Review Article

Theme: Kidney Transporters: Importance in Clearance, Disease and Drug-Drug Interactions

Guest Editors: Marilyn Morris and Colin Brown

Renal Organic Anion Transporters (SLC22 Family): Expression, Regulation, Roles in Toxicity, and Impact on Injury and Disease

Li Wang¹ and Douglas H. Sweet^{1,2}

Received 8 August 2012; accepted 12 September 2012; published online 9 October 2012

Abstract. Organic solute flux across the basolateral and apical membranes of renal proximal tubule cells is a key process for maintaining systemic homeostasis. It represents an important route for the elimination of metabolic waste products and xenobiotics, as well as for the reclamation of essential compounds. Members of the organic anion transporter (OAT, SLC22) family expressed in proximal tubules comprise one pathway mediating the active renal secretion and reabsorption of organic anions. Many drugs, pesticides, hormones, heavy metal conjugates, components of phytomedicines, and toxins are OAT substrates. Thus, through transporter activity, the kidney can be a target organ for their beneficial or detrimental effects. Detailed knowledge of the OATs expressed in the kidney, their membrane targeting, substrate specificity, and mechanisms of action is essential to understanding organ function and dysfunction. The intracellular processes controlling OAT expression and function, and that can thus modulate kidney transport capacity, are also critical to this understanding. Such knowledge is also providing insight to new areas such as renal transplant research. This review will provide an overview of the OATs for which transport activity has been demonstrated and expression/function in the kidney observed. Examples establishing a role for renal OATs in drug clearance, food/drug–drug interactions, and renal injury and pathology are presented. An update of the current information regarding the regulation of OAT expression is also provided.

KEY WORDS: kidney; nephrotoxicity; proximal tubule; solute carrier.

INTRODUCTION

Two vital functions performed by the kidney are the removal of substances from the blood followed by their elimination via the urine and the reabsorption of specific compounds from the glomerular filtrate back into the systemic circulation. Much of the secretion and reabsorption of charged organic solutes occurs in the proximal tubule. However, the plasma membrane of proximal tubule cells presents a barrier to the passive diffusion of such charged, hydrophilic molecules, and renal transcellular solute flux is a complex process involving dozens of membrane-bound transporter proteins. Two major superfamilies of transport proteins that have been identified are the ATP binding cassette transporters, which can directly bind and hydrolyze ATP as a driving force for the unidirectional transport of substrates across cell membranes, and the solute carriers (SLC), transport proteins that are indirectly coupled to cellular

energy and utilize the membrane potential difference and/or the stored energy of concentration gradients as driving forces (1–3). Currently, the human SLC superfamily has at least 55 separate gene families encompassing a combination of 362 confirmed and putative transporter proteins (1). The SLC22 family is comprised of the organic cation/anion/zwitterion transporters with ~30 identified/putative members and includes the organic cation transporters (OCTs and OCTNs), which mainly interact with cationic and zwitterionic organic molecules, and the organic anion transporters (OATs), which predominantly interact with anionic and zwitterionic organic molecules (2–4).

In vitro characterization of cloned transporters has demonstrated that OATs handle an enormous variety of structurally diverse organic anions. Many drugs, including diuretics, antihypertensives, antibiotics, antivirals, and anti-cancer agents, are organic anions at physiological pH and, thus, subject to active tubular secretion which, in turn, impacts their pharmacokinetic, pharmacodynamic, and toxic properties. In addition, endogenous substances (e.g., metabolic intermediates/by-products and hormones) and environmental toxins and toxicants (e.g., mycotoxins and pesticides) also exist as anionic species. Indeed, the attributes of known OAT substrates indicate that renal OAT function is central to organism homeostasis and the progression of certain disease

¹ Department of Pharmaceutics, Virginia Commonwealth University, Medical College of Virginia Campus, 410 N 12th Street, PO Box 980533, Richmond, VA 23298, USA.

² To whom correspondence should be addressed. (e-mail: dsweet@vcu.edu)

states. Further, interindividual variation in therapeutic response within human populations may be linked to altered renal OAT affinity, expression level, and/or spatial distribution as a result of dietary or genetic factors. Clearly, a more thorough understanding of OAT function is necessary if we wish to accurately assess their impact on drug efficacy and the extent of exposure to endogenous and xenobiotic toxins/toxicants.

This review will focus on the OATs of the SLC22 transporter family and in particular on those OATs for which transport activity has been demonstrated and expression/function in kidney established (Table I). Basic cloning, expression profile, and functional characterization information for each transporter has been summarized in great detail recently and will not be repeated here (3,4). Rather, an update of the current information regarding the regulation of OAT expression (Table II) and evidence supporting the involvement of renally expressed OATs in drug clearance, food/drug–drug interactions, and renal pathology (Table III) will be emphasized. Table IV provides a listing of compounds demonstrated to interact with various OATs and reported kinetic parameters (K_m , K_i , or IC_{50} values), when available, that serves as an update (2010–2012) to the comprehensive table from reference (3). Throughout this review, the convention of referring to human transporters with all capital letters (*i.e.*, OAT1) and nonhuman transporters with initial capital followed by lower case letters (*i.e.*, Oat1) is used.

ORGANIC ANION TRANSPORTERS IN RENAL PROXIMAL TUBULE

As indicated in Table I, species differences in known OAT family members do exist, with OAT4 (SLC22A11),

OAT5 (SLC22A10), and OAT7 (SLC22A9) being unique to humans and Oat5 (Slc22a19), Oat6 (Slc22a20), Oat8 (Slc22a25), and Oat9 (Slc22a27) being restricted to nonhuman species (most thoroughly explored in mice and rats). It is important to clarify that OAT5 (SLC22A10) and Oat5 (Slc22a19) are not orthologs. Of the 11 OATs for which transport activity has been demonstrated, OAT1 (SLC22A6), OAT2 (SLC22A7), OAT3 (SLC22A8), OAT4, and URAT1 (SLC22A12) proteins have been detected and localized in human kidney, and Oat1 (Slc22a6), Oat2 (Slc22a7), Oat3 (Slc22a8), Oat5, Oat8, Oat9, Oat10 (Slc22a13), and Urat1 (Slc22a12) proteins have been detected and localized in rodent kidney (2–5). OAT1/Oat1, OAT2/Oat2, and OAT3/Oat3 are targeted to the basolateral membrane of renal proximal tubule cells (Fig. 1) and mediate the movement of substrate molecules from the blood into the cells via dicarboxylate/organic anion exchange (2–4). In the apical membrane, OAT4 appears to mediate substrate reabsorption from urine through exchange with dicarboxylates or hydroxyl ions, and URAT1/Urat1 mediates reabsorption of urate and other substrates by monocarboxylate exchange (Fig. 1). In rodent proximal tubules, Oat5 may mediate substrate reabsorption via dicarboxylate exchange; however, no information is available regarding the driving forces and directionality of Oat9 (2–5). While Oat8 was identified as a dicarboxylate/organic anion exchanger, immunohistochemistry localized this transporter to the apical membrane of intercalated cells in the collecting ducts, with no signal in proximal tubule. Finally, organic anion transport by OAT10 (SLC22A13) was reported to be driven by exchange with dicarboxylates or hydroxyl ions, similar to OAT4; however, localization information is restricted to brush border membrane vesicles prepared from rat kidney (2–4).

Table I. Functional SLC22 Family Organic Anion Transporters

Transporter (gene designation)	Organic anion transport demonstrated	mRNA expression detected in the kidney	Protein expression confirmed in the kidney
Human			
OAT1 (SLC22A6)	✓	✓	✓
OAT2 (SLC22A7)	✓	✓	✓
OAT3 (SLC22A8)	✓	✓	✓
OAT4 (SLC22A11)	✓	✓	✓
OAT5 (SLC22A10) ^a	–	–	–
OAT7 (SLC22A9)	✓	–	–
OAT10 (SLC22A13)	✓	✓	–
URAT1 (SLC22A12)	✓	✓	✓
Rodent			
Oat1 (Slc22a6)	✓	✓	✓
Oat2 (Slc22a7)	✓	✓	✓
Oat3 (Slc22a8)	✓	✓	✓
Oat5 (Slc22a19) ^a	✓	✓	✓
Oat6 (Slc22a20)	✓	–	–
Oat8 (Slc22a25)	✓	✓	✓
Oat9 (Slc22a27)	✓	✓	✓
Oat10 (Slc22a13)	–	✓	✓
Urat1 (Slc22a12)	✓	✓	✓

^a Human OAT5 (SLC22A10) and rodent Oat5 (Slc22a19) are not orthologs, *i.e.*, are not coded for by the same gene and therefore do not represent the same transporter; rat and mouse Oat5 are paralogs, *i.e.*, represent the same gene across species and thus code for the same transporter

Table II. Summary of OAT Regulation

Regulation level	Regulating factor	Influence	Reference
mRNA	Activating transcription factor 1 (ATF1)/cAMP responsive element binding protein 1 (CREB1)	hOAT3 expression ↑	(6)
	Hepatocyte nuclear factor 1α/1β (HNF1α/1β)	HNF1α homodimer and HNF1α/1β heterodimer: hOAT1, mOat3, hOAT3, hOAT4, mUrat1, and hURAT1 expression ↑ HNF1β homodimer: mOat3, hOAT3, hOAT4, mUrat1, and hURAT1 expression ↑ HNF1α: hOAT5 and hOAT7 expression ↑	(7–10) (11)
	Hepatocyte nuclear factor 4α (HNF4α)	hOAT1 expression ↑ hOAT2 expression ↑	(12,13) (12,13)
	B cell lymphoma 6 protein (BCL6)	rOat1 and rOat3 expression ↑	(14)
Protein	Protein kinase C (PKC), nonspecific	OAT1/Oat1 and OAT3/Oat3 activity ↓ PKCα: hOAT1 and hOAT3 transport activity and cell surface expression ↓	(3) (15)
	Specific PKC isoform	PKCδ and PKCε: hOAT3 transport activity and cell surface expression ↑ PKCζ: rOat1, mOat1, rOat3, mOat3, and hOAT3 transport activity ↑	(16) (16,17)

↑ increased expression or transport activity, ↓ decreased expression or transport activity

REGULATION OF OATS

Mounting evidence suggests that OAT expression can be regulated at both the mRNA and protein level. Examination of the promoter regions of the *hOAT1*, *hOAT2*, *hOAT3*, *hOAT4*, and *hURAT1* genes identified putative binding sequences for several transcription factors including activating transcription factor 1 (ATF1), cAMP responsive element binding protein 1 (CREB1), hepatocyte nuclear factors 1α, 1β, and 4α (HNF1α, HNF1β, HNF4α), paired box protein Pax-1, B cell lymphoma 6 protein, and Wilm's tumor protein 1 (6,14,18,19). The presence of DNA binding sequence motifs for known transcription factors suggests that they might be involved in the regulation of OATs, and a number of recent studies have investigated this possibility. The information covered in this section is summarized in Table II.

Deletion analysis of the *hOAT3* promoter indicated that the sequence from −214 to −77 base pairs was required for basal level transcriptional activity, and this region was found to contain a cAMP-response element (CRE) (6). Mutation of this sequence resulted in reduced *hOAT3* promoter activity. Both ATF1 and CREB1 are known to bind to CRE, and

subsequent electrophoretic mobility shift assays (EMSA) confirmed binding of these transcription factors to the *hOAT3* promoter (6). Further, phosphorylation of CREB1 and ATF1 in response to activation of protein kinase A (PKA) appeared to induce increased hOAT3 expression, suggesting stimulatory effects of PKA on hOAT3 activity might be explained through increased transcriptional activity (6).

