Handling of Drugs, Metabolites, and Uremic Toxins by Kidney Proximal Tubule Drug Transporters

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Abstract

The proximal tubule of the kidney plays a crucial role in the renal handling of drugs (e.g., diuretics), uremic toxins (e.g., indoxyl sulfate), environmental toxins (e.g., mercury, aristolochic acid), metabolites (e.g., uric acid), dietary compounds, and signaling molecules. This process is dependent on many multispecific transporters of the solute carrier (SLC) superfamily, including organic anion transporter (OAT) and organic cation transporter (OCT) subfamilies, and the ATP-binding cassette (ABC) superfamily. We review the basic physiology of these SLC and ABC transporters, many of which are often called drug transporters. With an emphasis on OAT1 (SLC22A6), the closely related OAT3 (SLC22A8), and OCT2 (SLC22A2), we explore the implications of recent *in vitro, in vivo,* and clinical data pertinent to the kidney. The analysis of murine knockouts has revealed a key role for these transporters in the renal handling not only of drugs and toxins but also of gut microbiome products, as well as liver-derived phase 1 and phase 2 metabolites, including putative uremic toxins (among other molecules of metabolic and clinical importance). Functional activity of these transporters (and polymorphisms affecting it) plays a key role in drug handling and nephrotoxicity. These transporters may also play a role in remote sensing and signaling, as part of a versatile small molecule communication network operative throughout the body in normal and diseased states, such as AKI and CKD.

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The kidney proximal tubule is the site of elimination of a vast number of small molecules. These include drugs (*e.g.,* antibiotics, antivirals, diuretics, nonsteroidal anti-inflammatory drugs, and antidiabetic agents), physiologically important metabolites (*e.g.,* folate, α-ketoglutarate, urate, and carnitine), nutrients (*e.g.,* vitamins and flavonoids), signaling molecules (*e.g.,* odorants, cyclic nucleotides, and prostaglandins), exogenous toxins (*e.g.,* mercurial conjugates and aristolochic acid), gut microbiome products (*e.g.,* kynurenine), and endogenous toxins (so-called uremic toxins, such as indoxyl sulfate) (1–7). Apart from excreting unmodified small molecule drugs, the kidney handles many conjugated metabolites, most of which are produced by phase 1 and phase 2 metabolism in the liver (*e.g.,* products of hydroxylation, sulfation, and glucuronidation reactions) (8). Genes for phase 1 and 2 reactions are also expressed in the kidney and are likely to be very important in metabolic functions of the proximal tubule cells of kidney as well (9), although this area of research is underexplored.

Consider a hypothetical hospitalized patient with stage 3 CKD, who may have slightly elevated levels of circulating uremic toxins (*e.g.,* indoxyl sulfate) and is also being treated with β-lactam antibiotics, loop diuretics, statins, and antiviral agents. This scenario thus includes a host of drugs, metabolites, and molecules that are handled by proximal tubule transporters, which orchestrate their clearance from the blood and their elimination into the urine. The presence of gene variants of some of these transporters (possibly more

common given this patient's ethnic background) could cause differences in expression or function of the transporters in the proximal tubule of this patient compared with others on the ward. Furthermore, perhaps the patient's blood pH has varied considerably, thus altering the net charge of some of the aforementioned organic molecules, and thus their capacity to be transported into different body tissues and fluids or be eliminated. Many of these small molecules tend to be protein bound, and perhaps the patient's albumin concentration is low, which may further affect small molecule distribution and elimination.

While this patient is hypothetical, this type of scenario is not uncommon. The variables that affect serum, tissue, and body fluid levels of a single drug, toxin, or metabolite excreted by the transporters that handle small molecules is quite complicated; much more so if one simultaneously considers several small molecules. Nevertheless, a great deal of progress has been made in the past few decades on the basic biology of drug, toxin, and metabolite handling, including those functioning in the kidney proximal tubule. With these details at hand, integration of this information and application to clinical settings, such as the scenario presented in the preceding paragraph, should eventually be feasible.

Many of the small molecules of clinical interest are charged: organic anions, organic cations, or molecules that have a zwitterionic character (both positive and negative charges). Molecules that are too large or albumin bound have limited glomerular filtration, and excretion instead depends largely on tubular secretion. First,

the molecules flow through the peritubular capillaries, where they are extracted by multispecific drug transporters at the basolateral (blood) surface of the proximal tubule cell (Figure 1, A and B). For the most part, these molecules are secreted unchanged into the tubular lumen by a set of transporters at the apical (luminal or urine) surface of the proximal tubule cell. There appear to be more than two dozen types of transporters involved in the net transport of organic anion, organic cation, or organic zwitterions by the proximal tubule. In some cases (e.g., urate), the net transport may be the result of both secretion and absorption, but here we mostly focus on the role of proximal tubule transporters in net secretion in the setting of the normal kidney and in disease states.

