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Minireview

Organic Anion Transporter 2: An Enigmatic Human Solute Carrier

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ABSTRACT

As a member of the solute carrier 22A (SLC22A) family, organic anion transporter 2 (OAT2; SLC22A7) is emerging as an important drug transporter because of its expression in both the liver and kidney, two major eliminating organs, and its ability to transport not only a wide variety of xenobiotics but also numerous physiologically important endogenous compounds, like creatinine and cGMP. However, OAT2 has received relatively little attention compared with other OATs and solute carriers (SLCs), like organic cation transporters, sodium-dependent taurocholate cotransporting polypeptide, multidrug and toxin extrusion proteins, and organic anion-transporting polypeptides. Overall, the literature describing OAT2 is rapidly evolving, with numerous publications contradicting each other regarding the transport mechanism, tissue distribution, and transport of creatinine and cGMP, two important endogenous OAT2 substrates. Despite its status as a liver and kidney SLC, tools for assessing its activity and inhibition are lacking, and its role in drug disposition and elimination remains to be defined. The current review focuses on the available and emerging literature describing OAT2. We envision that OAT2 will gain more prominence as its expression, substrate, and inhibitor profile is investigated further and compared with other SLCs.

Introduction

It has long been established that an important function of the kidney and liver is the secretion of organic anions into the urine and bile, respectively, and it has been recognized that such secretion is an active and saturable process that can be modulated by competitive inhibitors. Seven human OATs (i.e., OAT1, SLC22A6; OAT2, SLC22A7; OAT3, SLC22A8; OAT4, SLC22A11; OAT7, SLC22A9; OAT10, SLC22A13; and urate anion exchanger 1 (URAT1), SLC22A12) have been identified and characterized functionally (Burckhardt, 2012; Nigam, 2015; Nigam et al., 2015). These transporters are part of the organic ion (solute carrier, SLC) transporter superfamily (i.e., SLC22). What is unique about these OATs is their ability to mediate the exchange (extracellular against intracellular) of an impressive number of endogenous molecules, anionic drugs, metabolites, toxins, and signaling molecules with differing molecular composition, polarity, and charge. The second intriguing feature relates to their principal expression in renal proximal tubule cells (RPTCs) and hepatocytes, the major sites of active compound secretion in the body (Fig. 1).

OAT2 was actually the first mammalian OAT to be cloned. It was originally called a *novel liver-specific transporter* because of the dominant mRNA expression in the rat liver (Simonson et al., 1994). Later, novel liver-specific transporter OAT2 was found to be expressed in the liver and kidney at comparable levels and renamed OAT2 because of its close homology with OAT1 and its interaction with organic anions (Sekine et al., 1998). OAT1 was the next OAT identified, which was initially cloned from a mouse kidney cDNA library (Lopez-Nieto et al., 1997). It was described as novel kidney transporter because it was almost exclusively expressed in kidney. In subsequent work, the OAT1 orthologs from other species—including humans, rats, monkeys, pigs, and rabbits—were cloned. In 1999, cloning and characterization of rat OAT3 were reported (Kusuhara et al., 1999). The human OAT3 gene, named SLC22A8, was cloned in the same year (Race et al., 1999). Functional characterization of OAT3 from human and other species was soon followed. Other OATs in the family, OAT4 (Cha et al., 2000), OAT7 (Shin et al., 2007), OAT10 (Bahn et al., 2008), and URAT1 (Enomoto et al., 2002a), have been cloned from a human cDNA library. The gene expression, physiologic function, and substrate specificity of these transporters are just beginning to be investigated and characterized.

OAT1 and OAT3 have been considered the primary OATs mediating renal secretion of anionic compounds. They are expressed at the basolateral membrane of RPTCs. Subsequent to the initial discovery of rodent OAT1 and OAT3, orthologs were also identified in humans, monkeys, pigs, and rabbits and intensively characterized with respect to their tissue distribution, membrane localization, substrate specificity and functional characteristics. This knowledge has been summarized in several excellent reviews (Burckhardt, 2012; Emami Riedmaier et al., 2012; Koepsell, 2013; Wang and Sweet, 2013; Nigam et al., 2015). Over the past few years, OAT2 has emerged as the primary transporter for

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ABBREVIATIONS: aa, amino acid; DHEA, dehydroepiandrosterone; E3S, estrone-3sulfate; GFR, glomerular filtration rate; HEK, human embryonic kidney; IMC, indomethacin; K_i, inhibition constant; MATE, multidrug and toxin extrusion protein; MDCK, Madin-Darby canine kidney; MRP, multidrug resistance protein; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; PAH, *para*-aminohippuric acid; PCV, penciclovir; PROB, probenecid; RPTC, renal proximal tubule cell; SLC, solute carrier; SNP, single nucleotide polymorphism; TMD, transmembrane domain; URAT1, urate anion exchanger 1.

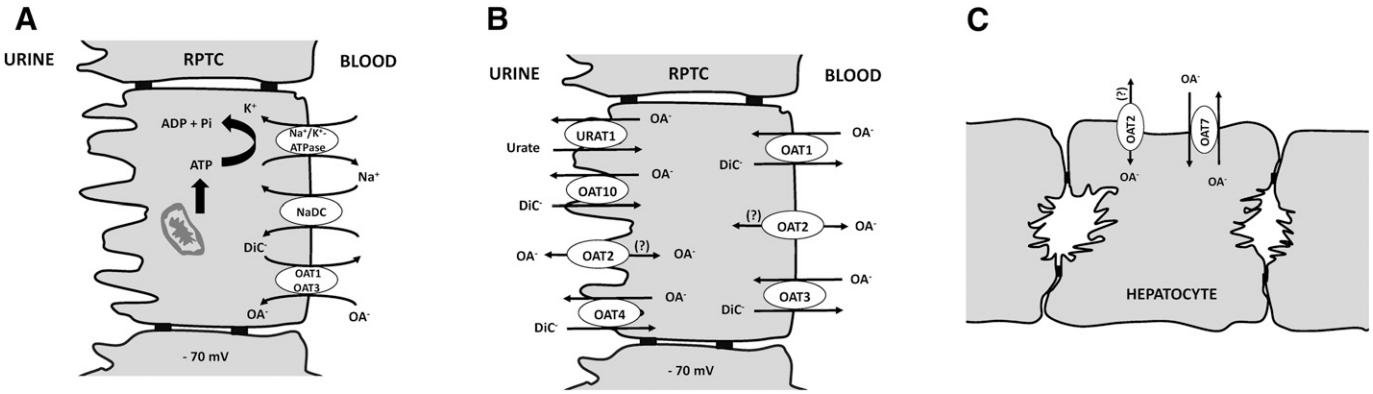

Fig. 1. Model of OAT1/OAT3 transport in a single epithelial cell of the kidney (A) and localization of OATs in RPTCs (B) and hepatocytes (C). Question marks indicate that the membrane localization of OAT2 in RPTCs and hepatocytes are unknown. NaDC, DiC^-, and OA^- represent sodium-dependent dicarboxylate transporter, dicarboxylate, and organic anion, respectively.

many endogenous and exogenous organic anions and cations, including a number of therapeutic drugs; however, conflicting data exist with regard to subcellular expression, substrate specificity, and driving force of OAT2. In addition, despite numerous publications, the physiologic, pharmacologic, and toxicologic importance of OAT2 remains ambiguous. In this review, with a focus on OAT2, we explore the implications of recent in vitro and in vivo data pertinent to OAT2 in comparison with OAT1 and OAT3.

Relatively Well Understood Aspects of OAT2

Splice Variant, Genomic, and Protein Structure. OAT2 shares many structural similarities with OATs including OAT1 and OAT3; however, there are also some differences to note as well. The human OAT proteins vary in size from 541 to 563 amino acids (aa) (Emami Riedmaier et al., 2012). Whereas the OAT1 and OAT3 genes encode 563 and 542 aa proteins, respectively, OAT2 proteins comprise 546, 548, and 539 aa, depending on splice variants (Table 1). OAT2 shares 37.3% identical aa with OAT1 and has 35.9% identity to OAT3. Overall, the most divergent region between OAT2, OAT1, and OAT3 is found on the C-terminal end. Whereas OAT2, OAT1, and OAT3 have high homology between species (about 80% aa identity in the rat, rabbit, human, and mouse), the homology between these three transporters for a given species is low (about 40%–50%) (Sun et al., 2001; Jacobsson et al., 2007).

