Chapter 10
Analysis of Renal Transporters

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Abstract The rapid technological advances in the drug transporter field have also greatly enhanced our knowledge on the expression, localization, function, and genetic variation of renal transporters. It is now widely acknowledged that carrier-mediated transport processes in the kidney proximal tubule are an important determinant of drug disposition and the extent to which drugs are accumulated in renal tissue. The study of renal transport has traditionally benefited a lot from physiological studies in isolated membrane vesicles, tubules, tissue slices, perfused kidneys, and intact animals. Together with molecular cloning and over-expression systems we now have a fairly good picture of the individual characteristics of the most important renal transporters. The next challenge will be to reconstruct the complexity of the interplay between the various uptake and efflux transporters of the proximal tubule in experimental and in silico models, in order to accurately predict renal drug clearance, drug–drug interactions, and the risk of nephrotoxicity in different populations. This chapter will give a critical review of current methods available for the exploration of renal drug transport.

Abbreviations

ABC	ATP-binding cassette
BBM	Brush border membrane
BLM	Basolateral membrane
CHO	Chinese hamster ovary cell line
ciPTEC	Human conditionally immortalized proximal tubule epithelial cell line

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COS-7	African green monkey cells
HEK293	Human embryonic kidney cells
HK-2	Human immortalized proximal tubule epithelial cell line
LLC_PK1	Pig kidney epithelial cells
MDCK	Madin-Darby canine kidney cells
PAH	*para*-Amino hippuric acid
pMEG	9-(2-phosphonylmethoxyethyl)guanine
Sf9	Spodeptera frugiperda (moth)
SLC	Solute carrier

10.1 Introduction

The mechanisms that contribute to the renal excretion of drugs and their biotransformation products are closely related to the physiological processes that take place in the nephrons, i.e., glomerular filtration, passive back diffusion, and transporter-mediated secretion and reabsorption. The major transport proteins that are relevant for the renal handling of drugs are mainly located in the proximal tubular cells. The same transporter families that play critical roles in drug influx and efflux in liver and intestine can also be found in the kidney (Degorter et al. 2012). From the solute carrier (*SLC*) gene superfamily, these are the oligopeptide transporters (PEPTs/*SLC15*), the organic anion/cation/zwitter ion transporters (OATs/OCTs/OCTNs/*SLC22*), the organic anion transporting polypeptides (OATPs/*SLCO*), and the multidrug and toxin extrusion transporters (MATE/*SLC47*). Members belonging to the ATP-binding cassette (*ABC*) superfamily important for renal drug efflux include P-glycoprotein (MDR1/*ABCB1*), the multidrug resistance-associated protein (MRP/*ABCC*) family, and breast cancer resistance protein (BCRP/*ABCG2*). An overview of the transporters currently considered to have a well-defined influence on renal drug clearance is given below and in Fig. 10.1. It is important to recognize that the interplay between these transporters located on the basolateral and luminal membrane in proximal tubular cells is critical in determining the extent and net direction of drug movement. Transport across the proximal tubule could be impeded or facilitated by the asymmetrical membrane distribution of influx and efflux transporters, which ultimately influences the plasma clearance and urinary excretion of a drug substrate.

The study of the mechanisms by which the kidney actively secretes compounds foreign to the body started with the pioneering paper in 1923 by Marshall and Vickers, who obtained the first conclusive evidence for this process with the anionic dye phenolsulphonphtalein (Marshall and Vickers 1923). Ever since, our understanding of the molecular and cellular mechanisms of renal drug excretion has been evolving by the advent of increasingly advanced techniques in the transporter field, including isolated renal cortical slices, isolated perfused tubules and kidneys, microperfusion, membrane vesicles, cell cultures and over-expression cell systems,

