Relevance of Transporters in Clinical Studies

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Contents

Abstract

It has become clear that drug disposition is not just a result of passive diffusion and metabolizing enzymes. Numerous transporters were identified in recent years to be involved in the absorption, distribution, and excretion of essentially all drugs. While transporters of the solute carrier (SLC) family are mainly involved in the uptake of drugs into cells, ATP-binding cassette (ABC) transporters are responsible for their efflux. Among the more than 420 SLC and 47 ABC transporters, only about 25 seem to be important for the disposition of over-the-counter and prescription drugs. Among these the Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) have identified seven transporters which need to be tested for investigational

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drugs and an additional five transporters that are considered to be important. Two of the seven transporters, the multidrug resistance protein 1 (MDR1) and the breast cancer resistance protein (BCRP), are ABC transporters. The other five, the organic cation transporter 2 (OCT2), the organic anion transporter 1 (OAT1) and 3 (OAT3), and the organic anion transporting polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3), are SLC transporters. If additional transporters become clinically relevant, they may be added by the regulatory agencies to the list or required transporters.

Introduction

Initially, drug disposition, which includes absorption, distribution, metabolism, and excretion, was thought to be mainly the result of passive diffusion and metabolizing enzymes. More recently, however, it became clear that drug transporters play an important role in absorption, distribution, and excretion of drugs and their metabolites (Giacomini et al. 2010; Hillgren et al. 2013). Most drugs are administered orally and have to cross initially a barrier built of polarized epithelial cells in the intestine, the enterocytes (Fig. 1a) (Drozdzik et al. 2014). Once in the portal blood, drugs first reach the liver where they can be removed by what is called first-pass metabolism, a combination of drug uptake into hepatocytes (Stieger and Hagenbuch 2016), metabolism by drug-metabolizing enzymes, and excretion into bile (Fig. 1b) (Pfeifer et al. 2014). Drugs that are not (completely) cleared by this first-pass effect will reach the systemic circulation and will be carried to their target organ where they bind to a receptor or are taken up into the cells to affect their drug target. To act in the brain, drugs also have to cross the blood-brain barrier, another polarized epithelial layer that contains drug uptake and efflux transporters in the plasma membranes facing the blood or the brain (Fig. 1c) (Abdullahi et al. 2017). Eventually, most drugs not cleared by the liver will be excreted via the kidneys by either filtration or secretion. Renal secretion also involves the transport of the drug across the basolateral membrane into the cell and then across the brush-border membrane into the tubule (Fig. 1d) (Liu et al. 2016).

In 2010 the International Transporter Consortium published the first recommendations on which transporters it considered to be important for drug absorption and disposition and extended this list in 2013 (Giacomini et al. 2010; Hillgren et al. 2013). In the meantime, the Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) have published guidelines for the pharmaceutical industry listing which drug transporters need to be tested when evaluating an investigational drug (Table 1).

This chapter will review the clinical importance of drug uptake and efflux transporters with an emphasis on those transporters that are highlighted by the regulatory agencies for investigational drugs.

Major Drug Transporters with Clinical Relevance

About 10% of all human genes are transporter related. Among these are ATP-binding cassette (ABC) transporters and solute carrier (SLC) transporters. ABC transporters are primary active efflux transporters that utilize the energy derived from the hydrolysis of ATP to transport their substrates against electrochemical concentration gradients. There are 7 ABC families that contain 47 members and 3 pseudogenes (https://www.genenames.org/cgi-bin/genefamilies/set/417). All the important drug efflux transporters are classified within three ABC families: in family ABCB there are the multidrug resistance protein 1 (MDR1; gene symbol *ABCB1*) and the bile salt export pump (BSEP; *ABCB11*); family ABCC contains the multidrug resistance-associated protein 2 (MRP2; *ABCC1*); and in family ABCG there is the breast cancer resistance protein (BCRP; *ABCG2*) (Table 1 and Fig. 1). The SLC transporters are in general uptake transporters, but some may also mediate the efflux of substrates out of the cell. SCL transporters can be secondary

