# 6 Solute Carrier (SLC) Family Transporters

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## 6.1 INTRODUCTION

Xenobiotic transporters are increasingly scrutinized for their contribution to the sub-optimal pharmacokinetics (PK) and safety of drug candidates that fail in clinical trials. Over the last decade, pharmaceutical industry has used structure–activity relationships (SARs) to develop compounds with half-lives conducive to no more than once-a-day dosing and a lower incidence of metabolism-based drug–drug interactions (DDIs), yet

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unexpected drug interactions were encountered in the clinic. Several of these interactions have now been attributed to drug transporters. We now know that higher metabolic stability often results in the unpredictable involvement of drug transporters, leading to transporter-mediated drug interactions. Any significant changes in transporter expression and function can contribute to variable PK, unexpected toxicities, and drug interactions.

DDIs involving membrane transporters can be the result of competition for the same substrate-binding site, tight or allosteric binding leading to inhibition of transporter activity, or changes in transporter expression. These interactions could alter the blood-concentration-time profiles of drugs, leading to altered levels of a coadministered compound. Evaluating the substrate potential of a drug candidate for specific transporters *in vitro* is beneficial, especially when the organ is also the drug target. For example, the hepatitis C drugs, α-interferon and S-acyl-2-thioethyl esters, or the HMG-CoA inhibitors, such as the statins, must achieve adequate concentrations in the liver for pharmacological activity. Inhibition of transporter activity could result in changes to hepatic availability of xenobiotics. Transporters can also play a role in toxicities, as inhibition of transporter activity could lead to increased drug concentrations or inhibition clearance (CL) of endogenous substrates, for example, bile salts, leading to cholestasis.

The solute carrier (SLC) transporter family includes over 300 membrane-bound transporters that comprise 55 gene families and at least 362 putatively functional protein-coding genes. These transporters control the uptake of endogenous compounds and xenobiotics including sugars, amino acids, nucleotides, inorganic ions, and drugs, in all living cells. According to their mode of action, as depicted in Fig. 6.1, SLC transporters can be divided into passive (facilitated) and active transporters (symporters and antiporters). Passive transporters allow the passage of solutes across membranes down their electrochemical gradients, and active transporters create ion/solute gradients across membranes, utilizing diverse energy-coupling mechanisms. While symporters transport the two moieties across the membrane in the same direction, antiporters exchange these in opposite directions.

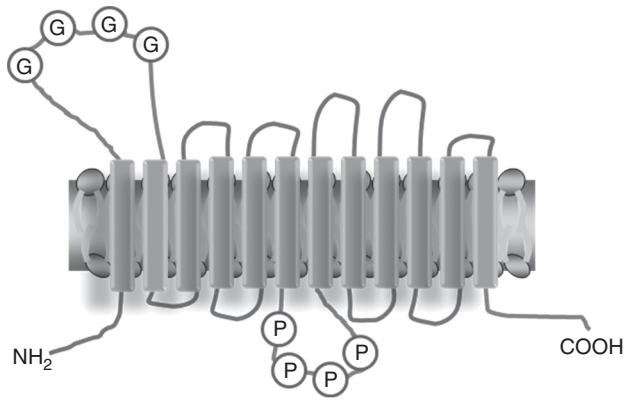


**Figure 6.1** Schematic of membrane topology for SLC transporters, based on predicted structure of OAT.

SLC transporters are located in all cellular and organelle membranes, with the exception of the nuclear membrane. Substrates of SLC membrane proteins include endogenous substances such as amino acids, glucose, inorganic cations and anions, bile salts, carboxylate, essential metals, biogenic amines, neurotransmitters, vitamins, fatty acids, lipids, nucleosides, hormones, urea, and xenobiotics. These transporters are therefore essential for the normal functioning of cells and organ systems.

Less than 20 of the known membrane-bound transporters have been characterized as relevant to drug-related interactions. These participate in the absorption, disposition, and/or excretion of drugs, xenobiotics, and endogenous compounds in organs including the intestine, liver and/or kidney, and brain and perform homeostatic functions. The families of SLC transporters related to drug transport are SLCO, SLC1A10, SLC47, SLC1A22, and SLC1A7 and are discussed in details in this review. Of the six SLCO gene families, four transport organic anion-transporting polypeptides (OATPs) are most relevant to drug transport. These transporters form a superfamily of sodium-independent transport systems that facilitate the transmembrane transport of amphipathic, endogenous, and exogenous organic compounds. Among this class, SLCO1 family is the best characterized and includes OATP1B1, 1B3, and 2B1. SLCO5 and SLCO6 are also believed to be important in drug transport. The SLC10 family participates in bile salt transport and has two well-characterized members in humans: the $Na^+$/taurocholate-cotransporting polypeptide (NTCP) and the apical $Na^+$-dependent bile salt transporter (ASBT), which are critical to the enterohepatic recirculation of bile salts, along with efflux transporter bile salt export pump (BSEP) on the canalicular member of hepatocytes. NTCP and ASBT are cotransporters that mediate sodium-dependent, electrogenic uptake of mainly bile salts into hepatocytes (NTCP), biliary epithelial cells, ileal enterocytes, and renal proximal tubular cells (ASBT). SLC47 includes the multidrug and toxicant extrusion-1 and -2 genes (MATE1 and MATE2). SLC1A22 is the major facilitator superfamily and includes organic cation transporters (OCTs), zwitterion/cation transporters (OCTNs), and organic anion transporters (OATs). Transporters of the SLC22 family function as uniporters that mediate facilitated diffusion in either direction (OCTs), anion exchange (OAT1 and OAT3) and Na+/l-carnitine cotransport (OCTN2), and play an important role in the detoxification of exogenous compounds. SLC7, with 14 members, represents cationic amino acid and glycoprotein transporters. This family is divided into two subgroups, the cationic amino acid transporters and the glycoprotein-associated amino acid transporters, also called *light chains* or *catalytic chains* of the hetero(di)meric amino acid transporters.

The interplay between chemicals and transporters is evaluated using multiple *in vitro* and *in vivo* experimental approaches. For *in vitro* evaluations, the method of choice has been stably transfected cell lines. The cell lines most commonly used for transporter transfections are the MDCK, HEK-293, and LLC-PK1, and in most cases, singly transfected cell lines suffice. To evaluate hepatic transport, fresh or cryopreserved primary hepatocytes provide a good model of hepatic uptake, efflux, biliary excretion, transporter-mediated drug interactions, induction, and hepatotoxicity. *In vivo*, transgenic rodents have been used to study drug transport, for example, the oatp2 and oatp4 single-knockout mice and the oct1/oct2 double-knockout mice.

Since transporters are widely distributed and have a broad substrate spectrum, altered transport kinetics due to the functional consequences of genetic variations (polymorphisms) can contribute to the interindividual variability of drug PK and pharmacodynamic (PD) effects, as well as to idiosyncratic toxicities. Owing to this, evaluations

of the effects of polymorphisms on ADME (absorption, metabolism, distribution, and elimination) should also be evaluated.

## 6.2 NOMENCLATURE AND GENERAL STRUCTURE OF SLC TRANSPORTERS INVOLVED IN XENOBIOTIC TRANSPORT

The SLC superfamily comprises more than 360 putatively functional protein-coding genes organized into 55 SLC families on the basis of alignment similarities and differences in transport mechanisms [1]. A website maintained by the HUGO Gene Nomenclature Committee provides the latest updates of SLC transporter nomenclature (http://www.genenames.org/genefamily/SLC.php). Generally, the root symbol of SLC is used to name the genes followed by a number or letter and then another number denoting the isoform, for example, SLC22 represents SLC family 22. The letters, A–E are used to specify subfamilies (Table 6.1). Owing to the new isoforms discovered within a given species and the rapid evolution of this family, the initial naming system underwent modifications to accommodate a unique, species-independent classification. For example, in the subclass originally named SLC21 that encodes OATP, the numeral "21" and the letter "A" were replaced by the letter "O," which stands for organic transporter [1]. It is also commonly accepted in transporter nomenclature that upper case letters denote the human protein, that is, OATP/SLCO and lower case letters indicate transporter proteins of preclinical species, for example, Oatp/Slco.

**TABLE 6.1 Major Human SLC Transport Proteins Involved in ADME**

| Transporter Symbol | Transporter Name | Synonyms | Chromosome |
|---|---|---|---|
| SLC7A5 | Solute carrier family 7 (amino acid transporter light chain, L system), member 5 | LAT1, E16, D16S469E, MPE16, CD98 | 16q24.3 |
| SLC7A5P1 | Solute carrier family 7 (amino acid transporter light chain, L system), member 5 pseudogene 1 | LAT1-3TM, MLAS | 16p11.2 |
| SLC10A1 | Solute carrier family 10 (sodium/bile acid cotransporter family), member 1 | NTCP | 14q24 |
| SLC10A2 | Solute carrier family 10 (sodium/bile acid cotransporter family), member 2 | ASBT, ISBT | 13q33 |
| SLC15A1 | Solute carrier family 15 (oligopeptide transporter), member 1 | PEPT1, HPECT1, HPEPT1 | 13q33-q34 |
| SLC15A2 | Solute carrier family 15 ($H^+$/peptide transporter), member 2 | PEPT2 | 3q21.1 |
| SLC16A1 | Solute carrier family 16, member 1 (monocarboxylic acid transporter 1) | MCT, MCT1 | 1p12 |
| SLC16A7 | Solute carrier family 16, member 7 (monocarboxylic acid transporter 2) | MCT2 | 12q13 |
| SLC22A1 | Solute carrier family 22 (organic cation transporter), member 1 | OCT1 | 6q25.3 |

