal clearance and (f) net tubular secretion of the same volunteers (shaded boxes) depicted in (b). The box plots (c–f) display the median and interquartile range (the 25th–75th percentile). The whiskers display lower and upper values within 1.5 times the interquartile range beyond the 25th and 75th percentile. The renal secretion of metformin was calculated by subtracting the clearance of creatinine from the renal clearance of metformin. The volunteers were given two doses of metformin (1,850 mg in total). The plasma metformin concentration–time curves after the second dose are shown.

Table 5. **Summary of the metformin pharmacokinetic parameters in healthy volunteers with known MATE1, OCT1, and OCT2 genotype and homozygous for the MATE2 reference allele (–130G/G) and heterozygous or homozygous for the MATE2 variant allele (–130G/A or –130A/A).**

	All		MATE1 reference (TT)		OCT1 reference (AA)		OCT2 reference (CC)	
	MATE2 reference (GG)	MATE2 variant (GA and AA)	MATE2 reference (GG)	MATE2 variant (GA and AA)	MATE2 reference (GG)	MATE2 variant (GA and AA)	MATE2 reference (GG)	MATE2 variant (GA and AA)
n	27	30	15	17	41	8	38	7
T_{max} (h)	1.73 ± 0.66	1.79 ± 0.64	1.95 ± 0.66	1.69 ± 0.69	1.67 ± 0.68	1.97 ± 0.43	1.72 ± 0.65	1.96 ± 0.47
C_{max} (ng/ml)	2,187 ± 490	2,182 ± 601	2,277 ± 501	2,087 ± 598	2,205 ± 470	2,088 ± 883	2,161 ± 509	2,195 ± 897
AUC_{0-24} (ng·h/ml)	13,400 ± 3,200	13,500 ± 2,800	14,000 ± 3,700	12,700 ± 2,400	13,700 ± 3,000	12,400 ± 3,400	13,300 ± 2,900	12,900 ± 3,300
AUC_{inf} (ng·h/ml)	14,100 ± 3,300	14,600 ± 2,900	15,00 ± 3,700	13,800 ± 2,500	14,600 ± 3,000	13,200 ± 3,600	14,300 ± 3,000	13,700 ± 3,600
V/F (l)	538 ± 152	598 ± 205	515 ± 172	648 ± 202	556 ± 184	642 ± 208	581 ± 185	585 ± 142
CL/F (ml/min)	1,056 ± 256	1,015 ± 224	1,033 ± 304	1,063 ± 205	1,020 ± 238	1,138 ± 283	1,091 ± 270	1,040 ± 263
$T_{1/2}$ (h)	5.87 ± 1.03	6.77 ± 1.71*	5.71 ± 0.86	7.05 ± 1.93*	6.29 ± 1.63	6.52 ± 1.22	6.45 ± 1.61	6.27 ± 1.09
Amount in urine$_{0-24}$ (mg)	375 ± 88	377 ± 101	350 ± 56	387 ± 117	376 ± 99	368 ± 80	375 ± 86	372 ± 85
CL_R (ml/min)	527 ± 162	533 ± 143	473 ± 145	579 ± 142*	518 ± 146	582 ± 133	533 ± 149	550 ± 128
CL_{CR} (ml/min)	119 ± 32	122 ± 41	108 ± 28	118 ± 34	117 ± 28	145 ± 58	120 ± 30	134 ± 52
CL_{SR} (ml/min)	407 ± 158	410 ± 137	365 ± 145	461 ± 144*	401 ± 146	437 ± 106	413 ± 149	427 ± 110

Data were obtained from healthy volunteers given two doses of metformin. The first dose (1,000 mg) was given at 1800 hr on study day 1 and the second dose (850 mg) was given at 0700 h on study day 2. Blood and urine samples for the pharmacokinetic analysis were collected 0–24 h after the second dose. *$P < 0.05$ as compared with reference.

$AUC0–24$, area under the concentration–time curve from 0 to 24 h time point; AUC_{inf}, area under the plasma concentration–time curve from 0 to infinity; CL/F, apparent oral clearance; CL_{CR}, creatinine clearance; CL_R, renal clearance; CL_{SR}, renal clearance by secretion; C_{max}, maximal plasma concentration; MATE, multidrug and toxin extrusion protein; OCT, organic cation transporter; $T_{1/2}$, plasma terminal elimination half-life; T_{max}, time to the maximal plasma concentration; V/F, apparent volume of distribution.

In the secondary analysis, we removed volunteers carrying at least one minor allele of the MATE1 polymorphism. In individuals who were homozygous for the reference MATE1 (n=32), the MATE2 variant was associated with lower plasma levels of metformin between 2 and 5 hours after metformin administration ($P < 0.05$, Figure 5b). Although most of the pharmacokinetic parameters remained similar between the MATE2 variant groups, the renal clearance (CL_R) and renal clearance by secretion (CL_{SR}) of metformin were significantly higher in volunteers carrying at least one MATE2 variant allele as compared with those homozygous for reference MATE2 ($P < 0.05$, Table 5, Figure 5).

The MATE2 Promoter Variant, g-130G>A, is Associated With Reduced Metformin Response in Healthy Volunteers

After metformin dosing, the glucose AUC was higher for volunteers homozygous for the MATE2 variant allele (333±37 mg/dL/h) as compared with those carrying at least one reference MATE2 allele (295±44 mg/dL/h; $P = 0.02$; Figure 6a-b), whereas no effect of the MATE2 variant was observed in baseline glucose AUC before metformin treatment. Removing individuals with variants in MATE1 (MATE2 reference, 301±38 mg/dL/h; MATE2 variant, 345±36 mg/dL/h; $P = 0.02$), OCT1 (MATE2 reference, 298±45 mg/dL/h; MATE2 variant, 333±37 mg/dL/h; $P = 0.04$) or OCT2 (MATE2 reference, 299±41 mg/dL/h; MATE2 variant, 342±30 mg/dL/h; $P = 0.02$) resulted in no change in the significance level of the glucose AUC between reference and variant MATE2 genotypes during metformin treatment.

Using linear regression, we determined if sex, age, BMI, fasting glucose, fasting insulin,
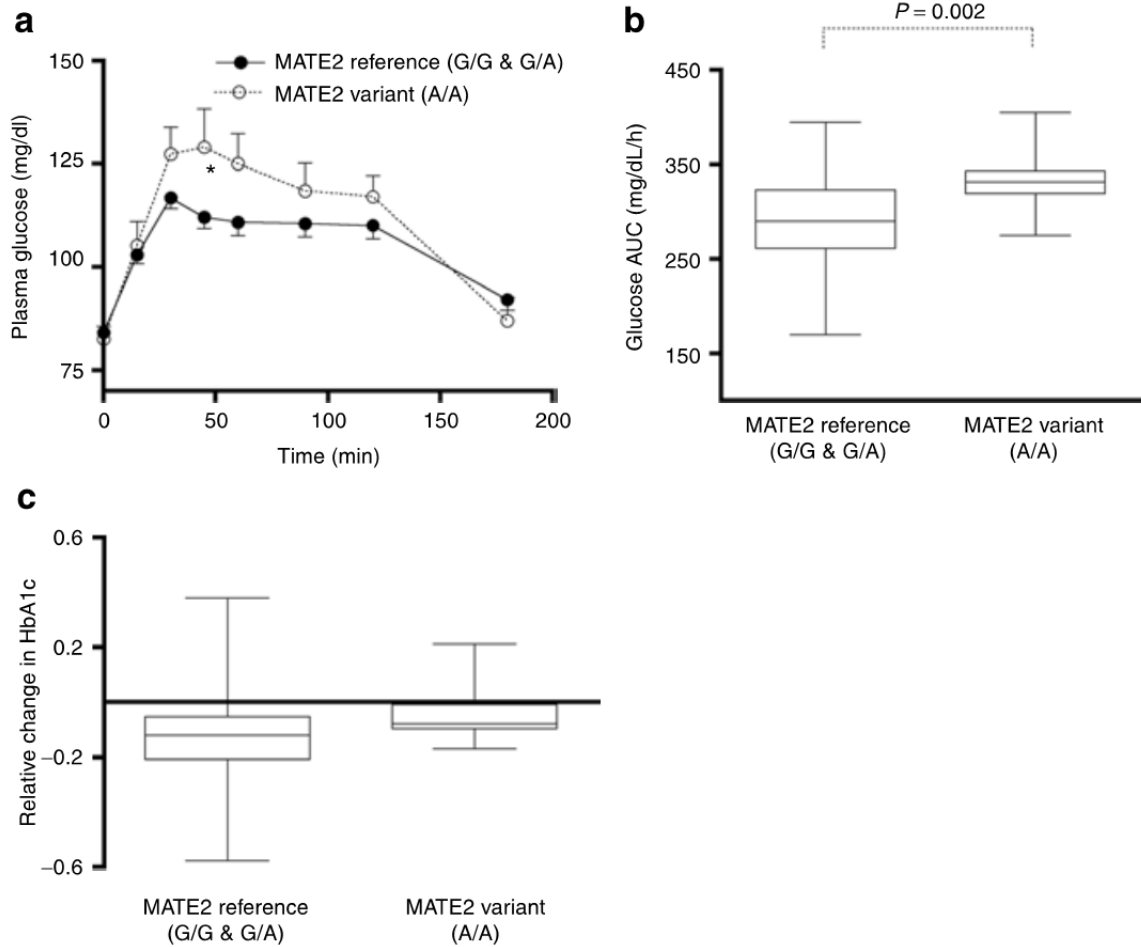
Figure 6. **Multidrug and toxin extrusion protein 2 (MATE2) genetic variants are associated with different response to metformin in healthy volunteers and patients with type 2 diabetes.** (a) The time course of plasma glucose concentrations after an oral glucose tolerance test (OGTT) during metformin treatment in healthy volunteers carrying at least one reference MATE2 allele ($n = 49$, filled circles) and those carrying two MATE2 variant alleles ($n = 8$, open circles). The data are expressed as mean $\pm$ SEM; $*P < 0.05$ as compared with volunteers with at least one reference allele. (b) The glucose exposure with OGTT (area under the curve) after metformin treatment in healthy volunteers. (c) The relative change in glycosylated hemoglobin (HbA1c) in Caucasian ($n = 189$) and African-American ($n = 64$) patients with type 2 diabetes receiving metformin

monotherapy who carry the reference allele for the MATE2 reduced–function coding variant (c.485C>T) and carry at least one reference MATE2 allele ($n = 232$) or are homozygous for the MATE1 variant allele ($n = 16$). Relative change in HbA1c was calculated as follows: (treatment minus baseline HbA1c)/baseline HbA1c. Relative change of –0.15 is interpreted as a decrease in HbA1c level by 15% from baseline. The box plots (b,c) display the median and interquartile range (the 25th–75th percentile). The whiskers display lower and upper values within 1.5 times the interquartile range beyond the 25th and 75th percentile.