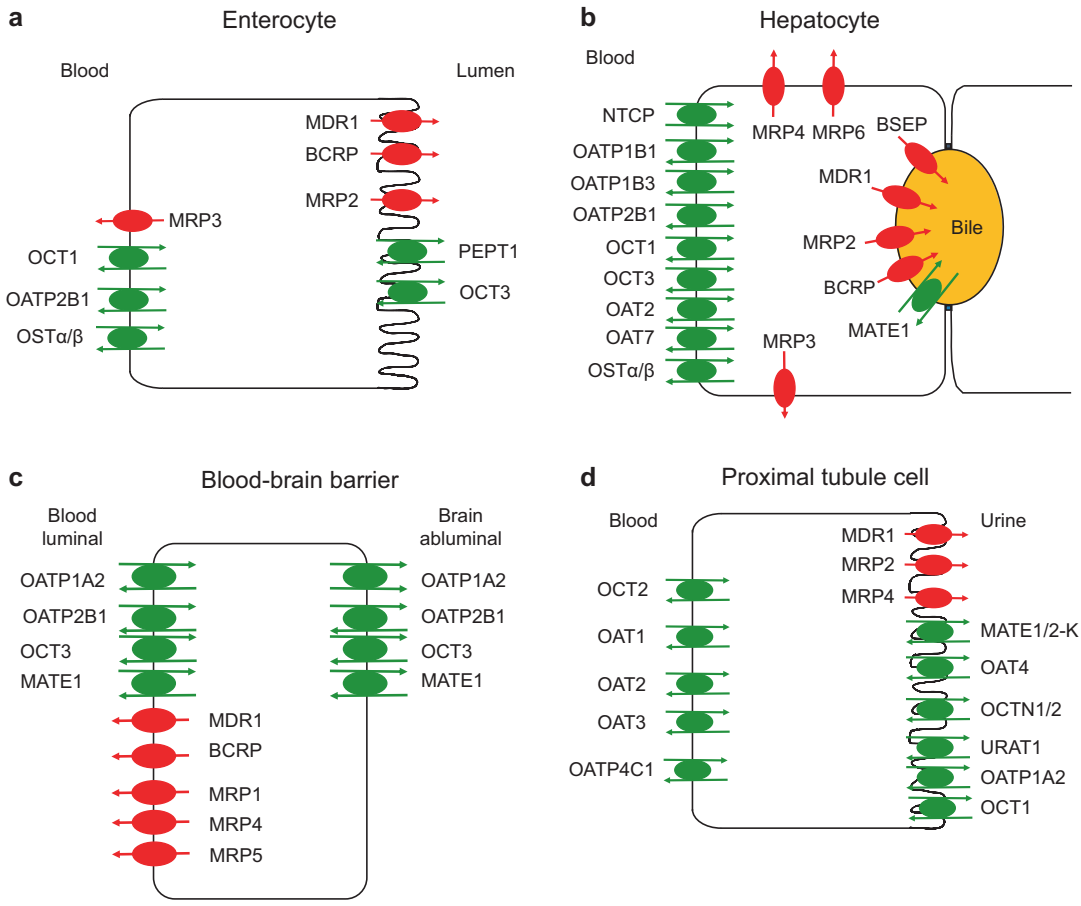

Fig. 1 Schematic localization of transporters involved in the disposition of drugs in the small intestine (**a**), hepatocytes (**b**), the blood-brain barrier (**c**), and the proximal tubule of the kidney (**d**)

active, transporting their substrates against an electrochemical gradient, or facilitated transporters, transporting their substrates along an electrochemical gradient. There are currently 65 SLC families that contain over 420 members and more than 20 pseudogenes (http://slc.bioparadigms.org/). All of the important drug uptake transporters that are recommended or considered by the regulatory agencies are classified within three SLC families: in the SLC22 family, there are the organic cation transporter 1 (OCT1; *SLC22A1*) and OCT2 (*SLC22A2*), as well as the organic anion transporter 1 (OAT1; *SLC22A6*) and OAT3 (*SLC22A8*); the SLC47 family consists of the multidrug and toxin extrusion protein 1 (MATE1; *SLC47A1*) and MATE2 (*SLC47A2*); and in the SLCO family, there are the organic anion transporting polypeptide 1B1 (OATP1B1; *SLCO1B1*) and OATP1B3 (*SLCO1B3*) (Table 1 and Fig. 1).

ATP-Binding Cassette (ABC) Transporters

The Multidrug Resistance Protein 1 (MDR1)

The gene for the human multidrug resistance protein 1 (MDR1; gene symbol *ABCB1*) was originally identified from multidrug-resistant carcinoma cells in 1986 (Roninson et al. 1986) as the gene encoding P-glycoprotein (Ueda et al. 1986), a glycoprotein of 170 kDa that was originally

Table 1 Human transporters involved in the uptake and efflux of endo- and xenobiotics

Protein name	Gene symbol	Recommended or considered by regulatory agencies
MDR1	*ABCB1*	FDA, EMA, PMDA
BSEP	*ABCB11*	Considered by FDA, EMA, PMDA
MRP1	*ABCC1*	
MRP2	*ABCC2*	Considered by PMDA
MRP3	*ABCC3*	
MRP4	*ABCC4*	
BCRP	*ABCG2*	FDA, EMA, PMDA
NTCP	*SLC10A1*	
ASBT	*SLC10A2*	
PEPT1	*SLC15A1*	
PEPT2	*SLC15A2*	
OCT1	*SLC22A1*	Considered by EMA and PMDA
OCT2	*SLC22A2*	FDA, EMA, PMDA
OAT1	*SLC22A6*	FDA, EMA, PMDA
OAT3	*SLC22A8*	FDA, EMA, PMDA
ENT1	*SLC29A1*	
ENT2	*SLC29A2*	
MATE1	*SLC47A1*	PMDA, considered by FDA and EMA
MATE2	*SLC47A2*	PMDA, considered by FDA and EMA
OSTα	*SLC51A*	
OSTβ	*SLC51B*	
OATP1A2	*SLCO1A2*	
OATP1B1	*SLCO1B1*	FDA, EMA, PMDA
OATP1B3	*SLCO1B3*	FDA, EMA, PMDA
OATP2B1	*SLCO2B1*	

Transporters are classified either as ATP-binding cassette (*ABC*) transporters or as solute carrier (*SLC*) transporters
EMA European Medicines Agency, *FDA* Food and Drug Administration, *PMDA* Pharmaceuticals and Medical Devices Agency

discovered in 1976 (Juliano and Ling 1976). Since then it has become clear that MDR1 is also expressed in normal cells of the body, in particular in epithelial cells (Schinkel and Jonker 2003).