**TABLE 6.1** (***continued***)

| Transporter Symbol | Transporter Name | Synonyms | Chromosome |
|---|---|---|---|
| SLC22A2 | Solute carrier family 22 (organic cation transporter), member 2 | OCT2 | 6q25.3 |
| SLC22A3 | Solute carrier family 22 (extraneuronal monoamine transporter), member 3 | OCT3, EMT | 6q25.3 |
| SLC22A4 | Solute carrier family 22 (organic cation/ergothioneine transporter), member 4 | OCTN1, MGC34546 | 5q23.3 |
| SLC22A5 | Solute carrier family 22 (organic cation/carnitine transporter), member 5 | OCTN2, SCD | 5q23.3 |
| SLC22A6 | Solute carrier family 22 (organic anion transporter), member 6 | ROAT1, PAHT, OAT1 | 11q12.3 |
| SLC22A7 | Solute carrier family 22 (organic anion transporter), member 7 | NLT, OAT2 | 6p21.1 |
| SLC22A8 | Solute carrier family 22 (organic anion transporter), member 8 | OAT3 | 11q12.3 |
| SLC22A9 | Solute carrier family 22 (organic anion transporter), member 9 | OAT4, FLJ23666, UST3, OAT7 | 11q12.3 |
| SLC47A2 | Solute carrier family 47, member 2 | FLJ31196, MATE2, MATE2-K | 17p11.2 |
| SLC28A1 | Solute carrier family 28 (sodium-coupled nucleoside transporter), member 1 | CNT1 | 15q25.3 |
| SLC28A2 | Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2 | CNT2, SPNT1, HCNT2, HsT17153 | 15q15 |
| SLC28A3 | Solute carrier family 28 (sodium-coupled nucleoside transporter), member 3 | CNT3 | 9q21.33 |
| SLC29A1 | Solute carrier family 29 (nucleoside transporters), member 1 | ENT1 | 6p21.1 |
| SLC29A2 | Solute carrier family 29 (nucleoside transporters), member 2 | DER12 | 11q13 |
| SLC29A3 | Solute carrier family 29 (nucleoside transporters), member 3 | ENT3, FLJ11160 | 10q22.2 |
| SLCO1A2 | Solute carrier organic anion transporter family, member 1A2 | OATP, OATP1A2, OATP-A | 12p12 |
| SLCO1B1 | Solute carrier organic anion transporter family, member 1B1 | OATP-C, LST-1, OATP1B1 | 12p12 |
| SLCO1B3 | Solute carrier organic anion transporter family, member 1B3 | OATP8, OATP1B3 | 12p12 |
| SLCO2B1 | Solute carrier organic anion transporter family, member 2B1 | OATP-B, OATP2B1 | 11q13 |

*Source*: Data derived from website of HUGO Gene Nomenclature Committee (HGNC) (http://www.genenames.org/genefamily/SLC.php).

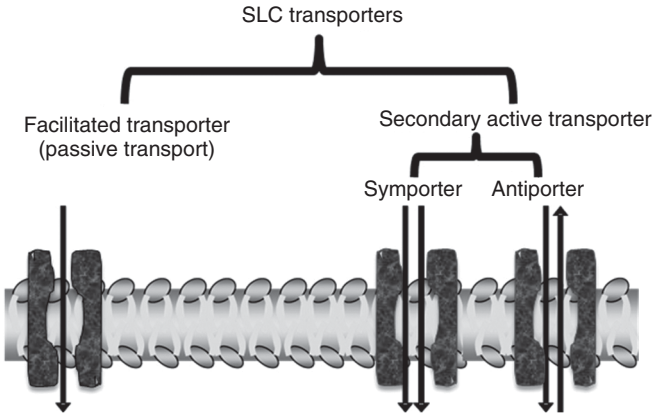


**Figure 6.2** Schematic depicting passive and active transport processes of SLC transporters.

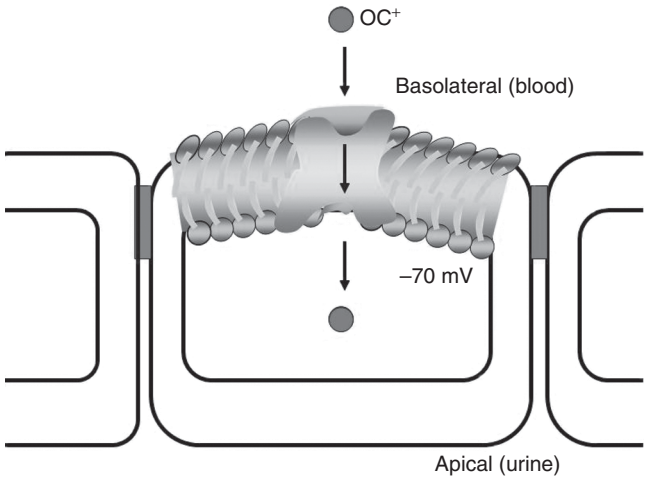


**Figure 6.3** Depiction of the carrier-mediated facilitated transport processes for transporters such as OCT.

The SLC family consists of proteins containing multiple transmembrane domains (Fig. 6.1) and facilitates the movement of a substrate across cellular membranes, either along or against its concentration gradient. Based on the transporting mechanism, the SLC superfamily is categorized into passive (uniporters or facilitative transporters) and secondary active transporters (Fig. 6.2). Passive transporters, also known as *facilitated transporters*, utilize an electrochemical potential difference caused by the respective substrates to move substrates down a concentration gradient (Fig. 6.3). In contrast, secondary active transporters translocate the substrates uphill against their electrochemical gradient by coupling with another ion or molecule that flows downhill with its concentration gradient (Fig. 6.4). Basically, these processes are free-energy changes. The secondary active transporter families, including the OATPs (SLC21A or OATP), peptide transporters (SLC15A or PEPT), novel-type OCT (SLC22A or OCTN), and

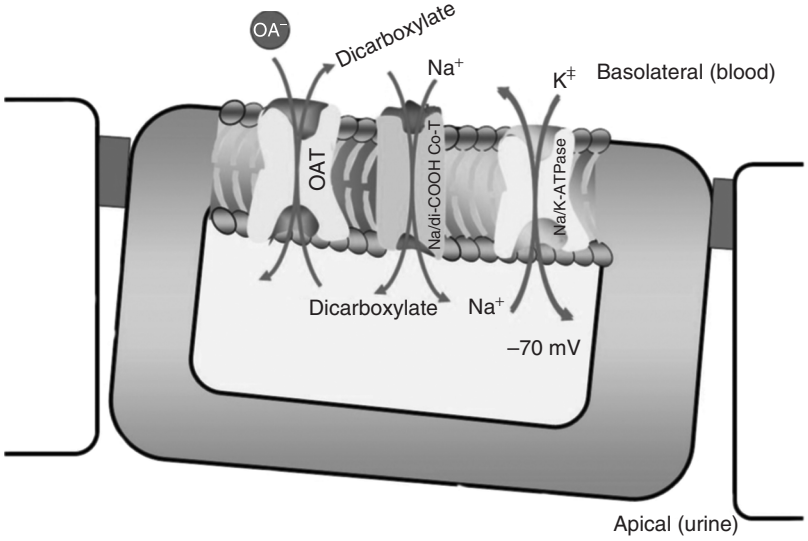


**Figure 6.4** Depiction of carrier-mediated active transport processes for transporters such as OAT.

sodium-dependent bile acid transporter (SLC10A1 or NTCP), either utilize ion gradients across membranes or cotransport with intracellular and/extracellular ions [2,3] (Fig. 6.4). On the basis of the direction of the coupling ion or molecule, the secondary active transporters are further categorized as symporters and antiporters (Fig. 6.2). Symporters move the transported molecule and coupled ion toward the same direction, while antiporters move the molecules and coupled ion in opposite directions. In addition to localization at the cellular plasma membranes, the SLC proteins are also expressed on membranes of cellular organelles such as mitochondria, endoplasmic reticulum, Golgi, and other vesicles. These SLC transporters are termed *mitochondrial* and *vesicular transporters* [1] and are not discussed in this chapter.

Relatively few of the SLC transporters have been established to be actively involved in disposition of market drugs or drug candidates. From a pharmaceutical industry perspective, these SLC transporters are of great importance, in that they may rate-limit drug exposure to metabolizing enzymes in metabolic tissues, thus serving as gatekeepers for drug disposition and as drug targets, facilitating drug delivery and being responsible for drug interactions. For example, the glucose and neurotransmitter transporters have been exploited as drug targets for the past two decades, and more recently, the oligopeptide transporter (PEPT1) is being investigated as an endogenous delivery mechanism for drugs, where translocation across the intestinal mucosa or blood–brain barrier (BBB) is challenging [4]. Considerable progress has been made in understanding SLC transporter-mediated DDI and toxicities [5]. In 2010, the International Transporter Consortium summarized several SLC carriers including OATPs/SLCO, OCTs/SLC22, and OATs/SLC22, which are clinically relevant to DDI, and made recommendations for evaluating the substrate and inhibition potential of candidate drugs with these transporters [6]. These transporters are discussed in more details below.

## 6.3 DISTRIBUTION AND PATHOPHYSIOLOGY OF SLC TRANSPORTERS

The SLC family is distributed in the epithelia of various organs and transports a variety of endogenous and exogenous substrates (Table 6.2). For example, OAT3 is expressed on the basolateral membrane of the proximal tubule in the kidney and mediates the secretion of organic anions into urine via carrier-mediated active transport (Fig. 6.8) [7–9]. To move a substrate across the cell membrane, the SLC transporters need to collaborate with the ATP-binding cassette (ABC) transporters or other SLC transporters in the same or opposite membrane of polarized cells. Recently, the human MATE2-K transporter was identified as a luminal membrane transporter that transports organic cations out of renal proximal tubular cells in coordination with OAT3 [10]. Diverse organ distribution of the SLC transporters and coexistence of multiple transporter isoforms in tissues suggest that collaborative and compensatory functions of transporters might play an important role in physiological communication network between organs, as well as tissue distribution and elimination of xenobiotics. Transporter pharmacology is a rapidly emerging subject in drug discovery and development and therefore is of particular interest to the pharmaceutical industry. The SLC members discussed below are clinically relevant in drug absorption, drug disposition, and/or DDI.

### 6.3.1 SLCO Superfamily: Organic Anion-Transporting Polypeptides (OATPs)

The SLCO subfamily proteins are predicted to have a 12-transmembrane-domain structure, similar to that depicted in Fig. 6.1. These transporters are expressed in organs such as the intestine, liver, muscle, and BBB. Members of the SLCO family include several OATP transporter isoforms that play an important role in uptake of structurally diverse endogenous compounds and xenobiotics, including clinical therapeutics. OATP transporters are involved in the entry of diverse drugs into organs. The inhibition or downregulation of OATP expression and/or function by a drug, or gene polymorphisms, may lead to PK alterations and an increase in the propensity for DDI [11].