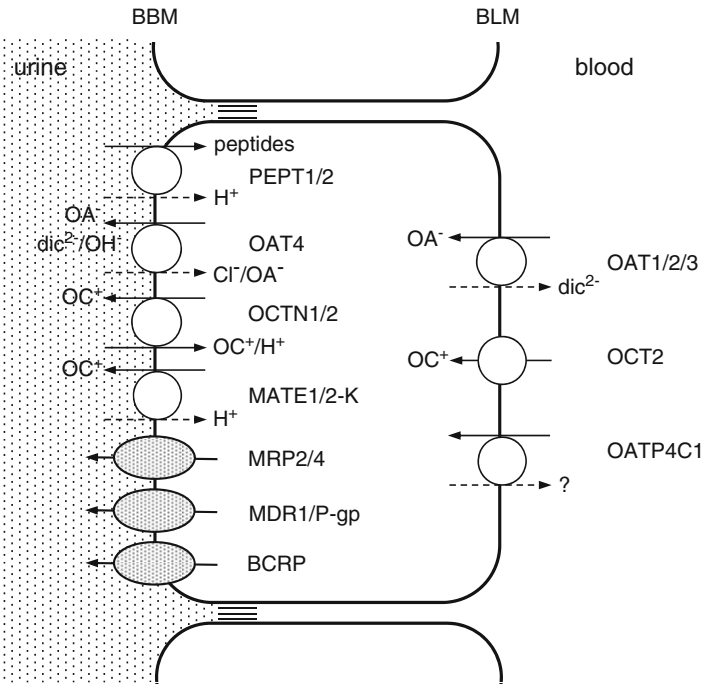

Fig. 10.1 Schematic model of the major drug transporters in human renal proximal tubular cells. SLC transporters are depicted by open circles and ABC transporters by shaded ovals. Solid arrows indicate the direction of drug transport. Dashed arrows depict the movement of driving ions. OCT2 is an electrogenic uniporter that transports organic cations (OC+) from blood into the cell driven by the inside-negative membrane potential. OCTN1 mediates luminal OC+ uptake as a H+/OC+ antiporter or can operate like OCTN2 as a bidirectional cation exchanger, mediating influx or efflux. MATE1 is a urinary OC+ efflux transporter that operates as a H+ antiporter. Peptidomimetic drugs are taken up by the H+/peptide symporters PEPT1 and PEPT2. Organic anions (OA^-) are taken up by the antiporters OAT1, OAT2, and OAT3, which are driven by the exchange with dicarboxylates (dic^{2-}), and released at the luminal side by OAT4 in exchange for Cl^-. OAT4 can also operate as a reabsorptive transporter coupled to cellular dicarboxylates or hydroxyl ions. A few amphipathic drugs are transported into the cell by the organic anion antiporter OATP4C1, for which the driving ion is unknown. The primary active ABC transporters MDR1/P-gp, MRP2, MRP4, and BCRP drive the efflux of a wide variety of amphipathic drugs and metabolites into urine

knockout mouse models, double transfected cell lines, physiologically based pharmacokinetic modeling, and simulation.

To date, no single method or model can accurately predict the contribution of renal transporters to overall drug clearance and disposition in humans in vivo. The purpose of this chapter is to discuss key technologies, including their strengths and limitations, and to examine some of the current challenges and future perspectives in studying renal drug transporters.

10.2 Important Human Transporters Involved in Renal Drug Handling

For most drugs that are handled by renal transporters elimination can be considered as a vectorial process, involving uptake from the blood across the basolateral membrane into proximal tubular cells, followed by efflux across the apical membrane into urine. At the basolateral membrane separate transporters are located for the influx of mainly hydrophilic, small molecular weight (MW < 400–500 Da) organic anions and cations (Masereeuw and Russel 2001). Because these systems are characterized by a high clearance capacity and wide substrate specificity, many drug substrates tend to accumulate in the cell sometimes causing kidney injury. To ensure the rapid efflux of potentially toxic compounds into urine, the apical membrane is equipped with a large number of efflux transporters belonging to different transporter families (Fig. 10.1, Table 10.1).

The organic anion transporters OAT1, OAT2, and OAT3 regulate the uptake of anionic drugs at the basolateral membrane of renal proximal tubule (Burckhardt 2012; Burckhardt and Burckhardt 2011). They operate as antiporters, actively driven by the inside>out concentration gradient of dicarboxylates. OAT1 and OAT3 have long been considered as the major uptake transporters, because of limited evidence for the expression of OAT2 and its unknown role in drug transport. OAT1 has highest affinity for hydrophilic organic anions with small molecular weights, like p-aminohippuric acid (PAH), adefovir, cidofovir, and tenofovir (Table 10.1). OAT3 also transports some larger amphipathic anions, including benzylpenicillin, pravastatin, and olmesartan, and even some cationic drugs, such as cimetidine and ranitidine (Table 10.1). A recent study showed that OAT2 probably mediates the active tubular secretion of the cGMP-like antiviral drugs acyclovir, ganciclovir, and penciclovir (Cheng et al. 2012). Whereas human OAT2 is expressed at the basolateral membrane, the mouse and rat orthologs are localized to the apical membrane of the proximal tubule (Burckhardt and Burckhardt 2011). The broader specificity of OAT3, as well as the relatively higher renal expression levels compared to OAT1 and OAT2 suggests a more pronounced role of OAT3 in human renal organic anion transport (El-Sheikh et al. 2008a; Masereeuw and Russel 2001, 2010). Serious drug–drug interactions have been reported between methotrexate and nonsteroidal anti-inflammatory drugs due to competition for OAT1- and OAT3-mediated uptake, although an interaction at the level of the apical efflux transporters MRP2 and MRP4 probably also contributes to this mechanism (El-Sheikh et al. 2007; Masereeuw and Russel 2010).