Classification of Organic Ion Transporters

Organic ion transporters in the proximal tubule are frequently collectively called multispecific drug transporters because of their multispecific nature and their crucial role in drug handling. But depending on the discipline (physiology, biochemistry, or pharmacology), or for historical reasons, a single transporter can sometimes be described by multiple different names in the literature (Table 1) (1). This can make it confusing, even for researchers in the field. These multispecific transporters fall into two general families: solute carrier (SLC) or ATP-binding cassette (ABC) transporters (1,3,10,11). The SLCs generally transport substances either down their concentration gradient or against their concentration gradient coupled with movement of a second substance down its concentration gradient. In the kidney, the most important multispecific SLC transporters appear to be the organic anion transporters (OATs), including OAT1 (SLC22A6, originally described as novel kidney transporter [NKT]) and OAT3 (SLC22A8), which appear to be the main transporters inhibited by the drug probenecid, and the organic cation transporters (OCTs) such as OCT2 (SLC22A2) (1). But increasing attention is being paid to other members of multiple SLC families, including organic anion transporting polypeptides (OATP, or SLCO family), multidrug and toxin extrusion proteins (MATEs or SLC47 family), peptide transporters (SLC15 family), and organic carnitine/zwitterionic transporters (SLC22A4 and SLC22A5) (1,12,13).

ABC transporters use energy generated by the hydrolysis of ATP to transport molecules across cell membranes. Several ABC transporters, including P-glycoprotein (P-gp; ABCB1), also known as multidrug-resistant protein 1 (MDR1), and breast cancer resistance protein (BCRP, also known as ABCG2) play key roles in tubular efflux. Other key family members involved in kidney proximal tubule transport are the multidrug-associated resistance proteins, (MRP2 [also known as ABCC2] and MRP4 [ABCC4]), located on the apical (urinary) surface of the cell (1,10).

Most substrates of medical importance (e.g., drugs, metabolites, and toxins) are eliminated primarily by more than one renal transporter in vivo. Para-aminohippurate (PAH), however, is largely extracted from the blood in vivo by OAT1, the classic PAH transporter (14–16). Many common drugs (e.g., antivirals) are known to interact with more than one SLC and/or ABC transporter expressed in the kidney,
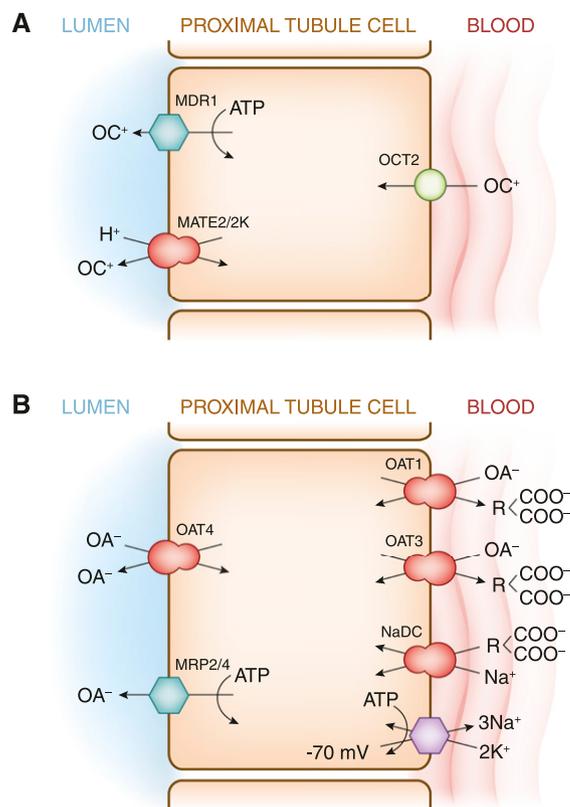

Figure 1. | Transcellular movement of organic cations (OCs) and organic anions (OAs) in kidney proximal tubule epithelial cells. (A) Transcellular movement of OCs in a kidney proximal tubule epithelial cell. Movement of OCs are facilitated by the negative potential difference within the cell, which is maintained by the basolateral Na^+,K^+-ATPase (not shown). OCs enter the cell across the basolateral membrane by organic cation transporters (OCTs), such as OCT2. The authors note that OCT2 is also sometimes depicted as an OC exchanger. Secretion across the apical membrane into the lumen occurs by electroneutral exchange with H^+ by solute carrier (SLC) transporters, including multidrug and toxin extrusion (MATE) protein 2/2K, and by ATP-binding cassette (ABC) transporters, such as multidrug-resistant protein 1 (MDR1), which use energy generated by ATP hydrolysis to transport molecules across the apical membrane. Only a fraction of the known transporters are shown. (B) OA transport via organic anion transporters (OATs) in a proximal tubule epithelial cell. Primary active transport of sodium out of the cell by the basolateral Na^+,K^+-ATPase creates the gradient that facilitates sodium dicarboxylate cotransporters (NaDCs) to move sodium and dicarboxylates [$R(COO^-)_2$], such as α-ketoglutarate, into the cell. The resulting high intracellular concentration of dicarboxylates promotes uptake of OA across the basolateral membrane in exchange for [$R(COO^-)_2$] by organic anion transporters (OAT1 and OAT3). Apical exit involves ABC transporters including multidrug-associated resistance proteins (MRP2, MRP4) and possibly other SLC transporters including OAT4 and urate transporter 1 (URAT1, not shown).

albeit with varying affinities and inhibitory constants (roughly 10 μM–1 mM); this makes it difficult to pin down the relative contributions of key transporters involved in renal elimination (1,17). Furthermore, while much of the data from in vitro transport assays and mouse knockout studies seems relevant to humans, caution must be exercised in extrapolating to human physiology.