Cropp et al. (2008) reported that OAT2 had two splice variants that differ in terms of two aa (OAT2-546aa, NM_006672; and OAT2-548aa, NM_153320) when cloning OAT2 gene from a human kidney cDNA library (Cropp et al., 2008). The difference between the two variants is a 6-bp insertion (TCCCAG) between exons 1 and 2 of the OAT2 gene. OAT2 detected in liver, kidney, and pancreas contained almost identical levels of the mRNA species of the two splice forms; however the protein localization of these two forms is different; OAT2-546 aa protein, detected by immunohistochemistry and immunoblotting studies, was localized to the plasma membrane, whereas OAT2-548aa protein was found in the intracellular compartment (Table 1). The former was able to transport cGMP, suggesting that it may be involved in cGMP signaling and play an important role in many physiologic processes, such as inflammation, angiogenesis, and cardiac hypertrophy. In contrast, OAT2-548aa exhibited a complete loss of transport function in terms of cellular uptake (Cropp et al., 2008; Hotchkiss et al., 2015). When expressed in three different cell lines [i.e., human embryonic kidney (HEK), Madin-Darby canine kidney (MDCK), and Chinese hamster ovary cells], OAT2-546aa was able to transport the guanosine analog antivirus drug penciclovir (PCV), which is a structurally similar molecule to cGMP. Furthermore, many previously identified substrates of OAT2-548aa, such as *para*-aminohippurate (PAH), estrone-3-sulfate (E3S), glutarate, succinate, paclitaxel, and dehydroepiandrosterone (DHEA) sulfate, were not transported by OAT2-546aa, suggesting different substrate specificity between the splice variants. The OAT2-546aa-mediated uptake of PCV

TABLE 1

Properties of human OAT1–3

Transporter	Gene Symbol	Alternative Name	Chromosomal Localization	Protein Length	mRNA Tissue Distribution	Protein Tissue Distribution	Membrane Localization	Transporter Mechanism	Major References
				aa					
OAT1	*SLC22A6*	NKT	11q12.3	563	Kidney > > brain, mammary gland, salivary gland, spleen, thymus, etc.	Kidney and choroid plexus	Basolateral	Organic anion-dicarboxylate exchanger	Burckhardt, 2012; Emami Riedmaier et al., 2012; Nigam et al., 2015
OAT2	*SLC22A7*	Novel liver-specific transporter	6p21.1	546, 548, 539	Liver > kidney > > brain, stomach, ileum, uterus, mammary gland, etc.	Liver and kidney	Basolateral and apical in the kidney and unknown in the liver	Unknown	Cropp et al., 2008; Burckhardt, 2012; Emami Riedmaier et al., 2012; Hotchkiss et al., 2015
OAT3	*SLC22A8*	ROCT	11q12.3	542	Kidney > > brain, spleen, uterus, etc.	Kidney, choroid plexus, and testes	Basolateral	Organic anion-dicarboxylate exchanger	Burckhardt, 2012; Emami Riedmaier et al., 2012; Nigam et al., 2015

was inhibited by various organic anions, cations, and neutral compounds with different molecular weights, suggesting broad ligand selectivity of OAT2-546aa.

Another variant OAT2-539aa was originally cloned from human kidney tissue by Enomoto and colleagues (Enomoto et al., 2002b) and assigned a GenBank accession number (AF210455) (Table 1). OAT2-539aa shares 97% aa sequence identity with OAT2-546aa and OAT2-548aa. This sequence differs greatly from OAT2-546aa and OAT2-548aa, primarily in the C-terminal end, which varies significantly among species (Hotchkiss et al., 2015). Human OAT2-539aa shares less homology to other species' Oat2 than OAT2-546AA, including rats, cattle, horses, rabbits, pigs, opossums, and chickens. Unfortunately, the OAT2-539aa sequence could not be found in the NHLBI Exome Sequencing Project Exome Variant Server database (http://evs.gs.washington.edu/EVS/). Moreover, Hotchkiss et al. (2015) failed to amplify cDNA of OAT-539aa from human kidney cDNA after several attempts (Hotchkiss et al., 2015). Taken together, the tissue expression of the OAT2-539aa splice variant requires confirmation. Overall, the prevalence of three splice variants in different ethnic groups, which may be critical to understand the physiologic, pharmacologic, and toxicologic importance of OAT2, is not known.

The gene SLC22A7 for human OAT2 is located on chromosome 6p21.1 (Kok et al., 2000) and that of murine Oat2 on chromosome 17qB3 (Table 1) (Kobayashi et al., 2002). OAT2 is not paired with any other gene from the SLC22 family and is phylogenetically distinct from OAT1 and OAT3 (Eraly et al., 2003; Cropp et al., 2008). Often, those transporter pair members are phylogenetically related and functionally similar. For example, OAT1 and OAT3 are a tightly linked pair with respect to genomic locus, and their functionalities appear to be similar, serving as broad-spectrum anion transporters with overlapping groups of substrates. In this regard, OAT2 is the most distantly related to OAT1, OAT3, and other human OATs (Sun et al., 2001; Sweet et al., 2001; Eraly et al., 2003).

Few reports have described the polymorphic nature of human OAT2. Xu et al. (2005) conducted analyses of coding region polymorphisms in OAT2 together with other OATs: OAT1, OAT3, OAT4, and URAT (Xu et al., 2005). Four OAT2 single nucleotide polymorphisms (SNPs) were identified. Of those, three were described as nonsynonymous. The synonymous OAT2 SNP (C12697) is commonly found in all ethnic groups. Later, Shin et al. (2010) added four new OAT2 SNPs, but no aa alteration was associated with the SNPs. In total, human OAT2 genomic sequences showed low variability. Although the authors found that there is a 10-fold variation in OAT2 protein expression in the liver specimens, genetic polymorphisms may not be a significant determinant to variation in the OAT2 expression and transport activity.

Since the crystal structures for mammalian OAT members are not available, structural information is still based mainly on hydropathical analysis of aa sequences. Similar to other OAT proteins, OAT2 contains 12 transmembrane domains (TMDs), both N and C termini localized intracellularly (OAT-548aa; Fig. 2). A large extracellular loop is found between TMD1 and TMD2 of OAT2, OAT1, and OAT3 proteins, carrying two to five N-glycosylation sites, which are important for targeting of transporter protein onto the plasma membrane (Tanaka et al., 2004). Cropp et al. (2008) reported that the addition of Ser and Gln, between Glu131 and Trp132 in the large extracellular loop 1 of OAT2-546aa, results in reduced stability and improper trafficking of OAT2-548aa. The OAT2-548aa–GFP-transfected cells exhibited much weaker fluorescent signals compared with those transfected with OAT2-546aa-GFP. The aa sequence near Glu131 of OAT2-546aa is highly conserved among members of the OAT subfamily, suggesting that this region may contain motifs that are critical to the stability and trafficking of OAT2. There are several conserved cysteine residues for the formation of disulfide bridges, which is important for stabilizing the tertiary structure. A second large loop located intracellularly between TMD6 and TMD7 carries consensus sites for phosphorylation by several protein kinases (two for protein kinase A (PKA) and two to six for protein kinase C (PKC)), which indicates that the transporters could be regulated by reversible phosphorylation (Srimaroeng et al., 2008).