OATP1A2/SLCO1A2 (formerly OATP-A) has been identified in the liver, brain, kidney, and testes [12] and transports diverse compounds, such as steroid sulfates, thyroid hormones, and opioid peptides [13]. In contrast to the distribution of OATP1A2, OATP1B3/SLCO1B3 and OATP1B1/SLCO1B1 are believed to be liver specific (Fig. 6.6) [14]. Immunohistochemical analysis indicates that OATP1B1, also referred to as *liver-specific transporter 1* (LST-1), OATP2, or OATP-C is localized to the basolateral (sinusoidal) membrane of hepatocytes [15]. Generally, OATP transporters exhibit a broad substrate selectivity that overlaps across isoforms. For example, the substrates for OATP1B1 or 1B3 include anionic (e.g., statins such as pravastatin, pitavastatin, and rosuvastatin), zwitterionic (e.g., rifampicin), and neutral lipophilic (e.g., paclitaxel) drugs. OATP1B1 and 1B3 also transport endogenous substances, such as bile acids, thyroid hormones, steroid sulfates, glucuronide conjugates, and peptides [12,16]. Comprehensive lists of human OATP substrates have been published and provide more details [17,18].

OATP2B1/SLCO2B1 (previously OATP-B) was initially cloned from the human brain and subsequently found in the liver, kidney, lung, heart, placenta, and gastrointestinal (GI) tract [19,20]. Functionally, OATP2B1 demonstrates higher transporter activity for estrone-3-sulfate and appears to have more limited substrate specificity relative to OATP1B1 or 1B3 [19].

**TABLE 6.2 Major Human SLC Transport Proteins, with Some Known Substrates and Inhibitors**

| Transporter | Organ, Cell, Membrane | Substrates Used Experimentally | Substrate Drugs | Endogenous Substrates | Inhibitors and Inhibitor Drugs |
|---|---|---|---|---|---|
| OATP1B1 | Liver, hepatocyte, basolateral membrane | BSP, E3S, E17β-G | Statins, repaglinide, olmesartan, enalapril, temocaprilat, valsatan | Bile acids, bilirubin, steroid hormones | Rifampicin, rifamycin SV, saquinavir, ritonavir, cyclosporine |
| OATP1B3 | Liver, hepatocyte, basolateral membrane | BSP, cholecystokinin 8, E17β-G | Statins, fexofenadine, telmisartan, valsartan, paclitaxel, enalapril, erythromycin, phalioidin | Bile acids, steroid hormones | Rifampicin, cyclosporine, ritonavir |
| OATP2B1 | Liver, hepatocyte, basolateral membrane. Endothelia | E3S, BSP | Statins, fexofenadine, glyburide, rifampicin | Bile acids, steroid hormones | Rifampicin, cyclosporine |
| OATP1A2 | Brain capillaries, endothelia. Liver, cholangiocytes. Kidney, distal nephron | E3S, BSP, fexofenadine | Methotrexate ouabain, levofloxacin, statins | Bile acids | Naringin, ritonavir, lopinavir, saquinavir, rifampicin, verapamil, dexamethasone, ketaconazole, lovastatin |
| OAT1 | Kidney, proximal tubule, basolateral membrane. Placenta | PAH | Adefovir, zidovudine, ciprofloxacin, methotrexate | Cyclic nucleotides, prostaglandin, uric acids | Probenecid, novobiocin |

(*continued overleaf*)

**TABLE 6.2** (*continued*)

| Transporter | Organ, Cell, Membrane | Substrates Used Experimentally | Substrate Drugs | Endogenous Substrates | Inhibitors and Inhibitor Drugs |
|---|---|---|---|---|---|
| OAT3 | Kidney, proximal tubule, basolateral membrane. Brain, choroid plexus, and blood–brain barrier | E3S, furosemide, bumetanide | NSAIDs, cefaclor, ceftizoxime, | Prostaglandin, uric acids, bile acids; conjugated hormones | Probenecid, novobiocin |
| OCT2 | Brain, capillaries. Endothelia. Liver, cholangiocytes. Kidney, distal nephron | E3S, BSP, dehydroepiandrosterone sulfate | Fexofenadine, methotrexate, digoxin, statins | Bile acids, choline, acetylcholine, and monoamine neurotransmitters | Naringin, ritonavir,rifampicin |
| OCT1 | Liver, hepatocytes, basolateral membrane. Intestine, enterocytes, apical membrane | TEA, MPP | Metformin, oxaliplatin | Choline, acetylcholine, and monoamine neurotransmitters | Quinine, quinidine, disopyramide |

| | | | | | |
|---|---|---|---|---|---|
| MATE1 | Kidney, proximal tubular cells, apical membrane. Liver, hepatocytes, apical membrane. Skeletal muscle | TEA, MPP | Metformin, cephalexin, acyclovir, ganciclovir | Peptides and nucleosides | Quinidine, cimetidine, procainamide, verapamil |
| MATE2-K | Kidney, proximal tubular cells, apical membrane | Metformin, MPP | MPP, TEA | Creatine, guanidine, thiamine | Cimetidine, quinidine, pramipexole |
| NTCP | Liver, hepatocytes, apical membrane | Bile acids (e.g., taurocholic acid, glycocholic acid) | Rosuvastatin, pitavastatin, atorvastatin | Bile acids, iodothyronines | Troglitazone, bosentan |

*Abbreviations*: BSP, bromosulfophthalein; E3S, estrone-3-sulfate; E17β-G, estradiol-17β-glucuronide; PAH, *para*-aminohippurate; TEA, tetraethylammonium; MPP, *N*-methylpyridinium.

*Source*: A significant part of this table is from the paper published by the International Transporter Consortium [6], with additions based on current literature cited elsewhere in the text.

### 6.3.2 SLC22 Superfamily: Organic Anion, Organic Cation, and Zwitterion Transporters (OATs/OCTs/OCTNs/MATEs)

Since Gründemann *et al*. discovered the rat Oct1 in 1994 [21], its orthologs were subsequently identified and found to be expressed in excretory organs such as the kidney. SLC22 transporters play a critical role in maintaining homeostasis of endogenous substances and urinary elimination of therapeutic agents by carrier-mediated facilitated transport (Fig. 6.3). The SLC22 transporters are also expressed in barrier organs such as the choroid plexus, retina, and placenta [22] and contribute to disposition and homeostasis of organic anions and cations, thereby modulating efficacy and toxicity of clinical therapeutics. SLC22 transporters interact with structurally diverse substrates. On the basis of the substrate specificity and mechanism of transport, SLC22 members are divided into several subgroups including OCTs, OATs, OCTNs, unknown soluble transporters (USTs), and fly-like putative transporters (FLIPTs) [23].

***6.3.2.1 OAT Subtypes 1–5.*** The transporters in this group translocate organic anions including xenobiotics, hormones, and biogenic amines against electrical and chemical gradients. OAT1 (SLC22A6) is expressed mainly at the basolateral membrane of proximal tubule cells and mediates the uptake of organic anions such as *p*-aminohippurate (PAH) from blood into renal proximal tubular cells. In *in vitro* functional assays, an outwardly directed concentration gradient of α-ketoglutarate or glutarate can improve the OAT1-mediated PAH transport, suggesting that α-ketoglutarate gradient provides the driving force for the uptake of organic anions [24]. OAT1 is thus identified as an organic anion/dicarboxylate exchanger. OAT2 (SLC22A7) is expressed abundantly on the basolateral membrane of the liver (sinusoidal membrane) and weakly in the kidney, with marked gender-based differences in expression [25]. Unlike OAT1, OAT2 transports substrates without dependence on sodium, dicarboxylate, glutarate, and α-ketoglutarate gradients and is not considered an organic/dicarboxylate exchanger [26]. Oat3 (SLC22A8) was initially cloned from a rat brain cDNA library and subsequently detected in the kidney, brain, liver, and at low concentrations in the eyes [27]. OAT3 is located on the basolateral membrane of the proximal tubule in the kidney and on the apical membrane of the choroid plexus in the brain [28]. Largely overlapping with the substrates of OAT1 and 2, OAT3 is capable of transporting PAH, methotrexate, cimetidine, and estrone sulfate.

Within the OAT family, OAT4 (SLC22A11) is the only transporter highly expressed in both the placenta and the kidney [22]. Expression of the OAT4 protein was confirmed on the apical membrane of the proximal renal tubular cells, but thus far, there is no consensus on its function. Similar to OAT1 and OAT3, OAT4 is reported to be responsible for reabsorption of substrates from the lumen, in exchange for dicarboxylates. The substrate spectrum of OAT4 is relatively narrow as compared with the other OAT isoforms. OAT4 substrates include estrone sulfate, dehydroepiandrosterone sulfate, ochratoxin A, prostaglandin $E_2$ ($PGE_2$), and prostaglandin F (PGF) [29].

Through computational analysis of the genome database, Oat5 (Slc22a19) was discovered [30]. The expression of Oat5 in mouse and rat is confirmed, and a human OAT5 ortholog is yet to be identified. Human OAT6 (SLC22A20), OAT7 (SLC22A9), OAT8 (SLC22A25), OAT10 (SLC22A13), FLIPT1 (SLC22A15), and their rat orthologs have been identified by searching genome databases or via PCR homology screening approaches [31–33]. However, the functional characteristics of this so-called orphan

transporter group are yet to be determined. To date, there is no evidence that these transporters play a major role in xenobiotic disposition.

***6.3.2.2 OCT Subtypes 1–3.*** Transporters in this class mainly translocate organic cations and weak bases and are electrogenic, sodium independent, and bidirectional [23]. OCT1 (SLC22A1) is predominantly expressed on the sinusoidal membrane of hepatocytes in human and rat liver and is also present in other preclinical species such as mouse and rabbit [34,35]. It is also found on the basolateral membrane of small intestinal enterocytes and the renal proximal tubular cells. OCT1 mediates $Na^+$-independent transport of type I organic cations (protonated molecules), such as tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium ($MPP^+$), *N*1-methylnicotinamide (NMN), dopamine, and choline [36], as well as type II cations (larger and bulkier cations) including methyl-quinine and quinidine. OCT1-mediated organic cations transport is potential, sensitive, and electrogenic.