The transcription factors HNF1α and HNF1β are expressed in the epithelia of many organs including the liver, kidney, and intestine (20). When explored in Hnf1α knockout mice, it was observed that renal mRNA expression of mOat1, mOat2, mOat3, and mUrat1 was reduced (7,21). Examination of the *mOat1* and *hOAT1* promoter regions revealed a HNF1 binding motif at −56 to −44 base pairs (8). Forced expression of HNF1α and HNF1β increased activity of both promoters, with HNF1α producing the greatest increase, followed by co-expression of HNF1α and HNF1β, and expression of HNF1β alone producing little to no effect. EMSA analysis detected binding of HNF1α homodimers and HNF1α/HNF1β heterodimers to the *hOAT1* promoter (8). The promoter sequence of *hOAT3* (−308 to +6 base pairs) also contains a HNF1α

Table III. Influence of Renal Injury and Disease on OAT Protein Localization and Expression Level

Renal injury/disease	Effect on OAT protein localization	Effect on total renal OAT protein level	Reference
Chronic renal failure (CRF)	?	Total rOat1 and rOat3 ↔	(22)
	Basolateral rOat1 ↓	Total rOat1 ↓	(23)
	Basolateral rOat3 ↔	Total rOat3 ↓	(23)
	?	Total rOat1, rOat2, rOat3, and rUrat1 ↓	(24)
Acute renal failure (ARF)	Basolateral hOAT1 ↓, cytoplasmic hOAT1 ↑	?	(25)
	Basolateral rOat1 ↓, cytoplasmic rOat1 ↑	Total rOat1 ↓	(26)
	?	Total rOat1 and rOat3 ↓	(27,28)
	Apical rOat5 ↓, urinary rOat5 ↑	Total rOat5 ↓	(29)
Acute kidney injury (AKI)	ERPTD: Urinary hOAT1 and hOAT3 ↑, Urinary hOAT4 ↓	?	(30)
	SAKI: Urinary hOAT1, hOAT3, and hOAT4 ↑		

? not determined, ↔ no change in expression, ↓ reduced level, ↑ increased level, *ERPTD* early reversible proximal tubular damage, *SAKI* severe acute kidney injury

Table IV. Organic Anion Transporter Substrates and Inhibitors

Compound	Transporter ^a	Interaction ^b	System ^c	K_m	Kinetics (μ M)		Reference
					K_i	IC_{50} (victim substrate) ^d	
1-Butanesulfonic acid	hOAT1	I	CHO			514 $\pm$ 72.1 (0.25 PAH)	(31)
	mOat1	I	CHO			313 $\pm$ 26.9 (0.25 PAH)	(31)
	rbOat1	I	CHO			421 $\pm$ 89.4 (0.25 PAH)	(31)
1-Propanesulfonic acid	hOAT1	I	CHO			2,036 $\pm$ 28.3 (0.25 PAH)	(31)
	mOat1	I	CHO			4,156 $\pm$ 1,079 (0.25 PAH)	(31)
	rbOat1	I	CHO				(31)
2,3-Dimercapto-1-propanesulfonic acid	hOAT1	I	CHO			83 $\pm$ 3 (0.25 PAH)	(31)
		I	HEK293			104.6 $\pm$ 13.1 (5 CF)	(32) ^e
						85.1 $\pm$ 8.8 (5 CF)	
	mOat1	I	CHO			55 $\pm$ 4.9 (0.25 PAH)	(31)
	rbOat1	I	CHO			72 $\pm$ 9.9 (0.25 PAH)	(31)
		I	HEK293			237.4 $\pm$ 23 (5 CF)	(32) ^e
						123 $\pm$ 14 (5 CF)	
3-Mercapto-1-propanesulfonic acid	hOAT1	I	CHO			204 $\pm$ 54.2 (0.25 PAH)	(31)
	mOat1	I	CHO			151 $\pm$ 19.8 (0.25 PAH)	(31)
	rbOat1	I	CHO			204 $\pm$ 34.9 (0.25 PAH)	(31)
3-Monoglucuronyl-glycyrrhetinic acid	hOAT1	S	HEK293	49.0 $\pm$ 18.3			(33)
Aminopterin	hOAT1	I	<i>X. laevis</i>			160 $\pm$ 58 (0.221 PAH)	(34)
Amitriptyline	hOAT1	I	S2		573.9	354 (5 PAH)	(35)
Arginine	mOat1	I	CHO		0.5	1,600 $\pm$ 300 (5 CF)	(36)
Aristolochic acid I	hOAT1	I	HEK293		0.80 $\pm$ 0.15	0.44 $\pm$ 0.08 (5 PAH)	(37)
	hOAT1	I	S2				(38)
	hOAT1	S	HEK293				(39)
	mOat1	S	CHO	0.79 $\pm$ 0.01			(40)
	rOat1	S	HEK293				(41)
Aristolochic acid II	hOAT1	I	S2			1.06 $\pm$ 0.09 (5 PAH)	(38)
	mOat1	S	CHO	1.50 $\pm$ 0.37			(40)
Bestatin	hOAT1	S	HEK293	679 $\pm$ 7			(42)
Caffeic acid	hOAT1	S/I	HEK293	28.6 $\pm$ 3.7		5.22 (25 PAH)	(43)
	hOAT1	I	<i>X. laevis</i>			16.6 $\pm$ 3.7 (0.221 PAH)	(44)
Caffeic acid-3- <i>O</i> -glucuronide	hOAT1	S/I	HEK293				(43)
Caffeic acid-4- <i>O</i> -glucuronide	hOAT1	S	HEK293				(43)
Caffeic acid-3- <i>O</i> -sulfate	hOAT1	S	HEK293	38.9 $\pm$ 3.3			(43)
Caffeic acid-4- <i>O</i> -sulfate	hOAT1	S	HEK293				(43)
Cephalexin	hOAT1	S	HEK293				(43)
Chlorpyrifosmethyl	hOAT1	I	S2		324.6	191.5 (5 PAH)	(45)
Cyclo- <i>trans</i> -4- <i>L</i> -hydroxypropyl-L-serine	hOAT1	S	HEK293				(35)
Daidzein-7- <i>O</i> -glucuronide	hOAT1	I	HEK293				(45)
Daidzein-7,4'- <i>O</i> -disulfate	hOAT1	I	HEK293				(46)
Diazepam	hOAT1	I	S2		83.5	133.3 (5 PAH)	(46)
Dihydrocaffeic acid	hOAT1	S/I	HEK293	21.4 $\pm$ 4.2			(35)
Dihydroferulic acid	hOAT1	S/I	HEK293				(43)

Table IV. (continued)

Compound	Transporter ^a	Interaction ^b	System ^c	Kinetics (μM)			Reference
				K_m	K_i	IC ₅₀ (victim substrate) ^d	
Dihydrocaffeic acid-3- <i>O</i> -glucuronide	hOAT1	S	HEK293				(43)
Dihydrocaffeic acid-4- <i>O</i> -glucuronide	hOAT1	S	HEK293				(43)
Dihydrocaffeic acid-3- <i>O</i> -sulfate	hOAT1	S/I	HEK293	76.7 ± 16.3			(43)
Dihydrocaffeic acid-4- <i>O</i> -sulfate	hOAT1	S/I	HEK293	115 ± 31			(43)
Dihydroferulic acid-4- <i>O</i> -sulfate	hOAT1	S/I	HEK293				(43)
Ferulic acid-4- <i>O</i> -sulfate	hOAT1	S	HEK293	54.2 ± 3.6			(43)
Fenitrothion	hOAT1	I	S2		51.2	26.6 (5 PAH)	(35)
Ferulic acid	hOAT1	S/I	HEK293			9.01 (25 PAH)	(43)
		I	CHO				(47)
Gaboxadol	rOat1	S	<i>X. laevis</i>	151 ± 58			(48)
Galic acid	hOAT1	I	CHO			1.24 ± 0.36 (1 PAH)	(47)
Genistein-4'- <i>O</i> -sulfate	hOAT1	S/I	HEK293	5.07 ± 1.81			(46)
Genistein-7- <i>O</i> -glucuronide	hOAT1	I	HEK293				(46)
Glycitein-7- <i>O</i> -glucuronide	hOAT1	I	HEK293				(46)
Indoxyl sulfate	mOat1	I	<i>X. laevis</i>			18 (30 CF)	(49)
Isoferulic acid	hOAT1	I	HEK293				(43)
Isoferulic acid-3- <i>O</i> -glucuronide	hOAT1	S	HEK293				(43)
Isoferulic acid-3- <i>O</i> -sulfate	hOAT1	S/I	HEK293	32.7 ± 5.3			(43)
Lithospermic acid	mOat1	I	CHO		14.9 ± 4.9		(50)
Lumiracoxib	hOAT1	I	<i>X. laevis</i>			3.3 ± 1.1 (0.221 PAH)	(51)
Ketoconazole	hOAT1	I	<i>X. laevis</i>			319 (1 PAH)	(52)
α-Ketoglutarate	mOat1	I	<i>X. laevis</i>			40 ± 3 (30 CF)	(36)
Malathion	hOAT1	I	S2		134.0	114.2 (5 PAH)	(35)
Mianserin	hOAT1	I	S2		99.0	312.6 (5 PAH)	(35)
Probenecid	hOAT1	I	<i>X. laevis</i>			17 (3 PAH); 14 (10 MTX)	(53)
Protocatechuic acid	hOAT1	I	CHO			18.08 ± 2.59 (1 PAH)	(47)
	mOat1	I	CHO				(50)
	mOat1	I	CHO			4,300 ± 600 (5 CF)	(36)
Pyruvate	hOAT1	S/I	HEK293				(46)
Quercetin-3- <i>O</i> -glucuronide	hOAT1	I	HEK293				(46)
Quercetin-3'- <i>O</i> -glucuronide	hOAT1	S/I	HEK293				(46)
Quercetin-3'- <i>O</i> -sulfate	hOAT1	S/I	HEK293	1.73 ± 0.38		1.22 (25 PAH)	(46)
Quercetin-7- <i>O</i> -glucuronide	hOAT1	I	HEK293				(46)
Rifampin	hOAT1	I	<i>X. laevis</i>		62.2	79.1 (1 PAH)	(52)
Rosmarinic acid	mOat1	I	CHO		5.5 ± 2.2		(50)
Salvianolic acid A	mOat1	I	CHO		4.9 ± 2.2		(50)
Salvianolic acid B	mOat1	I	CHO				(50)
Sinapinic acid	hOAT1	I	CHO			11.02 ± 1.55 (1 PAH)	(47)
Spermidine	mOat1	I	<i>X. laevis</i>			2,000 ± 700 (30 CF)	(36)
Spermine	mOat1	I	<i>X. laevis</i>			1,600 ± 600 (30 CF)	(36)
Sulfasalazine	hOAT1	I	<i>X. laevis</i>			4.6 (10 MTX)	(53)
Tanshinol	mOat1	I	CHO		86.0		(50)
Triazolam	hOAT1	I	S2			185.2 (5 PAH)	(35)

Table IV. (continued)