Table 1. Select transporters involved in organic ion transport in the kidney proximal tubule

Name	HGNC Gene Symbol[a]	Common Symbol	Alternative Symbol(s)	Other Name
Organic Anion Transporter 1	SLC22A6	OAT1	NKT	Novel Kidney Transporter
Organic Anion Transporter 3	SLC22A8	OAT3	ROCT	Reduced in Osteosclerosis Transporter
Organic Anion Transporter 4	SLC22A11	OAT4		
Urate Anion Exchanger 1	SLC22A12	URAT1	RST	Renal-Specific Transporter
Organic Cation Transporter 2	SLC22A2	OCT2		
Multidrug and Toxin Extrusion Protein 1	SLC47A1	MATE1		
Multidrug Resistance Protein 1	ABCB1	MDR1	P-gp	P-glycoprotein
Breast Cancer Resistance Protein	ABCG2	BCRP		
Multidrug Resistance Associated Protein 2	ABCC2	MRP2		
Multidrug Resistance Associated Protein 4	ABCC4	MRP4		

[a]For each solute carrier (SLC), a number (the family series) is followed by the letter A, which serves as a divider, and then the number of the particular transporter within that family; ATP-binding cassette (ABC) nomenclature includes a letter (A–G) to describe the subfamily, and then the number of the transporter member. By convention, transporters are displayed as all uppercase letters when referring to proteins or human genes. HGNC, Human Genome Organisation gene nomenclature committee; OAT, organic anion transporter; URAT1, urate transporter 1; OCT, organic cation transporter; MATE1, multidrug and toxin extrusion protein 1; MDR1, multidrug-resistant protein 1; BCRP, breast cancer resistance protein; MRP, multidrug-associated resistance protein.

Basic Organic Ion Transporter Physiology

Excretion of organic cations begins with transport on the basolateral surface of the proximal tubular cell (Figure 1A). This is primarily achieved by OCT2, a transporter of organic cations, which takes advantage of the negative potential difference within the cell maintained by the basolateral Na^+-K^+-ATPase. Several carriers on the apical surface subsequently transport organic cations across the apical membrane through electroneutral transport by exchange with proton (H^+), which capitalizes on the electrochemical gradient that favors movement of H^+ into the cells. The key apical transporters appear to be the MATEs (SLC47), but other SLC and ABC transporters may be involved.

OATs on the basolateral and apical membranes function in tandem to move organic anions from the blood, across the proximal tubule cells, and into the lumen (Figure 1B). The best-studied transporters on the basolateral membrane are OAT1 and OAT3, which have dozens of well established substrates. These transporters are organic anion/dicarboxylate exchangers, which use a tertiary active transport system on the basolateral side of the proximal tubule cell. The Na^+-K^+-ATPase pumps sodium out of the cell, while sodium/dicarboxylate cotransporters move sodium and dicarboxylate molecules into the cell. The OAT antiporters move organic anions into the cell and dicarboxylate molecules out of the cell because the gradient favors outward movement of dicarboxylates, such as α-ketoglutarate, to the peritubular capillary. The current thinking is that MRP2 (ABCC2) and MRP4 (ABCC4) are the main apical efflux transporters of many of the organic anions taken up by OAT1 and OAT3, but other transporters, such as OAT4 and the urate transporter urate anion exchanger 1 (URAT1 [SLC22A12]), may also be involved [18]. These transporters seem to work in concert to control the excretion of organic solutes [1–7,17].

Organic anion secretion has been recognized as an important function of the kidney for more than half a century.

In addition, it has long been known that secretion of organic anions by the kidney can be saturated, such that the addition of a second substance can inhibit secretion of the first. Since the mid-to-late 1990s, many of these transporters have been cloned [1,7,15,19,20] and a great deal of knowledge has accumulated as a result of transport studies in microinjected frog oocytes, transfected cells, and in vivo as well as ex vivo analysis of wild-type and knockout tissues [5,21,22]. These data may help explain drug-drug interactions (DDIs) but also may explain differences in pharmacokinetics in different patients, some of whom may have transporter single-nucleotide polymorphisms (SNPs) that lead to more rapid or relatively delayed transport. This represents a large body of work by many investigators, and it is impossible to cover each transporter [1,7,21,23–26]. In this review, however, we focus mainly on OAT1, OAT3, and OCT2, which have emerged as the primary transporters for many common drugs, toxins, and metabolites encountered in the clinical renal setting. To illustrate concepts, along the way we also highlight some interesting findings related to some of the other SLC and ABC transporters mentioned earlier. Additional details in the context of the broader field of drug transport can be found elsewhere [1].