OCT2 (SCL22A2) is expressed predominantly in kidney and also in placenta, lung, brain, and small intestine [23]. OCT2 is localized to the basolateral membrane of the distal tubule in the kidney and mediates uptake from the blood to the proximal tubular cells during the renal secretion of organic cations [37]. OCT2 transports many organic cations and plays an important role in the pharmacological, PK, and toxicological properties of therapeutics. In the brain, OCT2 is expressed on the apical (ventricular) membrane in epithelial cells of the choroid plexus. Since OCT2 transports monoamine neurotransmitters including dopamine, noradrenaline, adrenaline, and 5-hydroxytryptamine, tissue-dependent expression of OCT2 in tissues such as neurons, lung, and choroid plexus may participate in the regulation of interstitial and intracellular concentrations of monoamine neurotransmitters and cationic drugs [38].

OCT3 (SLC22A3) has a broader tissue distribution than OCT1 and OCT2 and is also detected in muscle, glial, cardiac, and cancer cell lines. OCT3 is localized on the basolateral membrane of the trophoblast in placenta, the epithelial cells of renal proximal tubules and hepatocytes, and the luminal membrane of bronchial epithelial cells and small intestinal enterocytes [39–41]. The broader distribution of OCT3 contributes to physiological functions such as regulation of the interstitial concentration of monoamine neurotransmitters and cationic drugs in the central nervous system and release of acetylcholine from placenta [42]. The substrate specificity of OCT3 is similar to that of OCT1 or OCT2, which is capable of transporting type I and II organic cations such as the neurotransmitters, namely, adrenaline, TEA, noradrenaline, and histamine, and the neurotoxin $MPP^+$.

OCT6 (SLC22A16), also named *CT2* or FLIPT2, is located predominantly in human testis but is also found in the embryonic liver, hematopoietic cells, and cancer cell lines. OCT6 transports carnitine with a high affinity and shows a greater selectivity for substrates. For example, OCT6 bidirectionally transports L-carnitine across cell membranes but does not interact with TEA and other organic cations [43].

***6.3.2.3 OCTN1–3 (SLC22A4–6).*** These transporter proteins function as organic cation uniporter or $H^+$/organic cation antiporters, or as uniporters for organic cations or $Na^+$/carnitine cotransporters. OCTN1 (SLC22A4) is expressed in kidney, muscle, placenta, prostate, and heart [44]. OCTN1 is an electroneutral $H^+$/organic cation antiporter and mediates the transport of monovalent organic cations including TEA, quinidine, pyrilamine, verapamil, and the zwitterion carnitine [45]. OCTN1 is expressed on the

luminal membrane of renal proximal tubular cells and mediates the cellular efflux of organic cations. The bidirectional function of this transporter indicates that it participates in reabsorption of organic cations [44]. There are significant species differences in the localization and transport mechanism of OCTN1 that is strongly expressed in the liver of rat, but not in humans [46].

OCTN2 (SLC22A5) is a $Na^+$-dependent transporter for TEA, choline, verapamil, pyrilamine, L-carnitine, and the zwitterionic β-lactam antibiotic cephaloridine. It also serves as a $Na^+$-independent cation transporter [41], mediating the $Na^+$-independent cotransport of short-chain acyl esters of carnitine, zwitterionic β-lactam antibiotics, L-lysine, and L-methionine. In the absence of $Na^+$, OCTN2 transports the organic cations and weak bases including quinidine, TEA, pyrilamine, verapamil, and choline [47]. Expression of OCTN2 is relatively ubiquitous and has been confirmed in kidney, muscle, heart, brain, lymphocyte, and sperm [23,46]. In kidney, luminal localization of OCTN2 in the proximal renal tubule provides the active process of reabsorption of L-carnitine, which can contribute to the secretion and reabsorption of organic cations. In the heart and muscles, OCTN2 mediates the uptake of L-carnitine into adipocytes and cardiac myocytes [23]. Polymorphic isoforms of OCTN1 and OCTN2 are linked to inflammatory bowel disease [48]. OCTN3 is found in the mouse testis and kidney and is believed to be less relevant for organic cation transport than OCTN1 and OCTN2.

***6.3.2.4 MATE Transporters.*** MATEs are OCTs with multiple isoforms. MATE1 (SLC47A1) is a human and mouse ortholog of the multidrug and toxin extrusion family conferring multidrug resistance to bacteria [49]. MATE1 is primarily expressed in the liver, kidney, and skeletal muscles and weakly in the heart [50]. Human MATE1 is a $Na^+$-independent transporter and transports TEA via a proton-cation antiport process. MATE1 interacts with $MPP^+$, serotonin, cimetidine, quinidine, and verapamil, demonstrating functional similarity to the OCTs and OCTNs. MATE1 is localized to the luminal membranes of proximal tubular cells in the kidney and on the canalicular membrane of hepatocytes in the liver and is thought to play a key role in the excretion of organic cations through exchange of protons in the kidney. Recently, MATE1-coordinated transport of antibiotics with OAT3 was reported [10].

MATE2 comprises two splice variants, MATE2-K and MATE2-B. MATE2-K is predominantly expressed in the human kidney, whereas MATE2-B was identified in the human brain. MATE2-K is thought to be the kidney-specific $H^+$/organic cation antiporter mediating the luminal efflux of a wide range of cationic compounds (endogenous substrates such as creatine, guanidine, and thiamine and drug substrates such as metformin and oxaliplatin) across the brush border membranes in the renal proximal tubular cells; however, the clear function of human MATE2-B is yet to be determined.

## 6.4 ROLE OF THE MAJOR SLC TRANSPORTERS IN DIFFERENT ORGANS

As described in the previous section, various drug transporters are expressed in the liver, kidney, intestine, placenta, and BBB. In most cases, individual SLC transporters are preferably expressed on either the apical or the basolateral membrane of polarized cells and work together to mediate the transport of xenobiotics. For example, in the liver, drugs are first taken up through the sinusoidal (basolateral or blood side)

membrane by hepatic uptake transporters. Once inside the cells, these drugs either are metabolized by P450 metabolism (Phase I) and/orconjugation (Phase II) and/or transported out of hepatocytes by transporter proteins located on the canalicular (apical) membrane and subsequently eliminated across bile canaliculi, or are transported back to the blood through sinusoidal (basolateral) transporters. The unidirectional transport process mediated by apical and basolateral membrane transporters guarantees the vectorial elimination of xenobiotics from the body. From a pharmaceutical perspective, organ distribution of SLC transporter proteins that is of the most interest with respect to absorption, disposition, and elimination is described below.

### 6.4.1 SLC Transporters in Intestine

Multiple parallel transport processes, such as passive transcellular diffusion, carrier-mediated absorption, and carrier-mediated efflux, exist in the intestinal barrier [51,52]. Absorption of therapeutics, nutrients, bile acids, and other compounds in the GI tract is often mediated by SLC transporters expressed in enterocytes (Fig. 6.5) [53]. Uptake transporters including PEPT1, ABST, concentrative or equilibrium nucleoside transporters (CNT1–3/SLC28A1–3 or ENT1–3/SLC29A1–3), OATP family, and organic cation/anion/zwitterion transporters (OAT/OCT/OCTN) facilitate drug absorption across the intestinal brush border membrane. These SLCs are critically involved in absorption, thereby contributing to the distribution and PK characteristics of many drugs. For example, β-lactam antibiotics and dipeptidelike anticancer drugs, such as bestatin, are absorbed by PEPT transporters [54,55].

Three subtypes of the SLC22 family have been identified in the GI tract: OCTs, OATs, and the OCTNs. OATPs of the SLCO family also have important functions in the intestine and transport structurally diverse substrates [56]. ASBT is critical for

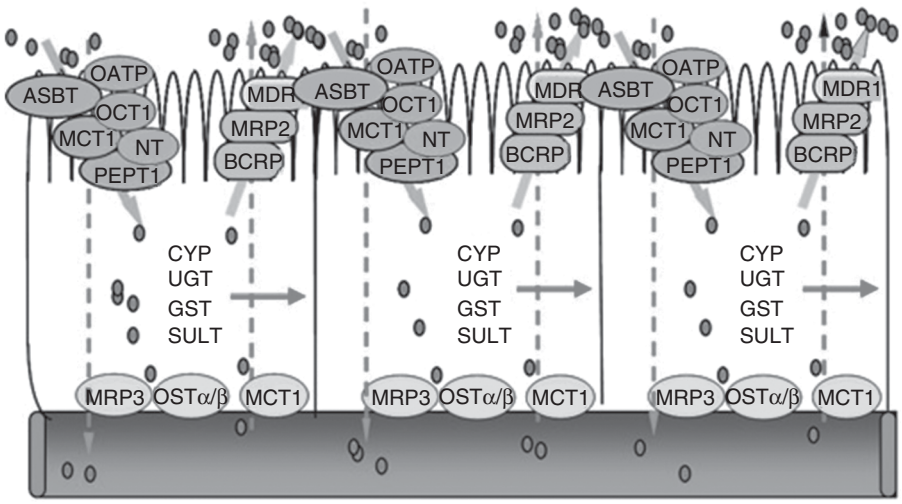


**Figure 6.5** Schematic of apical and basolateral membrane transporters in human enterocytes [50].

enterohepatic circulation of bile acids by mediating their absorption at the apical membranes of enterocytes [57]. A defect in ASBT function could result in primary bile acid malabsorption characterized by congenital diarrhea and reduced plasma cholesterol levels [58]. Nucleoside transporters belonging to the SLC28 family are essential for *de novo* nucleic acid synthesis and for cancer therapy by mediating uptake of anticancer and antiviral nucleoside analogs into tumor tissues. CNT1–3 (concentrative) are localized to the apical membranes of epithelial cells and mediate nucleoside uptake against concentration gradients, whereas ENT1–3 (equilibrium) translocate nucleoside substrates following concentration gradients and are believed to be responsible for nucleoside efflux at the basolateral membranes of enterocytes. The oligopeptide transporter PEPT1 is present at the apical membrane of enterocytes and transports nutrient-derived peptides, as well as peptidomimetic drugs [59].

Recently, targeting uptake transporters in the design of poorly absorbed drugs has been proposed as a strategy for improving bioavailability and therapeutic efficacy [4,60]. Because active uptake and efflux transporters in the GI tract may contribute to the bioavailability of orally administered drugs, modulation of their expression levels or activities may lead to potential drug transporter interactions during absorption.