In enterocytes, MDR1 is expressed in the apical membrane (Fig. 1a) where it protects the body from toxic xenobiotics. Pumping its substrates out of the cells, MDR1 can restrict substrate uptake and thus affect the bioavailability of numerous drugs. In general, MDR1 substrates are hydrophobic and amphipathic. They can be between 200 and over 4000 Da and are mostly uncharged or positively charged. They include numerous anticancer agents, antimicrobials, several HIV protease inhibitors, immunosuppressant drugs like cyclosporine A, and various cardiovascular drugs including calcium channel blockers and digoxin (Schinkel and Jonker 2003; Terada and Hira 2015; Saidijam et al. 2018). See Lund et al. (2017) for a compilation of drugs that modulate MDR1 activity and can lead to adverse drug-drug interactions. Given the broad substrate spectrum and the fact that several of these drugs often are prescribed together, there is a real possibility for adverse drug-drug interactions either because of inhibition or induction of MDR1. The quinidine-digoxin drug-drug interaction, e.g., could be explained by inhibition of MDR1-mediated efflux of digoxin from enterocytes by quinidine, resulting in increased absorption and thus increased plasma concentration of digoxin. Similar effects due to MDR1 inhibition were reported for amiodarone, dronedarone, and propafenone, three antiarrhythmics that are likely prescribed to patients that also take oral digoxin (Wessler et al. 2013). In contrast, rifampicin administration

induced the expression of MDR1 in the duodenum and decreased absorption and thus bioavailability of digoxin (Wessler et al. 2013).

MDR1 is also a key player in the protection of the brain from toxic xenobiotics. It is expressed in the luminal membrane (Fig. 1c) where it prevents the uptake of its substrates from blood into the endothelial cells of the blood-brain barrier (Schinkel and Jonker 2003). As a consequence, the brain penetration of numerous drugs is rather low but can be increased in the presence of MDR1 inhibitors or in the absence of a functional MDR1. This was nicely demonstrated using *mdr1a (−/−)* mice (Schinkel et al. 1996). These mice are lacking the mouse homolog of the human MDR1 protein, and therefore they accumulated drugs in the brain 10- to 100-fold above the levels of their wild-type controls (Schinkel and Jonker 2003).

In the liver (Fig. 1b), MDR1 is expressed at the canalicular membrane of hepatocytes and can impact the excretion of mainly cationic xenobiotics that have been taken up into hepatocytes via OCT1 and OCT3. In proximal tubule cells (Fig. 1d), MDR1 is expressed at the brush-border membrane and can affect the secretion of substrates that have been taken up into the tubular cells via OCT2.

Given that mutations or polymorphisms can lead to an inactive (or overactive) MDR1, it is also important to search for and characterize such gene variants. At least 390 sequence variants have been identified in the coding region of the *ABCB1* gene, but the majority of these variants only occur at low frequencies (<1%). Wolking et al. (2015) reviewed the literature and concluded that multiple studies with the three most frequent polymorphisms c.1236C > T (G412G), c.2677G > T/A (A893S/T), and c.3435C > T (I1145I) with different drug substrates have been performed. Some of them have been associated with effects on drug disposition, response, and toxicity, but overall the findings were conflicting and had limited clinical implications. These findings indicate that in the case of MDR1, inhibition due to drug-drug interactions and induction of expression are predominant and clinically more relevant than polymorphisms.

Because MDR1 can affect the oral bioavailability, the distribution, and the excretion of drugs, the regulatory agencies expect that investigational drugs are tested in vitro whether they are substrates of MDR1. Class I drugs (i.e., highly soluble and highly permeable) according to the biopharmaceutical classification system (Amidon et al. 1995) only need to be tested as MDR1 substrates if there are potential safety concerns regarding distribution into the brain or the kidneys. The in vitro test should either be performed by measuring the transepithelial flux, e.g., using Caco-2 cell layers or by inhibiting the transepithelial flux with at least one known MDR1 inhibitor at a concentration of more than ten times its K_i value. An efflux ratio of at least two or inhibition of the efflux ratio by more than 50% by the known inhibitor indicates that the investigational drug is an MDR1 substrate. If the drug is a substrate, an in vivo study might be necessary based on the safety margin of the drug, on its therapeutic index, and on the fact that a likely co-medication is an MDR1 inhibitor (Lund et al. 2017).

The Bile Salt Export Pump (BSEP)

The human bile salt export pump (BSEP; gene symbol *ABCB11*), a glycoprotein of 170 kDa, is expressed almost exclusively in the canalicular membrane of hepatocytes (Fig. 1b) where it is responsible for the export of mainly conjugated bile acids (Stieger 2011). Normal BSEP function is required because mutations in the *ABCB11* gene that result in either reduced or completely absent BSEP function result in cholestatic liver disease that can be mild (benign recurrent intrahepatic cholestasis type 2) or life threatening (progressive familial intrahepatic cholestasis type 2) (Stieger 2011). Numerous drugs and xenobiotics inhibit BSEP function in vitro, and a correlation of their IC_{50} values with reported hepatotoxicity revealed that if the IC_{50} value is below 25 μM, chances for drug-induced liver injury increased (Morgan et al. 2010; Stieger 2011). However, no correlation between maximal free plasma concentration and BSEP inhibition or liver injury could be

established. This is likely due to the fact that the uptake step for these drugs, transport across the basolateral membrane into hepatocytes, is rate limiting (Hillgren et al. 2013).

Sequencing of the *ABCB11* gene revealed numerous mutations and polymorphisms. For example, the c.1331T>C variant leading to an alanine at position 444 instead of a valine (V444A) is associated with low BSEP expression (Stieger 2011) and was overrepresented in a population with cholestatic liver disease, suggesting that it can contribute to or predispose for liver disease (Droge et al. 2017).

Although not required by the regulatory agencies, the International Transporter Consortium recommends that BSEP function should be tested under certain conditions. The European Medicines Agency states that BSEP inhibitory potential should be considered in particular if plasma bile acid levels are increased in animal studies. Furthermore, if a BSEP inhibitor is given to humans, their serum bile acid levels should be monitored along with liver serum markers because of the potential of drug-induced liver injury (Hillgren et al. 2013).