Drugs and Toxins

There are now US Food and Drug Administration (FDA) regulatory guidelines to examine the transport of new drugs with a view toward understanding DDIs at the transporter level [27]. Indeed, the FDA recommends that applications for new drugs and biologics in which renal secretion is significant be studied in vitro to determine whether these agents are substrates for OAT1, OAT3, or OCT2 [28]. When these transporters appear to play a role, additional studies may be required. The list of drugs known to interact with these renal transporters is extensive, and there are data in human, rodents, and other species [5,17]. This focus on drug

transporters promises to eventually improve understanding of pharmacokinetics in normal and diseased states (5).

The endogenous metabolite, creatinine, is a well described substrate for OCT2 and apical MATEs; competitive inhibition of these carriers can cause increases in serum creatinine by blocking its excretion (Figure 2A) (29–31). Drugs such as cimetidine and trimethoprim are examples. OATs, while mainly transporters of organic anions, can also transport some organic cations, including creatinine (32,33). In addition, newer agents, such as the integrase inhibitor dolutegravir and the novel pharmacoenhancer cobicistat, also block creatinine excretion (34,35). This effect appears to be largely from drug-metabolite interactions due to competition for OCTs, MATEs, and possibly OATs (29,32–35).

OCTs also transport organic cation drugs, such as metformin, and it has been postulated that polymorphisms in these transporters might account for the variability in clinical effect of this medication and the risk of toxicity. In the case of metformin, polymorphisms in OCT1, primarily expressed in hepatic cells, may explain variable efficacy, whereas polymorphisms in renal secretion initiated by OCT2 may contribute to variable pharmacokinetics (13).

The ABC transporter, P-gp (MDR1), which transports a wide range of molecules, is one of the best understood from the viewpoint of protein structure in relation to

transport function (1,36,37). P-gp has been widely studied in the context of cancer biology and its contribution to tumor resistance to chemotherapy (with increased expression of MDR1 on cancer cells associated with greater resistance). MDR1 also transports digoxin and interacts with quinidine.

OAT1, originally described as an NKT (15), and OAT3, also known in mice as reduced in osteosclerosis transporter) (38), are the main transporters of organic anions in the proximal tubule (Figure 2B) (1,7). When the *Oat1* or *Oat3* genes are deleted in mice, the knockout mice have markedly blunted responses to loop and thiazide diuretics (14,39). These diuretics must enter the proximal tubule cell from the blood (basolateral) side and then be secreted into the proximal tubular lumen (apical side) before they can act more distally in the nephron to inhibit sodium transport. In addition, *Oat3* knockout mice are deficient in penicillin excretion (40), and both the *Oat1* and *Oat3* knockout kidney tissue has defective handling of antiviral agents, such as those used to treat HIV (41,42). It is worth noting that the kidney may handle medications in the same class differently. For example, furosemide is filtered and secreted, whereas filtration appears to be the main route of bumetanide entry into the tubular lumen (43,44); these pharmacologic differences may be important when diuretics are used in the setting of certain types of acute kidney disease and CKD.

Recent metabolomics data from *Oat3* knockout mice, together with *in vitro* studies, support the view that OAT3 is a major route of elimination for many compounds that undergo phase 2 modifications by drug-metabolizing enzymes in the liver (*e.g.*, glucuronidation) (8). Although detailed studies need to be performed in knockout mice, such modified compounds might be expected to include dietary plant products along with drugs and toxins. Many of these hepatically metabolized compounds are not routinely measured. In addition, it is possible that competition by drugs (*e.g.*, antibiotics, nonsteroidal anti-inflammatory drugs [NSAIDs], and antivirals) for OATs can affect the levels of many other metabolized compounds in the blood. Historically, probenecid was used to limit renal penicillin elimination where there was a critically small supply (45). It was later used to augment the effect of penicillin in the treatment of gonorrhea and other systemic infections (46). Probenecid is also used to block uric acid reabsorption in the proximal tubule for the treatment of gout (47); as discussed below, uric acid is a substrate of several proximal tubule drug transporters, including BCRP (ABCG2), OAT1, OAT3, and the related OAT family member (URAT1).

Drug transporters also handle environmental toxins, which may contribute to their toxicity to the renal tubules. For example, mercury exists in the blood in thiol conjugates (with glutathione and cystathione), which effectively act like organic anions. OAT1 and OAT3 appear to be the major transporters involved in elimination of mercuric conjugates, which can lead to renal and central nervous system toxicity following mercurial exposure (48–51). The proximal tubule of the kidney of the *Oat1* knockout mouse is largely resistant to nephrotoxic damage that occurs from systemic administration of mercuric chloride (Figure 3) (52). Aristolochic acid (53) and ochratoxin A (54), the putative nephrotoxins involved in Balkan endemic nephropathy, are both
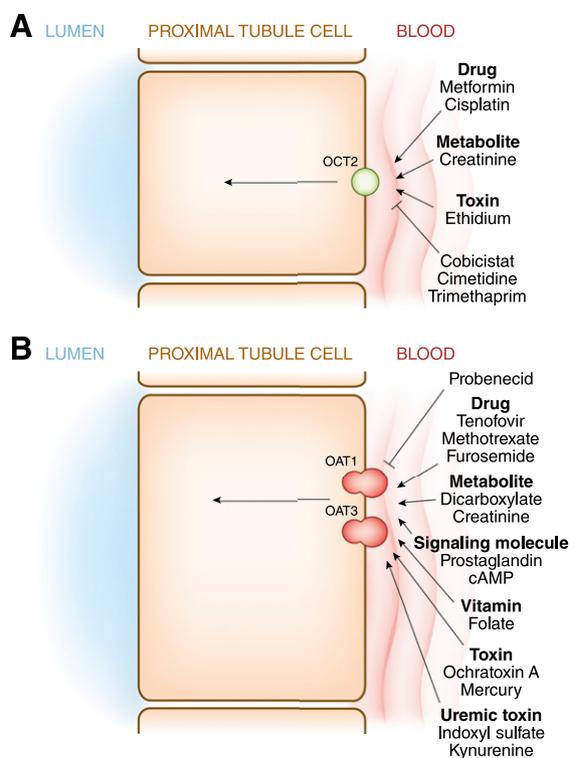