Compound	Transporter ^a	Interaction ^b	System ^c	Kinetics (μ M)			Reference
				K_m	K_i	IC_{50} (victim substrate) ^d	
Vanillic acid	hOAT1	I	CHO			7.65 $\pm$ 2.92 (1 PAH)	(47)
Aristolochic acid I	mOat2	S	CHO	0.36 $\pm$ 0.05			(40)
Aristolochic acid II	mOat2	S	CHO	0.67 $\pm$ 0.19			(40)
Orotic acid	hOAT2	S	HEK293				(54)
	rOAT2	S	HEK293				(54)
Uric acid	hOAT2	S	HEK293	1,168 $\pm$ 335			(55)
<i>N</i> -((5 <i>S</i>)-3-[4-(1,1-dioxidothiomorpholin-4-yl)-3,5-difluorophenyl]-2-oxo-1,3-oxazolidin-5-yl)methyl)acetamide 1-butanedisulfonic acid	hOAT3	S	HEK293	44 $\pm$ 5			(56)
	hOAT3	I	CHO			5,098 $\pm$ 184.3 (0.015 ES)	(31)
	mOat3	I	CHO			10,975 $\pm$ 2,647 (0.015 ES)	(31)
	rbOat3	I	CHO			5,640 $\pm$ 715.9 (0.015 ES)	(31)
	rbOat3	I	CHO				(31)
1-Propanedisulfonic acid	hOAT3	I	CHO			40 $\pm$ 7.4 (0.015 ES)	(31)
2,3-Dimercapto-1-propanedisulfonic acid	hOAT3	I	CHO			31.6 $\pm$ 6.6 (5 CF)	(32) ^e
		I	HEK293			172 $\pm$ 36 (5 CF)	
	mOat3	I	CHO			85 $\pm$ 16.9 (0.015 ES)	(31)
	rbOat3	I	CHO			18 $\pm$ 2.5 (0.015 ES)	(31)
		I	HEK293			52.4 $\pm$ 7.6 (5 CF)	(32) ^e
		I				172 $\pm$ 22 (5 CF)	
2,4-Dichlorophenoxyacetate	mOat3	I	CHO		2.0 $\pm$ 0.4		(57)
2,4-Dihydroxyphenylacetic acid	mOat3	I	CHO		347 $\pm$ 98		(57)
3-Mercapto-1-propanedisulfonic acid	hOAT3	I	CHO			2,139 $\pm$ 289 (0.015 ES)	(31)
	mOat3	I	CHO			2,325 $\pm$ 268 (0.015 ES)	(31)
	rbOat3	I	CHO			2,320 $\pm$ 785 (0.015 ES)	(31)
	rbOat3	I	CHO				(33)
3-Monogluconyl-glycyrrhetic acid	hOAT3	S	HEK293	30.1 $\pm$ 2.9			(47)
4-Hydroxybenzoic acid	hOAT3	I	HEK293				(57)
5-Hydroxyindoleacetic acid	mOat3	I	CHO		67.8 $\pm$ 7.2		(34)
Aminopterin	hOAT3	I	<i>X. laevis</i>			59.2 $\pm$ 14.9 (0.0184 ES)	(37)
Aristolochic acid I	hOAT3	I	HEK293		0.4		(38)
	hOAT3	I	S2		0.84 $\pm$ 0.10	0.65 $\pm$ 0.08 (0.05 ES)	(39)
	hOAT3	S	HEK293				(40)
	mOat3	S	CHO	0.51 $\pm$ 0.06			(38)
Aristolochic acid II	hOAT3	I	S2			1.28 $\pm$ 0.18 (0.05 ES)	(40)
Bestatin	mOat3	S	CHO	1.05 $\pm$ 0.36			(42)
Bumetanide	hOAT3	S	HEK293	632 $\pm$ 14			(53)
Caffeic acid	hOAT3	I	<i>X. laevis</i>			7.8 (2 ES); 5.4 (10 MTX)	(43)
	hOAT3	S/I	HEK293			30.8 (100 CF)	(44)
	hOAT3	I	<i>X. laevis</i>			5.4 $\pm$ 1.3 (0.0175 ES)	(43)
Caffeic acid-3- <i>O</i> -glucuronide	hOAT3	S	HEK293				(43)
Caffeic acid-4- <i>O</i> -glucuronide	hOAT3	S	HEK293				(43)
Caffeic acid-3- <i>O</i> -sulfate	hOAT3	S	HEK293				(43)

Table IV. (continued)

Compound	Transporter ^a	Interaction ^b	System ^c	Kinetics (μM)			Reference
				K_m	K_i	IC ₅₀ (victim substrate) ^d	
Caffeic acid-4- <i>O</i> -sulfate	hOAT3	S	HEK293				(43)
Celecoxib	hOAT3	I	S2		35.3		(58)
Cephalexin	hOAT3	S	HEK293				(45)
<i>p</i> -Coumaric acid	hOAT3	I	HEK293				(47)
Cyclo- <i>trans</i> -4-L-hydroxypropyl-L-serine	hOAT3	S	HEK293				(45)
Daidzein-7- <i>O</i> -glucuronide	hOAT3	S/I	HEK293	19.1 ± 1.9			(46)
Daidzein-7,4'- <i>O</i> -disulfate	hOAT3	S/I	HEK293				(46)
Dehydroepiandrosterone sulfate	mOat3	I	CHO		3.8 ± 1.1		(57)
		S/I	<i>X. laevis</i>				(57,59)
Dihydrocaffeic acid	hOAT3	S/I	HEK293	21.4 ± 4.2		14.2 (100 CF)	(43)
Dihydrocaffeic acid-3- <i>O</i> -glucuronide	hOAT3	S	HEK293				(43)
Dihydrocaffeic acid-4- <i>O</i> -glucuronide	hOAT3	S	HEK293				(43)
Dihydrocaffeic acid-3- <i>O</i> -sulfate	hOAT3	S	HEK293				(43)
Dihydrocaffeic acid-4- <i>O</i> -sulfate	hOAT3	S	HEK293				(43)
Dihydroferulic acid-4- <i>O</i> -glucuronide	hOAT3	S	HEK293				(43)
Dihydroferulic acid-4- <i>O</i> -sulfate	hOAT3	S	HEK293			7.35 ± 3.73 (1 ES)	(43)
Ferulic acid	hOAT3	I	HEK293				(47)
Ferulic acid-4- <i>O</i> -glucuronide	hOAT3	S	HEK293				(43)
Ferulic acid-4- <i>O</i> -sulfate	hOAT3	S	HEK293			9.02 ± 3.24 (1 ES)	(43)
Gallic acid	hOAT3	I	HEK293				(47)
Genistein-4'- <i>O</i> -glucuronide	hOAT3	S/I	HEK293				(46)
Genistein-7- <i>O</i> -glucuronide	hOAT3	S/I	HEK293	7.94 ± 1.42			(46)
Genisteic acid	hOAT3	I	HEK293	17.6 ± 3.9		86.81 ± 21.78 (1 ES)	(47)
Glycitein-7- <i>O</i> -glucuronide	hOAT3	S/I	HEK293				(46)
Hippuric acid	mOat3	I	CHO		79.3 ± 4.0		(57)
Homovanillic acid	mOat3	I	CHO		135 ± 27		(57)
Isoferulic acid-3- <i>O</i> -glucuronide	hOAT3	S	HEK293				(43)
Isoferulic acid-3- <i>O</i> -sulfate	hOAT3	S	HEK293				(43)
Lithospermic acid	mOat3	I	CHO		31.1 ± 7.0		(50)
Lumiracoxib	hOAT3	I	<i>X. laevis</i>			1.9 ± 0.4 (0.0184 ES)	(51)
Penicillin G	mOat3	I	CHO		60.7 ± 5.9		(57)
Probenecid	mOat3	I	CHO		0.8 ± 0.2		(57)
Protocatechuic acid	hOAT3	I	CHO			87.36 ± 28.57 (1 ES)	(47)
	mOat3	I	CHO				(50)
Quercetin-3- <i>O</i> -glucuronide	hOAT3	S/I	HEK293			0.43 (100 CF)	(46)
Quercetin-3'- <i>O</i> -glucuronide	hOAT3	S/I	HEK293	5.25 ± 0.86		1.31 (100 CF)	(46)
Quercetin-3'- <i>O</i> -sulfate	hOAT3	S/I	HEK293			0.75 (100 CF)	(46)
Quercetin-7- <i>O</i> -glucuronide	hOAT3	S/I	HEK293				(46)
Rosmarinic acid	mOat3	I	CHO		4.3 ± 0.2		(50)
Salicylate	mOat3	I	CHO		343 ± 93		(57)
Salvianolic acid A	mOat3	I	CHO		21.3 ± 7.7		(50)

Table IV. (continued)

Compound	Transporter ^a	Interaction ^b	System ^c	Kinetics (μ M)			Reference
				K_m	K_i	IC ₅₀ (victim substrate) ^d	
Salvianolic acid B	mOat3	I	CHO				(50)
Sinapinic acid	hOAT3	I	HEK293			25.74 $\pm$ 1.56 (1 ES)	(47)
Sulfasalazine	hOAT3	I	<i>X. laevis</i>			3.0 (10 MTX)	(53)
Syringic acid	hOAT3	I	HEK293				(47)
Tanshinol	mOat3	I	CHO				(50)
Vanillic acid	hOAT3	I	HEK293				(47)
Aristolochic acid I	hOAT4	I	HEK293		22.4		(37)
	hOAT4	I	S2			61.3 $\pm$ 8.35 (0.05 ES)	(38)
Aristolochic acid II	hOAT4	I	S2			72.0 $\pm$ 9.32 (0.05 ES)	(38)
Caffeic acid-3- <i>O</i> -sulfate	hOAT4	S	HEK293				(43)
Caffeic acid-4- <i>O</i> -sulfate	hOAT4	S	HEK293				(43)
Ferulic acid-4- <i>O</i> -sulfate	hOAT4	S	HEK293				(43)
Isoferulic acid-3- <i>O</i> -sulfate	hOAT4	S	HEK293				(43)
Perfluorooctanoate	hOAT4	S	CHO	172 $\pm$ 46 (pH 6); 310 $\pm$ 30 (pH 7.4)			(60)
Sinapinic acid	hOAT4	I	CHO				(47)
6-Hydroxybenzbromarone	hURAT1	I	MDCK			0.23 $\pm$ 0.05 (100 U)	(61)
Benzarone	hURAT1	I	MDCK			0.12 $\pm$ 0.03 (100 U)	(61)
Benzbromarone	hURAT1	I	MDCK			0.05 $\pm$ 0.02 (100 U)	(61)
Captopril	hURAT1	I	MDCK			>5,000 (100 U)	(61)
Enalapril	hURAT1	I	MDCK			648 $\pm$ 77 (100 U)	(61)
Fenofibric acid	hURAT1	I	HEK293			35.7 $\pm$ 3.9 (20 U)	(62)
Fenofibric acid (reduced)	hURAT1	I	HEK293			570 $\pm$ 25 (20 U)	(62)
Indomethacin	hURAT1	I	MDCK			40.7 $\pm$ 2.20 (100 U)	(61)
Irbesartan	hURAT1	I	<i>X. laevis</i>			121.7 (20 U)	(63)
Losartan	hURAT1	I	HEK293			571 $\pm$ 28 (20 U)	(62)
		I	<i>X. laevis</i>				(63)
Orotate	hURAT1	S	HEK293	5.2 $\pm$ 0.4			(64)
Perfluorooctanoate	hURAT1	S	HEK293	64.1 $\pm$ 30.5			(60)
Phenylbutazone	hURAT1	I	MDCK			197 $\pm$ 48.5 (100 U)	(61)
Probenecid	hURAT1	I	MDCK			86.39 $\pm$ 0.07 (10 U)	(65)
		I	MDCK			41.7 $\pm$ 3.70 (100 U)	(61)
Sulfapyrazone	hURAT1	I	MDCK			716 $\pm$ 34.3 (100 U)	(61)
Telmisartan	hURAT1	I	<i>X. laevis</i>				(63)
Valsartan	hURAT1	I	<i>X. laevis</i>				(63)
2,4-Dichlorophenoxyacetate	mOat6	I	CHO		15.7 $\pm$ 2.0		(57)
2,4-Dihydroxyphenylacetic acid	mOat6	I	CHO		61.4 $\pm$ 7.1		(57)
5-Hydroxyindoleacetic acid	mOat6	I	CHO		48.9 $\pm$ 10.3		(57)
Dehydroepiandrosterone sulfate	mOat6	I	CHO		38.8 $\pm$ 3.1		(57)

Table IV. (continued)

Compound	Transporter ^a	Interaction ^b	System ^c	Kinetics (μM)			Reference
				K_m	K_i	IC ₅₀ (victim substrate) ^d	
Hippuric acid	mOat6	I	CHO		59.9±4.9		(57)
Homovanillic acid	mOat6	I	CHO		3.0±0.5		(57)
Penicillin G	mOat6	I	CHO		1,450±480		(57)
Probenecid	mOat6	I	CHO		8.3±2.5		(57)
Salicylate	mOat6	I	CHO		49.0±4.4		(57)