### 6.4.2 SLC Transporters in Liver

Hepatic drug elimination generally constitutes a series of events including (i) transport of drug into the hepatocyte via passive diffusion or hepatic uptake transporters (phase 0); (ii) intracellular metabolism, including cytochrome P450 metabolism (phase I) and/or conjugation (phase II); and (iii) flux into blood or bile via the hepatic transporters (phase III). Recently, the role of hepatic SLC uptake transporters in drug disposition has received considerable attention [6], as these proteins play an important role in translocating endogenous compounds and drugs from blood into the liver. This function represents a rate-limiting step in the entry of substrates into hepatocytes and plays a key role in drug elimination, even for the compounds that are primarily metabolized by hepatic enzymes. The distribution and elimination of drugs in the liver is dependent on the degree of expression of each transporter protein and substrate affinity for a transporter. Uptake transporters in liver may contribute to drug exposure, DDI, and toxicity of a therapeutic agent. Hepatic uptake transporters can also be targeted to selectively deliver therapeutic agents to the liver.

Hepatic uptake systems are divided into two classes, $Na^+$-dependent and $Na^+$-independent influx transporters (Fig. 6.6). The $Na^+$-dependent bile acid uptake is mediated by the NTCP, while the OATP transporters, OATs, OCTs, and OCTNs transport a variety of organic anions through $Na^+$-independent processes. Among the SLC superfamily, SLCO family, SLC22 family, and SLC10 gene family represent the predominant transport proteins that act as active carriers of organic anions across the sinusoidal membrane. Of the 11 functional human OATP transporters identified, only 3 isoforms (OATP1B1, OATP1B3, and OATP2B1) are currently believed to play an important role in hepatic uptake of exogenous therapeutics and endogenous compounds in hepatocytes [6]. OATP1B1, OATP2B1, and OATP1B3 are colocalized on the same membrane domain and have overlapping substrates, suggesting that inhibition of any one of the OATP would theoretically not cause a significant change in drug PK. While it is not easy to assess the relative contribution of each of these three transporters,

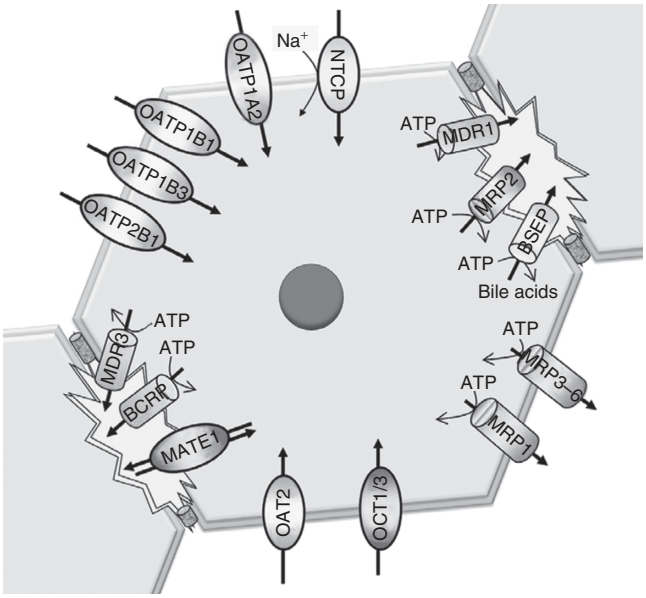


**Figure 6.6** Schematic of apical and basolateral membrane transporters in human hepatocytes.

it is known that net inhibition of hepatic uptake can result in drug interactions and changes in systemic exposure and even unexpected toxicity. For example, in kidney transplant recipients receiving cyclosporine, a 3.8-fold increase in the area under the curve (AUC) of cerivastatin was observed [61] because of the inhibition of OATP1B1-mediated cerivastatin uptake into hepatocytes [62]. Recently, selective OATP inhibitors, such as estrone-3-sulfate for OATP1B1 and CCK-8 for OATP1B3, were used successfully to assess the relative roles of these isoforms in the hepatic uptake of pitavastatin, fexofenadine, and rosuvastatin [63].

Of the six distinct OAT members cloned from the human liver and kidney, OAT2 and OAT7 are predominately expressed on the sinusoidal membrane of hepatocytes. OAT7 shows 35–46% identity to other OAT family members and mediates an exchange of organic anions similar to other members from OAT family, but OAT7 has narrow substrate specificity, mainly encompassing sulfate conjugates in exchange for the four-carbon short-chain fatty acid butyrate [64,65]. The transport of cationic drugs is predominantly catalyzed by the OCTs. Of the three OCTs characterized, OCT1 is significantly expressed in the liver. The expression of OAT and OCT proteins is regulated by drugs and disease states. The activation of nuclear transcriptional factors by their respective ligands, such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and nuclear factor erythroid 2-related factor 2 (Nrf2), could alter the expression of a number of transporters in the hepatocyte [66]. For example, phenobarbital (acting through CAR) effectively decreases Oat2 expression in liver [66]. On the other hand, hepatic mRNAs for Oct1 and Oat3, but not for Oat2, were decreased in rats following lipopolysaccharide treatment [67].

### 6.4.3 SLC Transporters in Blood–Brain Barrier

The BBB consists of brain capillary endothelial cells with complex tight junctions that separate circulating blood and cerebrospinal fluid in the central nervous system. The high density cell layers of the BBB serve to regulate the transport of nutrients, waste products, and clinical agents in and out of the brain. The passive diffusion of small molecules across the BBB has traditionally been dependent on lipophilicity, in which modifications of this physiochemical property is a strategy to increase BBB permeability. We now know that similar to other organs such as the liver and intestine, distinct transport systems expressed in the apical and/or basolateral membrane can influence the selectivity of compounds across the BBB. Physiologically, the mechanism of BBB transport is divided into three separate processes: (i) blood-to-brain uptake of drugs and nutrients (e.g., glucose, nucleotides, and amino acids) [68], (ii) efflux ABC transporters including P-glycoprotein (P-gp/ABCB2) and BCRP (ABCG2) that prevent entry of xenobiotics into the brain, and (iii) brain-to-blood transport systems for elimination of metabolites, neurotransmitters, and neurotoxins [69]. The interplay of transporters at the BBB makes it possible to selectively restrict the distribution of many drugs and therefore enhances our understanding of the molecular mechanism governing brain penetration [70].

While ABC efflux transporters with broad substrate specificity act as a functional barrier restricting drug entry into the brain, several SLC members in the BBB may play a key role on the uptake of neuroactive compounds (Fig. 6.7). Originally cloned from human liver, SLC21A3 (OATP-A), is highly expressed in the brain, followed by kidney, liver, lung, and testis [71]. Uptake assays with mRNA-injected *Xenopus laevis* oocytes indicate that OATP-A is involved in the transport of δ-opioid receptor agonists such as D-penicillamine (2,5)-enkephalin and deltorphin II, suggesting its involvement in the transport of centrally active opioids [72]. Other members of the SLCO family, such as SLC21A9 (OATP-B), SLCO3A1 (OATP-D), SLC21A10 (OATP-E), and SLCO4A1 (OATP-F), are also detected in human brain. However, the exact localization and functions of these transporters are yet to be determined [12]. Most OAT members of the SLC22 family are found in the brain. Among those, OAT3 is the most abundant

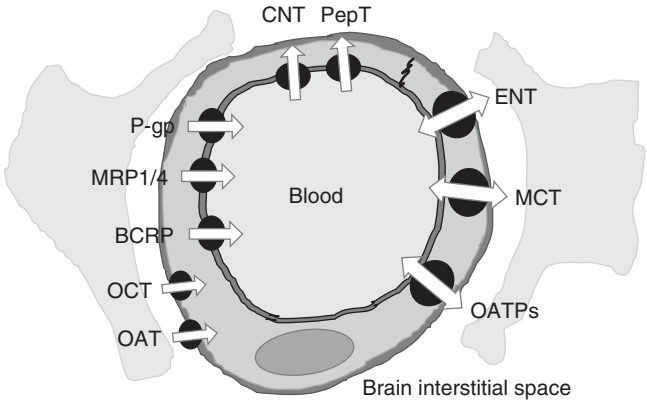


**Figure 6.7** Schematic of apical and basolateral membrane transporters in human BBB endothelial cells.

OAT localized to the basolateral membrane of brain capillaries [73]. Although OAT3 is also expressed in the liver, kidney, lung, and muscles [74], it appears to play a vital role in the entry of substrates into choroid plexus and in the exclusion of anionic metabolites of neurotransmitters from the brain [28,75].

Three OCTs OCT1–3 are reported expressed in the brain at varying levels. While OCT1 mRNA expression is believed to be negligible, OCT2 mRNA found in neurons and choroid plexus is reported to be involved in neuron uptake and reabsorption of substrates such as dopamine, norepinephrine, serotonin, histamine, and choline from the cerebrospinal fluid [38]. In the cerebral cortex and cerebellar and hippocampal neurons in rodents, OCT3 mRNA expression is greater than that of OCT1 and 2 [76,77]. Although there is no direct evidence, the ability to interact with cationic neurotoxins and neurotransmitters suggests that OCT3 may play a significant role in handling neuron active compounds in the brain [78]. In addition to OCT and OAT, OCTN1 and 2 are detected in neurons in hippocampus, cerebellum, spinal cord, and superior cervical ganglion [77]. Systemic and brain concentrations of acetyl-l-carnitine are decreased in Octn-2 gene knockout mice, suggesting that luminal expressed Octn-2 could assist the brain uptake of carnitine [79].

### 6.4.4 SLC Transporter in Kidney

The kidney is a principal excretory organ for endogenous substances and clinical drugs and their metabolites through the processes of filtration, tubular secretion, and selective or passive reabsorption. The active secretion and reabsorption of organic anion occur mainly in the proximal tubule region of the kidney, particularly in the renal proximal tubular cells. SLC transporters in these cells play an important role in several processes: (i) uptake of chemicals into the cells; (ii) drug efflux into the glomerular filtrate; (iii) reabsorption of drug from the filtrate; and (iv) drug efflux back into the blood. The active renal secretion of drugs is accomplished through a vectorial transport process in the renal proximal tubules, which consists of uptake from systemic circulation via the basolateral membrane and subsequent efflux into urine across the luminal membrane [3,80,81]. Renal tubular epithelial cells express a variety of transporter proteins that handle diverse substrates to mediate proximal tubular secretion and, in some cases, reabsorption to maintain body homeostasis. Renal transporters have been recognized as important determinants in drug elimination for many clinically used drugs [3]. There are two primary groups of carrier-mediated transporters (SLC22 family) for organic anions and cations. OATs, OCTs, and OCTNs play key roles in active renal proximal tubular secretion (Fig. 6.8). Additional kidney transporters include several members of the SLC family, such as the OATPs, sodium-phosphate transporter (SLC17A1, NPT), PepTs, ENT and CNT, and MATE1 and MATE2-K.