The first step in proximal tubular secretion of cationic drugs is mediated by OCT2, the predominant organic cation transporter in the basolateral membrane (Nies et al. 2011). OCT2 operates as a uniporter that facilitates the uptake of comparatively small monovalent cationic drugs by diffusion down the inside-negative electrochemical gradient of the proximal tubular cell (Table 10.1). In rodents, Oct1 is also expressed in the kidney, in addition to Oct2 (Grundemann et al. 1994). A clinically important OCT2 substrate is metformin, which is among the most widely prescribed

Table 10.1 Major human SLC and ABC drug transporters expressed in kidney

Gene	Protein	Mechanism	Membrane localization	Examples of drug substrates
SLC15 family				
SLC15A1	PEPT1	H^+/peptide symporter	Apical	Ampicillin, amoxicillin, bestatin, cefaclor, cefadroxil, cefixime, enalapril, temocapril, temocaprilat, midodrine, valacyclovir, valganciclovir (Brandsch et al. 2008; Dobson and Kell 2008; Rubio-Aliaga and Daniel 2008; Russel et al. 2002)
SLC15A2	PEPT2	H^+/peptide symporter	Apical	Amoxicillin, bestatin, cefaclor, cefadroxil, valganciclovir (Brandsch et al. 2008; Dobson and Kell 2008; Rubio-Aliaga and Daniel 2008; Russel et al. 2002)
SLC22 family				
SLC22A2	OCT2	OC uniporter	Basolateral	Mepiperphenidol, memantine, cimetidine, famotidine, ranitidine, metformin, propranolol, pancuronium, quinine, zidovudine, cisplatin (Ciarimboli 2008; Dobson and Kell 2008; Koepsell et al. 2007)
SLC22A4	OCTN1	H^+ or OC antiporter	Apical	Mepyramine, quinidine, verapamil, ergothioneine, gabapentin (Dobson and Kell 2008; Koepsell et al. 2007; Urban et al. 2008)
SLC22A5	OCTN2	OC antiporter Na^+ symporter (carnitine)	Apical	Mepyramine, quinidine, verapamil, valproate, cephaloridine, emetine (Dobson and Kell 2008; Koepsell et al. 2007)
SLC22A6	OAT1	DC/OA antiporter	Basolateral	Adefovir, cidofovir, acyclovir, didanosine, stavudine, trifluridine, ganciclovir, PMEG, PMEDAP, tenofovir, zalcitabine, zidovudine, tetracycline, methotrexate, bumetanide, furosemide, ibuprofen, indomethacin, ketoprofen, PAH, cimetidine, ranitidine (Burckhardt 2012; Burckhardt and Burckhardt 2011; Dobson and Kell 2008; Russel et al. 2002)
SLC22A7	OAT2	DC/OA antiporter	Basolateral	Acyclovir, ganciclovir, penciclovir, erythromycin, cimetidine, ranitidine, zidovudine, 6-fluorouracil, methotrexate, taxol, bumetanide, salicylate, PAH (Burckhardt 2012; Burckhardt and Burckhardt 2011; Cheng et al. 2012; Russel et al. 2002)

(continued)

Table 10.1 (continued)

Gene	Protein	Mechanism	Membrane localization	Examples of drug substrates
SLC22A8	OAT3	DC/OA antiporter	Basolateral	Benzylpenicillin, tetracycline, valacyclovir, zidovudine, cimetidine, ranitidine, methotrexate, furosemide, ibuprofen, indomethacin, ketoprofen, salicylate, PAH, pravastatin, olmesartan (Burckhardt 2012; Burckhardt and Burckhardt 2011; Dobson and Kell 2008; Kusuhara and Sugiyama 2009; Russel et al. 2002)
SLC22A9	OAT4	Cl^-/ OH^-/DC/ OA antiporter	Apical	Tetracycline, zidovudine, methotrexate, bumetanide, ketoprofen, salicylate, PAH (Burckhardt 2012; Burckhardt and Burckhardt 2011; Dobson and Kell 2008; Russel et al. 2002)
SLC47 family				
SLC47A1	MATE1	H^+/OC antiporter	Apical	Cimetidine, procainamide, metformin, cephalexin, cephadrine, fexofenadine (Koepsell et al. 2007; Matsushima et al. 2009; Moriyama et al. 2008; Tanihara et al. 2007; Terada and Inui 2008)
SLC47A2	MATE2-K	H^+/OC antiporter	Apical	Cimetidine, procainamide, metformin, fexofenadine, oxaliplatin (Ho and Kim 2005; Koepsell et al. 2007; Matsushima et al. 2009; Moriyama et al. 2008; Terada and Inui 2008)
SLCO family				
SLCO4C1	OATP4C1	ND	Basolateral	Digoxin, ouabain, methotrexate (Dobson and Kell 2008; Hagenbuch and Gui 2008; Hu et al. 2008)
ABC family				
ABCB1	MDR1/P-glycoprotein	primary active	Apical	Vinblastine, vincristine, daunorubicin, doxorubicin, colchicine, docetaxel, paclitaxel, ortataxel, etoposide, imatinib, methotrexate, bisantrene, mitoxantrone, paclitaxel, topotecan, digoxin, digitoxin, celiprolol, talinolol, indinavir, nelfinavir, ritonavir, saquinavir, levofloxacin, grepafloxacin, sparfloxacin, erythromycin, ivermectin, chloroquine, amiodarone, lidocaine, losartan, lovastatin, mibefradil, fexofenadine, terfenadine, carbamazepine, desipramine, loperamide, methadone, morphine, sumatriptan, vecuronium, cyclosporin A, tacrolimus, sirolimus (Dietrich et al. 2003; Hu et al. 2008; Murakami and Takano 2008; Oostendorp et al. 2009; Russel et al. 2002; Sarkadi et al. 2006; Zhou 2008)