^aTransporter species: *h* human, *m* mouse, *r* rat, *rb* rabbit^bInteraction: *I* inhibitor, *S* substrate^cExpression system: *CHO* Chinese hamster ovary cells, *HEK293* human embryonic kidney cell line, *MDCK* Madin-Darby canine kidney cell line, *S2* cell line derived from second segment of proximal tubule, *X. laevis* *Xenopus laevis* oocytes^dIC₅₀: concentration of inhibited substrate is shown in parentheses (in micromolars), substrate abbreviations: *CF* carboxyfluorescein, *ES* estrone sulfate, *MTX* methotrexate, *PAH* para-aminohippurate, *U* Urate; ^eThis reference reported values for both the oxidized and reduced forms of this substrate, respectively^eThis reference reported values for both the oxidized and reduced forms of this substrate, respectively

motif, as do the rat and mouse orthologs (9). Mutation of the HNF1α motif reduced basal *hOAT3* promoter activity. Similar to *hOAT1*, expression of HNF1α increased *hOAT3* and *mOat3* promoter activity more than co-expression of HNF1α and HNF1β; however, in contrast, expression of HNF1β alone also increased activity, although to a lesser extent (9). EMSA analysis confirmed binding of HNF1α homodimers and HNF1α/HNF1β heterodimers to the *hOAT3* promoter (9). Repression of endogenous HNF1α expression in a human-derived liver cell line (Huh7) with a small interfering RNA (siRNA) construct significantly decreased expression of *hOAT5* and *hOAT7* message, but was without effect on *hOAT2* mRNA expression level (11). Binding and upregulation of *hOAT5* and *hOAT7* promoter activity by HNF1α were also demonstrated, but as mentioned previously, these OATs are not expressed in the kidney (11). The *hURAT1* (−253 to +83 base pairs) and *mUrat1* (−261 to +80 base pairs) minimal promoter regions were found to contain a HNF1 motif, and mutation of the sequence in *hURAT1* abolished promoter activity (7). Both *hURAT1* and *mUrat1* responded to forced HNF1α and HNF1β expression like *hOAT3* and *mOat3*, suggesting HNF1α homodimers, HNF1α/HNF1β heterodimers, and HNF1β homodimers all stimulate promoter activity with the same respective order of potency as on *hOAT3/mOat3* (7). In contrast to the other OATs, EMSA analysis confirmed that HNF1β homodimers, in addition to HNF1α homodimers and HNF1α/HNF1β heterodimers, could bind to the promoter region of *hURAT1* (7). Lastly, two HNF1 motifs (at −97 to −85 and −41 to −29) were located in the *hOAT4* promoter (10). Overexpression of either HNF1α or HNF1β alone, or in combination, enhanced *hOAT4* promoter activity, and this enhanced activity was blunted by mutation of either motif and abolished when both motifs were mutated (10). When examined under conditions of endogenous HNF1α expression level (HepG2 cells), or endogenous HNF1α and HNF1β levels in combination (Caco-2 cells), promoter activity was only responsive to the HNF1 motif at the −97 to −85 position (10). Like *hURAT1*, EMSA analysis demonstrated that HNF1β and HNF1α homodimers, as well as HNF1α/HNF1β heterodimers, could bind this HNF1 motif; binding to the −41 to −29 motif was reported as barely detectable (10). Since it has been determined that, in renal proximal tubules, HNF1α/HNF1β heterodimers and HNF1β/HNF1β homodimers predominate, one or both of these complexes likely influences OAT expression *in vivo* (20).

Another HNF family member, HNF4α, was observed to enhance the activity of both *hOAT1* (−2747 to +88 base pairs) and *hOAT2* (−679 to +54 base pairs) promoter constructs (12,13). HNF4α is known to have several potential sequence motifs; a direct repeat of hexamers separated by one (DR-1) or two (DR-2) nucleotides, or an inverted repeat of hexamers separated by 8 nucleotides (IR-8). For *hOAT1*, promoter scanning revealed the presence of a DR-2 motif (at −875 to −862 base pairs) and an IR-8 motif (at −123 to −104 base pairs) (12). Co-expression of HNF4α with constructs containing either one or both of these motifs resulted in increased *hOAT1* promoter activity, which was diminished by mutation (12). Subsequent EMSA experiments confirmed binding of HNF4α homodimers to each of these motifs in the *hOAT1* promoter (12). The

Fig. 1. Organic anion transporters of the SLC22 family in human and rodent renal proximal tubule cells. As illustrated, human and rodent orthologs of OAT1/Oat1, OAT2/Oat2, OAT3/Oat3, OAT10/Oat10, and URAT1/Urut1 have been identified. Notable species differences are OAT4 having no identified rodent ortholog and Oat5 and Oat9 having no known human orthologs. Oat8 mRNA has been detected in rodent kidney; however, it is expressed in cortical collecting ducts, not in the proximal tubule; its membrane targeting is not known. The driving forces governing Oat9 function have not been elucidated

promoter region of *hOAT2* was found to contain a DR-1 type HNF4 α consensus motif at position -329 to -317 that is conserved in the rat ortholog (13). Presence of the wild-type sequence correlated with HNF4 α activated *hOAT2* promoter activity, and sequence mutation resulted in loss of HNF4 α activation (13). EMSA analysis demonstrated HNF4 α binding to *hOAT2* promoter fragments containing the motif and introduction of HNF4 α siRNA constructs markedly reduced endogenous *hOAT2* mRNA expression in Huh7 cells (13).

Recently, microarray analysis identified B cell lymphoma 6 protein (BCL6) as a transcription factor exhibiting male dominant expression in rats (14). Since *rOat1* and *rOat3* had previously been reported to exhibit male dominant expression that is modulated by steroid hormones (66), the investigators examined the promoter regions for BCL6 consensus binding motifs. Five putative BCL6 sequence motifs were identified in the promoter region of *rOat1* and six in the promoter of *rOat3*; no mention of whether these sites are found in the promoters of *hOAT1* and/or *hOAT3* was made (14). Expression activity of a construct corresponding to -2,252 to +113 base pairs of the *rOat1* promoter, which contained four of the identified BCL6 sites, was increased when BCL6 was co-expressed. Activity of a truncated construct (-1,266 to +113 base pairs) containing only two of the sites showed a similar level of activity, suggesting that either not all of the potential binding sites are functional or that response to binding of BCL6 is "all or none," and interaction with multiple sites does not further modulate expression level (14). A similar effect was observed for *rOat3*, with a clear increase in promoter activity upon co-expression of BCL6, but with no difference in the magnitude of effect between constructs containing six (-2,567 to +12 base pairs), three (-752 to +12 base pairs), or two (-444 to +12 base pairs) BCL6 binding motifs (14).

In addition to transcriptional regulation, there is evidence supporting posttranslational regulation of OATs by protein kinases. As recently reviewed (3), there appeared to be a general trend for decreased OAT1/Oat1 and OAT3/Oat3 activity via nonspecific protein kinase C (PKC) activation.

However, a reported exception to this was stimulation of rat and mouse Oat1- and rat and mouse Oat3-mediated transport upon activation of the atypical isoform PKC ζ (17). Increased transport of para-aminohippurate (PAH), estrone sulfate, and adefovir was observed in renal cortical slices in response to PKC ζ activation (17). The effect on estrone sulfate transport was not observed in slices prepared from Oat3 knockout mice. Direct binding of PKC ζ to *hOAT3* was demonstrated in a yeast two-hybrid system, and binding to *rOat3* was demonstrated by immunoprecipitation and immunoblotting (17). Recently, another study confirmed the binding and stimulatory effects of PKC ζ on *hOAT3* (16). In addition, the isoforms PKC δ and PKC ϵ , were observed to bind to *hOAT3* (16). This was associated with increased *hOAT3* at the cell surface, with no effect on total cellular *hOAT3*, suggesting redistribution of *hOAT3* from intracellular compartments as the mechanism behind upregulation of transport activity (16). In contrast, activation of the PKC α isoform by angiotensin II has been demonstrated to decrease transport activity of *hOAT1* and *hOAT3* in concert with decreased cell surface expression (15). Therefore, it appears as though the effects of PKC activation on OAT activity are PKC isoform dependent, and can lead to either upregulation or downregulation of OAT activity depending upon the specificity of PKC isoform activation. Thus, rapid short-term modulation of OAT activity appears possible through changes in transporter cell surface expression via cycling in and out of the membrane from a cytoplasmic pool of transporters under the control of kinases, whereas long-term changes might involve alteration of gene transcriptional activity and, presumably, protein synthesis.

OATS IN RENAL INJURY AND PROTECTION

Many clinical therapeutics, endogenous metabolic toxins, and xenobiotic toxins/toxicants accumulate in renal proximal tubules cells and induce renal injury. Cellular damage can take a variety of forms including oxidative stress, protein acylation, DNA adduct formation, mitochondrial dysfunction (impaired ATP synthesis), lipid peroxidation, and necrosis. In opposition, a number of molecules purported to exert

protective effects, such as antioxidant, anti-inflammatory, and anti-proliferative activities, also enter renal proximal tubule cells. There is growing evidence from both *in vitro* and *in vivo* systems implicating OATs in the renal targeting of these substances.

Inorganic mercury accumulates preferentially in the kidneys and can result in acute renal failure. Typical manifestations of mercury intoxication include renal vasoconstriction, reduced glomerular filtration rate (GFR), enzymuria, tubular dilatation, and formation of epithelial cell casts. Early *in vitro* and *in vivo* studies indicated that entry of mercury from the systemic circulation into proximal tubule cells occurred via the same transport pathway as PAH (recently reviewed in (2,4). Subsequent studies using Madin Darby canine kidney (MDCK) cells stably expressing hOAT1 indicated a direct correlation between hOAT1 expression and uptake and toxicity of mercury-thiol conjugates (67). Recently, direct *in vivo* evidence supporting the role of OAT1/Oat1 in mediating mercury nephrotoxicity was obtained in studies utilizing Oat1 knockout mice (68). Renal function and histopathology were examined in wild-type and Oat1 knockout mice 18 h post-intraperitoneal injection with HgCl_2 (4 mg/kg). Wild-type mice treated with HgCl_2 exhibited significantly increased kidney/body weight ratio (indicative of edema) and blood urea nitrogen level, as compared to saline injected wild-type animals (68). These indicators of renal injury and renal insufficiency were significantly blunted in Oat1 knockout mice treated with HgCl_2 (68). Furthermore, histopathological markers of nephrotoxicity observed in mercury-treated wild-type mice, including tubular dilatation and necrosis, and sloughing of the brush border membrane, were virtually undetectable in kidneys from Oat1 knockout mice (68). These findings strongly support a major role for OAT1/Oat1 in the pathology of renal mercury toxicity.