MATE1 genotype, MATE2 genotype or metformin exposure (AUC) predicted the glucose AUC during metformin treatment. The MATE1 (P = 0.02) and MATE2 genotypes (P = 0.02) were the only significant predictors of metformin response, with each genotype alone explaining 7% of the variability in response to metformin. When both MATE1 and MATE2 genotype were included in a multiple linear regression model, 15% of the variance in metformin response was explained (P = 0.005). The OCT2 genetic polymorphism has been previously associated with the renal clearance of metformin and nephrotoxicity of cisplatin (15; 35). In our study, the OCT2 genotype (rs316019) alone had no effect on variation in the pharmacokinetics or pharmacodynamics of metformin.

DISCUSSION

Previous reports on genetic variants of MATE1, MATE2, OCT1 and/or OCT2 on metformin pharmacokinetics (17) and/or pharmacodynamics (11; 12; 17; 21; 26; 36) have focused on synonymous or nonsynonymous single-nucleotide polymorphisms (SNPs) within nonregulatory regions of these genes. In this study, we determined the effects of two promoter variants of MATE1 (rs2252281) and MATE2 (rs12943590), discovered previously in our laboratory (28; 29), on the pharmacokinetics and pharmacological response to metformin in healthy volunteers and on the glycemic response to metformin in patients with type 2 diabetes. In our primary analysis, we considered each variant separately. Given the colocalization of MATE1 and OCT1 in hepatocytes, and MATE1, MATE2 and OCT2 in proximal tubule cells (Figure 2), polymorphisms affecting the accumulation and/or elimination of metformin in these tissues could confound the effects

of the individual MATE1 and MATE2 promoter variants on metformin disposition and response. Therefore, whenever possible, we performed secondary analyses excluding individuals who had OCT1 (rs72552763), OCT2 (rs316019) or the other MATE genotypes that could potentially confound the measured parameters. Our major findings include: (*i*) The MATE1 reduced-expression promoter variant is associated with increased response to metformin in healthy volunteers and patients with type 2 diabetes who were homozygous for the OCT1 reference allele. (*ii*) The MATE2 enhanced-expression promoter variant is associated with reduced response to metformin in healthy volunteers. (*iii*) The CL_R and CL_{SR} of metformin were significantly greater in volunteers carrying the promoter variant of MATE2 as compared with those homozygous for reference MATE2 in a subset of healthy individuals who were homozygous for the MATE1 reference allele. To our knowledge, this is the first study to show that functional promoter polymorphisms in MATE1 and MATE2 contribute to variation in the response and disposition of metformin in both healthy volunteers and type 2 diabetic patients.

Although the MATE1 polymorphism (g.-66T>C) did not influence metformin disposition, healthy volunteers who were homozygous for the MATE1 variant allele had a greater glucose-lowering response to metformin (Figure 4a-b). Similarly, diabetic patients without reduced-function alleles of OCT1 who were also homozygous for the MATE1 variant allele had a 15% greater relative reduction in Hb1Ac as compared with patients carrying the MATE1 reference allele (Figure 4c, Table 4). The finding that a MATE1 variant affects the pharmacodynamics, but not the pharmacokinetics of metformin, underscores the importance of transporters in tissue-specific drug distribution.

181

MATE1 is highly expressed in both the liver and kidney (16) (Figure 1), with the liver being a major site of pharmacologic action and the kidney being predominantly a site of metformin elimination (although effects on glucose may also occur). In the kidney, MATE1 is redundantly coexpressed with MATE2 and MATE2-K, and therefore, a variant in MATE1 would need to have a large effect size to have a measurable effect on the renal elimination of the drug. In contrast, in the liver, MATE1 appears to be the sole metformin transporter expressed on the bile canalicular membrane; therefore, a genetic variant in MATE1 may have a more measurable effect on the pharmacodynamics compared to the pharmacokinetics of metformin. Mechanistically, our results are consistent with MATE1 acting as a proton gradient-driven efflux pump in tissues of pharmacodynamic importance, such as the liver (37; 38). The reduced-expression promoter polymorphisms of MATE1 would presumably result in reduced transporter expression levels, leading to reduced efflux and correspondingly higher tissue levels of metformin. The higher tissue levels of metformin are predicted to associate with a greater pharmacologic response. Of note, when diabetic patients carrying the OCT1 reduced-function variant alleles were included in the analyses, we did not observe an effect of the MATE1 promoter variant on the response to metformin. It is possible that the reduced-function OCT1 variant masked the effects of the MATE1 variant. Particularly in the liver, the variant allele of OCT1 would result in lower drug levels and thus oppose the effects of the reduced-expression variant of MATE1. Our results also suggest that the MATE1 variant has a noticeable effect in patients with type 2 diabetes. For example, a typical diabetic patient with a baseline HbA1c of 8% (Table 2) receiving metformin monotherapy (1,000 mg/day) and a carrier of the reference allele for the OCT1

genotype would have their HbA1c decreased by an additional 1.2% if they were a carrier of the MATE1 variant allele instead of the reference MATE1. The magnitude of this effect is large and of clinical significance, given that, on average, metformin monotherapy lowers HbA1c by 1.12% within the first year of therapy (39; 40). If our results are replicated in other cohorts, genotyping for the MATE1 polymorphism as a basis for personalizing metformin hypoglycemic therapy should be considered.

The effect of genetic variants in MATEs on metformin pharmacokinetics and pharmacologic action also suggests the potential for clinically significant drug-drug interactions that may "phenocopy" the effects of genetic variants. In fact, in an in vitro cell system (Chinese Hamster Ovary), inhibitors of human MATEs were identified from a diverse set of drug classes (e.g., pyrimethamine, baclofen, ketoconazole, naloxone, propranolol) (41). In addition, recently Kusuhara et al. (42) demonstrated that pyrimethamine, an anti-protozoal drug and inhibitor of MATEs, reduced metformin renal clearance in human volunteers. This example highlights the importance of follow-up clinical studies to elucidate the clinical consequences of any *in vitro* drug-drug interactions identified and how such a drug-drug interaction may phenocopy the reduced-expression variant of MATE1.

Recent studies in our laboratory have shown that a common MATE2 promoter variant (g.-130G>A) associates with reduced response to metformin in diabetic patients (28). In the current study, we hypothesized that this variant is associated with reduced response to metformin as a result of a pharmacokinetic mechanism. Surprisingly, in healthy

volunteers, MATE2 g.-130G>A did not affect the CL_R and CL_{SR} of metformin (Figure 5 and Table 5). Because MATE1 and MATE2 are expressed on the apical membrane of the proximal tubule and are likely to work together to mediate metformin renal elimination (Figure 2), we hypothesized that the effect of the MATE2 variant may be masked by the opposing effect of the MATE1 variant. Indeed, when we removed the individuals with the reduced-expression promoter variant of MATE1 g.-66T>C, we observed that the CL_R and CL_{SR} of metformin were significantly greater in individuals carrying the MATE2 variant allele compared to the reference allele (Figure 5). Consistent with this finding, the mean metformin exposure (AUC) was lower and the mean amount of metformin excreted in urine was higher in volunteers with the variant MATE2 allele, although the difference was not statistically significant, possibly because of variability in the bioavailability of metformin among our participants (Table 5). Of note, metformin concentrations were significantly lower in volunteers carrying the variant MATE2 at time points after the maximal plasma concentration (C_{max}). Importantly, we observed a longer half-life in individuals with the MATE2 variant alleles ($P = 0.03$). As metformin exhibits apparent flip-flop kinetics (43), early time points reflect elimination, whereas, later time points predominantly reflect drug absorption. The lower plasma levels after the C_{max} and the longer half-life of the drug in individuals homozygous for the variant MATE2 allele are consistent with a reduced rate and extent of absorption of metformin. These results suggest that expression polymorphisms of MATE2 may alter the absorption of metformin from the gastrointestinal tract, as well as, its secretion (clearance) from the kidney. Although currently not implicated in the absorption of metformin, MATE2, is expressed in the small intestine (Figure 1) and could

184

contribute to the interindividual variation in the bioavailability of metformin. Current immunohistochemistry data suggests that MATE2 is expressed at a moderate level in the different sections of the gastrointestinal tract (e.g. duodenum, stomach, small intestine and colon) (44). Further studies are required to confirm the localization and determine the function of MATE2 in the gastrointestinal tract and delineate the impact of MATE2 on the absorption of metformin.