Figure 2. | Schematic of potential interactions between drugs, metabolites, and toxins due to competition for tubular secretion at the transporter level. (A) Some of the molecules that are transported by organic cation transporters (*e.g.*, OCT2). Transport is inhibited by cimetidine and trimethoprim and by the novel pharmacoenhancer, cobicistat. (B) Some of the substrates for the organic anion transporters OAT1 and/or OAT3. Transport is inhibited by probenecid.

transported into the proximal tubule by OATs. In this disorder, patients may present with evidence of tubular dysfunction or simply advanced CKD. The pathology is characterized by tubulointerstitial disease and fibrosis. Although the mechanism is not certain, these substances are clearly toxic to cultured renal epithelial cells (55). There is also a high incidence of uroepithelial cancer in these patients. Environmental toxins, such as the water-repellent polymer, perfluorooctanoic acid, are also substrates of OATs (56).

Uremic Toxins

Over 100 molecules have been implicated in the pathogenesis of uremic syndrome (57–60). Their precise roles are debated, but many of these are small organic anions, such as indoxyl sulfate, carboxy-methyl-propyl-furanpropionate, p-cresol sulfate, and kynurenine; molecules associated with CKD that can accumulate between dialysis sessions (57,59). These and many other potential uremic toxins are good OAT substrates. In *Oat1* knockout mice, some of these organic anions accumulate (61), although the mice do not appear ill and have normal life spans (14). Putative uremic toxins are also affected to varying degrees in *Oat3* knockout mice and *OATP4C1* transgenic rats (8,62,63). Uremic toxins interact with other SLC and ABC transporters as well. On the basis of *in vitro* transport data, the high levels of circulating organic anion uremic toxins have the possibility of competing, at the level of transporters, for elimination and distribution of drugs, metabolites, and toxins (64). To the extent that transporters such as OAT1 and OAT3 play central roles in the regulation of systemic and local metabolism, this could contribute to the abnormalities in metabolism seen in uremia (1,7,65). How these transporters handle various uremic toxins in the kidney and nonrenal tissues in the setting of both normal physiology and disease is a fertile area for future translational research and may provide important insights into how to both delay and treat the symptoms of uremia.

Gut Microbiome Products, Nutrients, and Natural Products

There is growing evidence that many of the potential uremic toxins have their origin in the gut microbiome. Indole, for example, is produced by gut bacteria, undergoes sulfation in the liver, and is then excreted as indoxyl sulfate by the kidney (66). One of the primary renal transporters involved appears to be OAT1, which transports several gut microbial products or their metabolites (61,67). OAT1, OAT3, and other SLC and ABC transporters are also necessary for the elimination of dietary natural products, including a wide range of flavonoids, as well as vitamins, and thus might indirectly regulate vitamin-dependent metabolic pathways (8,67).

Metabolites and Signaling Molecules

Metabolomic studies in knockout animals, particularly *Oat1* and *Oat3* knockout mice, have confirmed a central role for drug transporters in the transport of many important metabolites and signaling molecules (8,14,61,67,68). These include α-ketoglutarate, which plays a central role in the Krebs cycle (tricarboxylic acid cycle); vitamins; molecules with antioxidant properties (*e.g.,* urate and flavonoids); and the gut microbial derivatives already described. Signaling molecules, such as cyclic nucleotides, prostaglandins, odorants, and conjugated steroids, are also eliminated *via* the OATs and other SLC and ABC drug transporters. Recent systems biology and omics integration of metabolomics and transcriptomics data from *Oat* knockout mice suggests that OATs and possibly all multispecific drug transporters play a role in the regulation of systemic and tissue metabolic and signaling processes (1,7,8,65,67,69). This type of information has led to the remote sensing and signaling hypothesis discussed below (65,69).