Renewed interest in complementary and alternative therapies, particularly in Western cultures, has led to the increased use of herbal therapies. Often, these are complex mixtures of dried plants or plant extracts with poorly defined active components and unregulated manufacturing processes. As they are marketed for their beneficial health effects, such therapies are frequently viewed as being safe; however, a number of herbal medicines have produced unwanted toxicities and drug interactions (69). For example, *Aristolochia* plants, used in herbal remedies for thousands of years in both Eastern and Western countries, contain the nephrotoxic agent aristolochic acid (AA), identified as the causative agent in what is now referred to as aristolochic acid nephropathy (AAN) (70). The proximal tubule has been identified as the main target site for AAN in both humans and rodent models (71,72). AA collectively refers to a complex mixture of carboxylic acids, the major components of which are AA-I and AA-II. The only structural difference between the two compounds is the presence of an *O*-methoxy group at the 8-position of AA-I (40). Both AA-I and AA-II have metabolic intermediates capable of forming DNA adducts that produce carcinogenic effects; however, this property is not linked to their toxicity as only AA-I is nephrotoxic (73,74). When examined directly in overexpressing HEK-293 cell lines, AA-I was demonstrated to be a competitive inhibitor of hOAT1, hOAT3, and hOAT4 with K_i values of 0.63 ± 0.15 , 0.50 ± 0.10 , and 20.63 ± 1.7 μM , respectively (37). Significantly increased

AA-I DNA adducts were observed in all three transporter-expressing cell lines as compared to non-transporter-expressing cells, and this increase was reversed in the presence of probenecid, supporting hOAT-mediated accumulation of AA-I (37). In another *in vitro* study, both AA-I and AA-II were shown to inhibit hOAT1, hOAT3, and hOAT4, but produced no effect on hOAT2 activity (38). Reported IC_{50} values indicated that AA-I was a slightly better inhibitor than AA-II on all three transporters, although the differences were not great, (in micromolars) 0.44 ± 0.08 and 1.06 ± 0.09 (hOAT1), 0.65 ± 0.08 and 1.28 ± 0.18 (hOAT3), and 61.3 ± 8.35 and 72.0 ± 9.32 (hOAT4), for AA-I and AA-II, respectively (38). Recently, direct uptake of AA-I and AA-II was demonstrated for mOat1, mOat2, and mOat3 indicating both compounds are high-affinity substrates for these transporters (40). Michaelis constants (K_m values) correlated well with inhibitory values reported for the human orthologs, (in micromolars) 0.791 ± 0.007 and 1.498 ± 0.371 (mOat1), 0.356 ± 0.050 and 0.673 ± 0.1192 (mOat2), and 0.514 ± 0.057 and 1.047 ± 0.360 (mOat3), for AA-I and AA-II, respectively (40). Discrepancy of the results for mOat2 and hOAT2 in cellular uptake of AA-I and AA-II may represent species differences.

Analysis of structural determinant effects on uptake indicated that the carboxyl group of AA-I is key to high-affinity interaction with mOat1, mOat2, and mOat3, whereas the nitro group only impacted interactions with mOat1 (40). Accumulation of AA-I in mouse renal cortical slices was found to be probenecid-, PAH-, and estrone sulfate-sensitive, correlating with an OAT-mediated transport process (40). Furthermore, this accumulation in slices resulted in AA-I DNA adduct formation that was reversed by probenecid (40). In agreement with these findings, a recent *in vivo* study in mice demonstrated that probenecid reduced the urinary excretion of AA-I and protected the kidney from AA-I-induced nephrotoxicity (39). Results in mOat1 and mOat3 knockout mice indicated that loss of function of either transporter was associated with decreased renal accumulation of AA-I and lessened the severity of renal injury as compared to wild-type animals (39). Significant downregulation of rOat3, but not rOat1, protein also was observed after long-term (7 days) administration of an AA mixture (75). Taken together, these findings strongly suggest an OAT-mediated mechanism in the pathology of AAN and suggest inhibition of renal OAT activity might serve as a potential therapeutic strategy following AA exposure.

Phenolic acids represent a growing class of OAT substrates/inhibitors. They are common dietary constituents as they are produced from the amino acids phenylalanine and tyrosine in plants (76). Many phenolic acids are also metabolically derived from anthocyanidins and anthocyanins after consumption (77). They are purported to have beneficial health effects in chronic diseases, e.g., diabetes, cardiovascular disorders, and cancer, through free radical scavenging, anti-proliferative, and anti-inflammatory activities (76,78), hence their growing popularity as alternative medicines/dietary supplements. Interaction of the related dietary polyphenols and human OATs was being investigated as many as 7 years ago, when ellagic acid was identified as a substrate for hOAT1 ($\text{IC}_{50} = 207$ nM), rOat1, and hOAT4, but not mOat2, hOAT3, or mOat5 (79). More recently, the phenolic acids, lithospermic acid, protocatechuic acid, rosmarinic acid, sal-

vianolic acid A, salvianolic acid B, and tanshinol, identified as active components of the traditional Chinese medicine Danshen (*Salvia miltiorrhiza*) which is utilized in cardiovascular disease, were shown to inhibit mOat1- and mOat3-mediated transport (50). Lithospermic acid, rosmarinic acid, and salvianolic acid A were found to be potent competitive inhibitors; K_i constants were 14.9 ± 4.9 vs. 31.1 ± 7.0 μ M for lithospermic acid, 5.5 ± 2.2 vs. 4.3 ± 0.2 μ M for rosmarinic acid, and 4.9 ± 2.2 vs. 21.3 ± 7.7 μ M for salvianolic acid A, on mOat1 and mOat3, respectively (50). An extensive study showed the phenolic acids caffeic acid, dihydrocaffeic acid, dihydroferulic acid, and ferulic acid were substrates of hOAT1 and/or hOAT3, while a number of their glucuronide- and sulfate-conjugated metabolites were effective inhibitors (43). An independent study identified caffeic acid as a competitive inhibitor of both hOAT1 and hOAT3 (IC_{50} =16.6 and 5.4 μ M, respectively) and demonstrated the potential for food–drug interactions via inhibition of hOAT1 and hOAT3-mediated transport of antifolates and antivirals (44). Inhibition of hOAT1 by gallic acid, protocatechuic acid, sinapinic acid, and vanillic acid, and of hOAT3, by ferulic acid, gallic acid, gentisic acid, protocatechuic acid, and sinapinic acid has been observed (47). IC_{50} values ranged from 1.24 to 18.08 μ M on hOAT1 and from 7.53 to 87.36 μ M on hOAT3 (47). Inhibition of hOAT1 by gallic acid, and of hOAT3 by gallic or gentisic acid, was identified as having potential for clinically significant food/drug–drug interactions. Human OAT4 activity was not affected by any of these compounds (47). Whether or not any dietary polyphenols/phenolic acids actually promote human health or improve disease outcomes remains unresolved. However, these studies provide clear evidence for the potential of phenolic acids to interact with OATs and to produce food–drug interactions, emphasizing the need to better understand the impact of transport processes on the distribution and elimination of these compounds. Regardless of any health benefits through antioxidant or anti-inflammatory-type activities, these compounds might provide an alternative treatment in the management of OAT-mediated nephrotoxicity, without producing significant side effects.

OAT FUNCTION AND EXPRESSION IN MODELS OF RENAL INJURY AND DISEASE

Renal function plays a vital role in the clearance of drugs and endogenous/exogenous toxins and toxicants. The overall renal clearance of substances is determined by the net effect of glomerular filtration, tubular secretion, and reabsorption processes. Renal disease states that result in dysfunction or loss of function can greatly influence the rate of elimination and tissue level of transporter substrates (80,81). OATs, which are known to mediate the accumulation of organic anions from the blood and urine into proximal tubule cells, can be affected by renal failure and disease in terms of mRNA and protein expression level, transport activity, and membrane cycling, showing altered renal clearance of their substrates (80–82). A summary of the influence of renal injury and disease on OAT protein localization and expression level is provided in Table III.

Progression of chronic renal failure (CRF) leads to impaired activity and function of the proximal tubule. In the laboratory, this pathological condition is mimicked with the 5/

6 nephrectomy model, which replicates the onset of uremia and chronic kidney disease-related complications observed in humans (83). More recently, this model has been used to examine changes in OAT protein levels in response to CRF (22–24). When examined 14 days post-surgery, Ji *et al.* found a 50% decrease in the renal clearance of unbound PAH, but no significant changes in rOat1 or rOat3 protein levels by Western blot (22). In agreement, Torres and co-workers reported significant decreases in filtered, secreted, and excreted PAH at 168 days post-surgery in 5/6 nephrectomized animals vs. sham-operated controls (23). However, in this study changes in renal PAH handling correlated with an ~40% decrease in rOat1 protein expression in kidney homogenates and basolateral membrane preparations from CRF rats. Rat Oat3 protein levels were also significantly reduced in kidney homogenates, but not in the basolateral membranes (23). Finally, Naud, *et al.* detected reduced rOat1 (~40%), rOat2 (~40%), rOat3 (~87%), and rUrat1 (~38%) proteins in CRF vs. control animals 42 days after surgery, but renal clearance was not examined in these animals (24).

While this model has the potential to yield much information on the effects of CRF on renal OAT expression and function, several important differences between these studies need to be noted (22–24). First, while described generically as 5/6 nephrectomized animals, the methodologies used to establish the model varied considerably, with one study removing the right kidney and ligating the posterior and anterior segmental branches of the left renal artery in a single operation (22), and the others utilizing a “two-stage 5/6 nephrectomy” procedure wherein the two poles of the left kidney were removed in one operation, followed by removal of the right kidney during a second operation 1 week later (23,24). Second, as indicated, the interval post-surgery when the observations were made varied widely between the studies. Third, the origins of the antibodies used to visualize the OATs via immunoblotting, and the predicted weights of the protein bands detected, differed. Ji *et al.* developed custom antibodies that detected ~77 kD and ~72 kD bands for rOat1 and rOat3, respectively. These bands are bigger than the predicted molecular weights of ~61 kD for rOat1 and ~59 kD for rOat3; however, there is evidence supporting posttranslational glycosylation of OATs *in vivo*. Their findings are in good agreement with published results with a polyclonal antibody directed against hOAT1 which recognized an ~80-kD band in crude renal cortex homogenate that was reduced to ~60 kD upon treatment with glycosidase, and matched an ~60-kD band corresponding to an unglycosylated hOAT1 *in vitro* transcription/translation reaction product (84). The study by Torres *et al.* used a commercial rOat1 antibody (Alpha Diagnostics, Inc.) and reported a ~57-kD rOat1 band, and a custom rOat3 antibody and reported a ~130-kD band for rOat3 (23). As indicated above, the result for rOat1 would correspond nicely with its unglycosylated form; however, these were native kidney homogenates, and no glycosidase treatment was performed. Since the protein samples were reduced (boiled in the presence of mercaptoethanol and sodium dodecyl sulfate), the reported rOat3 signal seems unexpectedly large. Naud *et al.* used all commercial antibodies, with anti-rOat1 and anti-rUrat1 from Abbiotec and anti-rOat2 and anti-rOat3 from Alpha Diagnostics, Inc., and did not report the predicted molecular weight for any of

the detected bands (24). To aid interpretation of future studies, it may be useful to standardize the 5/6 nephrectomy surgical techniques used, optimize/standardize the time post-surgery experiments are conducted, and perform more rigorous antibody characterizations prior to use, *e.g.*, comparison of signal across preparations from transporter-expressing cell lines and/or transporter knockout mouse tissues to confirm specificity/lack of cross-reactivity and predicted molecular weight of detected protein(s).

In contrast to CRF, even a small reduction in renal blood flow can lead to hypoxic injury and/or acute renal failure (ARF). ARF is commonly seen in critically ill patients and exhibits a mortality rate exceeding 50% (85). Ischemia/reperfusion injury associated with disease or physical injury is a common trigger for ARF, and once induced, ARF can lead to a systemic buildup of uremic toxins via a combination of reduced GFR and impaired tubular transport. Ischemia/reperfusion injury is also observed in kidneys utilized for transplant and can result in marked impairment of PAH extraction (86). This observation led to immunohistochemical studies examining the cellular distribution of hOAT1 in biopsies obtained from cadaveric renal allografts 1 h post-reperfusion (25). While most allografts were seen to maintain some degree of basolaterally targeted hOAT1, it appeared to be greatly reduced as compared to control tissues and was accompanied by the appearance of fine and coarse “cytoplasmic aggregates” positive for hOAT1 (25). This correlated with depressed net tubular secretion of PAH in 15 out of 18 patients examined 3–7 days post-transplant (25). No clear association between total ischemic time, which varied from 13–32 h, could be made, and it should be noted that patients were taking a number of drugs known to interact with hOAT1 and hOAT3 when PAH secretion was measured. Nonetheless, these data support the contention that renal organic anion transport impairment observed in some instances of disease, injury, or transplant-induced ARF might be due to loss of OATs in the proximal tubule cell membranes.