Although the transport modes of OAT1 and OAT3 have been extensively studied and clarified over the past two decades, the transport

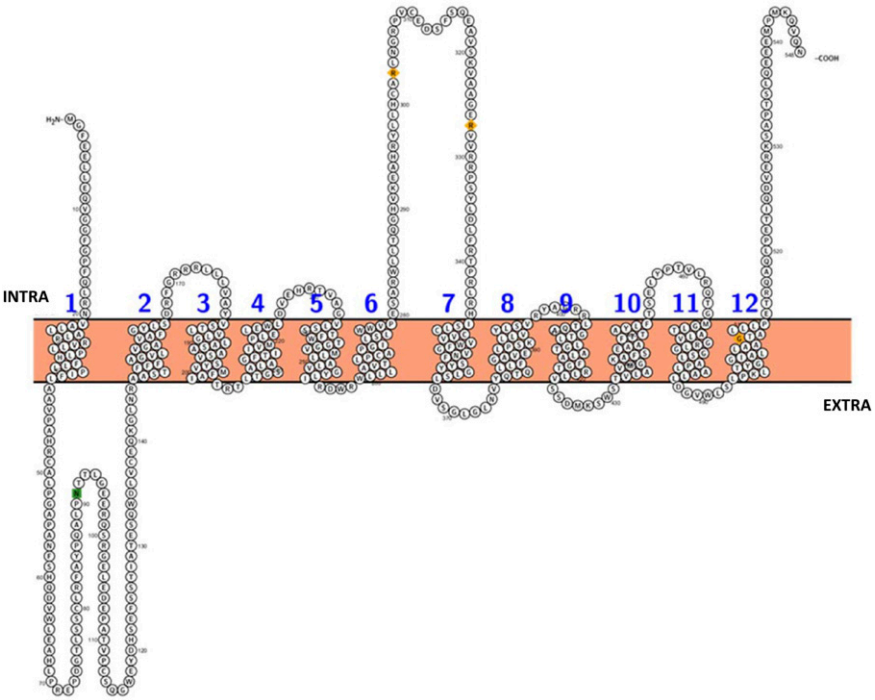

Fig. 2. The aa sequence and predicted membrane topology of human OAT2. The aa residues are designated by the single letter code. Topologic structure of OAT2-548aa was generated using Protter version 1.0 (Omasits et al., 2014). Hydropathic analysis shows 12 transmembrane segments with intracellular NH_2 and COOH termini. The large extracellular loop between transmembrane segments 1 and 2 contains one asparagine-X-serine/threonine (N-X-S/T) motif for potential N-linked glycosylation (N in green). Putative protein phosphorylation sites are located in the transmembrane segment 12 and large intracellular loop between transmembrane segments 6 and 7 (G and R in orange).

TABLE 2

In vitro substrates of human OAT2

Compound Type	Substrate	Cell System	Variant	K_m	OAT1 Substrate	OAT3 Substrate	Reference
				μM	K_m (μM)	K_m (μM)	
Therapeutics							
Antibiotics	Cefotaxime	HEK293 cells	OAT2-546aa		Unknown	Yes (549 and 717)	Yee et al., 2013
	Erythromycin	*X. laevis* oocytes	OAT2-548aa	18.5	Unknown	Unknown	Kobayashi et al., 2005b
	Tetracycline	Mouse S2 RPTCs	Unknown	440	Unknown	Yes (556)	Babu et al., 2002
Antigout and uricosuric agents	Allopurinol	*X. laevis* oocytes	OAT2-548aa		Unknown	Unknown	Kobayashi et al., 2005a
Antimetabolites	5-Fluorouracil	*X. laevis* oocytes	OAT2-548aa	0.0538	Unknown	Unknown	Kobayashi et al., 2005a
	Methotrexate	Mouse S2 RPTCs	Unknown		Yes (554 and 724)	Yes (10.9–70.6)	Sun et al., 2001
Antivirals	Acyclovir	HEK293 cells	OAT2-546aa	94	Yes (289–839)	Yes (416–772)	Cheng et al., 2012
	Ganciclovir	HEK293 cells	OAT2-546aa	264	Yes (896)	Unknown	Cheng et al., 2012
	PCV	HEK293 cells	OAT2-546aa	284	Unknown	Unknown	Cheng et al., 2012
Dietary Supplements and vitamins	Ascorbic acid (vitamin C)	*X. laevis* oocytes	OAT2-548aa		Unknown	Unknown	Kobayashi et al., 2005a
Diuretics	Bumetanide	*X. laevis* oocytes	OAT2-548aa	7.52	Yes	Yes (1.6)	Kobayashi et al., 2005a
H_2 receptor antagonists	Cimetidine	HEK293 cells	OAT2-546aa		Yes	Unknown	Tahara et al., 2005
	Ranitidine	HEK293 cells	OAT2-546aa	396	Yes	Unknown	Tahara et al., 2005
Herbal medications	Tanshinol	HEK293 cells	OAT2-546aa	859	Yes (121)	Yes (1,888)	Jia et al., 2015
Methylxanthines	Theophylline (1,3-DMX)	*X. laevis* oocytes	OAT2-548aa	12.6	Unknown	Unknown	Kobayashi et al., 2005b
	Xanthine	HEK293 cells	OAT2-546aa		Unknown	Unknown	Yamada et al., 2014
Nonsteroidal anti-inflammatory drugs	Diclofenac	HEK293 cells	OAT2-546aa	46.8	No	No	Zhang et al., 2016
	Diclofenac acyl glucuronide	HEK293 cells	OAT2-546aa	8.6	Yes (60.2)	Yes (114)	Zhang et al., 2016
	Salicylic acid	Mouse S2 RPTCs	OAT2-539aa		Yes	Yes	Igarashi et al., 2002
Nucleoside reverse transcriptase inhibitors	Zidovudine	Mouse S2 RPTCs	Unknown	26.8	Yes (45.9)	Yes (145)	Takeda et al., 2002
Taxanes	Paclitaxel	*X. laevis* oocytes	OAT2-548aa	0.143	Unknown	Unknown	Kobayashi et al., 2005a
Topoisomerase Inhibitors	Irinotecan	HEK293 cells	OAT2-546aa	42.4	Unknown	Unknown	Marada et al., 2015
Endogenous compounds							
Androgens	DHEA sulfate	*X. laevis* oocytes	OAT2-548aa		Unknown	Unknown	Kobayashi et al., 2005a
Estrogens	E3S	*X. laevis* oocytes	OAT2-548aa		Unknown	Unknown	Kobayashi et al., 2005a
None	1-Methyl-4-phenylpyridinium	HEK293 cells	OAT2-546aa		Unknown	Unknown	Shen et al., 2015
	cGMP	HEK293 cells	OAT2-546aa	101	Yes	Yes	Marada et al., 2015
	cGMP	HEK293 cells	OAT2-546aa	88			Cropp et al., 2008
	cGMP	HEK293 cells	OAT2-546aa				Henjakovic et al., 2015
	Creatinine	HEK293 cells	OAT2-546aa	795	Unknown	Yes	Shen et al., 2015
	Creatinine	HEK293 cells	OAT2-546aa	986			Lepist et al., 2014
	Orotic acid	HEK293 cells	OAT2-546aa	200 (rOat2)	Unknown	Unknown	Fork et al., 2011
	PAH	Other cells	Unknown		Yes (3.1–60.3)	Yes (7.4 and 87.2)	Sun et al., 2001
	Uric acid	HEK293 cells	OAT2-546aa		Unknown	Unknown	Henjakovic et al., 2015
Prostaglandins	Prostaglandin E_2	*X. laevis* oocytes	OAT2-548aa		Unknown	Unknown	Kobayashi et al., 2005a
	Prostaglandin F_2	Mouse S2 RPTCs	OAT2-539aa	0.425	Unknown	Unknown	Enomoto et al., 2002b
	Prostaglandin F_2	HEK293 cells	OAT2-546aa	45.5	Unknown	Unknown	Jia et al., 2015
	Prostaglandin F_2	Other cells	unknown		Unknown	Unknown	Nakagomi-Hagihara et al., 2007

mechanism of OAT2 is not yet fully understood. Located on the basolateral membrane of RPTCs, OAT1 and OAT3 function as tertiary active transporters and operate as organic anion-dicarboxylate exchangers (Fig. 1A; Table 1). These cotransported dicarboxylates then drive the exchange of dicarboxylates with anions into the cell via OAT1 and OAT3 because the gradient favors outward movement of dicarboxylates, such as α-ketoglutarate, to the peritubular capillary (Sekine et al., 1997). As a preferred physiologic counter ion of OAT1 and OAT3, the plasma concentration of α-ketoglutarate in humans is in the 8–12 μM range (Wagner et al., 2010; Halámková et al., 2012), whereas the intracellular concentration of α-ketoglutarate in rat RPTCs is much higher (approximately 265 μM). The gradient of α-ketoglutarate provides a driving force for taking up anions from blood (Pritchard, 1995; Sweet et al., 1997). Once inside the cell, organic ions may undergo export as intact molecules via the brush-border membrane transporters OAT4, OAT2, and URAT1) (Miyazaki et al., 2005; Shen et al., 2015). These transporters work in concert to mediate the renal secretion of anionic endogenous solutes and xenobiotics, although some anionic compounds that are taken up by these SLCs could be metabolized by intracellular enzymes.