The current study in healthy volunteers is consistent with previous studies in type 2 diabetic patients (28) that associate the MATE2 promoter variant with a reduced response to metformin and provides evidence that this effect may be the result of a pharmacokinetic mechanism. That is, plasma levels of metformin were significantly lower in the volunteers homozygous for the variant MATE2 allele (Figure 5) throughout the OGTT (2-5 h after metformin administration), potentially decreasing glucose uptake after the OGTT. Alternatively, by modulating metformin levels in a target tissue, the MATE2 variant may directly affect the pharmacodynamics of metformin. In particular, MATE2 is predominantly expressed in the kidney (Figure 1) (16), an organ increasingly recognized to play a significant role in both systemic glucose production (20-25%) and glucose utilization (10%) in the fasting state (45-48). In type 2 diabetes, renal gluconeogenesis and glucose uptake increases (49). Thus, individuals homozygous for the MATE2 enhanced-expression variant would achieve lower levels of metformin in the kidney, possibly translating into a reduced pharmacologic effect. A more comprehensive understanding of the peripheral targets of metformin is needed to completely interpret these results.

In conclusion, this study demonstrates that promoter variants of MATE1 and MATE2 contribute to the glycemic response to metformin in healthy volunteers and in patients with type 2 diabetes. Furthermore, the study provides evidence that MATE1 and MATE2 work in concert in the kidney to mediate metformin renal elimination and that genetic variants of MATEs and OCTs should be considered together when ascertaining the genetic determinants of renal elimination of metformin. Finally, the results of our study suggest an important role of MATE2 in the pharmacokinetics and pharmacodynamics of metformin.

REFERENCES

1.   Alexander GC, Sehgal NL, Moloney RM, Stafford RS. 2008. National trends in treatment of type 2 diabetes mellitus, 1994-2007. *Arch Intern Med* 168:2088-94

2.   Zhou G, Myers R, Li Y, Chen Y, Shen X, et al. 2001. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108:1167-74

3.   Cusi K, Consoli A, DeFronzo RA. 1996. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 81:4059-67

4.   Pentikainen PJ, Neuvonen PJ, Penttila A. 1979. Pharmacokinetics of metformin after intravenous and oral administration to man. *Eur J Clin Pharmacol* 16:195-202

5.   Tucker GT, Casey C, Phillips PJ, Connor H, Ward JD, Woods HF. 1981. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. *Br J Clin Pharmacol* 12:235-46

6.   Sirtori CR, Franceschini G, Galli-Kienle M, Cighetti G, Galli G, et al. 1978. Disposition of metformin (N,N-dimethylbiguanide) in man. *Clin Pharmacol Ther* 24:683-93

7.   Graham GG, Punt J, Arora M, Day RO, Doogue MP, et al. 2011. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet* 50:81-98

8.   Yin OQ, Tomlinson B, Chow MS. 2006. Variability in renal clearance of substrates for renal transporters in chinese subjects. *J Clin Pharmacol* 46:157-63

9.   Leabman MK, Giacomini KM. 2003. Estimating the contribution of genes and environment to variation in renal drug clearance. *Pharmacogenetics* 13:581-4

10. Cook MN, Girman CJ, Stein PP, Alexander CM. 2007. Initial monotherapy with either metformin or sulphonylureas often fails to achieve or maintain current glycaemic goals in patients with Type 2 diabetes in UK primary care. *Diabet Med* 24:350-8

11. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, et al. 2008. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther* 83:273-80

12. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, et al. 2007. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 117:1422-31

13. Tzvetkov MV, Vormfelde SV, Balen D, Meineke I, Schmidt T, et al. 2009. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. *Clin Pharmacol Ther* 86:299-306

14. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. 2010. Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response. *Pharmacogenet Genomics* 20:38-44

15. Chen Y, Li S, Brown C, Cheatham S, Castro RA, et al. 2009. Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. *Pharmacogenet Genomics* 19:497-504

16. Masuda S, Terada T, Yonezawa A, Tanihara Y, Kishimoto K, et al. 2006. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol* 17:2127-35

17. Christensen MM, Brasch-Andersen C, Green H, Nielsen F, Damkier P, et al. 2011. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. *Pharmacogenet Genomics* 21:837-50

18. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. 2009. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. *Pharmacogenomics J* 9:242-7

19. Konig J, Zolk O, Singer K, Hoffmann C, Fromm MF. 2011. Double-transfected MDCK cells expressing human OCT1/MATE1 or OCT2/MATE1: determinants

of uptake and transcellular translocation of organic cations. *Br J Pharmacol* 163:546-55

20. Sato T, Masuda S, Yonezawa A, Tanihara Y, Katsura T, Inui K. 2008. Transcellular transport of organic cations in double-transfected MDCK cells expressing human organic cation transporters hOCT1/hMATE1 and hOCT2/hMATE1. *Biochem Pharmacol* 76:894-903

21. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. 2009. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study. *Diabetes* 58:745-9

22. Meyer zu Schwabedissen HE, Verstuyft C, Kroemer HK, Becquemont L, Kim RB. 2010. Human multidrug and toxin extrusion 1 (MATE1/SLC47A1) transporter: functional characterization, interaction with OCT2 (SLC22A2), and single nucleotide polymorphisms. *Am J Physiol Renal Physiol* 298:F997-F1005

23. Wang ZJ, Yin OQ, Tomlinson B, Chow MS. 2008. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. *Pharmacogenet Genomics* 18:637-45

24. Song IS, Shin HJ, Shim EJ, Jung IS, Kim WY, et al. 2008. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. *Clin Pharmacol Ther* 84:559-62

25. Tsuda M, Terada T, Mizuno T, Katsura T, Shimakura J, Inui K. 2009. Targeted disruption of the multidrug and toxin extrusion 1 (mate1) gene in mice reduces renal secretion of metformin. *Mol Pharmacol* 75:1280-6

26.   Toyama K, Yonezawa A, Tsuda M, Masuda S, Yano I, et al. 2010. Heterozygous variants of multidrug and toxin extrusions (MATE1 and MATE2-K) have little influence on the disposition of metformin in diabetic patients. *Pharmacogenet Genomics* 20:135-8

27.   Chen L, Pawlikowski B, Schlessinger A, More SS, Stryke D, et al. 2010. Role of organic cation transporter 3 (SLC22A3) and its missense variants in the pharmacologic action of metformin. *Pharmacogenet Genomics* 20:687-99

28.   Choi JH, Yee SW, Ramirez AH, Morrissey KM, Jang GH, et al. 2011. A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. *Clin Pharmacol Ther* 90:674-84

29.   Ha Choi J, Wah Yee S, Kim MJ, Nguyen L, Ho Lee J, et al. 2009. Identification and characterization of novel polymorphisms in the basal promoter of the human transporter, MATE1. *Pharmacogenet Genomics* 19:770-80

30.   Roden DM, Pulley JM, Basford MA, Bernard GR, Clayton EW, et al. 2008. Development of a large-scale de-identified DNA biobank to enable personalized medicine. *Clin Pharmacol Ther* 84:362-9

31.   Timmins P, Donahue S, Meeker J, Marathe P. 2005. Steady-state pharmacokinetics of a novel extended-release metformin formulation. *Clin Pharmacokinet* 44:721-9

32.   Rao N, Chou T, Ventura D, Abramowitz W. 2005. Investigation of the pharmacokinetic and pharmacodynamic interactions between memantine and glyburide/metformin in healthy young subjects: a single-center, multiple-dose, open-label study. *Clin Ther* 27:1596-606

33. Sambol NC, Chiang J, O'Conner M, Liu CY, Lin ET, et al. 1996. Pharmacokinetics and pharmacodynamics of metformin in healthy subjects and patients with noninsulin-dependent diabetes mellitus. *J Clin Pharmacol* 36:1012-21

34. Sambol NC, Chiang J, Lin ET, Goodman AM, Liu CY, et al. 1995. Kidney function and age are both predictors of pharmacokinetics of metformin. *J Clin Pharmacol* 35:1094-102

35. Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, Sparreboom A. 2009. Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin Pharmacol Ther* 86:396-402

36. Jablonski KA, McAteer JB, de Bakker PI, Franks PW, Pollin TI, et al. 2010. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program. *Diabetes* 59:2672-81

37. Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. 2005. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci U S A* 102:17923-8

38. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. 2007. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol* 74:359-71

39. Little RR, Rohlfing CL. 2011. Analytical goals for HbA1c: are HbA1c results good enough for optimal use? *J Diabetes* 3:3-6

40. Hirst JA, Farmer AJ, Ali R, Roberts NW, Stevens RJ. 2012. Quantifying the effect of metformin treatment and dose on glycemic control. *Diabetes Care* 35:446-54

41. Astorga B, Ekins S, Morales M, Wright SH. 2012. Molecular determinants of ligand selectivity for the human multidrug and toxin extruder proteins MATE1 and MATE2-K. *J Pharmacol Exp Ther* 341:743-55

42. Kusuhara H, Ito S, Kumagai Y, Jiang M, Shiroshita T, et al. 2011. Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at oral microdose and at therapeutic dose in healthy subjects. *Clin Pharmacol Ther* 89:837-44

43. Scheen AJ. 1996. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet* 30:359-71

44. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, et al. 2010. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* 28:1248-50

45. McGuinness OP, Fugiwara T, Murrell S, Bracy D, Neal D, et al. 1993. Impact of chronic stress hormone infusion on hepatic carbohydrate metabolism in the conscious dog. *Am J Physiol* 265:E314-22

46. Gerich JE. 2010. Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: therapeutic implications. *Diabet Med* 27:136-42

47. Gerich JE. 2000. Physiology of glucose homeostasis. *Diabetes Obes Metab* 2:345-50

48. Meyer C, Dostou JM, Welle SL, Gerich JE. 2002. Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *Am J Physiol Endocrinol Metab* 282:E419-27

49. Meyer C, Woerle HJ, Dostou JM, Welle SL, Gerich JE. 2004. Abnormal renal, hepatic, and muscle glucose metabolism following glucose ingestion in type 2 diabetes. *Am J Physiol Endocrinol Metab* 287:E1049-56

CHAPTER 6

DEVELOPMENT AND CHARACTERIZATION OF A

HUMANIZED MATE2K MOUSE

INTRODUCTION

In drug development, the identification of drug transporters that play a role in the drug's disposition, safety and efficacy is a major challenge. *In vitro* assays are useful to inform drug developers whether a particular drug is a substrate and/or inhibitor of a given transporter. However, in most cases, drugs are substrates and inhibitors of multiple membrane transporters. In these situations, it is difficult to define the relative contribution of each transporter to the tissue accumulation and elimination of a drug.