Experimentally induced ARF, achieved via clamping of the renal arteries, is a common approach used to model this type of renal injury in the laboratory. When this same group examined localization of rOat1 in the induced ARF model, redistribution of rOat1 was observable with as little as 5 min of ischemia, but the amount of rOat1 protein did not diminish until 1 h post-reperfusion, and was still not fully recovered after 10 days (26). A similar study examined rOat1 and rOat3 expression for 14 days following ischemia-induced ARF (27). Both transporter proteins showed a similar response to ARF, exhibiting a significant reduction (~50%) from 6 h to 3 days post-ischemia, returning to control levels by 7 days post-injury (27). This loss and recovery of transporter protein levels correlated with effects on the net renal secretion of PAH (a measure insulated from changes in GFR), which was significantly reduced from 6 h to 3 days post-reperfusion as compared to sham-operated animals, returning to control levels by 7 days (27). Matsuzaki *et al.* also reported downregulation of rOat1 and rOat3 protein expression level in rat kidney 48 h after ischemia (28). Significant decline in accumulation of PAH and estrone sulfate in renal slices from ARF animals suggested reduced transport capacity mediated by rOat1 and rOat3 (28). Interestingly, administration of cobalt, a treatment proposed to stabilize hypoxia inducible

factor 1, an oxygen-sensitive transcription factor which serves to upregulate certain genes under low oxygen conditions, significantly blunted the downregulation of rOat3 protein, partially restoring estrone sulfate accumulation in renal slices (28). The treatment was without effect on rOat1 or PAH accumulation (28).

The apical transporter rOat5 also has been demonstrated to be downregulated in response to ischemia-induced ARF (29). When examined 1 h post-reperfusion after 5 min of ischemia, no change in rOat5 protein level was detected in renal homogenates or apical membrane preparations. However, if ischemia was maintained for 1 h, a significant reduction (~40%) in rOat5 was observed (29). Regardless, rOat5 protein levels detected in urine (relative to urinary creatinine) were significantly elevated 1 h after either 5 or 60 min of ischemia, leading the authors to suggest that urinary excretion of rOat5 protein may serve as a sensitive, noninvasive measure of renal functional and structural integrity following renal injury or during renal dysfunction (29). Since there is no identified human ortholog of rodent Oat5, the clinical utility of this finding is unclear. However, urinary hOAT1, hOAT3, and hOAT4 protein levels were recently examined in patients diagnosed with acute kidney injury (AKI) (30). Two AKI patient groups were identified, those who recovered without intervention (“early reversible proximal tubular damage”) and those who required intervention in the form of dialysis (“severe AKI”). Patients who suffered “early reversible proximal tubular damage” exhibited increased urinary levels of hOAT1 and hOAT3, but decreased levels of hOAT4, as compared to controls, and patients experiencing “severe AKI” exhibited increased urinary levels of all three transporters (30). The authors speculated that in patients with “early reversible proximal tubular damage,” the transporters became mistargeted, with basolateral hOAT1 and hOAT3 translocating to the apical membrane and sloughing into the urine with apical hOAT4 moving “inward,” whereas “severe AKI” resulted in proximal tubule cell apoptosis. Whether such a profound mechanism is the underlying cause of the observed patterns is unclear at this time.

There is clear evidence that prostaglandin E₂ (PGE₂) plays a role in the downregulation of OAT1/Oat1 and OAT3/Oat3 in response to ARF and CRF. Dose-dependent downregulation of both rOat1 and rOat3 protein expression by PGE₂ (IC₅₀ ~25 nM) was demonstrated in NRK-52E cells, which are a rat renal proximal tubular cell model (87). Cortical PGE₂ levels are elevated in response to ARF and CRF, and inhibition of the cyclooxygenase (COX) cascade was shown to exert protective effects (88,89). Since COX-1 and COX-2 mediate prostaglandin synthesis from arachidonic acid, it is thought that COX inhibitors work by preventing generation of cortical PEG₂ in response to injury. Accordingly, in the ischemia/reperfusion rat model, administration of the nonselective COX inhibitor indomethacin (1 mg/kg) immediately post-ischemia completely reversed ARF-induced downregulation of rOat1 and rOat3 protein expression levels within 24 h (90). Moreover, renal PAH net secretion was also recovered (90). In a different study, the COX-2-specific inhibitor, parecoxib, was found to produce similar effects when administered 1 h (20 mg/kg intraperitoneally) prior to the start of ischemia (91). When examined 12 h post-reperfusion, both rOat1 and rOat3 protein levels were

significantly higher than in ARF animals that did not receive parecoxib; however, the levels were still significantly reduced as compared to sham control animals (91). Similarly, PAH secretion was markedly restored in parecoxib animals, but still blunted as compared to sham controls (91). Whether this difference is due to the shorter duration post-reperfusion that the animals were examined (12 vs. 24 h) or to the more selective action of parecoxib vs. indomethacin is not known. Together, these data strongly implicate induced PGE₂ as being responsible for the downregulation of OATs observed in CRF, ARF, and human renal allografts.

OATS AND DRUG-DRUG INTERACTIONS

Following the development of probenecid as an agent to block the renal elimination of penicillin almost 70 years ago, clinical reports of probenecid-mediated DDIs resulting in altered distribution and renal elimination of a variety of drugs, including other β -lactams, cephalosporin antibiotics, fluoroquinolone antimicrobials, diuretics, and chemotherapeutic agents, began to appear (92–95). Modern studies using cloned transporters and knockout mouse lines have demonstrated a clear role for OATs in mediating, at least in part, a number of these DDIs (3,96). Recently, a clinical study using healthy volunteers demonstrated that when probenecid was administered concomitantly with mesna, a cancer chemotherapy adjuvant used to decrease the renal toxicity of some chemotherapeutic agents, the fraction of mesna secreted was reduced by 67%, and total mesna renal clearance was decreased by 55% (97). Moreover, hOAT1, hOAT3, and hOAT4 were demonstrated to mediate the probenecid-sensitive transport of mesna and dimensa *in vitro*, with inhibitory constants (K_i) in the ~15–30- μ M range (97). These K_i values are below the clinical concentrations for these compounds, strongly implicating OATs in this DDI as well as having a vital role in the effectiveness of this therapy (97).

Patients taking pravastatin and gemfibrozil in combination to control their cholesterol and triglyceride levels have experienced alterations in pravastatin pharmacokinetics (increased plasma levels and decreased renal clearance) and been reported to suffer from increased pravastatin-associated adverse events (98). Subsequent work indicated this DDI might be the result of gemfibrozil inhibition of hOAT3-mediated pravastatin transport in these patients (99). Another lipid altering agent, gemcabene, has been implicated in exacerbating blood pressure reduction when co-administered with the angiotensin-converting enzyme inhibitor, quinapril, in hypertensive patients (100). When examined *in vitro*, gemcabene was found to inhibit hOAT3 transport of quinaprilat, the pharmacologically active quinapril metabolite, in a dose-dependent manner, suggesting DDI on hOAT3 as the molecular basis for the observed increase in pharmacological activity (100).

Clearly, renally expressed OATs are poised to make great contributions to the pharmacokinetic profiles of many clinical therapeutics, having significant impact on their dosing regimens, pharmacodynamics, efficacy, and toxicity. OATs have great potential to be the sites for many clinically relevant DDIs, and the conclusive identification of their contributions to such interactions is expanding. In recognition of these facts, a recent whitepaper by the “International

Transporter Consortium” (101), in conjunction with guidance documents issued by the United States Food and Drug Administration (FDA) (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf>) and by the European Medicines Agency (http://www.ema.europa.eu/ema/index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500090112&url=menus/document_library/document_library.jsp&mid=WC0b01ac058009a3dc&jsenabled=true), have provided recommendations concerning which transporters are thought to have sufficient evidence regarding their impact on drug disposition and pharmacokinetics (e.g., hOAT1, hOAT2, and hOAT3) to warrant investigation of new molecular entities for interactions, as a substrate or inhibitor, during the drug development process. In the whitepaper, it was recommended that *in vitro* studies with transporter-expressing cell lines should be conducted to determine if the compound is a substrate, if the renal clearance is greater than or equal to 50% of the total clearance (considered to be substantial and clinically important), and greater than 1.5 times the unbound fraction times the GFR (101). The FDA guidance recommends that greater than twofold accumulation over background in transporter-expressing cells is necessary to regard the compound as a substrate. If these conditions are met, clinical DDI studies should be considered. For compounds found to be inhibitors of these transporters, if the ratio of the unbound maximum serum level (C_{max} or C_{ss}) divided by the compound-specific inhibition potency (K_i or IC_{50}) is greater than or equal to 0.1, clinical DDI studies are recommended. In 2010, VanWert *et al.* provided a comprehensive table on OATs summarizing the orthologs studied, compounds reported to interact with each of the OATs, whether they were confirmed substrates or only examined as inhibitors, and their affinities (reported K_m , K_i , or IC_{50} values) that is a useful reference tool when exploring such assessments (3). In Table IV of this work, we provide an update to the 2010 table, focusing on recent publications (2010–2012) in this area.

CONCLUSIONS

Over the last 15 years great progress has been made in identifying the biological properties, physiological roles, and biopharmaceutical significance of OATs. However, more effort is required to better define the role of OATs in “normal” physiology, their changing function across an organism’s lifespan, and their response to toxic exposure, whether environmental or as part of disease progression. While single nucleotide polymorphisms (SNPs) have been identified in both coding and regulatory regions of hOAT1, hOAT3, hOAT4, and hURAT1, most identified SNPs have failed to significantly impact OAT expression and function (102–105). Thus, modulations in OAT expression and transport activity through transcriptional and posttranslational mechanisms might exert greater influence on the individual variations in pharmacokinetics and drug efficacy observed in clinical investigations, at different developmental stages (e.g., pediatric vs. geriatric), and during progression of certain diseases (106–108). More thorough understanding of these processes, in addition to OAT substrate specificity, might lead to new approaches for protecting renal function after injury or toxic insult, or for manipulating renal secretory or reabsorptive pathways to more effectively remove or reclaim endogenous metabolites and xenobiotics. Greater insight to the

pathophysiological changes to normal renal proximal tubule cell functions vital to OAT activity, *e.g.*, production of the intracellular mono- and dicarboxylates utilized as counterions by exchangers, would also be invaluable. As we move forward in our study of renal OAT function and make determinations as to the likely significance of DDIs involving OATs, we need to remember that, while we have come a long way, there is still much we do not know, and this changing context (*e.g.*, developmental stage, long-term polypharmacy, disease) might transcend a previously trivial interaction to one of great significance.