Assumed to be an organic anion-dicarboxylate exchanger similar to OAT1 and OAT3, the interaction of OAT2 with α-ketoglutarate and other dicarboxylates as a potential physiologic counter ion of OAT2 have been investigated in a few studies. The conflicting results have been published with respect to the driving force for OAT2. α-Ketoglutarate, a dicarboxylate of five carbons, was initially identified as a substrate of human OAT2 expressed in EcR 293 cells that are derived from HEK293 cells (Sun et al., 2001); however, the transport of PAH by OAT2 was not blocked by α-ketoglutarate, in concert with the finding that α-ketoglutarate does not trans-stimulate OAT2-mediated E3S and

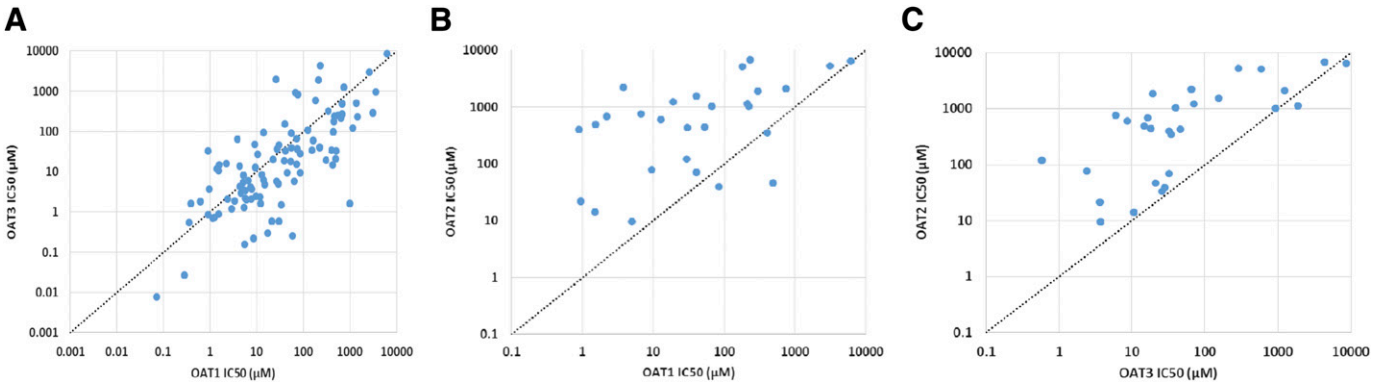

Fig. 3. Comparison of IC_{50} values of dual inhibitors of human OAT1 and OAT3 (A), OAT1 and OAT2 (B), and OAT2 and OAT3 (C), respectively. The diagonal is the line of identity. The IC_{50} data are collected from the University of Washington Metabolism and Transporter Drug Interaction Database (DIDB) and listed in Supplemental Table 1. To test for statistically significant differences among different transporters for IC_{50}, one-way analysis of variance was performed. When the F ratio showed that there were significant differences among groups, the Turkey's post-test was used to determine which groups differ.

cGMP uptake (Henjakovic et al., 2015). Consistently, although rat Oat2 mediates the uptake of α-ketoglutarate (Sekine et al., 1998), 1 mM α-ketoglutarate does not inhibit the uptake of salicylate mediated by rat Oat2 (Morita et al., 2001). These results suggest that OAT2 transport is unlikely to occur via exchange with α-ketoglutarate. On the other hand, 5 mM fumarate and succinate (4-carbon dicarboxylates), but not glutarate (five-carbon dicarboxylates), trans-stimulate OAT2-mediated uptake of E3S (Kobayashi et al., 2005a), suggesting that OAT2 may be an organic anion-dicarboxylate exchanger with preference for four-carbon dicarboxylates. Sato et al. (2010) failed in their attempt to repeat the trans-stimulation of OAT2-mediated urate uptake in HEK293 cells preloaded with succinate up to 100 mM (Sato et al., 2010). In addition, Hotchkiss et al. (2015) investigated the cis-inhibitory effect of various Krebs cycle intermediates, including fumarate, succinate, and α-ketoglutarate, on OAT2 transport; however, none of those Krebs cycle intermediates inhibited OAT2-mediated PCV uptake (Hotchkiss et al., 2015). They also showed that, indeed, succinate is not a substrate for OAT2. Likewise, Henjakovic et al. (2015) did not observe cis-inhibition of OAT2-mediated cGMP uptake by succinate and other dicarboxylates, whereas in the same study, four of six dicarboxylates (i.e., glutarate, adipate, pimelate, and suberate) were shown to inhibit OAT1- and OAT3-mediated transport. Moreover, they showed that none of the seven short-chain monocarboxylates inhibits OAT2-mediated cGMP transport. Given the high intracellular concentration of glutamate (2–20 mM) (Newsholme et al., 2003) and the outward direction of the glutamate gradient (Fork et al., 2011), glutamate may be the major physiologic substrate that enables OAT2-mediated translocation. Although human OAT2 has been shown to transport glutamate, cGMP-glutamate exchange by OAT2 has not been demonstrated (Henjakovic et al., 2015). Furthermore, whereas OAT1 and OAT3 exhibit pH- and chloride-dependence (higher transport at acidic pH and lower transport in the absence of chloride), no pH- and chloride-dependent transport has been observed with OAT2 (Henjakovic et al., 2015). Moreover, OAT2-mediated cGMP uptake is not affected by depolarization of membrane potential by high potassium. Taken together, the counter ion for OAT2 transport remains to be identified.

Potential Role of OAT2 in Drug Disposition. Like OAT1 and OAT3, OAT2 can accommodate a variety of organic anions and indeed has also been shown to transport a number of pharmacologically active agents. OAT2 has been increasingly recognized in terms of its role in drug disposition (Table 2). Well-characterized drug substrates of OAT2 include numerous antibiotics (cefotaxime, erythromycin, and tetracycline) (Babu et al., 2002; Kobayashi et al., 2005b; Yee et al., 2013), antivirals (acyclovir, PCV, and ganciclovir) (Cheng et al., 2012), antimetabolites (5-fluorouracil and methotrexate) (Sun et al., 2001; Kobayashi et al., 2005a), H-2 receptor antagonists (cimetidine and ranitidine) (Tahara et al., 2005), diuretics (bumetanide) (Kobayashi et al., 2005a), nonsteroidal anti-inflammatory drugs (e.g., diclofenac) (Zhang et al., 2016), topoisomerase inhibitor (irinotecan) (Marada et al., 2015), and endogenous prostaglandins and hormones (e.g., prostaglandin E_2, prostaglandin F_2, DHEA sulfate, and E3S) (Enomoto et al., 2002b; Kobayashi et al., 2005a, 2014; Jia et al., 2015) (Table 2). OAT2 has also been identified as a candidate diclofenac β-D-glucuronide transporter (Zhang et al., 2016). Similarly, theophylline has been identified as an OAT2 substrate. The apparent K_m value for the OAT2-mediated transport of theophylline was reported as 12.6 μM, and OAT2-mediated theophylline uptake is inhibited by erythromycin, suggesting that hepatic OAT2 may serve as the locus of the well described theophylline-erythromycin interaction (Table 2). Furthermore, it has been reported that OAT2 can transport a large variety of purine and pyrimidine nucleobases, nucleosides, and nucleotides, including cGMP (Cropp et al., 2008). Many substrates of OAT2 are also substrates of OAT1 and/or OAT3 (Table 2). It is worth noting that three OAT2 slice variants (OAT2-546aa, OAT-548aa, and OAT2-539aa), with different transport specificity, have been used by different laboratories, which has led to contradictory results.