The Multidrug Resistance-Associated Protein 2 (MRP2)

The multidrug resistance-associated protein 2 (MRP2; gene symbol *ABCC2*) is a 190 kDa glycoprotein expressed in the canalicular membrane of hepatocytes, as well as in the apical membrane of enterocytes and proximal tubular cells (Fig. 1). It was originally identified as a canalicular multispecific organic anion transporter mediating the efflux of conjugated anionic substrates including bilirubin glucuronides and numerous drug conjugates into bile. Its functional absence leads in humans to the Dubin-Johnson syndrome, a rare benign disorder characterized by conjugated hyperbilirubinemia (Schinkel and Jonker 2003). Numerous studies have characterized the role MRP2 plays in the hepatobiliary excretion of drugs, and with its strategic localization in the apical membrane of enterocytes, MRP2 and its inhibition could affect the bioavailability of its drug substrates. Furthermore, it also can contribute directly to the renal excretion of drugs. It seems that both MDR1 and MRP2 play an important role in protecting the human body from potentially toxic xenobiotics.

Given its broad substrate specificity, drug-drug interactions seem possible. However, only a few of these interactions have been described so far. In a recent screening of 124 natural compounds, only 3.2% were inhibitors of MRP2, while the breast cancer resistance protein (BCRP) was inhibited by 36% of the compounds (Sjostedt et al. 2017). Besides inhibitors also stimulators of MRP2 have been characterized in vitro, but so far only a few studies have investigated the stimulatory effect on MRP2 in vivo, and it could be verified in a rat model (Heredi-Szabo et al. 2009).

Numerous genetic polymorphisms have been identified in the *ABCC2* gene, but only a few lead to decreased MRP2 function, and conjugated hyperbilirubinemia is a possible consequence. Because hyperbilirubinemia could be a sign of hepatotoxicity, the International Transporter Consortium recommends that in cases of drug-induced hyperbilirubinemia, inhibition of MRP2 should be tested (Hillgren et al. 2013). Furthermore, the Japanese Pharmaceuticals and Medical Devices Agency suggests that inhibition of MRP2 could lead to increased drug concentrations in hepatocytes or drug-induced increases in plasma concentrations of endogenous compounds.

The Breast Cancer Resistance Protein (BCRP)

The breast cancer resistance protein (BCRP; gene symbol *ABCG2*) is an ABC half-transporter of 75 kDa that probably functions as a dimer. It was originally identified in the multidrug-resistant human breast cancer MCG-7 cell line. Initial characterization demonstrated that expression of BCRP conferred resistance to several anticancer agents (Saidijam et al. 2018). Later studies discovered that similar to MDR1, BCRP is expressed in the apical membrane of many epithelia including the enterocytes, hepatocytes, and endothelial cells of the blood-brain barrier

(Fig. 1) and protects the organism from numerous xenobiotics (Terada and Hira 2015).

Functional characterization revealed that besides anticancer agents, BCRP transports numerous drugs from many different classes including antivirals, antibiotics, tyrosine kinase inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs), and statins (Lee et al. 2015). Although BCRP transports such a wide variety of drugs, drug-drug interactions exclusively due to BCRP are rare, except for limiting oral absorption in the intestine, because of an overlap in substrate specificity with other transporters and drug-metabolizing enzymes. For example, curcumin, a natural polyphenol and the main curcuminoid of turmeric, increased AUC of the NSAID sulfasalazine in healthy volunteers between 2- and 3.2-fold (Lee et al. 2015). Similarly, the AUC of rosuvastatin increased in healthy volunteers in the presence of the immunosuppressant cyclosporine A or the protease inhibitors tipranavir and ritonavir. A suggested mechanism includes inhibition of uptake into hepatocytes via OATP1B1 (see below) and increased absorption due to BCRP inhibition in the intestine (Lee et al. 2015).

BCRP expressed at the blood-brain barrier, together with MDR1, protects the brain from potentially toxic xenobiotics. While this is good for the normal function of the brain, it also limits the brain penetration of drugs that have their target in the brain. One example is imatinib mesylate, for which there is some in vitro evidence that it could be used to treat malignant gliomas. However, a clinical study showed that imatinib mesylate had minimal activity in malignant gliomas, probably because it is a substrate of BCRP (Urquhart and Kim 2009). Therefore, inhibitors of BCRP, and potentially dual BCRP/MDR1 inhibitors, could be useful tools to increase drug delivery to the brain.

More than 80 polymorphisms have been documented in the *ABCG2* gene. However, most of these are rare and found in less than 1% of the population. The most frequent polymorphism, c.421C>A, results in a lysine at position 141 instead of a glutamine (Q141K). This mutation leads to a protein that is less stable than the wild-type and results in a reduced BCRP function, due to reduced plasma membrane expression levels. The allele frequency of this polymorphism is about 5–10% in Caucasians and African-Americans but between 30% and 60% in East Asians (Hira and Terada 2018). In addition, this polymorphism has been identified in a genome-wide association study as being associated with increased serum urate concentrations and an increased risk of gout. This demonstrates another important role BCRP plays in the elimination of uric acid in the intestine (Cleophas et al. 2017).

Because BCRP has the potential to affect the oral bioavailability, the tissue distribution, and the hepatic excretion of drugs, the regulatory agencies treat BCRP similar as MDR1 and expect that investigational drugs are tested in vitro whether they are substrates of BCRP following the same principles as outlined for MDR1 above. Like for MDR1, class I drugs according to the biopharmaceutical classification system are excluded.

Solute Carrier (SLC) Transporters

The Organic Cation Transporters (OCTs) of the SLC22 Family

Organic Cation Transporter 1 (OCT1)

The organic cation transporter 1 (OCT1; gene symbol *SLC22A1*) is a glycoprotein of approximately 70 kDa expressed mainly at the sinusoidal or basolateral membrane of human hepatocytes (Fig. 1b). In addition, OCT1 is expressed at the basolateral membrane of human enterocytes (Fig. 1a), at the apical membrane of proximal tubule cells (Fig. 1d), and in several additional tissues including the lung, heart, skeletal muscle, brain, mammary and adrenal gland, eye, adipose tissue, and immune cells (Koepsell 2013).