REFERENCES

- He L, Vasilou K, Nebert DW. Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genomics*. 2009;3:195–206.
- Sweet DH. Organic anion transporter (Slc22a) family members as mediators of toxicity. *Toxicol Appl Pharmacol*. 2005;204:198–215.
- VanWert AL, Gionfriddo MR, Sweet DH. Organic anion transporters: discovery, pharmacology, regulation and roles in pathophysiology. *Biopharm Drug Dispos*. 2010;31:1–71.
- Sweet DH. Renal organic cation and anion transport: from physiology to genes. In: McQueen CA, editor. *Comprehensive toxicology*, 2nd ed., vol. 7. Oxford: Elsevier; 2010. p. 23–53.
- Tsuchida H, Anzai N, Shin HJ, Wempe MF, Jutabha P, Enomoto A, *et al.* Identification of a novel organic anion transporter mediating carnitine transport in mouse liver and kidney. *Cell Physiol Biochem*. 2010;25:511–22.
- Ogasawara K, Terada T, Asaka J, Katsura T, Inui K. Human organic anion transporter 3 gene is regulated constitutively and inducibly via a cAMP-response element. *J Pharmacol Exp Ther*. 2006;319:317–22.
- Kikuchi R, Kusuhara H, Hattori N, Kim I, Shiota K, Gonzalez FJ, *et al.* Regulation of tissue-specific expression of the human and mouse urate transporter 1 gene by hepatocyte nuclear factor 1 alpha/beta and DNA methylation. *Mol Pharmacol*. 2007;72:1619–25.
- Saji T, Kikuchi R, Kusuhara H, Kim I, Gonzalez FJ, Sugiyama Y. Transcriptional regulation of human and mouse organic anion transporter 1 by hepatocyte nuclear factor 1 alpha/beta. *J Pharmacol Exp Ther*. 2008;324:784–90.
- Kikuchi R, Kusuhara H, Hattori N, Shiota K, Kim I, Gonzalez FJ, *et al.* Regulation of the expression of human organic anion transporter 3 by hepatocyte nuclear factor 1 alpha/beta and DNA methylation. *Mol Pharmacol*. 2006;70:887–96.
- Jin L, Kikuchi R, Saji T, Kusuhara H, Sugiyama Y. Regulation of tissue-specific expression of renal organic anion transporters by hepatocyte nuclear factor 1 alpha/beta and DNA methylation. *J Pharmacol Exp Ther*. 2012;340:648–55.
- Klein K, Jungst C, Mwinyi J, Stieger B, Krempler F, Patsch W, *et al.* The human organic anion transporter genes OAT5 and OAT7 are transactivated by hepatocyte nuclear factor-1alpha (HNF-1alpha). *Mol Pharmacol*. 2010;78:1079–87.
- Ogasawara K, Terada T, Asaka J, Katsura T, Inui K. Hepatocyte nuclear factor-4[alpha] regulates the human organic anion transporter 1 gene in the kidney. *Am J Physiol Renal Physiol*. 2007;292:F1819–26.
- Popowski K, Eloranta JJ, Saborowski M, Fried M, Meier PJ, Kullak-Ublick GA. The human organic anion transporter 2 gene is transactivated by hepatocyte nuclear factor-4 alpha and suppressed by bile acids. *Mol Pharmacol*. 2005;67:1629–38.
- Wegner W, Burckhardt BC, Burckhardt G, Henjakovic M. Male-dominant activation of rat renal organic anion transporter 1 (Oat1) and 3 (Oat3) expression by transcription factor BCL6. *PLoS One*. 2012;7:e35556.
- Duan P, Li S, You G. Angiotensin II inhibits activity of human organic anion transporter 3 through activation of protein kinase Calpha: accelerating endocytosis of the transporter. *Eur J Pharmacol*. 2009;627:49–55.
- Li S, Duan P, You G. Regulation of human organic anion transporter 3 by peptide hormone bradykinin. *J Pharmacol Exp Ther*. 2010;333:970–5.
- Barros SA, Srimaroeng C, Perry JL, Walden R, Dembla-Rajpal N, Sweet DH, *et al.* Activation of protein kinase Ceta increases OAT1 (SLC22A6)- and OAT3 (SLC22A8)-mediated transport. *J Biol Chem*. 2009;284:2672–9.
- Eraly SA, Hamilton BA, Nigam SK. Organic anion and cation transporters occur in pairs of similar and similarly expressed genes. *Biochem Biophys Res Commun*. 2003;300:333–42.
- Li T, Walsh JR, Ghishan FK, Bai L. Molecular cloning and characterization of a human urate transporter (hURAT1) gene promoter. *Biochim Biophys Acta*. 2004;1681:53–8.
- Lazzaro D, De Simone V, De Magistris L, Lehtonen E, Cortese R. LFB1 and LFB3 homeoproteins are sequentially expressed during kidney development. *Development*. 1992;114:469–79.
- Maier JM, Slitt AL, Callaghan TN, Cheng X, Cheung C, Gonzalez FJ, *et al.* Alterations in transporter expression in liver, kidney, and duodenum after targeted disruption of the transcription factor HNF1alpha. *Biochem Pharmacol*. 2006;72:512–22.
- Ji L, Masuda S, Saito H, Inui K. Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy. *Kidney Int*. 2002;62:514–24.
- Monica Torres A, Mac Laughlin M, Muller A, Brandoni A, Anzai N, Endou H. Altered renal elimination of organic anions in rats with chronic renal failure. *Biochim Biophys Acta*. 2005;1740:29–37.
- Naud J, Michaud J, Beauchemin S, Hebert MJ, Roger M, Lefrancois S, *et al.* Effects of chronic renal failure on kidney drug transporters and cytochrome P450 in rats. *Drug Metab Dispos*. 2011;39:1363–9.
- Kwon O, Hong SM, Blouch K. Alteration in renal organic anion transporter 1 after ischemia/reperfusion in cadaveric renal allografts. *J Histochem Cytochem*. 2007;55:575–84.
- Kwon O, Wang WW, Miller S. Renal organic anion transporter 1 is maldistributed and diminishes in proximal tubule cells but increases in vasculature after ischemia and reperfusion. *Am J Physiol Renal Physiol*. 2008;295:F1807–16.
- Schneider R, Sauvant C, Betz B, Otremba M, Fischer D, Holzinger H, *et al.* Downregulation of organic anion transporters OAT1 and OAT3 correlates with impaired secretion of para-aminohippurate after ischemic acute renal failure in rats. *Am J Physiol Renal Physiol*. 2007;292:F1599–605.
- Matsuzaki T, Watanabe H, Yoshitome K, Morisaki T, Hamada A, Nonoguchi H, *et al.* Downregulation of organic anion transporters in rat kidney under ischemia/reperfusion-induced acute renal failure. *Kidney Int*. 2007;71:539–47.
- Di Giusto G, Anzai N, Endou H, Torres AM. Oat5 and NaDC1 protein abundance in kidney and urine after renal ischemic reperfusion injury. *J Histochem Cytochem*. 2009;57:17–27.
- Kunin M, Holtzman EJ, Melnikov S, Dinour D. Urinary organic anion transporter protein profiles in AKI. *Nephrol Dial Transplant*. 2012;27:1387–95.
- Astorga B, Wunz TM, Morales M, Wright SH, Pelis RM. Differences in the substrate binding regions of renal organic anion transporters 1 (OAT1) and 3 (OAT3). *Am J Physiol Renal Physiol*. 2011;301:F378–86.
- Rodiger M, Zhang X, Ugele B, Gersdorff N, Wright SH, Burckhardt G, *et al.* Organic anion transporter 3 (OAT3) and renal transport of the metal chelator 2,3-dimercapto-1-propanesulfonic acid (DMPS). *Can J Physiol Pharmacol*. 2010;88:141–6.
- Makino T, Okajima K, Uebayashi R, Ohtake N, Inoue K, Mizukami H. 3-Monoglucuronyl-glycylrhretinic acid is a substrate of organic anion transporters expressed in tubular epithelial cells and plays important roles in licorice-induced pseudoaldosteronism by inhibiting 11beta-hydroxysteroid dehydrogenase 2. *J Pharmacol Exp Ther*. 2012;342:297–304.
- Uwai Y, Iwamoto K. Transport of aminopterin by human organic anion transporters hOAT1 and hOAT3: comparison with methotrexate. *Drug Metab Pharmacokin*. 2010;25:163–9.
- Chiba S, Ikawa T, Takeshita H, Ichiba K, Sagi M, Mukai T, *et al.* Interactions of human organic anion transporter 1 (hOAT1) with substances associated with forensic toxicology. *Leg Med (Tokyo)*. 2011;13:180–5.

36. Ahn SY, Jamshidi N, Mo ML, Wu W, Eraly SA, Dnyanmote A, *et al.* Linkage of organic anion transporter-1 to metabolic pathways through integrated “omics”-driven network and functional analysis. *J Biol Chem.* 2011;286:31522–31.
37. Bakhiya N, Arlt VM, Bahn A, Burckhardt G, Phillips DH, Glatt H. Molecular evidence for an involvement of organic anion transporters (OATs) in aristolochic acid nephropathy. *Toxicology.* 2009;264:74–9.
38. Babu E, Takeda M, Nishida R, Noshiro-Kofuji R, Yoshida M, Ueda S, *et al.* Interactions of human organic anion transporters with aristolochic acids. *J Pharmacol Sci.* 2010;113:192–6.
39. Xue X, Gong LK, Maeda K, Luan Y, Qi XM, Sugiyama Y, *et al.* Critical role of organic anion transporters 1 and 3 in kidney accumulation and toxicity of aristolochic acid I. *Mol Pharm.* 2011;8:2183–92.
40. Dickman KG, Sweet DH, Bonala R, Ray T, Wu A. Physiological and molecular characterization of aristolochic acid transport by the kidney. *J Pharmacol Exp Ther.* 2011;338:588–97.
41. Zeng Y, Zhang R, Wu J, Liu M, Peng W, Yu X, *et al.* Organic anion transporter 1 (OAT1) involved in renal cell transport of aristolochic acid I. *Hum Exp Toxicol* 2012; 31: 759–70.
42. Zhu Y, Meng Q, Wang C, Liu Q, Sun H, Kaku T, *et al.* Organic anion transporters involved in the excretion of bestatin in the kidney. *Peptides.* 2012;33:265–71.
43. Wong CC, Barron D, Orfila C, Dionisi F, Krajcsi P, Williamson G. Interaction of hydroxycinnamic acids and their conjugates with organic anion transporters and ATP-binding cassette transporters. *Mol Nutr Food Res.* 2011;55:979–88.
44. Uwai Y, Ozeki Y, Isaka T, Honjo H, Iwamoto K. Inhibitory effect of caffeic acid on human organic anion transporters hOAT1 and hOAT3: a novel candidate for food–drug interaction. *Drug Metab Pharmacokinet.* 2011;26:486–93.
45. Zhang J, Wang C, Liu Q, Meng Q, Cang J, Sun H, *et al.* Pharmacokinetic interaction between JBP485 and cephalexin in rats. *Drug Metab Dispos.* 2010;38:930–8.
46. Wong CC, Botting NP, Orfila C, Al-Maharik N, Williamson G. Flavonoid conjugates interact with organic anion transporters (OATs) and attenuate cytotoxicity of adefovir mediated by organic anion transporter 1 (OAT1/SLC22A6). *Biochem Pharmacol.* 2011;81:942–9.
47. Wang L, Sweet DH. Potential for food–drug interactions by dietary phenolic acids on human organic anion transporters 1 (SLC22A6), 3 (SLC22A8), and 4 (SLC22A11). *Biochem Pharmacol.* 2012;84:1088–95.
48. Larsen M, Holm R, Jensen KG, Sveigaard C, Brodin B, Nielsen CU. 5-Hydroxy-L-tryptophan alters gaboxadol pharmacokinetics in rats: involvement of PAT1 and rOat1 in gaboxadol absorption and elimination. *Eur J Pharm Sci.* 2010;39:68–75.
49. Wikoff WR, Nagle MA, Kouznetsova VL, Tsigelny IF, Nigam SK. Untargeted metabolomics identifies enterobiome metabolites and putative uremic toxins as substrates of organic anion transporter 1 (Oat1). *J Proteome Res.* 2011;10:2842–51.
50. Wang L, Sweet DH. Active hydrophilic components of the medicinal herb *salvia miltiorrhiza* (Danshen) potentially inhibit organic anion transporters 1 (Slc22a6) and 3 (Slc22a8). *Evid Based Complement Alternat Med.* 2012;2012:(Article ID: 872458).
51. Uwai Y, Honjo H, Iwamoto K. Inhibitory effect of selective cyclooxygenase-2 inhibitor lumiracoxib on human organic anion transporters hOAT1 and hOAT3. *Drug Metab Pharmacokinet.* 2010;25:450–5.
52. Choi MK, Jin QR, Choi YL, Ahn SH, Bae MA, Song IS. Inhibitory effects of ketoconazole and rifampin on OAT1 and OATP1B1 transport activities: considerations on drug–drug interactions. *Biopharm Drug Dispos.* 2011;32:175–84.
53. Elsby R, Fox L, Stresser D, Layton M, Butters C, Sharma P, *et al.* *In vitro* risk assessment of AZD9056 perpetrating a transporter-mediated drug–drug interaction with methotrexate. *Eur J Pharm Sci.* 2011;43:41–9.
54. Fork C, Bauer T, Golz S, Geerts A, Weiland J, Del Turco D, *et al.* OAT2 catalyses efflux of glutamate and uptake of orotic acid. *Biochem J.* 2011;436:305–12.
55. Sato M, Mamada H, Anzai N, Shirasaka Y, Nakanishi T, Tamai I. Renal secretion of uric acid by organic anion transporter 2 (OAT2/SLC22A7) in human. *Biol Pharm Bull.* 2010;33:498–503.
56. Lai Y, Sampson KE, Balogh LM, Brayman TG, Cox SR, Adams WJ, *et al.* 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.* 2010;334:936–44.
57. Schnabolk GW, Gupta B, Mulgaonkar A, Kulkarni M, Sweet DH. Organic anion transporter 6 (Slc22a20) specificity and Sertoli cell-specific expression provide new insight on potential endogenous roles. *J Pharmacol Exp Ther.* 2010;334:927–35.
58. Maeda A, Tsuruoka S, Ushijima K, Kanai Y, Endou H, Saito K, *et al.* Drug interaction between celecoxib and methotrexate in organic anion transporter 3-transfected renal cells and in rats *in vivo*. *Eur J Pharmacol.* 2010;640:168–71.
59. Miyajima M, Kusuhashi H, Fujishima M, Adachi Y, Sugiyama Y. Organic anion transporter 3 mediates the efflux transport of an amphipathic organic anion, dehydroepiandrosterone sulfate, across the blood–brain barrier in mice. *Drug Metab Dispos.* 2011;39:814–9.
60. Yang CH, Glover KP, Han X. Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. *Toxicol Sci.* 2010;117:294–302.
61. Shin HJ, Takeda M, Enomoto A, Fujimura M, Miyazaki H, Anzai N, *et al.* Interactions of urate transporter URAT1 in human kidney with uricosuric drugs. *Nephrology (Carlton).* 2011;16:156–62.
62. Uetake D, Ohno I, Ichida K, Yamaguchi Y, Saikawa H, Endou H, *et al.* Effect of fenofibrate on uric acid metabolism and urate transporter 1. *Intern Med.* 2010;49:89–94.
63. Nakamura M, Anzai N, Jutabha P, Sato H, Sakurai H, Ichida K. Concentration-dependent inhibitory effect of irbesartan on renal uric acid transporters. *J Pharmacol Sci.* 2010;114:115–8.
64. Miura D, Anzai N, Jutabha P, Chanluang S, He X, Fukutomi T, *et al.* Human urate transporter 1 (hURAT1) mediates the transport of orotate. *J Physiol Sci.* 2011;61:253–7.
65. Wempe MF, Jutabha P, Quade B, Iwen TJ, Frick MM, Ross IR, *et al.* Developing potent human uric acid transporter 1 (hURAT1) inhibitors. *J Med Chem.* 2011;54:2701–13.
66. Ljubojevic M, Herak-Kramberger CM, Hagos Y, Bahn A, Endou H, Burckhardt G, *et al.* Rat renal cortical OAT1 and OAT3 exhibit gender differences determined by both androgen stimulation and estrogen inhibition. *Am J Physiol Renal Physiol.* 2004;287:F124–38.
67. Aslamkhan AG, Han YH, Yang XP, Zalups RK, Pritchard JB. Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. *Mol Pharmacol.* 2003;63:590–6.
68. Torres AM, Dnyanmote AV, Bush KT, Wu W, Nigam SK. Deletion of multispecific organic anion transporter Oat1/Slc22a6 protects against mercury-induced kidney injury. *J Biol Chem.* 2011;286:26391–5.
69. Wojcikowski K, Johnson DW, Gobe G. Medicinal herbal extracts—renal friend or foe? Part one: the toxicities of medicinal herbs. *Nephrology (Carlton).* 2004;9:313–8.
70. Debelle FD, Vanherweghem JL, Nortier JL. Aristolochic acid nephropathy: a worldwide problem. *Kidney Int.* 2008;74:158–69.
71. Lebeau C, Debelle FD, Arlt VM, Pozdzik A, De Prez EG, Phillips DH, *et al.* Early proximal tubule injury in experimental aristolochic acid nephropathy: functional and histological studies. *Nephrol Dial Transplant.* 2005;20:2321–32.
72. Pozdzik AA, Salmon IJ, Debelle FD, Decaestecker C, Van den Branden C, Verbeelen D, *et al.* Aristolochic acid induces proximal tubule apoptosis and epithelial to mesenchymal transformation. *Kidney Int.* 2008;73:595–607.
73. Sato N, Takahashi D, Chen SM, Tsuchiya R, Mukoyama T, Yamagata S, *et al.* Acute nephrotoxicity of aristolochic acids in mice. *J Pharm Pharmacol.* 2004;56:221–9.