A great number of drugs that inhibit transport by OAT1, OAT2, and OAT3 have been identified, and the IC_{50} or inhibition constant (K_i) determined (Burckhardt, 2012; Nigam et al., 2015). Although OAT2 has distinct inhibitor specificity compared with OAT1 and OAT3, they share overlapping specificities for numerous inhibitors (Fig. 3) (Supplemental Table 1). The basolateral OAT1 and OAT3 transporters generally share comparable affinities with various inhibitors ($P > 0.05$), whereas the inhibition potential of these inhibitors against OAT2 is significantly lower (Fig. 3, B and C) ($P < 0.0001$) (Supplemental Table 1). For example, probenecid (PROB) and ketoprofen are less potent inhibitors of human OAT2 compared with OAT1 and OAT3 (IC_{50} values of 393 μM vs. 4.57 and 10.9 μM and 400 μM vs. 4.34 and 5.98 μM, respectively) (Khamdang et al., 2002; Jia et al., 2015); however, indomethacin (IMC) exhibited similar inhibitory potency against OAT2 (IC_{50} values 2.1–6.5 μM) (Shen et al., 2015; Zhang et al., 2015), OAT1 (IC_{50} values 3–10 μM), and OAT3 (IC_{50} values 0.61–5.95 μM) (Khamdang et al., 2002). We envision that inhibitors with marked differences in inhibition potency might be used to determine the relative contributions of each OAT to organic anion uptake and support transporter phenotyping using isolated primary RTPCs.

Role of OAT2 in the Transport of Endogenous Compounds. OAT2 facilitates the transport of various endogenous compounds across biologic membranes in the liver and kidney and thus regulates the intracellular concentrations of transported substances. Cyclic nucleotides cAMP and cGMP are OAT2 substrates (Sun et al., 2001). Human OAT2 transports endogenous nucleobases, nucleosides, and nucleotides such as adenine, cytidine, adenosine, inosine, and guanidine, but not thymine, thymidine, and cytosine. It also transports GMP, GDP, and GTP (Cropp et al., 2008). OAT2 also mediates the low K_m (K_m of 0.71 μM) transport of prostaglandin E2 (Kimura et al., 2002). Conflicting results have been published in OAT2 mediated transport of sulfated steroid hormones. For example, Sun et al. (2001) reported DHEA sulfate is not an OAT2 substrate (Sun et al., 2001), whereas Kobayashi et al. (2005b) showed that OAT2 does transport the compound (Kobayashi et al., 2005b) (Table 2).

cGMP plays a critical role in many physiologic processes involved in mediating cellular response to various stimuli and is a key regulator of biologic processes, including cell proliferation, differentiation, and apoptosis, through the activation of cGMP-dependent protein kinase signaling pathway (Sager, 2004). Several multidrug resistance proteins, such as MRP4, MRP5, and MRP8, have been identified as efflux pumps mediating the extrusion of cGMP from cells (Sager, 2004). On the other hand, OAT2 has been identified as a highly efficient, bidirectional facilitative transporter that can regulate both intracellular and extracellular levels of cGMP and may be involved in cGMP signaling pathways for gene regulation (Cropp et al., 2008); however, how OAT2 inhibition modulates cGMP intracellular concentrations has not been studied in great detail.

Creatinine is actively secreted into the urine from blood, and recent investigations have shown that the efficiency of creatinine uptake by OAT2 is greater than that of other renal transporters, such as organic cation transporter (OCT)2, multidrug and toxin extrusion protein (MATE)1, and MATE2-K (Lepist et al., 2014; Shen et al., 2015). A more complete overview of the role of OAT2 in creatinine elimination is presented in the following section.

Enigmatic Aspects of OAT2

Despite the fact that singularly transfected OAT2 mediates the active transport of numerous xenobiotics and endobiotics, its expression profile in human tissues has received relatively little attention compared with other SLCs. OAT2 scaling factors (relative activity and relative expression) have not been developed, and there are virtually no reports of in vitro-in vivo extrapolation exercises involving the transporter.

Underinvestigated Liver Solute Carrier

Uncertain OAT2 Expression in the Liver. A high level of OAT2 mRNA expression is observed in the liver versus other tissues, such as testis, choroid plexus, stomach, ileum, and uterus in humans (Fork et al., 2011). The higher level of OAT2 mRNA expression in the liver than in the kidney was observed in humans and rats by others (Sekine et al., 1998; Sun et al., 2001; Hilgendorf et al., 2007) (Table 1); however, surprisingly, Oat2 mRNA expression was reported at the highest level in the kidney rather than in the liver in rats by another group (Buist et al., 2002). Regarding the expression level of OAT2 relative to other SLC transporters in the liver, in fact, OAT2 mRNA levels in the liver are comparable to those of organic anion-transporting polypeptide (OATP)1B1, OATP1B3, OATP2B1, sodium-dependent taurocholate cotransporting polypeptide (NTCP), and OCT1. Ohtsuki et al. (2012) simultaneously quantified the protein amount and the mRNA expression and activity of 19 drug-metabolizing enzymes and 22 drug transporters in 17 human liver samples. They compared the protein expression levels with corresponding mRNA expression levels and activities and found that the OAT2 protein expression did not correlate with the corresponding mRNA expression. The differences between mRNA and protein expression are thought to be due to post-transcriptional modification, intracellular trafficking, and membrane sorting. As a result, mRNA expression may not serve as a surrogate for transporter function and actually may be rather misleading since transmembrane proteins are partly localized to the membrane of intracellular organelles. Vildhede et al. (2015) quantified various SLCs and compared the membrane proteomic signature of freshly isolated hepatocytes to that of human liver tissue. The expression of OAT2 protein was 30- to 100-fold lower than that of other hepatic uptake transporters, such as OATP1B1, OATP1B3, and OCT1. The low expression of OAT2 may contribute to its low activity in human liver described in the following section. Nakamura et al. (2016) used an even larger-scale proteomics approach and determined that the hepatic expression of OAT2 was more comparable to that of the OATPs (~1–2 fmol/μg of microsomal protein) (Prasad et al., 2016).

In regard to OAT2 liver tissue distribution, the expression of rat Oat2 mRNA is homogeneous across periportal and pericentral regions (Fork et al., 2011). Rat Oat2 protein has been specifically localized to the basolateral (sinusoidal) membrane of hepatocytes (Simonson et al., 1994), like other hepatic uptake transporters, such as OATP1B1, OATP1B3, and OCT1. Species-dependent localization of OAT2 in the liver is possible, however. Surprisingly, although it is assumed that human OAT2 is localized in the sinusoidal membrane of hepatocytes, the immunohistochemistry of OAT2 protein in human liver, which is the main organ of its expression, has not been demonstrated.