OCT1 transports a wide variety of substrates that in general have a molecular weight of less than 500 Da, are mainly hydrophobic, and carry a positive charge. Common model substrates for in vitro studies are 1-methyl-4-phenylpyridinium (MPP^+), *N*-methylquinine, and tetraethylammonium (TEA) (Koepsell

2013). Besides these model substrates, numerous endogenous cationic substrates including the neurotransmitters acetylcholine, dopamine, norepinephrine, and serotonin are transported by OCT1. Among the drug substrates are antiarrhythmics, antibiotics, anticholinergics, the antidiabetic metformin, antihypertensives, anticancer agents, antivirals, β2-agonists, diuretics, and H2-antagonists (Nies et al. 2011). The importance of OCT1 for metformin uptake into its target cells, human hepatocytes, was demonstrated when in healthy volunteers metformin was co-administered with verapamil, an OCT1 inhibitor. After co-administration with verapamil, metformin did not reduce maximal blood glucose concentrations to the same degree as when the volunteers only received metformin (Patel et al. 2016). In contrast, metformin exhibited a larger reduction in blood glucose levels after healthy volunteers were treated with rifampicin, an agonist of the pregnane X receptor (PXR). As a result OCT1 mRNA levels were increased about four-fold in peripheral blood cells. This study suggests that increased expression of OCT1 resulted in increased hepatic uptake and activity of metformin (Patel et al. 2016).

Numerous single nucleotide polymorphisms were identified in the *SLC22A1* gene, several of which affect metformin efficacy. Reduced function polymorphisms c.181C>T (R81C), c.1201G>A (G401S), c.1260GAT>del (420del), and c.1393G>A (G465R) resulted in higher metformin AUC, higher maximal plasma concentrations, and reduced glucose-lowering effects during oral glucose tolerance testing (Wagner et al. 2016). These findings are consistent with reduced uptake of metformin into hepatocytes. OCT1 is not only important for metformin uptake but mediates the uptake of many additional clinical important drugs. For example, lamivudine, a drug used to treat HIV infection and a substrate of OCT1, was transported less efficiently by the above polymorphic variants, and carriers of these polymorphisms would have lower drug efficiency.

The International Transporter Consortium has listed OCT1 as a clinical relevant transporter because OCT1 activity positively correlates with how patients with chronic myeloid leukemia respond to imatinib and because OCT1 seems to be involved in the interindividual response to metformin (Giacomini et al. 2010). However, so far the regulatory agencies do not require OCT1 to be tested for drug interactions. The European Medicines Agency and the Japanese Pharmaceuticals and Medical Devices Agency consider to add OCT1 to the required drug transporters to be tested in the future.

Organic Cation Transporter 2 (OCT2)

The organic cation transporter 2 (OCT2; gene symbol *SLC22A2*) is a glycoprotein of 555 amino acids and is mainly expressed at the basolateral membrane of proximal tubule cells (Fig. 1d) (Koepsell 2013). There it plays a crucial role in the secretion of organic cations by mediating the first step, uptake across the basolateral membrane into the tubular cells, before these cations are excreted across the brush-border membranes by MATE1 and MATE2K (see below). Additional tissues with minor OCT2 expression are the small intestine, lung, placenta, thymus, brain, and inner ear. Similar to OCT1, also OCT2 has a broad substrate specificity and transports numerous OCT1 substrates but also some distinct compounds (Nies et al. 2011). Model OCT2 substrates include 1-methyl-4-phenylpyridinium (MPP^+); tetraethylammonium (TEA); endogenous monoamines like norepinephrine, dopamine, and serotonin; the antineoplastic drug oxaliplatin; the antiviral lamivudine; the antidiabetic drug metformin; and the antihypertensive atenolol (Yin and Wang 2016).

The combined uptake by OCT2 across the basolateral membrane and the secretion by MATE1/2-K across the brush-border membrane are crucial functions for the renal elimination of metformin. Several drug-drug interactions at OCT2 affect renal metformin secretion. Cimetidine, a histamine H_2-receptor antagonist, is well known to reduce renal clearance of metformin. Early studies in healthy volunteers suggested that the OCT2 substrate and inhibitor cimetidine would inhibit OCT2-mediated uptake of metformin into tubular cells and thus reduce renal clearance (Yin and Wang 2016). Similarly, the antiviral

dolutegravir, when co-administered with metformin, increased metformin AUC by 2.5-fold. Based on in vitro studies where dolutegravir was identified as a weak OCT2 inhibitor, its effect is probably only partially due to OCT2 inhibition (Yin and Wang 2016). The anticancer drug cisplatin leads to dose-limiting nephrotoxicity because the kidney accumulates this drug to a higher degree than other organs. In vitro studies demonstrated that cisplatin is a good substrate of OCT2. In addition, cancer patients with the polymorphism c.808G>T (S270A) which results in lower OCT2-mediated uptake had reduced cisplatin-induced nephrotoxicity (Yin and Wang 2016). Thus, selective OCT2 inhibitors might be useful in protecting cancer patients from cisplatin toxicity.