In the past two decades, the development of genetically modified mouse models has been a valuable tool to illustrate and identify the *in vivo* impact of membrane transporters on pharmacokinetics and response. Knockout and transgenic mouse models are available for many transporters (*e.g.*, Mdr1a, Bcrp, Mrp2, Oct1, Oct2, Mate1) (1-7), and these have played enormous roles in understanding the role of transporters in mediating pharmacokinetics and pharmacodynamics.

Species differences in the tissue distribution of organic cation transporters have been demonstrated (8-10). For MATE2, the ortholog of human MATE2 has not been found in rodents and the ortholog of rodent Mate2 has not been identified in humans (11; 12). In fact, recent phylogenic analyses have classified human MATE2 and rodent Mate2 in

entirely separate classes (9; 13) and some investigators have suggested that it would be appropriate to rename rodent Mate2 to Mate3 (13). Therefore, a wild type mouse can be considered to be essentially a Mate2 knockout mouse.

To provide an *in vivo* tool that will aid in defining the role of human MATE2K in mediating drug disposition, safety and efficacy, we attempted to develop a mouse that selectively expresses human MATE2K in the proximal tubular cells. We selected to the gamma glutamyl transpeptidase 1 type II promoter as it has been successfully used to generate other transgenic mice with proximal tubular cell specific expression without any known regulation by hormones or xenobiotics (14-16). In this study, a mouse that has MATE2K transgene integrated into its genome was developed. However, this transgene did not demonstrate transgene expression in the kidney or any other tissue analyzed.

MATERIALS AND METHODS

Design and Construction of the Transgenic Construct

The transgene used included the proximal tubule-specific promoter of mouse gamma glutamyl transpeptidase 1 type II (GGT1; -346 to +70 bp, relative to start codon), Kozak sequence (GCCACC), the coding sequence for human MATE2K and the bovine growth hormone polyadenylation signal (bGH polyA). Briefly, GGT1 and MATE2K were cloned from human kidney cDNA and inserted into the pcDNA3.1 (+) vector (Life Technologies, Grand Island, NY) in the *Nhe*I/*Kpn*I and *Xho*I/*Xba*I restriction sites. At the end of the bGH polyA (native to the pcDNA3.1 (+) vector), residues (5'-GCTGGG-3') were mutated to engineer a *Sma*I restriction site (5'-CCCGGG-3'). Primers used for

cloning are listed in Table 1. A schematic of the transgene is shown in Figure 1.

Validation of GGT1 Promoter Activity in HEK293 Cells

The GGT1 promoter was cloned into pGL3 (Promega, Madison, WI) and transfected into human embryonic kidney 293 (HEK293) cells using lipofectamine LTX (Life Technologies), according to the manufacturer's protocol. HEK293 cells were cultured in Dulbecco's modified eagle medium (DMEM) H-21 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 U/mL streptomycin and 2 mM glutamine. Approximately 32 hours after transfection, the reporter assay was measured using the Dual-luciferase® reporter assay system (Promega) according to the manufacturer's protocol and quantified using the Glomax 96-well plate luminometer (Promega). The firefly luciferase to renilla luciferase ratios were determined and expressed as relative luciferase activity.

Transgenic Mouse Line Generation

MATE2K humanized mice (hMATE2K) were generated in a wild-type C57BL6 background by the Gladstone Institute's Transgenic Gene Targeting Core (San Francisco, CA). Briefly, the transgene was linearized by sequential restriction digest with *Nhe*I and *Sma*I and purified by gel extraction. Linearized transgene was then injected into a pronucleus of a fertilized on-cell host embryo and the microinjected embryos were implanted into the oviducts of recipient female mice. After birth, founders were identified by PCR of DNA derived from mouse tails using GoTaq Green Master Mix (Promega) and transgene specific primers (Table 1). Founders were then mated with

Table 1. PCR primers used for cloning of the MATE2K transgene and for the detection of the transgene in humanized MATE2K mice.

Primer name	Sequence (5' --> 3')
NheI-GGT1 fwd	CTAGCTAGCTAGAGATCTAAGCTATGGTCTAGTGCCTGG
GGT1-KpnI rvs	GGGGTACCCCGGCAAGAGGTCAGCTAAGGGG
XhoI-MATE2K fwd	CCGCTCGAGCGGGCCACCATGGACAGCCTCCAGGACAC
MATE2K-XbaI rvs	GCTCTAGAGCCTAGTGCCTGGTGGCTAGGATC
bGH polyA SmaI fwd	CTGAGGCGGAAAGAACCACCCGGGGCTCTAGGGGGTATC
bGH polyA SmaI rvs	GATACCCCCTAGAGCCCCGGGTGGTTCTTTCCGCCTCAG
GGT1-MATE2K genotype fwd	GTGGGGGAGCCCCTTTCCCAG
GGT1-MATE2K genotype rvs	GCCGCCACTGTGCTGGATATCTG
MATE2K ORF genotype fwd	TCATGATCTGTGTTGAGTGGTGGG
MATE2K ORF genotype rvs	TGACCACAAAGGTCAGAAGGATGC

fwd, forward (sense) primer; rvs, reverse (anti-sense) primer; GGT1, gamma glutamyl transpeptidase 1 type II; hMATE2K, humanized multidrug and toxic extrusion protein 2K; bGH poly A, bovine growth hormone poly adenylation signal; ORF, open reading frame.
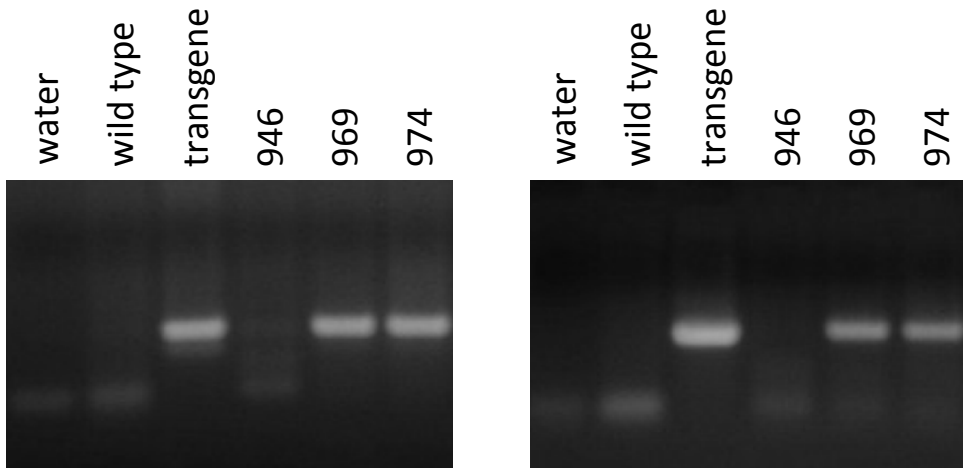
Figure 1a

Figure 1b
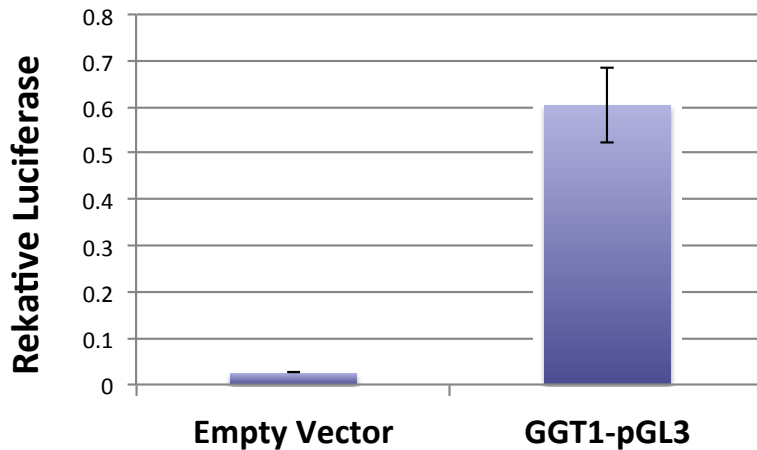
Figure 1c

Figure 1.   Generation of MATE2K humanized mice. (a) Schematic of hMATE2K transgene. (b) Validation of GGT1 promoter activity in HEK293 cells.   The firefly luciferase to renilla luciferase ratios were determined and expressed as relative luciferase activity.   Data shown represent mean values and SD from triplicate wells in a

representative experiment. (c) PCR identification of hMATE2K transgene in tail DNA from founders 969 and 944 using primers spanning the GGT1-hMATE2K junction (left) and MATE2K cDNA (right) of the transgene.

hMATE2K, humanized multidrug and toxic extrusion protein 2K; GGT1, gamma glutamyl transpeptidase 1 type II; ORF, open reading frame; HEK293, human embryonic kidney 293; PCR, polymerase chain reaction.

wild-type C57BL6 mice and lines were expanded (only founder 969 produced pups). The presence of the transgene was verified in all new pups of subsequent generations. These experiments were approved by the Institutional Animal Care and Use Committee of University of California, San Francisco.

Total Body and Organ Weights of hMATE2K Mice

Age matched 15 week old male C57BL6 WT (n=3) and hMATE2K (n=3) mice were fasted overnight, weighed, anesthetized with isoflurane and sacrificed by cervical dislocation. Tissues (kidney, liver, skeletal muscle, gonadal depot fat, brain, small intestine, large intestine, stomach, heart, spleen and lung) were weighed and snap frozen in liquid nitrogen for RNA extraction.