ABCC2	MRP2	primary active	Apical	Vinblastine, vincristine, doxorubicin, etoposide, cisplatin, methotrexate, indinavir, ritonavir, saquinavir, grepafloxacin, glutathione conjugates, PAH (Nies and Keppler 2007; Russel et al. 2002; van de Water et al. 2005; Zhou et al. 2008)
ABCC4	MRP4	primary active	Apical	Methotrexate, leucovorin, topotecan, 6-mercaptopurine, 6-thioguanine, adefovir, tenofovir, ceftrizoxime, cefazolin, cefotaxime, cefmetazole, hydrochlorothiazide, furosemide, olmesartan, edaravone glucuronide, PAH (Borst et al. 2007; Russel et al. 2002, 2008; van de Water et al. 2005; Zhou et al. 2008)
ABCG2	BCRP	primary active	Apical	Mitoxantrone, flavopiridol, topotecan, SN-38, camptothecin, methotrexate, imatinib, gefitinib, erlotinib, abacavir, lamivudine, zidovudine, nelfinavir, cerivastatin, pitavastatin, rosuvastatin, glibenclamide, olmesartan, dipyridamole, cimetidine, edaravone sulfate, albendazole sulfoxide, oxfendazole, ciprofloxacin, norfloxacin, ofloxacin, sulfasalazine, nitrofurantoin (Cusatis and Sparreboom 2008; Robey et al. 2009; Sarkadi et al. 2006; van Herwaarden and Schinkel 2006)

PMEG 9-(2-phosphonylmethoxyethyl)guanine, *PMEDAP* 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine, *DC* dicarboxylate, *PAH* p-aminohippuric acid, *SN*-38 active metabolite of irinotecan, *ND* not determined

drugs for the treatment of type 2 diabetes. Genetic polymorphisms of OCT2 have been associated with a decrease in renal metformin clearance (Song et al. 2008). Using metabolomics, tryptophan was recently identified as a specific endogenous substrate of OCT2 related to metformin disposition, and consequently a potential biomarker of genetic variability in transporter activity (Song et al. 2012). Coadministration of cimetidine with metformin has been shown to reduce the renal clearance of metformin, leading to a clinically relevant increase in plasma concentrations (Wang et al. 2008). It has been suggested from in vitro studies that cimetidine is an inhibitor of OCT2; however, because of its relatively low inhibitory constant this seems unlikely at therapeutic plasma concentrations (Lepist and Ray 2012).

OATP4C1 is the only OATP family member expressed in human proximal tubular cells (Obaidat et al. 2012). The transporter is located in the basolateral membrane and substrate specificity is restricted to a few drugs that are mainly excreted by the kidney, i.e., methotrexate, the cardiac glycosides, digoxin, and ouabain, as well as thyroid hormones (Table 10.1). The mechanism by which OATP4C1 translocates drugs across the membrane and the counter ion it exchanges its substrates for are not yet identified. OATP1A2 expression was identified in the apical membrane of distal nephrons, but its role in renal drug handling is unclear (Lee et al. 2005). The renal expression of OATPs is remarkably different in rodents. Except for the ortholog Oatp4c1 in the basolateral membrane, at least three different Oatps are located in the brush border membrane of rodent kidney, none of which are expressed in humans (Sekine et al. 2006).

At the apical membrane of the proximal tubule, the ABC transporters P-glycoprotein, MRP2, MRP4, and BCRP mediate the primary active efflux of drugs. P-glycoprotein is likely involved in the urinary excretion of digoxin and a number of hydrophobic cationic drugs (Masereeuw and Russel 2001, 2012; Zhou 2008). The *ABCB1* gene encoding for P-glycoprotein is highly polymorphic, and a relationship has been suggested with calcineurin inhibitor efficacy and toxicity in renal transplant patients. However, data on the clinical relevance of these polymorphisms are not unequivocal (Cascorbi 2011). Anionic drugs, including glucuronide, glutathione, and sulfate conjugates, formed in the proximal tubular cells or taken up from the circulation, are pumped into urine via MRP2 and MRP4 (van de Water et al. 2005). As compared to MRP2, MRP4 appears to have a higher affinity for small organic anions and its protein expression is approximately fivefold higher (Russel et al. 2008; Smeets et al. 2004). BCRP was only recently identified in the apical membrane of the proximal tubule (Huls et al. 2008). The overlap in substrate specificity with P-glycoprotein and the MRPs suggests its potential involvement in renal drug excretion (Masereeuw and Russel 2012).