The transporter-mediated regulation of uric acid, mainly by renal transporters but also by nonrenal (*e.g.,* intestinal) transporters, appears quite complex in both humans and mice. Genome-wide association studies, *in vitro* transport data, and studies on knockout mice indicate that earlier models for uric acid handling were oversimplified. Several transporters have been implicated in renal urate handling to date; apical URAT1 (originally known as the renal-specific transporter in mice [70]) and SLC2A9 appear to play important roles in urate reabsorption, whereas apical ABCG2 (BCRP) and basolateral OAT1 and OAT3 are likely to play key roles in urate secretion (71,72). The complex regulation of uric acid may reflect a functional role that is
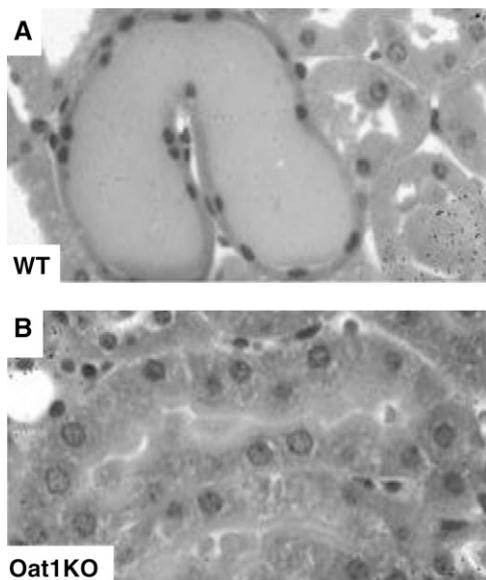

Figure 3. | **Photomicrographs demonstrating tubular and cellular changes in wild-type (WT) and organic anion transporter 1 (*Oat1*) knockout kidneys upon exposure to mercuric chloride, HgCl$_2$.** (A) In the wild-type kidney, substantial tubular damage was seen, as indicated by the dilated tubular lumen and flattened tubule epithelial cells, after a single dose of HgCl$_2$ (4 mg/kg body wt, intraperitoneal). (B) In contrast, the *Oat1* knockout (Oat1KO) mice had normal tubules that appeared unaffected by mercury exposure. Although not shown, serum urea nitrogen levels increased significantly in the wild-type mouse but not in the knockout mouse with preserved tubules. (Adapted with permission from Torres *et al.* 52.)

not yet fully appreciated. Further study is clearly needed because uric acid has been implicated as both an antioxidant and a pro-oxidant and, in the case of the latter, a potential culprit in a range of disorders from atherosclerosis to hypertension and renal disease (73).

The details of transcriptional regulation of drug transporters in the proximal tubule are just beginning to unfold. Through a combination of systems-biology approaches involving analysis of transcriptomics and combining chromatin immunoprecipitation with DNA sequencing (ChIP-seq) data, it has been possible to implicate, and then show experimentally, that hepatocyte nuclear factor-4α and hepatocyte nuclear factor-1α are major regulators of OAT and OCT expression, as well as other proximal tubule transporters and drug-metabolizing enzymes (9,74,75). Unlike the extensive modern understanding of drug metabolism in the liver, the role of proximal tubule drug-metabolizing enzymes, in the context of both the proximal tubule cell and systemic physiology, is not well understood and is probably underappreciated. Future studies of the regulation of renal drug transporter expression by pharmaceuticals, uremic toxins, and environmental agents are likely to have a major effect on our understanding of the pathophysiology of CKD, as well as renal pharmacology and toxicology. It is conceivable that the transcription factors in the proximal tubule coordinate the regulation of drug transporter gene expression by sensing levels of signaling molecules, metabolites, drugs, and toxins, and responding by producing and deploying additional transporters as needed (1,7,65,69).

DDIs and Drug-Metabolite Interactions

Knowledge of DDIs at the level of organic ion transport in the kidney can also influence care. Some DDIs, such as penicillin and probenecid, are well established and have been put to clinical use; the half-life of penicillin is greatly prolonged by coadministration of probenecid, an OAT inhibitor (45–47). In contrast, some DDIs can lead to dire consequences. Methotrexate is taken up from the blood *via* OATs. NSAIDs can inhibit OATs, and when methotrexate and NSAIDs are used together, methotrexate toxicity can occur, manifesting as severe bone marrow suppression (76,77). DDIs at the level of P-glycoprotein (MDR1/ABCB1) in the proximal tubule and elsewhere are thought to explain the well known digoxin-quinidine interaction resulting in digoxin toxicity, including arrhythmias (78). Poor renal function can further complicate the clinical picture (79).

Whereas DDI *via* substrate competition has been well recognized (80), drug-metabolite interaction by a similar mechanism has received comparatively less attention. Considerable *in vitro* and substrate modeling (*e.g.,* pharmacophore) data suggest that drug-metabolite interaction could be a substantial problem because the drugs and metabolites handled by OATs and other drug transporters have structural similarities (61,81). This may be especially important in a setting such as CKD, in which circulating organic anion levels (*e.g.,* uremic toxins such as indoxyl sulfate) are high. With the broader application of metabolomics methods, this should become clearer in the near future.

Pharmacogenomic and Toxicogenomic Considerations

Early studies of transporter polymorphisms raised the possibility that multiple SNPs in basolateral (*e.g.,* OAT1 and OAT3) (82,83) and apical (*e.g.,* URAT1, OAT4, MRP2, and MRP4) drug transporters may affect the net transport of drugs, toxins, and metabolites from blood to urine (50,84). In addition, noncoding SNPs that regulate drug transporter expression might be particularly important (82,85). These polymorphisms may explain differences in drug response and toxicity among individuals. While emerging data seem to be consistent with these notions, there is much to be done in order to improve understanding of how coding or noncoding SNPs in renal drug transporter genes, and particularly combinations of these SNPs, affect overall renal handling of drugs, metabolites, and toxins (83).