Phenotyping of hMATE2K Mice

Age matched 23 week old male C57BL6 WT (n=3) and hMATE2K (n=3) mice were placed in metabolic cages for 24 h and urine was collected. After 24 h, animals were anesthetized with isoflurane, and blood (500-1000 μl) was collected by heart puncture and transferred to Vacutainer serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Tubes were inverted five times, allowed to clot for 30 minutes and centrifuged for 10 minutes at 1000-1300xg. Animals were perfused with 1x phosphate buffered saline, followed by 4% paraformaldehyde (PFA). Gonadal depot fat and kidneys were fixed for 18 hours in 4% PFA, embedded in paraffin, cut into 7 micron sections and mounted on glass slides. The tissue sections were stained with hematoxylin and eosin for histologic analysis. Serum and urine were sent for chemistry evaluation.

Serum and Urine Chemistry Evaluation

Serum and urine chemistry were conducted by the Clinical Laboratory at San Francisco General Hospital. Sodium, potassium, chloride, carbon dioxide, creatinine, blood urea nitrogen, glucose, calcium, albumin, phosphate, alanine transaminase, aspartate transaminase, uric acid and lactate dehydrogenase were measured in serum and sodium, potassium, chloride, creatinine, urea nitrogen, glucose, phosphate, uric acid and protein were measured in urine.

RNA Isolation and Gene Expression Analysis

Total RNA from kidney, liver, brain, small intestine, large intestine, stomach, heart, spleen and lung was extracted using Qiagen's RNeasy Kit (Valencia, CA), according to the manufacturer's protocol. Total RNA from skeletal muscle and gonadal depot fat was extracted using TRIzol (Life Technologies), following the manufacturer's protocol. RNA was quantified using a Nanodrop 1000 (ThermoFisher Scientific, Wilmington, DE) and stored at -80°C until use. Total RNA from each sample was reverse transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen), according to the manufacturer's protocol. Quantitative RT-PCR was carried out in 96-well or 382-well plates in a total volume of 10 μl reaction solution that includes a cDNA equivalent of up to 100 ng total RNA, specific probe and Taqman Universal Master Mix (Life Technologies). Reactions were run on an ABI 7900 HT Fast Real-Time PCR machine (Life Technologies) using the default thermal cycling conditions. The following TaqMan Gene Expression Assays were used: mouse Oct1, Mm00456303_m1; mouse Oct2, Mm00457295_m1; mouse Oct3, Mm00488294_m1; mouse Mate1, Mm00840361; mouse

Pmat, Mm00525575; human MATE2K, Hs00945650_m1; mouse Ppia, Mm03302254_g1; mouse Gapdh, Mm99999915_g1. The delta cycle threshold (C_T) values for all the genes in each sample were calculated by subtracting the mean C_T values for two housekeeping genes (Ppia and Gapdh) from the C_T for each target gene. The relative quantity of each gene was then determined by calculating the $2^{-\Delta Ct}$ value.

Statistical Analysis

Data are presented as mean ± SD. Unpaired nonparametric Student's *t*-tests were used to analyze the differences between MATE2K and C57BL6 WT mice using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). A statistically significant result was defined when $p < 0.05$.

RESULTS

Generation of Humanized MATE2K Mice

Humanized MATE2K mice were generated to exclusively express the human MATE2K gene in the proximal tubule cells of the kidney. A schematic of the transgene is presented in Figure 1a. To validate the *in vitro* activity of the GGT1-promoter, luciferase gene reporter assays were performed with GGT1-pGL3. In HEK293 cells, GGT1-pGL3 transfected cells demonstrated a 22-fold increase in relative luciferase expression above pGL3-transfected cells (Figure 1b). Humanized MATE2K mice were generated by pronuclear microinjection using a GGT1-MATE2K-bGH polyA transgene. Thirty-three pups were evaluated for transgene integration by PCR analysis of tail DNA. Of these pups, two (969 and 974) were identified as founders and lines were expanded (Figure 1c).

Total Body and Organ Weights of hMATE2K Mice

Age-matched 15-week old hMATE2K mice (*n*=3, per group) had a greater total body weight than wild type mice (*n*=3, per group; *p*=0.02; Figure 2a). In addition, hMATE2K mice also had significantly larger gonadal depot fat than wild type mice (*n*=3, per group; *p*<0.0005; Figure 2b). There was no significant difference in the weights of kidney, liver, skeletal muscle, brain, small intestine, large intestine, stomach, heart, spleen and lung organs between wild type and hMATE2K mice.

Kidney and Gonadal Depot Fat Histology

Hematoxylin and eosin staining of wild type and hMATE2K mouse kidneys reveled no differences in kidney morphology (Figure 2c). Analysis of hematoxylin and eosin staining of gonadal depot fat revealed that hMATE2K mice have larger adipocytes than age-matched wild type mice (Figure 2d).

Urine and Serum Chemistry of Wild Type and hMATE2K Mice

Urine and serum were collected over 24 h from fasted 23-week old age-matched wild type and hMATE2K mice. Compared to wild type mice, hMATE2K mice have a 64% reduction in urine volume over a 24 h time interval (*n*=3, per group; *p*<0.01; Figure 2e). In addition, hMATE2K mice have a reduction in uric acid (*p*=0.02) and the urinary electrolytes sodium (*p*=0.01), chloride (*p*=0.03) and phosphate (*p*=0.02) (*n*=3, per group; Table 2). There was no difference in the 24 h urinary amounts of potassium, creatinine, urea nitrogen, glucose and calcium between the two groups of mice (Table 2). In serum, there was a significant decrease in blood urea nitrogen (*p*=0.04) and a significant increase
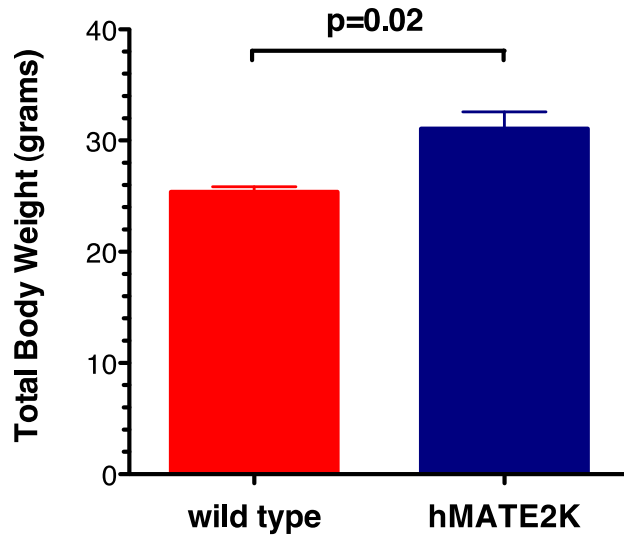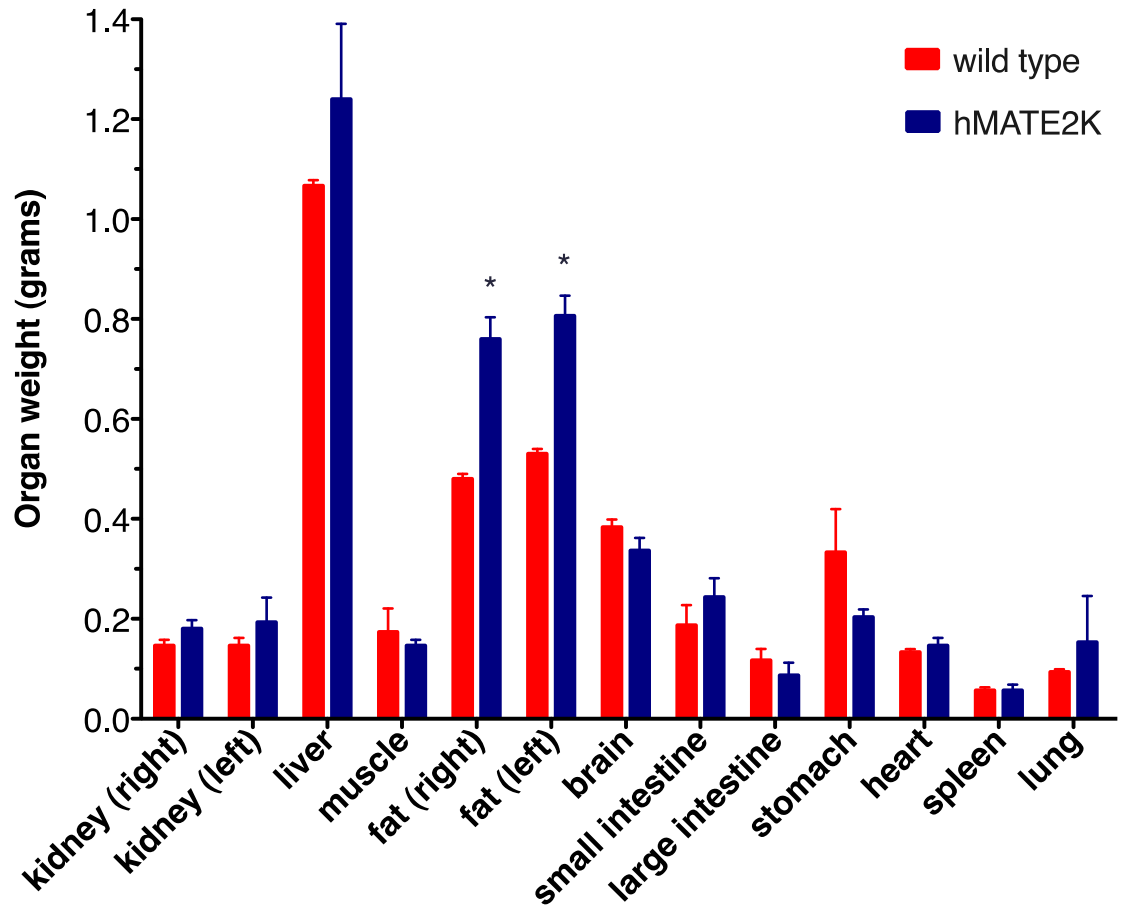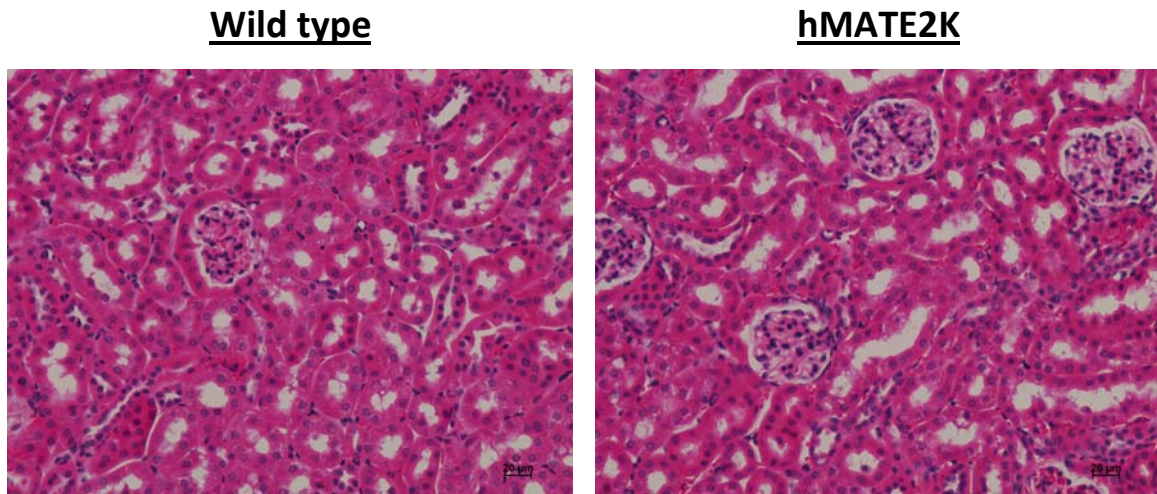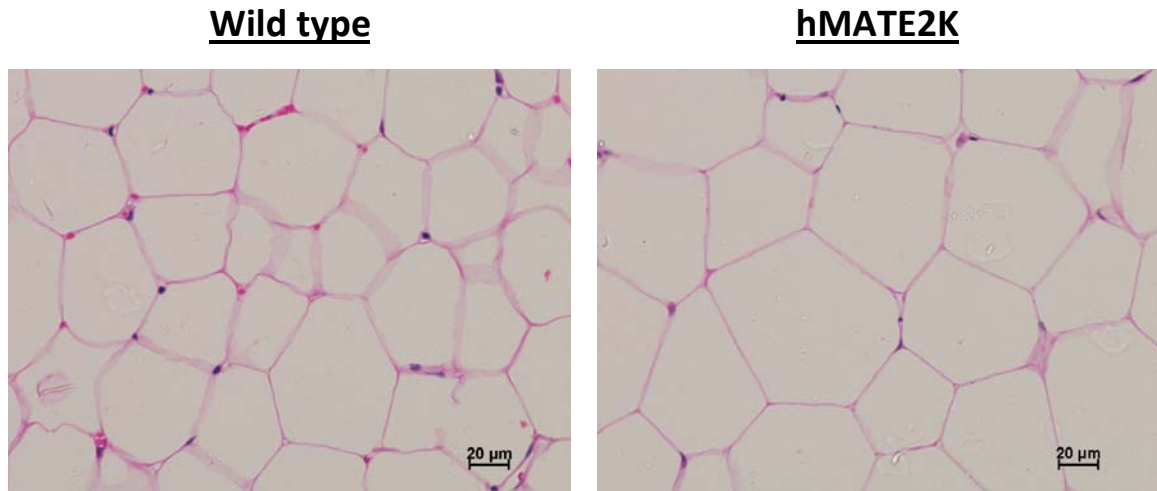
Figure 2a