The organic cation transporters MATE1, MATE2-K, OCTN1, and OCTN2 mediate the secondary active efflux of cationic drugs across the luminal membrane. The steep outside>in transmembrane H^+ gradient provides a powerful driving force for the MATE transporters. MATE1 is expressed throughout the body, but predominantly in liver and kidneys, whereas MATE2-K is exclusively located in kidney proximal tubules (Nies et al. 2011). Genetic polymorphisms of MATE1 and MATE2-K have been linked to the variability in renal handling of cationic drugs

like metformin and to accumulation of oxaliplatin, causing drug-induced nephrotoxicity (Kajiwara et al. 2009). Cimetidine appears to be a potent inhibitor of both transporters and there is increasing evidence that the inhibition of MATEs rather than OCT2 is a likely mechanism underlying the renal drug–drug interaction of cimetidine with metformin and other cationic drugs (Ito et al. 2012; Lepist and Ray 2012). The carnitine/organic cation transporters, OCTN1 and OCTN2, are also driven by H^+/organic cation antiport or organic cation/organic cation antiport. Their substrate specificity is comparable to the MATEs, and because of their bidirectional mode, OCTNs could also be involved in organic cation reabsorption (Tamai 2013).

PEPT1 and PEPT2 are H^+-coupled peptide symporters that mediate the active reabsorption of antiviral drugs, beta-lactam antibiotics, and angiotensin-converting enzyme inhibitors from the primary urine. They are both expressed in a sequential order along the renal proximal tubule (Brandsch et al. 2008). PEPT2 has the highest affinity and appears to be the major player in the renal reabsorption of peptide-like drugs (Kamal et al. 2008).

OAT4 is only expressed in humans; there exists no ortholog in rodents or other species (Burckhardt and Burckhardt 2011). The transporter is able to operate as a bidirectional asymmetric antiporter mediating the influx and efflux of organic anions. As an influx transporter, OAT4 couples the luminal uptake of endogenous substrates like urate and estrone sulfate to the release of dicarboxylates or hydroxyl ions from the proximal tubular cell. In the efflux mode, anionic drugs are excreted into urine in exchange with luminal Cl^-. The number of drugs accepted by OAT4 seems somewhat smaller than for OAT1 and OAT3 (Burckhardt 2012; Rizwan and Burckhardt 2007).

10.3 Methods to Analyze Renal Drug Transport

This paragraph discusses key methods and new technical developments to study renal drug transport, including discussion of their advantages and disadvantages, which are summarized in Table 10.2.

10.3.1 Mechanistic Understanding Through Molecular Models

The need for robust in vitro assays in preclinical drug development to optimize the pharmacokinetic properties of drug candidates has led to numerous cell-based and membrane vesicle-based assays. Both approaches include transfection of yeast, insect, or mammalian cells with cDNA, using viral vectors, physical methods, or biochemical agents, leading to the functional over-expression of a specific transport protein.

For SLC transporters, uptake assays have been developed by incubating transporter-transfected cells in Petri dishes or multi-well plates with potential substrates.

Table 10.2 Summary of key technologies used to study renal drug transport

	Molecular models	Cell systems	Organotypic systems	Perfused kidney systems	Preclinical
Techniques used	(1) Membrane vesicles (2) Transfected nonpolarized cells (3) Oocyte expression system	(1) Freshly isolated cells (2) Cryopreserved cells (3) Cell cultures in 2-D (4) Transfected polarized cells in 2-D (5) 3-D cell cultures	(1) Killifish renal tubules (2) Kidney-on-chip (3) Kidney slices (4) Isolated (perfused) mammalian tubules	(1) Isolated perfused kidney (2) Nonfiltering perfused kidney (3) Single-pass perfused kidney (4) Stopped-flow capillary microperfusion	(1) Spontaneous mutants (2) Transgenic or knockout animals (3) Physiological-based models
Advantages	(1–3) Substrate specificities/ molecular characterization	(1–5) Vectorial transport (1–3, 5) Suitable for regulatory processes and protein routing	(1, 2, 4) Suitable for vectorial transport, regulatory processes and protein routing (3) Uptake kinetics	(1–4) Accurate determination of overall renal drug clearance	(1–3) Overall drug clearance (3) Includes all functional characteristics

Disadvantages	(1–3) Regulation of transport proteins (almost) not possible	(1–5) Transport rates slow as compared to in vivo (1) Short viability, transporter retraction from plasma membrane (2) Dedifferentiation after immortalization, uptake activity insufficiently conserved during the isolation/cryopreservation process (4) Misrouting, regulatory pathways often absent	(1, 3, 4) Short viability (1) Comparative model (2) Selection of cell types (3) Efflux cannot be measured (3) Lumen often collapses	(1–4) Sophisticated equipment; difficult to distinguish between transport proteins	(3) Molecular data needed as input
Relevant references	Astorga et al. (2011), Cihlar and Ho (2000), El-Sheikh et al. (2007), El-Sheikh et al. (2008c), Hagmann et al. (1999), Ishikawa et al. (2004), Kuze et al. (1999), Mutsaers et al. (2011a), Robey et al. (2011), Shukla et al. (2012), and Soreq and Seidman (1992)	Brown et al. (2008), Jenkinson et al. (2012), Konig et al. (2011), Miller (1992), Mutsaers et al. (2011b), Ryan et al. (1994), Sato et al. (2008), Terlouw et al. (2001), Wilmer et al. (2010), and Windass et al. (2007)	Asthana and Kisaalita (2012), Long et al. (2011), Miller (2002), Oo et al. (2011), Wever et al. (2007), and Wright and Dantzler (2004)	De Kanter et al. (2002), Heemskerk et al. (2007, 2008), Hori et al. (1988), Huh et al. (2012), Jung et al. (2011), Maack (1980), Masereeuw et al. (2003), Tsuruoka et al. (2001), and Ullrich et al. (1984)	Johnson et al. (2010), Jonker et al. (2002), Rowland et al. (2011), Russel et al. (1987), and Zhao et al. (2011)