As noted earlier, OAT1 and OAT3 are the major transporters of loop and thiazide diuretics, and secretion into the urinary space by the proximal tubular cells is necessary for these diuretics to induce the desired natriuresis by inhibiting sodium transport in later tubule segments. A noncoding region polymorphism has been identified in patients with diuretic resistance that could affect OAT1 and/or OAT3 expression (86). Polymorphisms in OAT1, OAT3, and MRP2 are more common among miners exhibiting toxicity from mercury-containing vapors (50). Additionally, OAT3 polymorphisms have been associated with altered cephalosporin handling (87), and suggestive evidence indicates the possible involvement of OATs in antiviral and methotrexate elimination (88,89). One of the better-studied examples of drug transporter polymorphisms affecting renal drug elimination is that of OCT2 (SLC22A2). Patients with certain SNPs in this transporter have altered metformin handling (90). In addition, polymorphisms in MATE1, on the apical membrane, may also affect metformin handling (91). OCT2 polymorphisms that affect transport function also may play a role in determining whether cisplatin nephrotoxicity occurs, as cisplatin gains entry into proximal tubule cells from the basolateral membrane (92).

Overall, results from *in vitro* transport studies in cells that overexpress transporters, together with results from studies in knockout animals (or tissues derived from them), and rat experiments (*e.g.,* after probenecid treatment) seem reasonably concordant with available human clinical data. Although there are sure to be many caveats and exceptions, this overall concordance is a very important point from an experimental and translational standpoint; it indicates that the considerable *in vitro*, *ex vivo,* and *in vivo* knockout mouse and rat data can continue to be used to help guide our clinical understanding.

Pediatric Developmental Pharmacology and Drug Elimination in the Aging Population

Most of our understanding of renal drug elimination comes from analysis of adult patients and adult animals. In addition, most studies have only limited consideration of ethnicity, sex, and the extremes of age (3,93). Human pediatric kidney data remain limited, so what we currently know, especially from a mechanistic standpoint, comes largely from a limited number of rodent studies. For example, it is known that drug transporter expression occurs

early in embryogenesis; indeed, several renal drug transporters are expressed transiently in the developing central nervous system and other tissues (94). Late in rodent gestation, the expression is largely limited to the future proximal tubules of the kidney (15,95); thereafter, there is a burst in renal expression around the time of birth, eventually reaching (and perhaps overshooting for a short time) adult expression (9,96). This is paralleled by functional changes, such as increasing PAH clearance (96). There is evidence in animals for a developmental inducibility window, during which renal drug transporters may, during the early postnatal period, be induced by substrates or by hormones (97,98). If this is true in humans, substrate induction could theoretically enhance the ability of a premature infant's kidney to excrete potentially deleterious drugs and toxins. A coordinated response by the postnatal kidneys with the liver is also needed to excrete drugs and metabolites during the continuing period of maturation (3). Furthermore, many OAT1 and OAT3 substrates are drugs, toxins, and metabolites that have been modified by phase 1 (*e.g.,* cytochrome-dependent) or phase 2 (conjugation, such as glucuronidation) reactions. For example, OAT3 is responsible for the elimination of many glucuronidated compounds (8). How this liver-kidney coordination is achieved during postnatal maturation, or during recovery from organ injury, is not yet well understood.

Adverse drug reactions in the elderly are also a major clinical concern. Some of these adverse drug reactions may be partly related to altered expression or function of drug transporters in the aging kidney (99,100). However, data on this important issue are limited.

AKI and CKD

Following AKI from ischemia or toxins, substantial changes have been observed in transcript and protein expression of many drug transporters. Initially, expression of certain transporters seems to decrease, followed by upregulation during recovery (24). The extent to which this is reflected in functional handling of specific drugs, metabolites, and toxins is not well understood. As discussed earlier, many of the putative uremic toxins, including indoxyl sulfate and kynurenine, are excellent substrates of OATs and other drug transporters, such as OATPs, and they accumulate in the knockout or transgenic models of these transporters. These substances may themselves be toxic to the tubule cell and may also contribute to the progression of CKD (8,61–63,101).

Although drug transporters on the basolateral and apical membranes of the proximal tubule protect from systemic toxicity by enhancing drug and toxin elimination, they are, in some instances, the mechanism by which substances toxic to the proximal tubule gain entry. Cephaloridine, a first-generation cephalosporin that has largely been replaced by newer agents with better bioavailability and less nephrotoxicity, is one such example. Cephaloridine, like other cephalosporins, is excreted unchanged by renal tubular secretion. Yet this agent accumulates in the proximal tubule; after basolateral OAT3 uptake, secretion by apical membrane transporters may not be sufficiently rapid to avoid toxicity, perhaps because of the zwitterionic nature of this agent (102). Toxicity appears to be related to oxidative stress from depletion of reduced glutathione (103). This imbalance in

proximal tubular cell entry and exit has also been implicated in the proximal tubule defect produced by the antiviral agent tenofovir. Indeed, *Oat1* knockout mice appear protected from tenofovir-induced proximal tubular damage, whereas those with loss of apical efflux of tenofovir seen in *Mrp4* knockout mice were particularly susceptible (104).