Figure 2b

Figure 2c
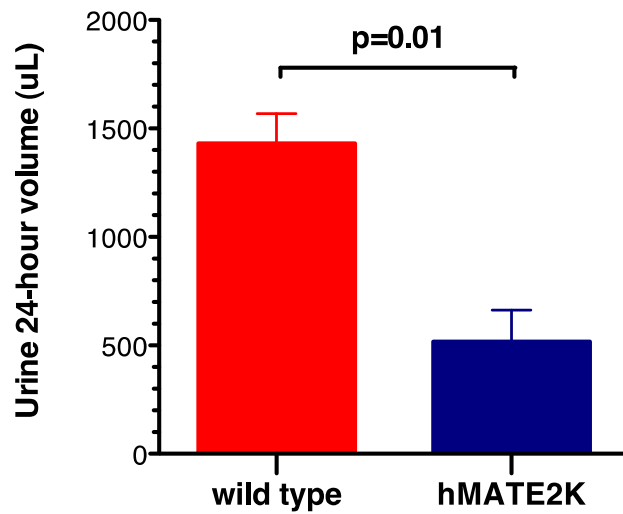
Figure 2d

Figure 2e

Figure 2f
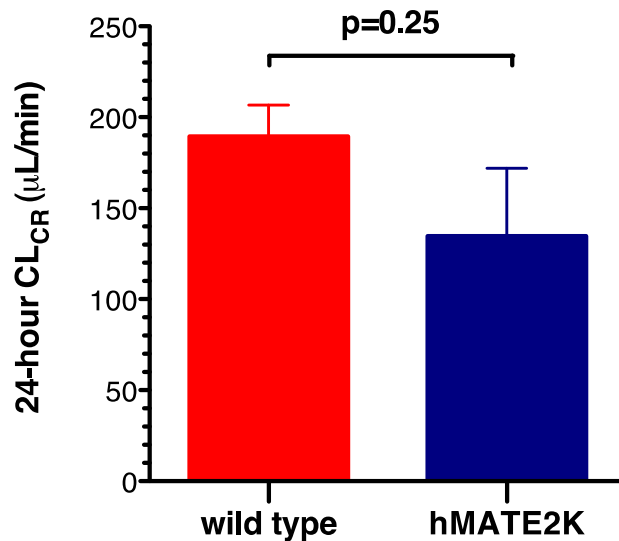
Figure 2. **Phenotyping of humanized MATE2K mice.** (a) Total body and (b) organ weights of 15-week old age matched wild type and hMATE2K mice. Hematoxylin and eosin staining of (c) kidney and (d) gonadal fat deposit tissue sections of 23-week old age matched wild type and hMATE2K mice. (e) 24-hour urine volume and (f) 24-hour creatinine clearance of 23-week old age matched wild type and hMATE2K mice. Data represent mean and standard deviation of 3 mice per group. *$p < 0.0005$ as compared to wild type mice.

hMATE2K, humanized multidrug and toxic extrusion protein 2K; CL_{CR}, creatinine clearance.

Table 2. 24-hour urine chemistry of wild type and humanized MATE2K mice.

Parameter (unit)	C57BL6 WT	hMATE2K	p-value
Sodium (mmol)	**0.13±0.02**	**0.03±0.02***	**0.01**
Potassium (mmol)	0.20±0.04	0.10±0.06	0.07
Chloride (mmol)	**0.12±0.03**	**0.05±0.03***	**0.03**
Creatinine (mg)	0.36±0.06	0.23±0.11	0.13
Urea nitrogen (mg)	27.0±5.9	13.4±7.1	0.06
Glucose (mg)	0.21±0.06	0.11±0.06	0.13
Calcuim (mg)	0.08±0.02	0.07±0.03	0.66
Phosphate (mg)	**3.2±0.4**	**1.4±0.8***	**0.02**
Uric acid (mg)	**0.14±0.01**	**0.07±0.03***	**0.02**

Data is as reported mean ± SD of 3 animals per group. *$p < 0.05$ as compared to wild type mice.

WT, wild type mouse; hMATE2K, humanized multidrug and toxic extrusion protein 2K mouse.

in albumin (p=0.02) and lactate dehydrogenase (p=0.01) in hMATE2K mice, as compared to wild type mice (n=3, per group; Table 3). There was no difference in serum concentrations of sodium, potassium, chloride, carbon dioxide, creatinine, glucose, calcium, phosphate, alanine transaminase, aspartate transaminase and uric acid between the two groups of mice (Table 3). In addition, there was no difference in the 24 h clearances of creatinine, a marker of kidney function, between hMATE2K and wild type mice (Figure 2f).

Gene Expression Analysis of Mouse Transporters and hMATE2K in Kidneys

The expression of mouse organic cation transporters (Oct1, Oct2, Oct3, Mate1, Pmat) and human MATE2K was examined in kidneys from hMATE2K and wildtype mice. There was no difference in the expression of mouse Oct1, Oct2, Oct3, Mate1 or Pmat between hMATE2K and wildtype mice (Figure 3). Surprisingly and importantly, there was no difference in the levels of hMATE2K transcripts in hMATE2K mice as compared to wild type mice (Figure 3) and the expression levels were at the limit of detection. There was also no difference in hMATE2K expression in dissected cortex from mouse kidneys (data not shown). In addition, Western blot analysis using a hMATE2K-specific antibody revealed that hMAT2K expression was absent from the kidneys of hMATE2K mice (data not shown). Human MATE2K mRNA was also absent from peripheral tissues including the liver, skeletal muscle, gonadal depot fat, brain, small intestine, large intestine, stomach, heart, spleen and lung (data not shown).

DISCUSSION

Table 3. Serum chemistry of fasted wild type and humanized MATE2K mice.