Limited Evidence for OAT2 Function in Liver. Limited information is available regarding the role of OAT2 in the uptake of compounds into hepatocytes and drug disposition as a result of the lack of experimental tools and reagents for studying OAT2. Specific substrates for human OAT2 that are not transported by other hepatic uptake transporters likely have not yet been identified. A compound that can selectively inhibit the basolateral influx mediated by OAT2 without blocking the uptake processes mediated by OCT1 and OATPs may be important with respect to validating the function of OAT2 in primary hepatocytes. OAT2 expressed in *Xenopus* oocytes and HEK293 cells is known to mediate transport of E3S (Kobayashi et al., 2005a, 2014), but E3S, an organic anion, is also a substrate for OATP1B1 and OATP2B1. Several early studies showed that PAH is a human OAT2 substrate, but Cropp et al. (2008) observed only appreciable uptake of PAH by HEK293 cells stably transfected with OAT2 compared with the control cells. Also, Hotchkiss et al. (2015) failed to observe the transport of PAH by HEK-OAT2 and CHO-OAT2 cells, although the uptake of PAH was approximately 2-fold higher in MDCK-OAT2 cells compared with parental cells. Because PROB is known to be a potent inhibitor of OAT1 and OAT3 with IC_{50} values ranging from 3 to 28 μM (Chu et al., 2007; Juhász et al., 2013; Shen et al., 2013), Enomoto et al. (2002b) examined its inhibitory effect on PGF2α uptake by mouse second segment (S2) proximal tubule cells stably expressing OAT2 (S2-OAT2). PROB inhibited the OAT2-mediated uptake in concentration-dependent manner with an IC_{50} value of 393–766 μM, which is almost equivalent to that against OATP1B1 (IC_{50} of 79.4–740 μM) (Izumi et al., 2013; Jia et al., 2015). IMC is a potent inhibitor of OAT2-mediated creatinine-d3 uptake with an apparent IC_{50} of 2.1 $\pm$ 0.4 μM (Shen et al., 2015). Although IMC had no effect on MATE1 and OCT1 (Badolo et al., 2010; Shen et al., 2015), 20 μM IMC reduced OATP1B1-mediated estradiol-17-β-glucuronide uptake by 88.6% (Karlgren et al., 2012). After careful review of the existing literature, it is apparent that tools for

assessing OAT2 inhibition and activity in human primary hepatocytes are lacking.

Conflicting Literature Describing Renal OAT2

Expression and Localization of OAT2 in Kidney. To date, few reports have described the localization of OAT2 protein in human renal tissue; however, OAT2 is unique in having different expression patterns in the kidney compared with OAT1 and OAT3, in addition to exhibiting species and sex differences in the expression.

Enomoto et al. (2002b) developed a custom antibody against the 14 aa of the carboxyl terminus of human OAT2 in rabbits and showed that human OAT2 was expressed at the basolateral membrane of RPTCs. Cheng et al. (2012) also localized OAT2 protein to the basolateral membrane of RPTCs in the human renal cortex; however, OAT2 has been recently reported to show a different expression pattern: whereas OAT1 and OAT3 are expressed similarly along the basolateral membrane of RPTCs, the expression of OAT2 is weaker at the basolateral membrane. In fact, OAT2 immunoactivity is more diffuse across the cytoplasm and even the luminal membrane of renal tubule epithelial cells (Ikarashi et al., 2013). The localization of OAT2 limited to the basolateral membrane of RPTCs has been further challenged by the results of a recent study showing that the transporter is located at the basolateral, cytoplasmic and apical side of RPTCs of both humans and monkeys. In contrast, rat Oat2 is localized at the apical membrane RPTCs only (Shen et al., 2015). The expression of rodent Oat2 at the luminal membrane in RPTCs, or even the connecting duct, has been reported previously by a few laboratories (Kojima et al., 2002; Ljubojević et al., 2007; Zlender et al., 2009). These results suggest species difference in localization of human, monkey, and rodent OAT2. The species-dependent cellular localization of OAT2 in RPTCs may be related to its driving force(s), which has not been fully understood, as described previously. Although the simultaneous presence of a transporter at both the apical and basolateral membranes of cells is not uncommon (Farrell et al., 1992; Harris et al., 1992; Gu et al., 2001; Godoy et al., 2014), the exact physiologic role and function of OAT2 apically and basolaterally expressed in the tubule cells remain to be elucidated.

OAT2 expression appears to be sex-dependent, at least in the rat and mouse. In adult male Sprague-Dawley rats, significantly higher Oat2 levels were observed in the liver compared with levels in the kidney. In contrast, Oat2 mRNA expression is kidney-predominant as considerably higher Oat2 levels were seen in the female kidney as compared with the liver (Buist et al., 2002); however, no sex difference in hepatic Oat2 mRNA expression has been reported for the rat (Buist et al., 2002). The female growth hormone secretion pattern is likely responsible for the Oat2 mRNA gender difference in the kidney as gonadectomy decreased renal Oat2 expression and growth hormone increased it in female rats (Buist et al., 2003). The gender differences and sex hormone regulation of Oat2 have been also observed at the protein level, as female rats exhibit more immunoreactive protein in the brush-border membrane of the proximal tubule S3 segment compared with levels in male rats. (Ljubojević et al., 2007). Female rats exhibited more immunoreactive protein in the brush-border membrane of the proximal tubule S3 segment compared with male rats. Expression of oat2 protein in castrated male rats exhibits an approximately 6-fold increase compared with sham-operated male rats, whereas the protein was decreased by 28% after ovariectomy in female rats. Testosterone treatment caused a remarkable decrease of Oat2 protein in castrated male rats to the levels observed in sham-operated rats, whereas estradiol and progesterone treatment caused a limited additional upregulation of the protein (Ljubojević et al., 2007). In mice, the expression pattern largely resembles that of rats (Buist and Klaassen, 2004; Ljubojević et al., 2007; Cheng et al., 2008). Because species differences may exist, more information is needed regarding sex differences in human kidney OAT2 expression. Unfortunately, the recent studies of Prasad et al. (2016) and Nakamura et al. (2016) did not include a proteomic analysis of human male versus female kidney cortex membrane preparations; however, both groups did conclude that OAT2 protein is present and expressed at lower levels versus OAT1, OAT3, and OCT2: 5.3 (OAT1), 0.9 (OAT2), 3.5 (OAT3), and 7.4 (OCT2) pmol/mg membrane of protein (mean of $n = 41$ different organ donors) (Prasad et al., 2016); and 10 (OAT1), 1.5 (OAT2), 5 (OAT3), and 5 (OCT2) pmol/mg membrane protein (pool of kidney samples) (Nakamura et al., 2016).

Role of OAT2 in Creatinine Renal Clearance. It has been a common practice to use glomerular filtration rate (GFR) as an index of kidney function, and the measurement of creatinine concentration in the plasma or serum is most widely used to estimate GFR (eGFR). Although creatinine, an organic cation with a small molecular weight, is freely filtered through the renal glomerulus, active secretion accounts for 10%–40% of total creatinine output in urine of human subjects (Levey et al., 1988). In RPTCs, the tubular secretion of creatinine from blood into urine is an active process and is thought to be mediated (vectorially) by basolateral OCT2 and apical MATEs (Lepist et al., 2014; Shen et al., 2015). In fact, drugs that are inhibitors of renal OCTs (i.e., OCT2/MATEs), including cimetidine, dolutegravir, pyrimethamine, trimethoprim, and vandetanib, inhibit creatinine secretion, resulting in increased creatinine concentrations in the blood, without causing kidney injury. Recently, Chu et al. (2016) collected OCT2, MATE1, and MATE2-K inhibition data for 16 drugs and conducted in vitro-in vivo extrapolation analysis by comparing total (C_{max}) and unbound maximum plasma concentrations with in vitro IC_{50} values. Because the relative contribution of individual transporters to the renal active tubular secretion of creatinine is unknown, OCT2, MATE1, and MATE2-K were assumed to contribute equally to creatinine transport. As a result, the lowest IC_{50} for OCT2, MATE1, and MATE2-K was used in the risk assessment exercise as the worst-case scenario. The use of an unbound C_{max}/IC_{50} ratio ($\geq$0.1), recommended by the Food and Drug Administration and International Transporter Consortium, resulted in four false-negative predictions (dronedarone, cobicistat, rilpivirine, and telaprevir) (Chu et al., 2016). The exact reason(s) for the underextrapolation for these four drugs is unclear. The inhibition of other transporters involved in the renal elimination of creatinine by the four drugs may contribute to their underprediction. In contrast, using C_{max}/IC_{50} ratios ($\geq$0.1), the authors were able to make a good prediction for creatinine increase for the set of 16 compounds.