After termination of cellular uptake, cells should be washed to remove the substrate and after cell lysation the intracellular content can be analyzed. The mammalian vector technology by Invitrogen is often used to over-express SLC proteins for this purpose in nonpolarized cell lines, including African green monkey cells (COS-7) or the Chinese Hamster overay cell line (CHO) (e.g. Astorga et al. 2011; Cihlar and Ho 2000; Kuze et al. 1999). Furthermore, human embryonal kidney cells (HEK293) have proven validity in studying SLC transporters (Han et al. 2010), even in high-throughput optimization assays (Lohmann et al. 2007). However, cell lines will remain heterogeneous after transfection, and to obtain lines stably expressing the transporter of interest, clonal selection has to be performed usually by serial dilution of the clone mixes and followed by propagation of clonal cell lines. These cell lines are also widely commercially available. In addition, expression of SLC transporters in oocytes of *Xenopus laevis* by cRNA injection has shown to be a promising method to elucidate the molecular characteristics of transporters. Important requirements for this technique are that the endogenous transport activity of the oocytes must be low and the assay used to assess transport activity must be sensitive enough to monitor in a few oocytes at least a twofold increase in transport signal above background (Soreq and Seidman 1992). Oocyte systems expressing some transporters are commercially available as well.

Baculovirus-transduced cell lines have proven their suitability, especially for expression of ABC transporters in insect cells or in mammalian cells. Expression in insect cells, such as cells from the moth *Spodoptera frugiperda* (Sf9), is valuable for structural studies as large quantities of purified proteins can be obtained (e.g., Ishikawa et al. 2004; Radanovic et al. 2003). ABC transporter expression in mammalian cells, on the other hand, allows for functional characterization of the transporters (e.g., Hagmann et al. 1999), and evaluating drug interactions (e.g., in studying the effect of nonsteroidal anti-inflammatory drugs with MRP2 and MRP4-mediated methotrexate transport (El-Sheikh et al. 2007) or interactions of uremic toxins on MRP4 and BCRP-mediated transport (Mutsaers et al. 2011a), and for mutational analysis (e.g., of MRP4 El-Sheikh et al. 2008c; Wittgen et al. 2012b). Functional studies with these transporters are particularly well performed in vesicular assays using isolated inside-out crude membrane fractions or membrane vesicles derived from transduced cells. Major advantages of this method are that metabolism is eliminated and that the composition of solutions on both sides of the membrane can be controlled. But the transporter over-expressing cell lines can also be used in whole cell-based studies (Robey et al. 2011). These cell lines can be used for efflux assays as well as for drug accumulation assays in which the difference in absence and presence of a specific inhibitor of the ABC transporter reflects the activity of the efflux pump (Wittgen et al. 2012a). This approach is also valuable for studying kinetics and interactions of lipophilic substrates for which the vesicular transport assays are hampered by technological difficulties. The baculovirus system also proved to be suitable for studying SLC transporter function and interactions in cell-based systems (El-Sheikh et al. 2008b), although these transporters are generally more difficult to over-express and often stable transfections (as described earlier) are necessary to detect significant transport. Despite high transduction efficiencies and controllable batch-to-batch variations by applying the histone deacetylase

inhibitor butyrate to increase protein expression (Shukla et al. 2012), a drawback of the baculovirus system is that expression is transient which hampers studying regulatory aspects of the transporters and their function in disease models.

10.3.2 Proximal Tubule Cell Systems for Transepithelial Transport Determinations

Freshly isolated renal proximal tubule cells are useful in studying overall cellular uptake kinetics and accumulation, but uptake is a hybrid parameter determined by both influx and efflux rates. Overall transport characteristics of isolated cells in suspension seem to resemble basolateral to luminal flux as compared to cells on filters or isolated perfused kidneys, although primary active transporters, like MRP2, were found to be retracted from the plasma membrane (Terlouw et al. 2001).

Primary cultures of cells grown as monolayers on permeable supports (filters) have several technically important advantages and allow studying cellular kinetics (Brown et al. 2008; Windass et al. 2007), but a major obstacle is dedifferentiation resulting in a selective loss of transporter systems, as is shown for renal organic anion uptake (Miller 1992). To overcome these problems, carcinoma cell lines have been characterized and proven to be suitable for studying drug transport, such as the human conditionally immortalized proximal tubule epithelial cell line (ciPTEC) (Wilmer et al. 2010) and HK-2 (Ryan et al. 1994), although the use of the latter cell line in studying drug transport seems rather limited (Jenkinson et al. 2012; Mutsaers et al. 2011b). Furthermore, cell lines have been developed that over-express one or more transport proteins. Polarized cells used for transporter transfection are, among others, Madin-Darby Canine Kindey cells (MDCK) or pig kidney cells (LLC-PK1). For example the double transfected MDCK II cell line, which expresses both hOCT2 and hMATE1, provides a useful model for studying renal vectorial transport (Konig et al. 2011; Sato et al. 2008). Important advantages are that transport mechanisms remain functional upon culturing, allowing the study of vectorial transport and regulation of transport proteins, and the preparation can be maintained for long term use. A major disadvantage of all cell cultures described is that transport rates are rather low as compared to in vivo kinetics.