Several polymorphisms have been identified in the *SLC22A2* gene with four of the non-synonymous variants showing ethnic-specific allele frequencies of more than 1%. These polymorphisms are c.495G>A (M165I), c.808G>T (A270S), c.1198C>T (R400C), and c.1294A>C (K432Q) (Fujita et al. 2006). When these four variants where tested in vitro using the *Xenopus laevis* oocyte expression system, all four variants were able to transport MPP^+, but differences in kinetics and in inhibition studies were observed, suggesting substrate-dependent effects. Such substrate-dependent inhibition was further characterized by comparing IC_{50} values obtained for the inhibition of the fluorescent substrate, *N*,*N*,*N*-trimethyl-2-[methyl(7-nitrobenzo[c][l,2,5] oxadiazol-4-yl)amino]ethanaminium iodide (NBD-MTMA), of MPP^+ and of metformin (Belzer et al. 2013). The results demonstrated that inhibition of OCT2-mediated metformin transport was about ten times more effective than for OCT2-mediated MPP^+ uptake. Thus, when drug-drug interactions at OCT2 are investigated, it might be more predictive if more than one transport substrate is used and ideally the most likely co-medication is included.

The regulatory agencies recommend that investigational drugs with significant renal secretion (active secretion of parent drug by the kidney is ≥25%) should be tested as substrates for OCT2. If the in vitro results show that the investigational drug is an OCT2 substrate (transport of at least twofold above the negative control) and an OCT2 inhibitor, i.e., inhibits OCT2-mediated transport of the investigational drug by ≥50% at a concentration of at least ten times the K_i value, in vivo studies might be necessary (Giacomini et al. 2010; Hillgren et al. 2013).

The Organic Anion Transporters (OATs) of the SLC22 Family

Organic Anion Transporter 1 (OAT1) and 3 (OAT3)

The organic anion transporter 1 (OAT1, gene symbol *SLC22A6*) is a glycoprotein of 550 amino acids and is mainly expressed at the basolateral membrane of the proximal tubule cells (Fig. 1d) (Koepsell 2013). It plays an important role in the renal secretion of numerous organic anions. OAT1 seems to work as an organic anion/α-ketoglutarate exchanger, exchanging the intracellular α-ketoglutarate for mainly hydrophilic and small (less than 300 kDa) organic anion. Besides various endogenous compounds including different dicarboxylates and prostaglandins, OAT1 mediates the basolateral uptake of numerous drugs such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, antibiotics, antivirals, histamine H2-receptor antagonists, and NSAIDs (Burckhardt and Burckhardt 2011). In in vitro assays, the model organic anion p-aminohippurate (PAH) is frequently used to characterize OAT1. The prototypical inhibitor of OAT1 is probenecid, although it is not an OAT1-selective inhibitor. Besides probenecid several NSAIDs inhibit OAT1-mediated transport (Burckhardt and Burckhardt 2011).

The organic anion transporter 3 (OAT3, gene symbol *SLC22A8*) is a 542-amino acid, and similar to OAT1, it is mainly expressed at the basolateral membrane of the proximal tubule cells (Fig. 1d) (Koepsell 2013). Together with OAT1 it is involved in the renal secretion of various endogenous and exogenous organic anions. Like OAT1, OAT3 seems to work as an organic anion/α-ketoglutarate exchanger. Thus,

both OATs are expressed in the same basolateral membrane and use the same mode of transport. Similarly, the substrate specificities of the two transporters overlap but are not identical. While OAT1 transports mainly small and hydrophilic organic anions, OAT3 substrates are in general larger and more hydrophobic. Endogenous OAT3 substrates include bile acids like cholate and taurocholate, sulfated hormones like dehydroepiandrosterone sulfate and estrone-3-sulfate, prostaglandins, and urate. In addition, OAT3 transports numerous drugs including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, antibiotics, histamine H2-receptor antagonists, and several statins (Burckhardt and Burckhardt 2011). In in vitro assays, the model organic anion used to characterize OAT3 function is estrone-3-sulfate. Probenecid is also an inhibitor of OAT3. In addition, NSAIDs, cimetidine, bumetanide, and some dicarboxylates were reported to inhibit OAT3.

Because both, OAT1 and OAT3, handle a broad range of substrates, several drug-drug interactions have been described. The organic anion transport inhibitor probenecid inhibits both OAT1 and OAT3. Probenecid is a uricosuric drug which inhibits the reabsorption of uric acid in the tubules and is used to treat hyperuricemia associated with gout. Probenecid can also be used to prolong penicillin serum levels because both OAT1 and OAT3 can transport penicillin and are involved in the secretion of this β-lactam antibiotic. However, based on in vitro data, it seems that the major effect is due to inhibition of OAT3, while the effect on cephalosporins is probably due to inhibition of both OATs (Burckhardt and Burckhardt 2011). This drug-drug interaction actually is used as a beneficial side effect of the drug and not an adverse effect. Similarly, probenecid is used to prevent nephrotoxicity of cidofovir, an antiviral drug used to treat cytomegalovirus-induced eye infections in people with AIDS. The underlying mechanism is inhibition of OAT1-mediated accumulation of cidofovir in the proximal tubule cells (Yin and Wang 2016).

In the *SLC22A6* gene encoding OAT1 and the *SLC22A8* gene encoding OAT3, only very few amino acid changing polymorphisms have been identified. For OAT1, the polymorphism c.149G>A (R50H) resulted in increased affinities of the mutated protein for adefovir, cidofovir, and tenofovir. This might lead to increased nephrotoxicity for patients carrying this polymorphism (Burckhardt and Burckhardt 2011). For OAT3, the polymorphism c.913A>T (I305F) which is found at a frequency of 3.5% in Asian-Americans showed reduced estrone-3-sulfate transport compared to the wild-type OAT3 (Burckhardt and Burckhardt 2011). In a recent report, it was shown that Asians with this variant had reduced renal secretion and clearance of the cephalosporin antibiotic cefotaxime (Yee et al. 2013). All of the other identified reduced-function variants are found at less than 3% allele frequency. Thus, the R50H mutation in OAT1 and the I305F mutation in OAT3 could potentially impact renal drug elimination and increase drug concentrations in subjects carrying these mutations.