OAT2 can also transport creatinine, although reports have been rather contradictory. For example, using S2-OAT2 cells, the OAT2-mediated transport of creatinine was low, and the authors concluded that creatinine is not an OAT2 substrate (Imamura et al., 2011). In contrast, Ciarimboli et al. (2012) used OAT2 expressed in HEK293 cells and showed that creatinine uptake was increased 2.3-fold (vs. mock HEK293 cells). Therefore, they concluded that OAT2 may play a role in creatinine clearance, although OCT2 plays a major role in creatinine tubular secretion. Similarly, Lepist et al. (2014) reported that creatinine was a high Km (K_m = 986 μM) OAT2 substrate in MDCK cells with the highest maximal velocity (V_{max}=117 $\times$ 10^4 pmol/min per mRNA copy number) versus OCT2 and OCT3. The transport rate at a physiologic creatinine concentration (100 μM) for OAT2 was 4.7- and 2.3-fold higher than that estimated for OCT2 and OCT3, respectively. Recently, we confirmed the findings of Lepist et al. and showed that OAT2 is a more efficient creatinine transporter compared with OCT2, MATE1, and MATE2-K (Shen et al., 2015); the creatinine transport efficiency by OAT2, the intrinsic transport clearance when normalized to the transporter protein, was significantly greater than that of OCT2 and MATEs (37- to 1850-fold). Moreover, potent OAT2 inhibitors (i.e., 50 μM BSP and 100 μM IMC) significantly inhibited (~50%) the intracellular

uptake and transepithelial transport of creatinine by human RPTCs. These data suggest that OAT2 may play an important role in creatinine renal excretion. IMC was demonstrated to be a potent inhibitor for OAT2 over other known creatinine transporters OCT2, MATE1, and MATE2-K (IC_{50} values of 2.1 μM for OAT2 vs. $>$ 200 μM for OCT2, MATE1, or MATE2-K) (Shen et al., 2015). Notably, IMC decreased creatinine tubular secretion from blood to urine, which was accompanied by an increase in creatinine plasma concentration in various clinical studies (Berg and Talseth, 1985; Al-Waili, 2002). In addition, the inhibitory effects disappeared after the cessation of IMC therapy in neonates (Kang et al., 1999; Walker et al., 2011). IMC is an inhibitor of prostaglandin synthesis; however, the transient effects might be caused by other mechanism(s) in addition to renal transporter inhibition. At least one report states that IMC does not affect creatinine renal secretion clearance in humans, although the results of the study may be confounded by the consumption of a high-protein meal (Levey et al., 1988). The inhibition of renal OAT2 may help explain the false-negative in vitro-in vivo extrapolations using OCT2 and MATE inhibition data for dronedarone, cobicistat, rilpivirine, and telaprevir (Chu et al., 2016). Unfortunately, the in vitro OAT2 inhibition data for dronedarone, rilpivirine, and telaprevir are currently not available; but cobicistat showed weak inhibition of OAT2 compared with MATEs and/or OCT2. Taken together, additional clinical investigations regarding the inhibition of creatinine clearance by other OAT2 inhibitors are needed.

Conclusions and Outlook

Although the expression of OAT2 has been confirmed in the liver, kidney, and many other tissues, its role in the disposition of drugs and endogenous molecules, species and gender differences, and genotype-phenotype associations requires further study. Such a lack of knowledge is best typified by the conflicting results obtained with creatinine in vitro and the conclusions of various investigators after attempting to localize the SLC within RTPCs. Unfortunately, the lack of selective OAT2 substrates and inhibitors, the paucity of freshly prepared human primary RPTCs, and limited information about the genetic polymorphisms of the human OAT2 gene and their relationship to OAT2 function have hindered progress to date. On the other hand, there is a consensus that OAT2 can mediate cGMP transport, thus potentially regulating the intracellular and extracellular levels of the important signaling cyclic nucleotide. How OAT2 functions coordinately with various multidrug resistance proteins (e.g., MRP4, MRP5, and MRP8) and the impact of OAT2 inhibition on cGMP signaling is not known. Despite the lack of knowledge, the fact that the in vitro OAT2-mediated transport efficiencies of certain drugs (i.e., acyclovir and ganciclovir) and important endogenous substrates (i.e., cGMP and creatinine) are relatively high, in addition to OAT2 expression in both liver and kidney, provides additional impetus for continued study of this enigmatic SLC. Such issues, in addition to efforts to sort out the roles of other uncharacterized OAT transporters, redundantly expressed on the plasma membrane of RPTCs and hepatocytes, are challenges for the future.

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Authorship Contributions

Performed data analysis: Shen, Lai, Rodrigues.

Wrote or contributed to the writing of the manuscript: Shen, Lai, Rodrigues.

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Supplementary Table

Organic Anion Transporter 2 (OAT2): An Enigmatic Human Solute Carrier

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Supplementary Table 1

Comparison of inhibition potency of various compounds on OTA1, OAT2 and OAT3. The IC_{50} geometric means of 102 compounds were obtained from University of Washington Metabolism and Transport Drug Interaction Database (Empty cells in IC_{50} field mean no data; PAH, p-aminohippuric acid; E3S, estrone-3-sulfate).

Inhibitor	OAT1		OAT2		OAT3	
	IC_{50} (μM)	Number of studies (probe substrates used)	IC_{50} (μM)	Number of studies (probe substrates used)	IC_{50} (μM)	Number of studies (probe substrates used)
(R)-flurbiprofen	2.35	1 (PAH)			2.13	1 (E3S)
(R)-ibuprofen	6.14	1 (PAH)			2.04	1 (E3S)
(R)-naproxen	5.26	1 (PAH)			8.09	1 (E3S)
(S)-flurbiprofen	0.62	1 (PAH)			1.8	1 (E3S)
(S)-ibuprofen	2.84	1 (PAH)			1.2	1 (E3S)
acetazolamide	75	2 (PAH)			816	1 (E3S)
acetylsalicylic acid	428	3 (PAH)			15	2 (E3S and cilostazol)
AK106-001616	33.6	1 (methotrexate)			1.49	1 (methotrexate)
aminopterin	160	1 (PAH)			59.2	1 (E3S)
baicalein	11.8	1 (PAH)			2.4	1 (E3S)
betamipron	9.09	5 (PAH, cidofovir, and adefovir)			48.3	1 (E3S)
bumetanide	9.51	2 (PAH)	77.5	2 (prostaglandin F2 and tetracycline)	2.42	2 (E3S)
cabozantinib 6-desmethyl amide cleavage product sulfate (EXEL-1644)	4.3	1 (PAH)			4.3	1 (PAH)
caffeic acid	9.31	2 (PAH)			12.9	2 (E3S and 5-carboxyfluorescein)
candesartan	17	9 (uric acid)			0.3	1 (uric acid)

cefaclor	1150	2 (PAH)			120.2	1 (E3S)
cefadroxil	6140	1 (PAH)	6410	1 (prostaglandin F2)	8620	1 (E3S)
cefamandole	30	1 (PAH)	430	1 (prostaglandin F2)	46	1 (E3S)
cefazolin	180	1 (PAH)	5090	1 (prostaglandin F2)	598	2 (E3S and pemetrexed)
cefdinir	692	1 (PAH)			272	1 (E3S)
cefoperazone	210	1 (PAH)	1140	1 (prostaglandin F2)	1890	1 (E3S)
cefoselis	2601	1 (PAH)			2925	1 (E3S)
cefotaxime	3130	1 (PAH)	5210	1 (prostaglandin F2)	290	1 (E3S)
cefotiam	640	1 (PAH)			213	1 (E3S)
ceftibuten	563	1 (PAH)			247	1 (E3S)
ceftizoxime	3599	1 (PAH)			957	1 (E3S)
ceftriaxone	230	3 (PAH)	6760	1 (prostaglandin F2)	4390	1 (E3S)
cephaloridine	740	2 (PAH and 6-carboxyfluorescein)	2090	1 (prostaglandin F2)	1241	1 (E3S)
cephalothin	220	2 (PAH and cidofovir)	1040	1 (prostaglandin F2)	40	1 (E3S)
chlorambucil	44.3	1 (PAH)			9.5	1 (E3S)
chlorothiazide	3.78	1 (PAH)	2205	1 (prostaglandin F2)	65.3	1 (E3S)
cilastatin	1470	1 (PAH)			231	1 (E3S)
cimetidine	492	1 (PAH)	46.4	3 (creatinine and cGMP)	21.2	6 (E3S, sitagliptin, and zonampanel))
CP-778875	57.8	1 (PAH)			0.26	1 (E3S)
cyclothiazide	84.3	1 (PAH)	39.2	1 (prostaglandin F2)	27.9	1 (E3S)
deoxycholic acid	85.4	2 (PAH)			9.57	1 (E3S)
diclofenac	1.52	4 (PAH,chlorothiazide, and adefovir)	14.3	1 (prostaglandin F2)	10.6	5 (E3S, pemetrexed, chlorothiazide, methotrexate, and penicillin G)
diflunisal	1.13	2 (PAH and cidofovir)			0.72	1 (6-carboxyfluorescein)
DX-619	473	2 (PAH and E3S)			239	1 (6-beta-hydroxycortisol)
ethacrynic acid	29.6	1 (PAH)	121	1 (prostaglandin F2)	0.58	2 (E3S and 6-carboxyfluorescein)