10.3.3 An Optimal Microenvironment Allows Functional Transport

As proximal tubule cells are highly polarized, maintenance of this polarity is critical for optimal functioning and responsiveness to environmental signals. This is dependent on communication between cells, which include features such as paracrine and autocrine signals but also biomechanic, haptotactic, and chemotactic processes, all influencing cell proliferation, migration, and differentiation.

With respect to transporter activities in more physiological models, signaling information can be important in transepithelial fluxes under normal but also under pathological circumstances. When cultured in 2-D, the functional polarity is only partially retained. Advances in 3-D platforms showed a benefit for tubular epithelial cells to grow in spheroids or tubule-like structures (Asthana and Kisaalita 2012). These platforms contain polymeric scaffolds or hydrogels, both without and with scaffolds to put some restraints on the size of the microtissue formed. One concern is, however, the threshold for oxygen diffusion into the tissue, as hypoxia can result in gene expression perturbation leading to a wide variety of changes in protein levels (Brooks et al. 2007). Most likely also drug transporters will be affected, as differences in expression levels were found in ischemic mouse kidneys (Huls et al. 2006).

More recently, bioreactors have been developed that allow proximal tubule cells to grow on hollow fibers in 3-D configuration under flow and oxygen-rich conditions. With these reactors, both uptake and secretion can be studied in one system. Although drug transport studies have, as yet, not been reported for hollow fiber cultures, clearly different transporter expression levels were determined when cells were cultured in a bioreactor under flow conditions as compared to static cultures (Oo et al. 2011). This emphasizes that the microenvironment indeed might influence proximal tubule cell transport function. The hollow fibers clearly have advantages over isolated renal tubules from different animal species, as these cell cultures are less fragile, can be of human origin, and potentially reduce the number of animals needed for drug testing. Moreover, in mammalian tubules the lumens collapse quickly after isolation, which makes this preparation unsuitable for investigating tubular secretion. Hence, techniques for perfusion of single, isolated tubules have been developed, exhibiting a high viability and allowing determining cellular uptake and tubular secretion rates with high accuracy (Wright and Dantzler 2004), processes which are in general faster in primary tissue as compared to cell cultures. Furthermore, nonmammalian vertebrates such as killifish (*Fundulus heteroclitus*) and zebrafish (*Danio rerio*) proximal tubules as a comparative models are very suitable for studying both uptake and efflux steps of renal tubular excretion (Long et al. 2011; Wever et al. 2007). By using fluorescent substrates and confocal microscopy it was shown that multiple drug transport mechanisms identified in mammalian models are present. With this transporter-based assay system not only substrate characteristics but also (hormonal) regulation of transporter proteins could be investigated (Miller 2002).

10.3.4 Multiple Cell Types for Overall Renal Drug Handling Assessments

The latest developments in 3-D culture technologies concern the microchips and microfluidics approaches to create cell-culture microenvironments for tissue differentiation and reconstitution of the microenvironments of living kidneys by using

two or more cell types (Jung et al. 2011). With these "organs-on-chips," human physiology can be studied in a tissue-specific context and potentially might replace animal studies in drug development (Huh et al. 2012), although a large number of (technical) hurdles need to be taken until a prototype kidney with its multiple cell systems can be mimicked on a chip.

Traditionally, tissue models used for drug transport studies or drug–drug interaction determinations include kidney slices (isolated) perfused kidneys. Kidney slices in studying drug transport was reintroduced in the last decade with new cryopreservation methods that allow an accurate in vitro tool for prediction of in vivo renal drug uptake and metabolism (De Kanter et al. 2002). Disadvantages are that rates of uptake are much lower than those observed in vivo and this tissue appeared to be unsuitable for studying drug efflux. In the 1980s, Ullrich and coworkers contributed significantly to the knowledge on structure-transport relation of renal organic anion and organic cation transport by using in vivo stopped-flow capillary microperfusion studies of rat kidney (Ullrich et al. 1984). The ex vivo isolated perfused kidney allows accurate determination of drug clearance under controlled conditions and in the absence of non-renal effects (Maack 1980). The viability of 3–4 h for both preparations is acceptable and the model is also suitable for studying transport under disease conditions (Heemskerk et al. 2007, 2008). A nonfiltering isolated perfused rat kidney model, with preserved renal perfusate flow and cellular integrity, also permits the study of proximal tubular transport independent of luminal events (Maack 1980). Furthermore, by using a single-pass perfusion system, the different membrane transport rates involved in excretion, viz. passive or facilitated diffusion, carrier-mediated uptake, intracellular accumulation, and secretion, can be determined by indicator dilution (Hori et al. 1988). Perfused kidney has shown its use in studying pharmacokinetics in transporter mutant animals as well (Masereeuw et al. 2003). In addition, an in situ mouse kidney perfusion model has been described, with a carotid artery cannula for measurement of blood pressure and for blood sampling, and cannulated bladder for urine sampling. In this way, blood pressure, renal plasma flow, and renal clearance of drugs can be determined in anesthetized mice (Tsuruoka et al. 2001). While technically challenging, the mouse perfusion model offers the great advantage of using the single and multiple transporter knockout models currently available.