74. Shibutani S, Dong H, Suzuki N, Ueda S, Miller F, Grollman AP. Selective toxicity of aristolochic acids I and II. *Drug Metab Dispos.* 2007;35:1217–22.
75. Lou Y, Li J, Lu Y, Wang X, Jiao R, Wang S, *et al.* Aristolochic acid-induced destruction of organic ion transporters and fatty acid metabolic disorder in the kidney of rats. *Toxicol Lett.* 2011;201:72–9.
76. Russell W, Duthie G. Plant secondary metabolites and gut health: the case for phenolic acids. *Proc Nutr Soc.* 2011;70:389–96.
77. Fleschhut J, Kratzer F, Reckemmer G, Kulling SE. Stability and biotransformation of various dietary anthocyanins *in vitro*. *Eur J Nutr.* 2006;45:7–18.
78. Rababah TM, Ereifej KI, Esah RB, Al-u'datt MH, Alrababah MA, Yang W. Antioxidant activities, total phenolics and HPLC analyses of the phenolic compounds of extracts from common Mediterranean plants. *Nat Prod Res.* 2011;25:596–605.
79. Whitley AC, Sweet DH, Walle T. The dietary polyphenol ellagic acid is a potent inhibitor of hOAT1. *Drug Metab Dispos.* 2005;33:1097–100.
80. Saito H. Pathophysiological regulation of renal SLC22A organic ion transporters in acute kidney injury: pharmacological and toxicological implications. *Pharmacol Ther.* 2009;125:79–91.
81. Sun H, Frassetto L, Benet LZ. Effects of renal failure on drug transport and metabolism. *Pharmacol Ther.* 2006;109:1–11.
82. Enomoto A, Endou H. Roles of organic anion transporters (OATs) and a urate transporter (URAT1) in the pathophysiology of human disease. *Clin Exp Nephrol.* 2005;9:195–205.
83. Shobeiri N, Adams MA, Holden RM. Vascular calcification in animal models of CKD: a review. *Am J Nephrol.* 2010;31:471–81.
84. Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, Sweet DH. The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol Pharmacol.* 1999;56:570–80.
85. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, *et al.* Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA.* 2005;294:813–8.
86. Corrigan G, Ramaswamy D, Kwon O, Sommer FG, Alfrey EJ, Dafoe DC, *et al.* PAH extraction and estimation of plasma flow in human postischemic acute renal failure. *Am J Physiol.* 1999;277:F312–8.
87. Sauvant C, Holzinger H, Gekle M. Prostaglandin E2 inhibits its own renal transport by downregulation of organic anion transporters rOAT1 and rOAT3. *J Am Soc Nephrol.* 2006;17:46–53.
88. Myers SI, Wang L, Liu F, Bartula LL. Suprarenal aortic clamping and reperfusion decreases medullary and cortical blood flow by decreased endogenous renal nitric oxide and PGE2 synthesis. *J Vasc Surg.* 2005;42:524–31.
89. Tokuyama H, Hayashi K, Matsuda H, Kubota E, Honda M, Okubo K, *et al.* Stenosis-dependent role of nitric oxide and prostaglandins in chronic renal ischemia. *Am J Physiol Renal Physiol.* 2002;282:F859–65.
90. Schneider R, Meusel M, Renker S, Bauer C, Holzinger H, Roeder M, *et al.* Low-dose indomethacin after ischemic acute kidney injury prevents downregulation of Oat1/3 and improves renal outcome. *Am J Physiol Renal Physiol.* 2009;297:F1614–21.
91. Hoehnerl K, Schmidt C, Bucher M. COX-2 inhibition attenuates endotoxin-induced downregulation of organic anion transporters in the rat renal cortex. *Kidney Int.* 2009;75:373–80.
92. Welling PG, Dean S, Selen A, Kendall MJ, Wise R. Probenecid: an unexplained effect on cephalosporin pharmacology. *Br J Clin Pharmacol.* 1979;8:491–5.
93. Bourke RS, Chheda G, Bremer A, Watanabe O, Tower DB. Inhibition of renal tubular transport of methotrexate by probenecid. *Cancer Res.* 1975;35:110–6.
94. Jaehde U, Sorgel F, Reiter A, Sigl G, Naber KG, Schunack W. Effect of probenecid on the distribution and elimination of ciprofloxacin in humans. *Clin Pharmacol Ther.* 1995;58:532–41.
95. Honari J, Blair AD, Cutler RE. Effects of probenecid on furosemide kinetics and natriuresis in man. *Clin Pharmacol Ther.* 1977;22:395–401.
96. Mulgaonkar A, Venitz J, Sweet DH. Fluoroquinolone disposition: identification of the contribution of renal secretory and reabsorptive drug transporters. *Expert Opin Drug Metab Toxicol.* 2012;8:553–69.
97. Cutler MJ, Urquhart BL, Velenosi TJ, Meyer Zu Schwabedissen HE, Dresser GK, Leake BF, *et al.* *In vitro* and *in vivo* assessment of renal drug transporters in the disposition of mesna and dimesna. *J Clin Pharmacol.* 2012;52:530–42.
98. Kyrklund C, Backman JT, Neuvonen M, Neuvonen PJ. Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. *Clin Pharmacol Ther.* 2003;73:538–44.
99. Nakagomi-Hagihara R, Nakai D, Tokui T. Inhibition of human organic anion transporter 3 mediated pravastatin transport by gemfibrozil and the metabolites in humans. *Xenobiotica.* 2007;37:416–26.
100. Yuan H, Feng B, Yu Y, Chupka J, Zheng JY, Heath TG, *et al.* Renal organic anion transporter-mediated drug–drug interaction between gemcabene and quinapril. *J Pharmacol Exp Ther.* 2009;330:191–7.
101. Giacomini KM, Huang SM, Tweedie DJ. Membrane transporters in drug development. *Nat Rev Drug Discov.* 2010;9:215–36.
102. Bhatnagar V, Xu G, Hamilton BA, Truong DM, Eraly SA, Wu W, *et al.* Analyses of 5' regulatory region polymorphisms in human SLC22A6 (OAT1) and SLC22A8 (OAT3). *J Hum Genet.* 2006;51:575–80.
103. Shima JE, Komori T, Taylor TR, Stryke D, Kawamoto M, Johns SJ, *et al.* Genetic variants of human organic anion transporter 4 demonstrate altered transport of endogenous substrates. *Am J Physiol Renal Physiol.* 2010;299:F767–75.
104. Zhou F, Zhu L, Cui PH, Church WB, Murray M. Functional characterization of nonsynonymous single nucleotide polymorphisms in the human organic anion transporter 4 (hOAT4). *Br J Pharmacol.* 2010;159:419–27.
105. Rizwan AN, Burckhardt G. Organic anion transporters of the SLC22 family: biopharmaceutical, physiological, and pathological roles. *Pharm Res.* 2007;24:450–70.
106. Han YF, Fan XH, Wang XJ, Sun K, Xue H, Li WJ, *et al.* Association of intergenic polymorphism of organic anion transporter 1 and 3 genes with hypertension and blood pressure response to hydrochlorothiazide. *Am J Hypertens.* 2011;24:340–6.
107. Sakurai Y, Motohashi H, Ueo H, Masuda S, Saito H, Okuda M, *et al.* Expression levels of renal organic anion transporters (OATs) and their correlation with anionic drug excretion in patients with renal diseases. *Pharm Res.* 2004;21:61–7.
108. Sweet DH, Eraly SA, Vaughn DA, Bush KT, Nigam SK. Organic anion and cation transporter expression and function during embryonic kidney development and in organ culture models. *Kidney Int.* 2006;69:837–45.