etodolac	71.8	2 (PAH and cidofovir)			65.7	2 (methotrexate and penicillin G)
fenofibric acid	5.77	2 (PAH)			2.2	1 (sitagliptin)
fluvastatin	26.3	1 (PAH)			5.79	
furosemide	12.8	6 (PAH, fluorescein, cidofovir, and chlorothiazide)	603	1 (prostaglandin F2)	8.6	3 (E3S, chlorothiazide, and sitagliptin)
hydrochlorothiazide	67.3	1 (PAH)	1023	1 (prostaglandin F2)	942	1 (E3S)
ibuprofen	2.2	6 (PAH and adefovir)	692	1 (prostaglandin F2)	16.4	
indomethacin	4.93	5 (PAH and adefovir)	9.56	3 (prostaglandin F2 and creatinine)	3.76	5 (E3S, pemetrexed, chlorothiazide, methotrexate, sitagliptin, and penicillin G)
JBP485	337	2 (ubenimex and acyclovir)			326	2 (ubenimex and cilostazol)
ketoprofen	0.89	5 (PAH, 6-carboxyfluorescein, and adefovir)	400	1 (prostaglandin F2)	32.7	3 (E3S, methotrexate, and penicillin G)
lansoprazole	7.58	1 (PAH)			2.12	2 (E3S and methotrexate)
lenvatinib	7.36	1 (PAH)			4.11	1 (E3S)
lithospermic acid	20.8	1 (PAH)			0.59	1 (E3S)
lobeglitazone	151	1 (PAH)			34.3	1 (E3S)
losartan	12	1 (uric acid)			1.6	1 (uric acid)
loxoprofen	27.1	1 (methotrexate)			37	3 (methotrexate)
lumiracoxib	3.3	1 (PAH)			1.9	1 (E3S)
mefenamic acid	0.95	2 (PAH and 6-carboxyfluorescein)	21.7	1 (prostaglandin F2)	3.66	3 (E3S, methotrexate, and 6-carboxyfluorescein)
methazolamide	438	1 (PAH)			97.5	1 (E3S)
methotrexate	998	1 (PAH)			1.61	2 (E3S)
mycophenolic acid	1.52	2 (PAH)			0.88	2 (E3S)
mycophenolic acid 7-O-glucuronide	512	1 (PAH)			32.4	2 (E3S)

mycophenolic acid phenyl-glucuronide	223	1 (PAH)			41.5	2 (E3S)
N-acetyl glutamic acid	26	1 (PAH)			1990	1 (E3S)
naproxen (S-naproxen)	1.54	8 (PAH, cidofovir, and adefovir)	486	1 (prostaglandin F2)	14.7	5 (E3S, pemetrexed, methotrexate, and penicillin G)
novobiocin	14.9	1 (PAH)			4.77	1 (E3S)
olmesartan	0.28	2 (PAH and uric acid)			0.03	1 (uric acid)
omeprazole	4.32	1 (PAH)			13.7	2 (E3 and methotrexate)
oxaprozin	0.89	1 (6-carboxyfluorescein)			0.87	1 (6-carboxyfluorescein)
p-aminohippuric acid (PAH)	29.4	6 (6-carboxyfluorescein, fluorescein, ubenimex, acyclovir, and adefovir)			5.06	2 (methotrexate, and penicillin G)
pantoprazole	63.2	1 (PAH)			5.93	2 (E3S and methotrexate)
paritaprevir	14	1 (unknown)			95	1 (unknown)
p-cresyl sulfate	690	1 (PAH)			485	1 (E3S)
phenacetin	299	3 (PAH and adefovir)	1878	1 (prostaglandin F2)	19.4	1 (E3S)
phenylbutazone	71.6	1 (PAH)			15.4	2 (methotrexate, and penicillin G)
piroxicam	40.6	3 (PAH and adefovir)	70.3	1 (prostaglandin F2)	32.2	3 (methotrexate, and penicillin G)
pravastatin	408	2 (PAH)	352	1 (prostaglandin F2)	34.3	4 (E3S and pemetrexed)
probenecid	6.67	22 (PAH, cidofovir, tanshinol, chlorothiazide, cidofovir, 6-carboxyfluorescein, adefovir, uric acid, dimesna, Tc-99m-mercaptoacetyl-triglycine)	766	2 (prostaglandin F2 and tanshinol)	5.93	10 (E3S, daidzein-7-O-glucuronide, dimesna,famotidine, pemetrexed, fexofenadine, Tc-99m-mercaptoacetyl-triglycine, methotrexate, zonampanel, and penicillin G)

puerarin	10.6	2 (PAH and methotrexate)			26.7	2 (methotrexate and penicillin G)
quercetin-3-O-sulfate	1.22	1 (PAH)			0.75	1 (5-carboxyfluorescein)
raltegravir	38.9	2 (tenofovir and cidofovir)			18.8	1 (E3S)
rhein	0.07	1 (PAH)			0.01	1 (E3S)
ritonavir	14	1 (unknown)			6.31	4 (tenofovir, PAH, and penicillin G)
rolofylline (KW-3902)	7.82	1 (PAH)			3.7	1 (E3S)
rosmarinic acid	0.35	1 (PAH)			0.55	1 (E3S)
salicylic acid	457	5 (PAH and uric acid)			178	3 (E3S, methotrexate, and penicillin G)
salvianolic acid A	5.6	1 (PAH)			0.16	1 (E3S)
salvianolic acid B	22.2	1 (PAH)			19.8	1 (E3S)
simvastatin	55.3	2 (PAH)			39.4	2 (E3S)
sulfasalazine	4.6	1 (methotrexate)			3	1 (methotrexate)
sulindac	53.1	2 (PAH)	440	1 (prostaglandin F2)	18.1	3 (E3S, methotrexate, and penicillin G)
tanshinol	40.4	2 (PAH)	1528	1 (prostaglandin F2)	155	2 (E3S)
tasimelteon oxidative metabolite M13	72.3	1 (unknown)			37.2	1 (unknown)
telmisartan	0.39	2 (PAH and uric acid)			1.6	1 (uric acid)
tolmetin	5.08	1 (PAH)			5.32	1 (penicillin G)
torsemide	55.2	1 (PAH)			89.9	1 (E3S)
torsemide M1 metabolite	125	1 (PAH)			109	1 (E3S)
trichlormethiazide	19.2	1 (PAH)	1220	1 (prostaglandin F2)	71.2	1 (E3S)
valsartan	8.58	3 (PAH, cidofovir, and uric acid)			0.22	2 (6-carboxyfluorescein and uric acid)
veliparib (ABT-888)	1371	1 (PAH)			505	1 (E3S)
wogonin	5.4	1 (PAH)			1.3	1 (E3S)
YM90K	5.8	1 (PAH)			3.4	1 (E3S)
zonampanel	1.4	1 (PAH)			11.8	1 (E3S)