Among the renal transporters detected, OAT1, OAT3, and OCT2 are located on the basolateral membrane of renal proximal tubule cells [37,82] and mediate the uptake of a variety of organic cations or organic anions from systemic circulation. The OATs and OCTs transport a broad range of substrates, including endogenous metabolites, anionic compounds such as nonsteroidal anti-inflammatory drugs, and the β-lactam antibiotics. Three OCT isoforms, OCT1–3, are identified as the primary transporters involved in renal secretion of several marketed drugs such as metformin, amantadine, and memantadine [3,83–85]. OATs and OCTs mediate the uptake of organic cationic and anionic compounds in concert with efflux carriers on the luminal membrane to

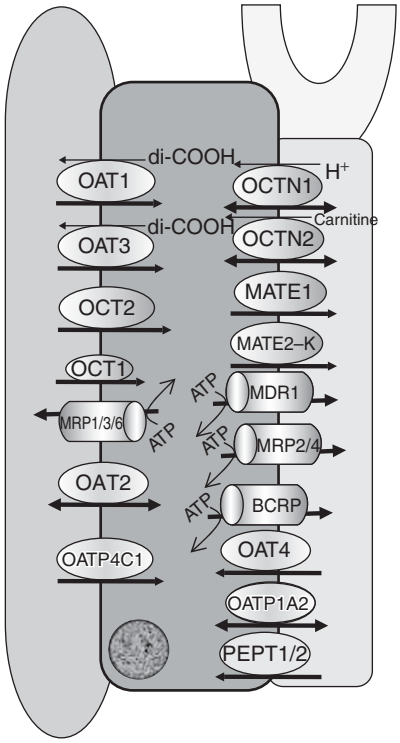


**Figure 6.8** Schematic of apical and basolateral membrane transporters in human renal proximal tubular cells.

translocate xenobiotics into urine. Decrease in renal secretion and CL may produce an increase in systemic drug exposure, thereby resulting in clinically significant changes in the overall drug PK. Competitive inhibition of proximal tubular secretion is the most common type of renal DDI, for example, probenecid (an OAT substrate and inhibitor) prevents excessive accumulation of cidofovir or cephaloridine in proximal tubular cells, resulting in reduced nephrotoxicity [60,86]. Similar to renal OATs, DDI may also result from the modification of OCT1–3 functions. For example, cimetidine reduces the renal CL of metformin and thus increases exposure (increase in AUC) of metformin in patients via inhibition of OCT2 activity [87]. Substrates taken up from the systemic circulation may subsequently undergo efflux across the brush border membrane of the proximal tubule cells by various ABC efflux transporters such as P-gp and BCRP [88]. MATE1 and MATE2-K are located in the brush border membrane of proximal tubular cells in humans and preclinical species, and facilitate renal secretion of structurally diverse compounds [89]. MATE2-K works collaboratively with OAT3 for vectorial transport of substrates in proximal tubular cells [10]. OAT expression may be modulated by disease states, for example, increased Oat1 expression is found in rats with bilateral ureteral obstruction [90]. Chronic renal failure induced by nephrectomy can result in a decrease of OAT1 expression [91]. Modulation of transporter expression under disease conditions can potentially modify the renal excretion of substrate drugs.

## 6.5 *IN VITRO* MODELS TO ASSESS SLC TRANSPORTERS

Cell- and membrane-based systems are used *in vitro* to characterize transporter-mediated interactions. Whole-cell models use transformed/immortalized cell lines or primary cells, while membrane-based models include membrane fragments or vesicles.

### 6.5.1 Cell-Based Models

***6.5.1.1 Immortalized Cell Lines.*** These cell lines are used to assess the absorption and permeability potential of compounds in drug discovery. The cells are referred to as *wild type* when used as a control for the cells overexpressing specific exogenous transporter genes. Caco-2 and MDCK cell lines are the most widely used system in ADME research for evaluating the substrate and inhibition potential of drug candidates. Although synonymous with efflux transporters, particularly MDR1 (P-gp), these cell lines are routinely used to assess the extent of transport across the epithelium because of the presence of well-developed and functional tight junctions. Caco-2 and MDCK cells can be cultured on Transwell® permeable supports to establish confluent monolayers with high transcellular resistance, allowing for measurement of uptake (apical to basolateral) and efflux (basolateral to apical) of test compounds through the monolayer. The advantage of the Caco-2 cells is that these are of human colonic origin and have many enterocytelike characteristics, while the MDCK cells are from the canine kidney. However, while the Caco-2 cells require three weeks to establish adequate tight junctions to form a resistant monolayer, the MDCK cells can do this within a week, making these more cost-effective, while essentially providing equivalent data [92]. Caco-2 and MDCK cells express multiple endogenous transporters such as ASBT; amino acid (e.g., LAT2), peptide (e.g., PEPT1), and monocarboxylate (MCT-1) transporters for uptake [93–95]; and MDR1, BCRP, and MRP1–3 for efflux [96]. Consequently, these cell lines (and others, e.g., LLC-PK1 and HK-2) are widely used for evaluation of oral bioavailability and predictions of BBB and tumor permeability. Although under appropriate culture conditions these wild-type cell lines produce polarized monolayers, with transporters correctly inserted in the cell plasma membrane, these are likely not present in the same quantities or ratios as those in the cells *in vivo*. Owing to the overlapping substrate specificities of many SLC transporters and the lack of transporter-specific inhibitors and substrates, an appropriate strategy is to use wild-type cell lines for the initial screening of test compounds, and if significant transport is observed, overexpressed/recombinant transporter systems are utilized to identify the specific transporters.

***6.5.1.2 Stably Transfected Cell Lines.*** These are the most widely used system for drug-transporter-based studies. The cell lines most commonly used for drug transporter transfections are MDCK and HEK-293. For uptake transporters, singly transfected cell lines suffice, for example, OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, and OCT2 [97,98]; however, if evaluating efflux, in some cases, the coexpression of uptake transporter(s) along with the efflux transporter may be necessary to fully evaluate efflux transport. Since MDR1 transports a wide variety of lipophilic substrates that can diffuse through the cell membrane, an uptake transporter is generally not required. However, MRP2 transports organic anions and conjugates and an uptake transporter, for example, OATP1B1 may be required to facilitate the uptake of MRP2 substrate compounds into

the cell [99]. Similarly, to assess efflux of bile salts via BSEP, a vectorial coexpression system may be needed in which cells are transfected with bile salt uptake transporter, NTCP, and the efflux transporter, BSEP [97]. Studies have shown that multiple uptake transporters can be transfected simultaneously, for example, the coexpression of major hepatic uptake transporters OATP1B1 and OATP1B3 or the renal uptake transporters OAT1, OAT3, and OCT2 to evaluate substrate uptake involving several transporters [100].

***6.5.1.3 Transiently Transfected Cell Systems.*** The advantage of the transiently transfected systems is that only one cell line is propagated in culture and the transporter of interest is transfected when needed, reducing the burden of maintaining multiple stable cell lines. Transient transfection systems are comparatively cost and resource sparing, especially when supporting large drug discovery programs. The vaccinia virus system is a widely used model [101] that yields high expression levels, as the virus shuts down the host cell protein synthesis by hijacking the host cell resources to generate recombinant viral proteins. Alternatively, *Xenopus* oocytes can be used as a recombinant system in which mRNA is injected into the cells to express the transporter of interest. In general, the overexpression of the transporter protein in this system is tedious and low throughput. This system has been used to express uptake transporters and has been applied to evaluate drug CL and address drug–transporter interactions [102].

***6.5.1.4 Primary Cells.*** A majority of transporter studies in primary cells have been conducted with rat or human hepatocytes. Both suspension [103] and plated [104,105] primary hepatocytes are effective to evaluate drug uptake, biliary excretion, and drug–transporter interactions. In culture, when sandwiched between two layers of extracellular matrix, these cells polarize and establish functional bile canaliculi, in which transporters localize correctly to the apical and basolateral membranes [106]. Cryopreserved hepatocytes preserve both drug-metabolizing enzymes and the major uptake and biliary transporters [107,108], although levels of these change over time in culture. *In vitro–in vivo* correlations of uptake CL have been demonstrated using primary hepatocyte cultures utilizing the well-stirred model [109]. Cultured human and rat hepatocytes can be used to evaluate the hepatotoxic potential of drugs due to inhibition of bile acid excretion [104] and OATP-mediated transport [110], providing insight toward the prediction of drug disposition and DDI. On the other hand, limited success has been observed for primary human renal proximal tubular cells for transporter-mediated studies. While uptake can be evaluated effectively in fresh suspensions, these cells tend to lose membrane transporter activity when placed in primary culture on plastic culture plates, possibly because of internalization of the transporter proteins (authors' unpublished observations). However, when these cells are plated on permeable supports, they establish transepithelial resistance of 100–200 $ohm^2$, enabling their use to assess bidirectional transport across the monolayer [111]. Typically, the cells are cultured for 8–10 days as monolayers on permeable filter supports. To date, mRNA has been detected for OCT2, OCTN2, OAT1, OAT3, OAT4, MDR1, MRP2, and BCRP and protein for OAT1 and OAT3 [112]. Functional activity studies have been conducted demonstrating that PAH is transported (likely via uptake across the basolateral membrane by OAT1 and OAT3) and creatinine is secreted by OCT2-mediated uptake at the basolateral membrane

and efflux by MDR1 at the apical membrane. Similarly, there are a limited number of studies using primary brain endothelial cells and none to our knowledge that investigated the SLC transporters.