Regarding regulatory agencies, OAT1 and OAT3 are treated similar as OCT2 because they also play a role in renal secretion of numerous drugs. Thus, the recommendations described above for OCT2 are the same for OAT1 and OAT3 (Giacomini et al. 2010; Hillgren et al. 2013).

The Multidrug and Toxin Extrusion (MATE) Proteins of the SLC47 Family

Multidrug and Toxin Extrusion 1 (MATE1) and 2 (MATE2)

The SLC47 family contains two genes, *SLC47A1* and *SLC47A2* encoding multidrug and toxin extrusion 1 (MATE1) and MATE2 as well as the splice variant MATE2-K. MATE1 is composed of 570 amino acids, MATE2 has 602 amino acids, and the splice variant MATE2-K which is missing part of exon 7 has 566 amino acids. MATE1 is expressed at the apical membrane of the proximal and distal tubule and at the canalicular membrane of hepatocytes. MATE2-K is also expressed in the apical membrane of the proximal tubule, while MATE2 mRNA was not detected in the kidney. Both MATE1 and MATE2-K are involved in the tubular secretion of organic

cations that were transported into tubular cells by OCT2. A potentially similar mechanism is proposed for hepatocytes where the basolateral OCT1 takes up cationic substrates into the cells and the canalicular MATE1 secretes them into bile. The transport mechanism for MATE-mediated secretion is H^+-coupled electroneutral organic cation exchange (Motohashi and Inui 2013). Substrates transported by MATE1/2-K include well-known OCT substrates including TEA, MPP^+, cimetidine, metformin, guanidine, procainamide, quinine, topotecan, cisplatin, oxaliplatin, but also some anionic compounds like estrone-3-sulfate, acyclovir, and ganciclovir. While some of these compounds including TEA, MPP^+, and quinidine had similar affinities for both, MATE1 and MATE2-K, affinities for choline and cimetidine were very different for the two transporters, suggesting that they are multispecific transporters with overlapping but also distinct substrate specificities.

Initially drug interactions with metformin, such as the above-described cimetidine-metformin interaction, were explained as cimetidine inhibition of OCT2-mediated metformin uptake across the basolateral membrane. However, in vitro studies revealed that cimetidine is a much stronger inhibitor of MATE1/2-K than of OCT2 with a K_i value more than 20-fold lower, suggesting that the main inhibitory effect is at the secretory step mediated by MATE1/2-K (Motohashi and Inui 2013). Pyrimethamine, an antiparasitic compound, is a selective inhibitor of MATE1/2-K. When co-administered with metformin, increased metformin AUC and decreased renal clearance of metformin were reported, demonstrating that inhibition of MAT1/2-K could lead to clinically relevant drug-drug interactions (Yin and Wang 2016).

The genetic variant c.-66T>C in the 5′UTR and the intronic c.922-158G>A in the *SLC47A1* gene are associated with a higher glucose-lowering effect of metformin, suggesting that they result in lower expression levels MATE1. The variant c.-130G>A in the 5′UTR of the *SLC47A2* gene has been associated with a decreased glucose-lowering effect of metformin (Staud et al. 2013). So far no non-synonymous polymorphisms in MATE1/2-K have been reported.

Because of observed drug-drug interactions and the potential for adverse effects of new drugs, the regulatory agencies recommend the same procedures for MATE1/2-K as for OCT2 and OAT1/OAT3 described above (Giacomini et al. 2010; Hillgren et al. 2013).

The Organic Anion Transporting Polypeptides (OATPs) of the SLCO Family

Organic Anion Transporting Polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3)

The organic anion transporting polypeptide 1B1 (OATP1B1, gene symbol *SLCO1B1*) is a glycoprotein of 691 amino acids with a molecular weight of about 85 kDa. It is exclusively and evenly expressed at the basolateral (or sinusoidal) membrane of human hepatocytes throughout the liver lobule. OATP1B3 (*SLCO1B3*) is also a glycoprotein with 702 amino acids and a molecular weight of about 120 kDa. In the liver, OATP1B3 is expressed at the basolateral membrane mainly around the central vein with less expression toward the portal vein (Hagenbuch and Stieger 2013). Besides the liver, OATP1B3 has also been documented in several cancers, but it seems that outside the liver the cancer-type OATP1B3 is mainly expressed, which is missing the N-terminal 28 amino acids and is hardly expressed at the plasma membrane. As a consequence its transport activity is strongly reduced (Chun et al. 2017).

OATP1B1 and OATP1B3 have a broad and partially overlapping substrate specificity. They transport various endogenous compounds including bile acids, bilirubin and its conjugates, thyroid hormones, and several steroid conjugates (Hagenbuch and Stieger 2013). Besides these endogenous substrates, both OATPs also transport numerous drugs including statins, antihypertensives, antibiotics, and anticancer agents (Roth et al. 2012). Given that the two proteins share 80% amino acid identity, it is not astonishing that they share most of the substrates. However, there are

some substrates that are specifically transported only or mainly by one of the two liver OATPs. Estrone-3-sulfate at low concentrations (nanomolar range) is preferentially transported by OATP1B1, but at higher concentrations the low affinity high capacity OATP1B3 can take over. For OATP1B3 at least two selective substrates have been documented: cholecystokinin-8 (CCK-8) and telmisartan (Roth et al. 2012). In addition, fluorescein-containing substrates like fluorescein-methotrexate or 8-fluorescein-cAMP are in general better substrates of OATP1B3 (Bednarczyk 2010; Gui et al. 2010).