The role of drug transporters in proximal tubule metabolism raises some interesting questions in the context of renal ischemia and other types of injury. The proximal tubule exhibits some unique metabolic characteristics (105). Although glycolytic enzymes are present in the proximal tubule, the cells are largely obligate oxidative in metabolic character; they are relatively incapable of glycolytic metabolism and poorly utilize glucose as a preferred substrate (77,106). Thus, in the setting of cell stress, the proximal tubule cells might be expected to depend more on gluconeogenesis, whereby glucose is synthesized from substrates such as lactate, a compound not produced by proximal tubules but potentially taken up by proximal tubule transporters. Kidney gluconeogenesis then differs from hepatic gluconeogenesis by using lactate as a primary substrate. Because proximal tubules are unable to shift to glycolytic activity, the proximal tubule cells are a potential victim of hypoxic damage in comparison to renal cells located more distally in the nephron, which retain considerable glycolytic capacity, in keeping with the often lower oxygen levels in the environment. The proximal tubule does require substrates and coupling OAT transport with α-ketoglutarate as the intracellular counter-transporting molecule, plays a role in regulating the intracellular levels of citric acid cycle molecules involved in oxidative metabolism. This also raises a question as to whether changes in net proximal tubule OAT activity as a result of altered expression levels or competing anions could affect proximal tubular metabolism and ability to maintain normal oxidative substrate supply when the proximal tubule dynamics are altered in the context of extremes of age, stress, injury, or recovery from injury. It may thus be very interesting to study whether resistance to hypoxia and ATP depletion can be conditioned by altering OAT function.

Remote Sensing and Signaling Hypothesis

In general, the application of multiple omics methods (*e.g.,* metabolomics and transcriptomics in wild-type and knockout animals) combined with systems biology approaches to reconstruct drug transporter–dependent metabolism is beginning to yield a new perspective on the role of renal and nonrenal SLC and ABC multispecific drug transporters. Transport of molecules by various SLC and ABC drug transporters in and out of cells could play a role in remote communication between cells and tissues as well as interfacing body fluids. Because SLC and ABC drug transporters are selectively expressed in different cells (*e.g.,* kidney, choroid plexus, intestine, biliary tract, liver, brain capillary endothelium, olfactory mucosa, placenta, mammary gland, and testes) that interface with body fluids that bathe other cells, these so-called drug transporters may be part of a remote sensing and signaling system (1,7,65,69) involved in cell, tissue, and organ crosstalk. These transporters are also well situated to play a role in mediating

and/or restoring homeostasis after injury; certain aspects of the pathophysiology of AKI and CKD may be considered disordered remote sensing and signaling. The broader remote sensing and signaling hypothesis has been explained in more detail in other articles (1,7,65,69).

Several OAT isoforms appear somewhat specialized for transport of classic signaling molecules and antioxidants and therefore well suited for signaling: liver-expressed OAT2 transports cyclic guanosine monophosphate (107), placental-expressed OAT4 transports conjugated estrogens (108), olfactory-expressed OAT6 has high selectivity for odorants (109), and OATPG seems specialized for prostaglandins (110). The drug transporters mediating the influx and efflux through tissues and body fluid compartments (*e.g.*, blood, urine, bile, cerebrospinal fluid, and amniotic fluid) could regulate remote communication *via* transport of key (potentially rate-limiting) metabolites and signaling molecules, hence regulating the movement of these molecules across tissue barriers in the body and also at the cellular level. Various types of data suggest that these SLC and ABC drug transporters are also involved in communication between gut bacteria and the body, and also across the maternal-fetal and maternal-neonate barriers *via* breast milk (1, 7).

In addition to a potential contribution to signaling in their own right, various drug transporters are directly affected by signaling molecules, which may govern transporter density on the cellular membrane. Drug transporters, like other transporters in the cell, may traffic from an internal pool, which might be deployed as needed or internalized (111) under stimulation by signaling pathways. Internalization or abnormal trafficking may affect drug excretion.

Summary

The hypothetical clinical vignette presented at the outset of this article represents the challenges that can be seen in many patients. The ballooning data regarding handling of small molecule drugs, metabolites, nutrients, and toxins by multispecific transporters expressed in the proximal tubule still do not explain the complexity of organic ion transport at play in the patient. While the discussion here has tended to focus more on renal drug transporters of the SLC22 family (especially OAT1 and OAT3), it is important to emphasize that many of the same principles and considerations are likely to apply to other SLC and ABC drug transporters in the kidney. Moreover, by focusing on the differences in tissue expression patterns, substrate specificities, regulation in development, evolutionary biology, and disease states, the field is just beginning to understand the role these drug transporters play not only in drug and toxin handling but in normal physiology and pathophysiology (24,65,69,112,113). Viewing uremia partly as a disorder of remote sensing and signaling may lead to new avenues of research and, possibly, novel therapeutic approaches.

Disclosures

None.

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