Parameter (unit)	C57BL6 WT	hMATE2K	p-value
Sodium (mM)	158±1	157±1	0.74
Potassium (mM)	6.5±0.5	6.9±0.2	0.24
Chloride (mM)	117±2	118±1	0.27
Carbon dioxide (mM)	12.7±1.5	12.0±2.0	0.67
Creatinine (mg/dL)	0.13±0.01	0.12±0.01	0.09
Blood urea nitrogen (mg/dL)	**33.0±3.6**	**24.0±3.6***	**0.04**
Glucose (mg/dL)	203±32	241±31	0.22
Calcium (mg/dL)	10.5±0.1	10.6±0.3	0.36
Albumin (g/dL)	**3.5±0.1**	**3.8±0.1***	**0.02**
Phosphate (mg/dL)	9.1±1.4	8.2±1.9	0.54
Alanine transaminase (U/L)	32.0±2.6	51.3±16.5	0.12
Aspartate transaminase (U/L)	109±5	322±141	0.06
Uric acid (mg/dL)	4.1±0.7	3.9±1.0	0.76
Lactate dehydrogenase (U/L)	**352±20**	**939±244***	**0.01**

Data is reported mean ± SD of 3 animals per group. $*p < 0.05$ as compared to wild type mice.

WT, wild type mouse; hMATE2K, humanized multidrug and toxic extrusion protein 2K mouse.
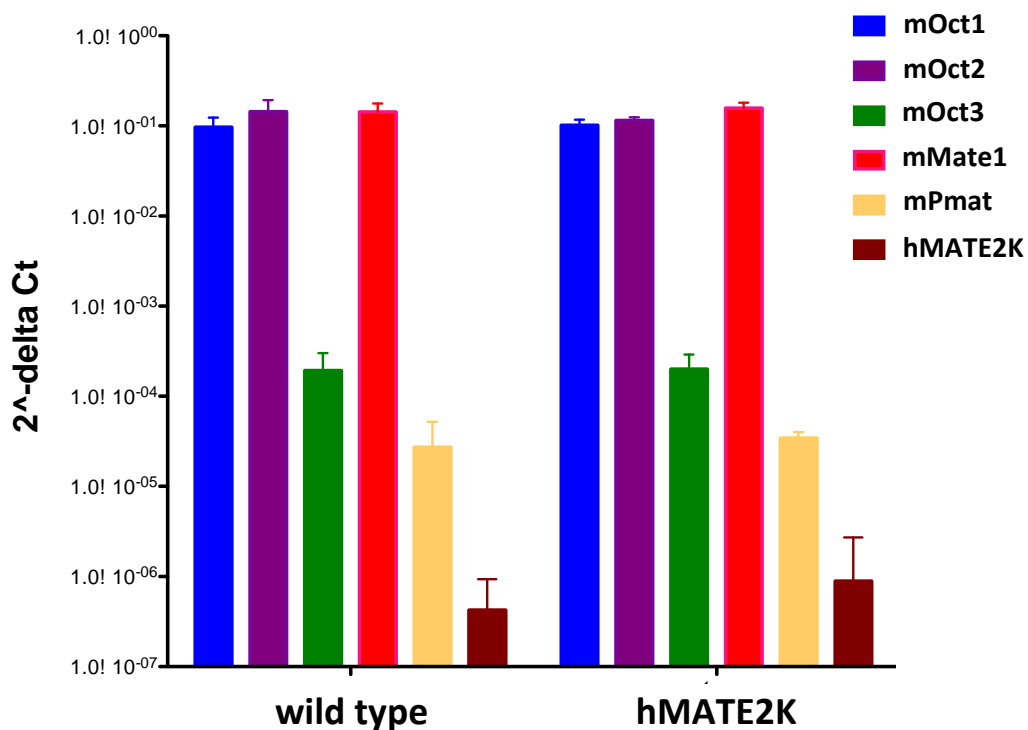
Figure 3. Expression of mouse organic cation transporters and the hMATE2K transgene in wild type and hMATE2K mice. The delta cycle threshold (C_T) values for all the genes in each sample were calculated by subtracting the mean C_T values for two housekeeping genes (Ppia and Gapdh) from the C_T for each target gene. The relative quantity of each gene was then determined by calculating the $2^{-\Delta Ct}$ value. Data shown represent mean values and standard deviation from triplicate wells in a representative experiment.

C_T, cycle threshold; mOct, mouse organic cation transporter; Mate1, multidrug and toxic extrusion protein 1; Pmat, plasma membrane monoamine transporter; hMATE2K, humanized multidrug and toxic extrusion protein 2K; Ppia, peptidylprolyl isomerase A; Gapdh, glyceraldehyde 3-phosphate dehydrogenase.

Since the development of transgenic mouse technology in 1982, transgenic mice have enabled scientists to observe the roles of genes in development, physiology and disease in a live animal. In Chapters 4 and 5, the role of MATE2K was examined in human subjects by altering function by a chemical agent and by gene expression changes from a hMATE2K promoter variant. However, in human subjects it is not possible to directly study any alterations in the tissue levels of drugs as a result of a drug-drug interaction or genetic alteration of transporter expression. A mouse that expresses the human MATE2K gene would help to define the role of MATE2K in renal drug accumulation and elimination. In addition, a transgenic mouse would provide important information about the physiologic function of the transporter in humans. As MATE2K expression is absent in rodents, an attempt was made to develop a transgenic mouse model that expresses the human MATE2K gene in a wild type background. Although, hMATE2K mice displayed transgene integration in the genome of the founder mice and their offspring, there was no evidence of hMATE2K expression in their kidneys or any peripheral tissues.

Typically, the rate of transgenesis (the number of pups that are founders) is 10-15% (17). In this study, we observed a 6% rate of transgenesis, with only one founder capable of producing pups. It is possible that fetal expression of MATE2K may be counter-selecting transgenic animals, thus lowering the rate of transgenesis. In addition to a low rate of transgenesis, the most common reasons for failure to develop a transgenic mouse include (*i*) poor transgene design, (*ii*) no transmission of transgene to progeny or (*iii*) the site of transgene integration (17). As similar transgenes using GGT1 as a proximal tubule

specific promoter and the bovine growth hormone polyadenylation signal have been successfully used in the past (14-16), it is unlikely that poor transgene design would be the cause for the failure of the hMATE2K mice to express the transgene. However, because of publication biases favoring positive results, it is not known whether others have attempted and failed to express genes with this construct. Also, it is unlikely that the founder was incapable of transmission to its offspring as the transgene was present in the DNA of each generation of progeny. It is possible that the site of transgene integration could explain why the hMATE2K mice do not express MATE2K. In these mice, the transgene was allowed to randomly integrate and did so into at least four distinct locations in the mouse genome (unpublished observations). This small number of integrations may have been insufficient to express detectable levels of hMATE2K protein. Such integrations are often key to overexpression of genes and detectable changes in phenotypes. Previous studies have also reported that tandem repeat of transgenes in the mouse genome are particularly prone to silencing (18-20). If the hMATE2K mouse had this type of integration, the hMATE2K transgene may have been transcriptionally silenced. In addition, the transgene may have integrated into an area of chromatin that is transcriptionally inactive or near genomic sequences reported to have insulating properties. Future attempts to generate a hMATE2K mouse should consider the incorporation of transgene sequences that will directly integrate into a specific locus in the mouse genome (*e.g.*, Rosa26).

In this study, a mouse that has the hMATE2K transgene incorporated into its genome was developed. This mouse is heavier and has larger gonadal depot fat than wild type mice.

Further, this mouse also has altered urine flow and serum and urinary electrolytes. However, these differences cannot be explained by hMATE2K expression, as mRNA and protein were absent in all tissues. The results of the study suggest that the observed phenotypes may have been due to disruption of other genes rather than expression of hMATE2K.

REFERENCES

1.	Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, et al. 1994. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 77:491-502

2.	Jonker JW, Wagenaar E, Mol CA, Buitelaar M, Koepsell H, et al. 2001. Reduced hepatic uptake and intestinal excretion of organic cations in mice with a targeted disruption of the organic cation transporter 1 (Oct1 [Slc22a1]) gene. *Mol Cell Biol* 21:5471-7

3.	Jonker JW, Buitelaar M, Wagenaar E, Van Der Valk MA, Scheffer GL, et al. 2002. The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. *Proc Natl Acad Sci U S A* 99:15649-54

4.	Jonker JW, Wagenaar E, Van Eijl S, Schinkel AH. 2003. Deficiency in the organic cation transporters 1 and 2 (Oct1/Oct2 [Slc22a1/Slc22a2]) in mice abolishes renal secretion of organic cations. *Mol Cell Biol* 23:7902-8

5. Vlaming ML, Mohrmann K, Wagenaar E, de Waart DR, Elferink RP, et al. 2006. Carcinogen and anticancer drug transport by Mrp2 in vivo: studies using Mrp2 (Abcc2) knockout mice. *J Pharmacol Exp Ther* 318:319-27

6. Tsuda M, Terada T, Mizuno T, Katsura T, Shimakura J, Inui K. 2009. Targeted disruption of the multidrug and toxin extrusion 1 (mate1) gene in mice reduces renal secretion of metformin. *Mol Pharmacol* 75:1280-6

7. Scheer N, Balimane P, Hayward MD, Buechel S, Kauselmann G, Wolf CR. 2012. Generation and characterization of a novel multidrug resistance protein 2 humanized mouse line. *Drug Metab Dispos* 40:2212-8

8. Alnouti Y, Petrick JS, Klaassen CD. 2006. Tissue distribution and ontogeny of organic cation transporters in mice. *Drug Metab Dispos* 34:477-82

9. Hiasa M, Matsumoto T, Komatsu T, Omote H, Moriyama Y. 2007. Functional characterization of testis-specific rodent multidrug and toxic compound extrusion 2, a class III MATE-type polyspecific H+/organic cation exporter. *Am J Physiol Cell Physiol* 293:C1437-44

10. Lickteig AJ, Cheng X, Augustine LM, Klaassen CD, Cherrington NJ. 2008. Tissue distribution, ontogeny and induction of the transporters Multidrug and toxin extrusion (MATE) 1 and MATE2 mRNA expression levels in mice. *Life Sci* 83:59-64