There are a number of clinically relevant drug-drug interactions that involve the liver-specific OATPs. Because polymorphisms that result in an inactive or less active transporter mainly in the *SLCO1B1* gene have been linked to altered drug disposition, it is in general assumed that OATP1B1 plays the more important role than OATP1B3 for the disposition of most drug substrates. In 2008, the SEARCH collaborative group was able to link the *SLCO1B1*variant c.521T>C (V174A, also known as OATP1B1*5) to an increased risk of statin-induced myopathy (Link et al. 2008). Several studies demonstrated that the immunosuppressant cyclosporine A, a known inhibitor of several OATPs, affected drug bioavailability in transplant patients that were treated with statins, repaglinide or bosentan (Patel et al. 2016). In several studies, statin AUC was increased in the presence of cyclosporine A between 3.5 and almost tenfold. Similar increases in AUCs for statins were also observed in patients treated with gemfibrozil, rifampicin, and HIV protease inhibitors. One of the common properties of all these drugs is that they are known inhibitors of OATP1B1 and OATP1B3. Because increased plasma levels of statins have been associated with rhabdomyolysis, any drug-drug interactions that can increase statin plasma concentrations have to be carefully monitored (Patel et al. 2016).

As indicated above, polymorphisms that lead to an inactive or less active transporter have been identified in the *SLCO1B1* gene. Reports regarding the variant c.388A>G (N130D, OATP1B1*1b) are conflicting with increased, decreased, or unchanged effects, also depending on the drug substrate (Gong and Kim 2013). When assessed in vitro, OATP1B1*5 expression at the plasma membrane is reduced to about 35% of wild-type OATP1B1. Consistent with this reduced expression level, the majority of the studies reported an increase in AUC for patients with this polymorphism. Similarly, OATP1B1*15 which consists of c.388A>G plus c.521T>C is associated with increased AUCs for several drugs including pravastatin, pitavastatin, and rosuvastatin as well as some antihypertensives, anticancer drugs, and the cholesterol-lowering ezetimibe (Gong and Kim 2013). For OATP1B3, only a few polymorphisms have been identified. The most frequent variants are c.334T>G (S112A) and c.699G>A (M233I), both showing similar expression levels in in vitro experiments. OATP1B3-M233I was associated with reduce uptake of CCK-8 and rosuvastatin (Gong and Kim 2013). The AUC of mycophenolic acid glucuronide in renal transplant patients was increased in patients with the c.334T>G and c.699G>A haplotype although in addition conflicting results were reported. Given that only a few studies are available that investigated OATP1B3 polymorphisms, additional studies are required to see whether, e.g., OATP1B1 function could compensate for decreased OATP1B3 activity. Patients with Rotor syndrome, a disorder with conjugated hyperbilirubinemia and coproporphyrinuria, were compared to their normal family members. In addition, studies in OATP1A/OATP1B knockout mice were performed. These studies revealed that only a combination of a defective OATP1B1 with a defective OATP1B3 lead to the disease, demonstrating that either a functional OATP1B1 or functional OATP1B3 was sufficient for normal bilirubin disposition (van de Steeg et al. 2012).

With regard to OATP1B1/OATP1B3 inhibitors, several compounds have been identified that inhibit uptake mediated by both transporters. The most frequently used inhibitors are rifampicin, cyclosporine A, rifamycin SV, bromosulfophthalein, and MK-571. However, many of these inhibitors also interact with other drug transporters. For example, MK-571 was originally established as an MRP2 inhibitor, but later experiments revealed that it also inhibited uptake

mediated by both, OATP1B1 and OATP1B3 (Brouwer et al. 2013). Estropipate (also known as piperazine estrone sulfate) is a somewhat selective inhibitor for OATP1B1 with an IC_{50} of 0.06 μM as compared to 19.3 μM for OATP1B3. In contrast, ursolic acid was identified as a somewhat selective OATP1B3 inhibitor with an IC_{50} of 2.3 μM as compared to 12.5 μM for OATP1B1 (Gui et al. 2010). However, given that both OATPs have shown substrate-dependent inhibition patterns, more detailed research is needed to elucidate the underlying mechanisms and to hopefully identify really selective OATP1B1 and OATP1B3 inhibitors. Such selective inhibitors might be useful as co-medication with new chemical entities that are good drugs but have a low bioavailability due to extensive liver first-pass metabolism.

OATP1B1 and OATP1B3 are key uptake transporters for numerous drugs and are expressed at the basolateral membrane of human hepatocytes. The regulatory agencies expect that investigational drugs are tested in vitro to examine whether they are substrates of OATP1B1 and/or OATP1B3 in cases where ADME studies indicate that the hepatic uptake or elimination of the investigational drug is significant (≥25% of total drug clearance) or if the hepatic uptake is clinically important (for biotransformation or if the drug target is in the liver). If the in vitro results show that the investigational drug is an OATP1B1 or OATP1B3 substrate (transport of at least twofold above the negative control), and a known OATP inhibitor such as rifampicin can decrease OATP1B1 and/or OATP1B3-mediated uptake by more than 50% at a concentration of at least ten times the K_i value, the investigational drug is considered an OATP1B1 or OATP1B3 substrate. If the investigational drug is a substrate, in vivo studies might be necessary (Giacomini et al. 2010; Hillgren et al. 2013).

Summary and Outlook

So far seven transporters, which are known to be involved in adverse drug-drug interactions due to inhibition of the transporters or due to polymorphisms, are required by the regulatory agencies to be tested for investigational drugs. Five additional transporters either are already required by certain agencies or are considered by some or all of the three major agencies. However, because some of the drug-drug interactions involve multiple transporters, and because there are at least another 13 transporters that are known to be involved in drug disposition, the list of required transporters can increase, and it is even possible that certain transporters that are currently not considered will be required to be tested in the future. The most likely candidates are listed in Table 1, but several other transporters that are drug targets or mediate the transport of only a few drugs with so far no reported significant adverse effects could become relevant in the future.

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