10.3.5 Translational Models

In man, pharmacokinetic studies are usually limited to analysis of plasma disappearance curves and urinary excretion data due to obvious ethical reasons. Therefore, the majority of in vivo transport studies are performed in laboratory animals, such as rats and mice, but larger animals are used as well especially when metabolism has to be taken into account. Various animal models have been developed and/or evaluated to study drug transport in absence or malfunction of a transporter protein. These may be provided by spontaneous mutation of a gene

resulting in disturbances, but also transgenic and gene-knockout manipulations have provided new and potentially powerful approaches for studying the functional and pathologic roles of transporter proteins, as described for example in *Bcrp* knockout mice (Jonker et al. 2002).

Drug excretion in vivo is described best by a physiologically based pharmacokinetic model, which includes all functional characteristics of the kidney that determine the excretion of drugs. These include protein binding, renal plasma flow, urine flow, glomerular filtration, tubular secretion, and cellular retention (Russel et al. 1987). Integration of in vitro findings are required for a better insight in renal drug handling, drug interactions, (hormonal) regulation of drug transport, and interindividual variability. In addition, all individual compartments of biological systems can be incorporated into multi-compartment models by using data empirically obtained from in vitro and animal studies (Zhao et al. 2011).

Novel systems models such as the Simcyp simulator (www.simcyp.com) have been developed to simulate drug pharmacokinetics and pharmacodynamics in virtual populations, with which drug–drug interactions and pharmacokinetic outcomes in clinical populations can be predicted. This platform uses databases that contain human physiological, genetic, and epidemiological information, which can be integrated with in vitro and clinical data to allow predictions (Johnson et al. 2010; Rowland et al. 2011).

10.4 Conclusion

The last decade has witnessed rapid technological progress in the field of transporter research, which has also greatly accelerated the gain of knowledge on renal drug transporters. A wealth of information has been generated about individual transporters by using molecular cloning techniques and functional characterization in over-expression systems, but much remains to be resolved regarding the coordinated action and regulation of the influx and efflux transporters in a proximal tubular cell as an integrated system. Although knockout mice have provided valuable insight into the in vivo role of different renal transporters, these studies need to be interpreted with some caution because of compensatory mechanisms and species-related differences in transporter expression and substrate specificity.

There is still a long way to go before we will be able to make accurate predictions of the renal clearance and exposure of drugs on the basis of the kinetic characteristics of individual transport proteins. Quantitative information on activity, substrate specificity, interindividual variation and abundance of transport proteins, as already available for many drug metabolizing enzymes, is required for physiologically based pharmacokinetic modeling and simulation of drug handling by the kidney. An important step has been made by the recent development of proteomics-based LC-MS/MS methods that enable the successful determination of absolute protein concentration levels of transporters in over-expression systems, proximal tubular cells, and human kidney tissue, which are useful to feed into the computer models

as in vitro-in vivo scaling factors (Ohtsuki et al. 2011). Currently, the lack of availability of a comprehensive in vitro model system of the proximal tubule, as predictive as for example Caco-2 cells are for intestinal transport, is a major limitation. While technically challenging, there is a great deal of promise in 3-D renal proximal tubular cell-culture systems with the potential of reconstructing the complex dynamic interplay among all the different transporters.

Personalized medicine through individualization of drug therapy is an important challenge for the future. The activity of transporter proteins may be influenced by genetic variation, which can be investigated by over-expression of the variants in a cellular system. But to get a picture of the actual impact on renal drug clearance, in vivo studies are required in human subjects with genetic polymorphisms to define the clinical relevance of certain transporters and to refine and validate the in silico models. As compared to the redundancy in renal organic anion transporters, the transporter-mediated renal excretion of cationic drugs seems to be more susceptible to drug–drug interactions and genetic variation. Based on current insights, it is expected that combined genetic polymorphisms in OCT2 and MATE1/MATE2-K variants could have important implications for cationic drug clearance and renal toxicity (Nies et al. 2011).

In summary, important advances have been made in the study of renal drug transporters. Whereas the functional characteristics of individual transporters have been relatively well-defined, there is a great need for comprehensive proximal tubular cell models and improved extrapolation of in vitro data to the clinical situation. Technical developments in molecular biology, tissue engineering, and systems pharmacology will provide new approaches to reach the ultimate goal of accurately predicting renal drug clearance, toxicity, and drug–drug interactions in an individual patient before the drug is actually administered.

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