***6.5.1.5 Hepatic Couplets.*** The hepatocyte couplet model allows for the evaluation of uptake and biliary elimination of test compounds. The advantage of the couplets is that these are polarized cells with a distinct basolateral membrane interacting with the media/buffer and an apical bile canaliculus, which is the sealed lumen between the two adjacent hepatocytes. Rat couplets have been used to evaluate bile transport and Bsep function by monitoring the uptake of taurocholate [113], canalicular transport of the organic cation daunorubicin [114], and electroneutral uptake and electrogenic secretion of the fluorescent bile salt 7β-NBD-NCT [115]. Although the data obtained from these studies is valuable, isolating and working with hepatic couplets requires high technical skill and is low throughput, likely accounting for the limited ADME studies with this model.

### 6.5.2 Membranes and Vesicles

Both membranes and vesicles are made by overexpressing the transporter of interest by transfection of a cell line, or from organ tissues, for example, liver canalicular membranes. In the case of cell lines, once the transporter is expressed, the cell membranes are isolated and the transporter can be purified from the cell membranes [116,117]. The cell membranes can be used as is or made into vesicles to assess transporter-mediated uptake into the vesicular space. At present, only the ABC transporters have been successfully assessed in cell membranes or vesicles. It is more challenging to assess SLC activity in these systems, as the transporters are either cotransporters, antiporters, or work down a pH or concentration gradient. At present, these conditions have not been worked out in isolated membrane vesicles.

### 6.5.3 Small Interference RNA (siRNA/RNAi)

The mRNA of interest is specifically knocked out by the introduction of antisense RNA into the cells, resulting in degradation of the corresponding complementary mRNAs in a gene-dependent manner and loss of expression of the target protein [118]. In cell lines, RNAi has been used successfully to silence activities of specific transporters, to identify the transporters responsible for L-DOPA renal transport (rBAT and LAT2) [119], and to identify ABCA1 as the transporter mediating the secretion of the Aβ peptide [120]. Partial knockdown of Mrp2 (50% expression) in rat primary hepatocytes resulted in a 45% decrease in the biliary excretion index of carboxy-dichlorofluorescein without effecting Mrp3 expression [121]. In the absence of specific substrates and inhibitors for transporters, siRNA may be an appropriate system to assess the contribution of specific transporters to test compound disposition in primary cells and wild-type cell lines.

## 6.6 *IN VIVO* MODELS OF TRANSPORT

Transporter studies can also be conducted *in situ*, using isolated perfused organs or *in vivo* in wild-type, genetic knockout, genetic knockin, transgenic, or humanized/chimeric animals. Combining *in vivo* and *in vitro* data, the model provides additional insights toward potential transporter-related issues in humans.

### 6.6.1 Isolated Perfused Organ Systems

Compared to the *in vitro* assays, the isolated perfused intestine, liver, brain, and kidney provide a relatively more physiological determination of transporter functions in absorption, biliary elimination, brain penetration, and renal excretion, while also allowing for evaluation of the contribution of drug-metabolizing enzymes. For *in situ* studies, the organ (typically rodent) is directly infused with a physiological buffer, artificial blood, plasma, or saline via a major blood vessel entering the organ (e.g., hepatic artery for the liver) and the test compound is added to perfusate and infused for predetermined times. Concentrations of test compound and metabolites are determined in the organ and at the point of exit from the organ. The advantage of the isolated perfused organ system is that the concentration of a drug in the organ of interest can be controlled [122]. An example of such a study is using the rat-isolated liver perfusion model to determine the AUC of digoxin in the presence and absence of the Oatp2 inhibitor rifampicin and the Mdr1 inhibitor quinidine. Concentrations of digoxin in the perfusate were increased by rifampicin and decreased by quinidine, indicating that rifampicin limits enzyme exposure to digoxin by inhibiting Oatp2-mediated uptake, whereas quinidine increases enzyme exposure to digoxin by inhibiting the Mdr1-mediated efflux [123].

### 6.6.2 Knockout and Transgenic Models

Since transporters have broad substrate specificity, knocking out one or more related transporters at a time can theoretically result in a more clear understanding of their *in vivo* function. Care has to be taken when extrapolating these predictions to humans, as there are major differences in the rodent and human transporters in terms of substrate recognition as well as the lack of orthologs, for example, in the kidney, rodents express both Oct1 and Oct2, while humans express only OCT2.

***6.6.2.1 Oatp Transporters.*** The hepatic transporter Oatp was first studied in a knockout mouse model of Oatp1b2 using the Oatp1b substrates pravastatin and rifampin. The liver-to-plasma ratios were lower in the knockouts as compared to wild type, indicating that Oatp1b2 is involved in the hepatic uptake and CL of these drugs [124]. The *Slco1b2*$-/-$ mice also exhibited lower liver-to-plasma ratios of the HMG-CoA reductase inhibitor lovastatin, but not of cerivastatin or simvastatin acid, suggesting that Oatp1b2 contribution in hepatic statin uptake is variable [125]. The *Slco1b2*$-/-$ mice were protected from hepatotoxicity induced by phalloidin and microcystin-LR, indicating a role for Oatp1b2 in transporting these toxins into the liver [126]. Transgenic mice expressing human OATP1B1 were created by feeding the mice a semisynthetic diet to downregulate endogenous *Slco* genes. Methotrexate PK revealed a 1.5-fold lower AUC in OATP1B1-expressing mice as compared to the wild types [127]. *Slco1a/1b*$-/-$ mice demonstrate significantly reduced hepatic uptake and elevated exposure to methotrexate and fexofenadine after IV or PO administration [128].

***6.6.2.2 OATs.*** Oat1$^{-/-}$ and Oat3$^{-/-}$ mice have demonstrated the *in vivo* relevance of these transporters in renal elimination of overlapping substrates. In kidney slices from Oat3$^{-/-}$ mice, taurocholate, estrone sulfate, and PAH concentrations were reduced

relative to those from wild-type, while there was no difference observed in the liver, which does not express Oat3 [28]. Penicillin G PK was reduced by one-half in Oat1$^{-/-}$ and Oat3$^{-/-}$ male mice and by two-thirds in female Oat3$^{-/-}$ mice [129]. Gender-related differences were also observed in methotrexate CL [130].

***6.6.2.3 OCTs.*** Knockout mouse models for all three OCTs have been developed. In the Oct1$^{-/-}$ mice, TEA accumulation was reduced significantly as compared with wild-type mice, indicating that Oct1 is the main sinusoidal uptake system for TEA in the liver [131]. Small intestinal excretion of TEA was reduced by about 50%, indicating that Oct1 also mediates the basolateral uptake of TEA into enterocytes [132]. Knocking out Oct1 from the mouse liver resulted in a shift in the elimination of Oct1 substrate drugs from hepatic to renal elimination, and consequently, renal excretion of drugs was increased [132]. Since the Octs have overlapping substrates, knocking out all three Octs could result in more definitive data. To assess this, Oct2 single-knockout and Oct1/2 double-knockout mice were generated [131]. While removal of Oct2 did not have a significant effect on elimination of TEA, in the double-knockout mice, renal secretion of TEA was completely eliminated and higher plasma levels were observed. Oct3-deficient mice showed significant reduction in $MPP^+$ accumulation in the heart as compared with wild-type mice [133]. This demonstrates that when extrapolating data with genetically manipulated preclinical species, it is advisable to consider the effects on all organs and not just on the one of interest to that particular study.

***6.6.2.4 MATE Transporters.*** Knockout models show that as compared to the wild-type animals, Mate1$^{-/-}$ mice exhibit a twofold increase in systemic exposure to metformin as a result of the reduced renal CL [134]. Mate1$^{-/-}$ mice demonstrate increased cisplatin-induced nephrotoxicity and increased plasma and renal concentrations relative to wild-type mice [135]. Reduced renal CL of cephalexin was also observed in Mate1$^{-/-}$ mice relative to that in the wild-type mice [136]. These studies showed that, in humans, the interplay between OCT2 and MATE1 may likely affect the net renal secretion of shared drug substrates.

***6.6.2.5 Limitations of knockout and transgenic murine models.*** Although some of the murine transporter orthologs being studied are eliminated in transgenic models, the presence of other murine-specific transporters with overlapping substrate affinities are still present, and therefore, data obtained is the result of a combination of human and murine events. Another problem is that deletion of one transporter can cause alteration in expression of other transporters and/or enzymes. For example, Mdr1a/1b−/− and Mdr1a−/− knockout mice had significantly increased levels of CYP3A, 2B, and 1A proteins and activities [137]. Moreover, many of the SLC transporters, for example, OATPs do not have animal and human orthologs. Moreover, these models do not recapitulate the role of regulatory proteins such as PXR, liver X receptor α (LXRα), and farnesoid X receptor (FXR), which are involved in regulating the key nuclear receptors governing expression of these transporters. While these models are useful in studying a particular transport protein, the results should be extrapolated with caution since the SLC transporter being assessed may not have a human ortholog or other elimination pathways may be up- or downregulated.

### 6.6.3 Chimeric/Humanized Murine Models

Potential future models that could reduce deficiencies in the transgenic models because of the lack of animal and human orthologs are "humanized" mouse liver animal models. Typically, human hepatocytes are transplanted into immunodeficient mice with compromised livers (e.g., SCID mice) and between 70% and 90% replacement of murine hepatocytes with human hepatocytes is reported [138,139]. Hepatic mRNA expression of human drug-metabolizing enzymes and transporters in these chimeric mice revealed that 21 of the 23 transporters found in the wild-type mice were expressed. These mice have mainly been utilized for PK studies and have not gained widespread use because of the problems associated with using immunodeficient animals, high costs, and highly variable data, likely because of differences in hepatocyte repopulation between the mice. More recently, a humanized mouse model has been developed in immunocompetent mice with normal liver function, using facile and ectopic implantation of a tissue-engineered human liver [140]. The juxtacrine and paracrine signals in the polymeric scaffolds appear to stabilize the function of cryopreserved primary human hepatocytes. These mice exhibit humanized liver functions that persist for weeks, enabling the evaluation of drug metabolism, DDI, and drug-induced hepatotoxicity. It is likely that these models will continue to improve and that "humanized" mice will prove to be an effective *in vivo* model for transporter-based evaluations.