11. Omote H, Hiasa M, Matsumoto T, Otsuka M, Moriyama Y. 2006. The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. *Trends Pharmacol Sci* 27:587-93

12. Terada T, Inui K. 2008. Physiological and pharmacokinetic roles of H+/organic cation antiporters (MATE/SLC47A). *Biochem Pharmacol* 75:1689-96

13. Yonezawa A, Inui K. 2011. Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxicodynamics and pharmacogenomics. *Br J Pharmacol* 164:1817-25

14. Sepulveda AR, Huang SL, Lebovitz RM, Lieberman MW. 1997. A 346-base pair region of the mouse gamma-glutamyl transpeptidase type II promoter contains sufficient cis-acting elements for kidney-restricted expression in transgenic mice. *J Biol Chem* 272:11959-67

15. Terzi F, Burtin M, Hekmati M, Federici P, Grimber G, et al. 2000. Targeted expression of a dominant-negative EGF-R in the kidney reduces tubulo-interstitial lesions after renal injury. *J Clin Invest* 106:225-34

16. Fiaschi-Taesch NM, Santos S, Reddy V, Van Why SK, Philbrick WF, et al. 2004. Prevention of acute ischemic renal failure by targeted delivery of growth factors to the proximal tubule in transgenic mice: the efficacy of parathyroid hormone-related protein and hepatocyte growth factor. *J Am Soc Nephrol* 15:112-25

17. 2003. Refinement and reduction in production of genetically modified mice. *Lab Anim* 37 Suppl 1:S1-S49

18. Garrick D, Fiering S, Martin DI, Whitelaw E. 1998. Repeat-induced gene silencing in mammals. *Nat Genet* 18:56-9

19. Muskens MW, Vissers AP, Mol JN, Kooter JM. 2000. Role of inverted DNA repeats in transcriptional and post-transcriptional gene silencing. *Plant Mol Biol* 43:243-60

20.   McBurney MW, Mai T, Yang X, Jardine K. 2002. Evidence for repeat-induced gene silencing in cultured Mammalian cells: inactivation of tandem repeats of transfected genes. *Exp Cell Res* 274:1-8

CHAPTER 7

CONCLUSIONS AND PERSPECTIVES

Renal organic ion transporters mediate the tubular secretion of many compounds, including xenobiotics and their metabolic products. Current data suggest that in the proximal tubule of the kidney, organic cations are transported from the blood to the lumen of the kidney by organic cation transporter 2 (OCT2) and are eliminated to the urine by the concerted action of the H^+/organic cation antiporters, multidrug and toxin extrusion 1 (MATE1) and 2K (MATE2K). The primary goal of this dissertation was to determine the clinical impact of renal organic cation transporters on drug disposition and response.

At the initiation of this dissertation research, we compiled literature and developed the UCSF-FDA TransPortal (Chapter 2). This database includes information from ~300 primary literature sources and drug labels on drug transporter expression, tissue distribution, direction of transport, substrate and inhibitor *in vitro* kinetics and clinical drug-drug interactions of 31 transporters from the ABC and SLC superfamilies.

From the development of the UCSF-FDA TransPortal, it was apparent that clinically potent and selective inhibitors of transporters in the renal organic cation transporter system had not yet been identified. Therefore, a strategic cell-based screen using clinically relevant concentrations was conducted to identify selective inhibitors of renal organic cation transporters that are capable of inhibiting at clinically relevant

concentrations (Chapter 3). Through this screen, we identified the histamine 2 receptor antagonist, nizatidine, as a clinically potent and selective inhibitor of MATE2K-mediated transport *in vitro*.

To evaluate the clinical impact of selective inhibition of MATE2K-mediated drug elimination, a clinical drug-drug interaction (DDI) study with the antidiabetic drug, metformin as the victim drug and nizatidine as the perpetrating drug was conducted (Chapter 4). To our knowledge, this was the first clinical study to investigate the impact of selective renal transporter inhibition on the pharmacokinetics and pharmacodynamics of a victim drug. In healthy volunteers, nizatidine increases the apparent volume of distribution and hypoglycemic activity of metformin. However, despite achieving unbound maximum concentrations that are greater than the *in vitro* inhibition potency of MATE2K-mediated transport, nizatidine did not change metformin's renal clearance or net secretory clearance. This study provides data that challenge the current guidelines that rely on *in vitro* predictions to inform the decision to conduct transporter-mediated clinical DDI studies (1-3). Further, our studies suggest that *in vitro* methods need to be improved and standardized to obtain more accurate IC_{50} values that predict *in vivo* inhibitory concentrations of perpetrator drugs.

We also investigated whether carriers of MATE1 and MATE2/2K promoter variants have an altered disposition and response of metformin (Chapter 5, collaboration with Dr. Sophie Stocker). Carriers of MATE1 g.-66T>C, a decrease in expression promoter variant, had no difference in the pharmacokinetics of metformin, but exhibited an

enhanced glycemic response. Interestingly, carriers of MATE2/2K g.-130G>A had a greater renal clearance of metformin and a poorer hypoglycemic response. These data (combined with data from Chapter 4) suggest that the kidney may be an important site for the glycemic action of metformin.

Our clinical studies (Chapters 4 and 5) suggest that there may be alterations in the kidney levels of metformin. However, in human subjects it is not possible to directly study any alterations in the tissue levels of drugs as a result of a drug-drug interaction or genetic alteration of transporter expression. To aid in our understanding of the importance of MATE2K in renal drug handling, we attempted to develop a humanized MATE2K mouse (Chapter 6). However, this mouse did not express MATE2K in the kidney or in any tissues.

Renal secretory transporters are implicated in numerous clinically significant drug-drug interactions, generally leading to increased plasma levels of drugs, alterations in tissue levels and potential safety issues (4-11). These safety issues may be underestimated if only evaluating differences in C_{max} and AUC. Understanding whether the interaction will potentiate or reduce possible nephrotoxicity requires knowledge of the specific site (apical or basolateral membrane) of the interaction. This requires the identification and clinical validation of selective substrates and inhibitors. While nizatidine was determined to be a clinically potent inhibitor of MATE2K *in vitro*, its impact on the pharmacokinetics of metformin was minimal. Further clinical evaluation (e.g., with

multiple dosing or continuous infusion of nizatidine) would be required to validate the utility of nizatidine as a selective inhibitor of MATE2K-mediated transport.

In summary, this dissertation provides evidence to support that MATE1 and MATE2K play an important role in renal drug handling and the hypoglycemic activity of metformin. However, the studies raise a number of questions about conventional methods used in *in vitro* transporter studies, extrapolation of *in vitro* results to clinical studies, and the criteria included in current Food and Drug Administration (FDA) and European Medicines Agency (EMA) DDI guidances. Further, as drug-drug interactions and genetic variation may occur in the same individual it is important to understand the contribution and interplay of both of these factors on renal drug handling. Clearly, future studies are needed to develop and standardize robust *in vitro* methods that accurately predict clinical DDIs and to understand the complex interplay between genetic variants of renal transporters and DDIs.

REFERENCES

1. *Guidance for Industry (Draft): Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm> Accessed May 2013

2. *European Medicines Agency's Guideline on the Investigation of Drug Interactions*.

<http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf> Accessed May 2013

3.   Hillgren KM, Keppler D, Zur AA, Giacomini KM, Stieger B, et al. 2013. Emerging Transporters of Clinical Importance: An Update From the International Transporter Consortium. *Clin Pharmacol Ther* e-pub before print

4.   Abel S, Nichols DJ, Brearley CJ, Eve MD. 2000. Effect of cimetidine and ranitidine on pharmacokinetics and pharmacodynamics of a single dose of dofetilide. *Br J Clin Pharmacol* 49:64-71

5.   Feng B, Obach RS, Burstein AH, Clark DJ, de Morais SM, Faessel HM. 2008. Effect of human renal cationic transporter inhibition on the pharmacokinetics of varenicline, a new therapy for smoking cessation: an in vitro-in vivo study. *Clin Pharmacol Ther* 83:567-76

6.   Kusuhara H, Ito S, Kumagai Y, Jiang M, Shiroshita T, et al. 2011. Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at oral microdose and at therapeutic dose in healthy subjects. *Clin Pharmacol Ther* 89:837-44

7.   Shiga T, Hashiguchi M, Urae A, Kasanuki H, Rikihisa T. 2000. Effect of cimetidine and probenecid on pilsicainide renal clearance in humans. *Clin Pharmacol Ther* 67:222-8

8.   Somogyi A, McLean A, Heinzow B. 1983. Cimetidine-procainamide pharmacokinetic interaction in man: evidence of competition for tubular secretion of basic drugs. *Eur J Clin Pharmacol* 25:339-45

9.   Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. 1987. Reduction of metformin renal tubular secretion by cimetidine in man. *Br J Clin Pharmacol* 23:545-51

10.   Somogyi AA, Bochner F, Sallustio BC. 1992. Stereoselective inhibition of pindolol renal clearance by cimetidine in humans. *Clin Pharmacol Ther* 51:379-87

11.   van Crugten J, Bochner F, Keal J, Somogyi A. 1986. Selectivity of the cimetidine-induced alterations in the renal handling of organic substrates in humans. Studies with anionic, cationic and zwitterionic drugs. *J Pharmacol Exp Ther* 236:481-7

Publishing Agreement

It is the policy of the University to encourage the distribution of all theses, dissertations, and manuscripts. Copies of all UCSF theses, dissertations, and manuscripts will be routed to the library via the Graduate Division. The library will make all theses, dissertations, and manuscripts accessible to the public and will preserve these to the best of their abilities, in perpetuity.

Please sign the following statement:

I hereby grant permission to the Graduate Division of the University of California, San Francisco to release copies of my thesis, dissertation, or manuscript to the Campus Library to provide access and preservation, in whole or in part, in perpetuity.

Kari Mornsley
_____
Author Signature

6/11/13
_____
Date