## 6.7 CLINICAL EXTRAPOLATIONS

Relatively limited work has been published thus far on successful quantitative correlations. One method published by Hirano *et al*. proposes correlation methodologies and predicts the degree of inhibition for OATP-transporter-mediated drug interactions [141]. If the compound inhibits a probe substrate with an $IC_{50}$ value $<10\times$ the unbound inhibitor concentration, then the compound has the potential to illicit a clinical drug–transporter interaction with the OATPs. These authors have furthered the drug interaction prediction by developing an algorithm based on *in vitro* and *in vivo* data, known as the *Degree of Interaction* ($R$) and estimated by the following equation:

$$R = 1 + \frac{f_u \times I_{in,\,max}}{K_i}$$

$I_{in,max}$ is the unbound inhibitor concentration ($C_{max}$) at the entry point of the organ (e.g., in the hepatic portal vein for the liver or the renal artery for the kidney), $f_u$ is the blood unbound fraction, and $K_i$ is the inhibition constant. However, an $IC_{50}$ value is typically utilized instead of the $K_i$. The $I_{in,max}$ is determined as

$$I_{in,\,max} = I_{max} + \frac{F_a \times \text{Dose} \times k_a}{Q}$$

where $I_{max}$ is the maximum circulating plasma concentration, $F_a$ represents the absorbed fraction of inhibitor, $k_a$ is the absorption rate constant in the intestine, and $Q$ is the hepatic blood flow rate in humans (1500 mL/min) [142]. As the ratio of $(f_u \times I_{in,max})/IC50$ increases, the likelihood of an interaction will increase as well. The potential for drug interaction is minimal if the calculated $R$-value is close to

one and drug interaction potential is likely with $R$ values >2. These extrapolations are confounded by the fact that many drugs that actively enter the liver are also metabolized, and thus far, there is no effective method to account for the interplay between transport and metabolism toward quantitative predictions of drug interactions.

In the case of the kidney, the quantitative extrapolations must also factor in the glomerular filtration rate (GFR) of the test article. When CL > GFR, it indicates active elimination via renal transporters, and when CL < GFR, it indicates active reabsorption is present. When CL = GFR, it may indicate no active transporter interaction or the potential for simultaneous active efflux and reabsorption, in which the two mechanism negate each other but transporter-mediated drug interactions may still occur.

While for the CYP450 enzymes there are sufficient *in vitro – in vivo* correlations to classify *in vitro* $IC_{50}$ and $K_i$ data as low-, medium-, and high-drug interaction liability, this is not the case with the SLC transporters. For hepatic transporters, the $K_m$ and $K_i$ values that appear to be relevant to a clinical endpoint are quantitatively similar to the CYP enzymes, for example, $K_i$ values in single digits for the OATP transporters [105]. However, in the case of the renal secretory transporters, in some cases, the $K_m$ and $K_i$ values that appear to be relevant to a clinical endpoint [143,144] can be relatively high compared to those reported for the hepatic transporters or CYP enzymes and are closer to what is reported for the UDP-glucuronosyltransferase (UGTs) [145]. For example, the CL for acyclovir in humans is higher than the GFR at 2.6 mL/min/kg and the affinity ($K_m$) for OAT1 is 242 μM [146]. Similarly, hydrochlorothiazide, which is actively transported predominantly via renal OATs, also has a CL > GFR (4.9 mL/min/kg) and a high $K_i$ value (150 μM) for the renal transporter OAT1 [147]. Until there are better methodologies developed to relate *in vitro* $K_m$ and $K_i$ values, the potential to predict *in vivo* transporter-mediated interactions will be weak. An effective strategy is to include benchmark marketed compounds, preferably similar chemotypes, as a comparator, to help guide predictions of potential interactions.

## 6.8 INTERPLAY BETWEEN SLC TRANSPORTERS AND DRUG-METABOLIZING ENZYMES

Transporters expressed in various tissues contribute to the distribution of physiological substances (e.g., bile acids and cholesterol) and nutrients and removal of metabolic waste [148]. The extent of drug movement across membranes is generally affected by physicochemical properties such as size, lipophilicity, charge, and degree of ionization. As transporters contribute to the ADME, they invariably influence the PK properties of drugs. The SLC/SLCO uptake transporters facilitate the distribution of hydrophilic drugs into tissues for greater access to drug-metabolizing enzymes. However, a majority of drugs are lipophilic and do not require an active transport process, as they can traverse the lipid bilayer of the cell membrane passively by diffusion.

Drug bioavailability and hepatic disposition can be greatly influenced by transporter–metabolism interplay. The interplay between drug-metabolizing enzymes and drug transporters was first identified in the 1990s from cyclosporine interaction studies with ketoconazole (CYP3A inhibitor) and rifampin (CYP3A4 inducer), in which changes in cyclosporine PK were not consistent with alterations of gut and hepatic metabolism by these two modulators [149–152]. The interplay became more

**TABLE 6.3 Drugs Displaying Potential SLC Transporter and Drug-Metabolizing Enzyme Interplay**

| Object | Transporter(s) | Drug-Metabolizing Enzymes | References |
|---|---|---|---|
| Rosuvastatin | OATP1B1 | $CL_{hep} = 10\%$; metabolism by CYP2C9; metabolism by UGT1A1 and UGT1A3 | 155 |
| Atorvastatin | P-gp, OATP1B1, BCRP | $CL_{hep} \sim 100\%$; CYP3A4 extensive, metabolism by UGT1A3 | 156–158 |
| Cerivastatin | P-gp, OATP1B1 | $CL_{hep} \sim 100\%$; CYP3A4 and CYP2C8 | 159 |
| Fluvastatin | OATP1B1 | $CL_{hep} = 95\%$; metabolism by CYP2C9 (75%), but also by 3A4 and 2 C8 | 160,161 |
| Simvastatin | OATP1B1 | $CL_{hep} \sim 87\%$; CYP3A4 extensive first-pass metabolism, with only 5% available systemically | 162 |
| Bosentan | OATP1B1, OATP1B3 | $CL_{hep} \sim 97\%$; metabolized by CYP2C9 and CYP3A4 | 163 |
| Bromocriptine | OATP1B1 | $CL_{hep} \sim 100\%$; CYP3A4, extensively metabolized | 164 |
| Repaglinide | OATP1B1 | $CL_{hep} \sim 98\%$; CYP2C8 and CYP3A approximately equal in contribution | 165,166 |

lucid when overlap of substrate specificity for CYP3A4 and the ABC transporter MDR1 was identified. In addition to MDR1, enzyme–transporter interplay was also observed with SLC transporters, in which the transporter facilitated drug uptake into tissues (GI tract and liver) and exposed the drug to the metabolizing enzyme (Table 6.3). After metabolism, transporters, such as MDR1, BCRP, and MRP2, may contribute to the efflux of the metabolite to bile and urine [153]. Thus, transporters can be viewed as aiding the access of drugs to drug-metabolizing enzymes, thereby facilitating metabolism to protect the body from a buildup of endogenous and exogenous substances, whereas drug efflux decreases the load on detoxification enzymes [154].

### 6.8.1 OATP Transporters

The most widely evaluated anionic drugs are statins (e.g., fluvastatin, cerivastatin, simvastatin, and pravastatin) and diabetes drugs such as troglitazone and repaglinide. Many of the statins undergo significant phase I and phase II metabolism (fluvastatin, simvastatin, lovastatin), while pravastatin is excreted largely unchanged [167–169]. Clinical evidence based on genetic polymorphism studies for SLCO1B1 (encoding OATP1B1) of c.521 T>C (p.Val174Ala) reveal increases in the plasma concentrations

of simvastatin acid and pravastatin, but no significant effect on fluvastatin [160], consistent with hepatic uptake being rate limited. As shown in Table 6.3, there are several examples that highlight transporter–enzyme interplay in drug disposition: fluvastatin (OATP1B1 and CYP2C9, and to a minor extent CYP3A4 and CYP2C8), repaglinide (OATP1B1, MDR1, and CYP2C8 and CYP3A4), and bosentan (OATP1B1, OATP1B3, and CYP3A4 and CYP2C9) [163,165,170]. In addition to drugs with uptake and CYP metabolism, atorvastatin and rosuvastatin are also metabolized by the UGT enzymes [156–158]. The combination of transporter–drug metabolism interplay results in significant and complex drug interactions that are difficult to predict using *in vitro* systems.

### 6.8.2 OATs/OCTs

There are limited publications on drug transporter–enzyme interplay for OAT and OCT substrates. Many of the anti-infective drugs are predominately eliminated by renal OATs, for example, Tamiflu and olmesartan. These two prodrugs undergo metabolism that requires esterase cleavage for activity, and subsequently, their carboxylate metabolites are exclusively renally eliminated [171,172]. Ciprofloxacin and dicloxacillin undergo hepatic metabolism; however, renal CL is the predominant pathway. Methotrexate has some involvement with xanthine oxidase and aldehyde oxidase [174], and zidovudine is metabolized by UGT2B7 in the kidney [175], but there is no drug transporter–metabolizing enzyme interplay linkage reported clinically. For the OCTs, procainamide (an antiarrhythmic agent) undergoes CYP2D6 metabolism, and 65% of the dose is excreted unchanged in the urine [176], and theophylline is predominately metabolized by CYP1A2 [177]. Similarly, no reported transporter–metabolism interplay has been reported for the OCTs.

## 6.9 TRANSPORTER-MEDIATED DRUG INTERACTIONS DUE TO INHIBITION

### 6.9.1 OATP Transporters

The interplay between SLC/SLCO transporters and drug-metabolizing enzymes can be quite complex when multiple drugs are coadministered. The cotherapeutics may enhance or reduce active transport across membranes, as well as modulate the activity of metabolic enzymes, resulting in larger than expected drug interactions [178]. The uptake of HMG-CoA reductase inhibitors pravastatin, pitavastatin, atorvastatin, and fluvastatin is saturable and has been shown to be rate limiting in rats and human hepatocytes [179]. As shown in Table 6.3, statins are metabolized by CYP3A4 (simvastatin and lovastatin) and have up to 20-fold increases in plasma concentrations in the presence of strong CYP3A4 inhibitors such as itraconazole and ritonavir. Potent inducers of CYP3A can greatly decrease plasma concentrations of simvastatin and simvastatin acid, and probably those of lovastatin and lovastatin acid, while pravastatin plasma concentrations are not significantly affected by CYP inhibition and only slightly affected by CYP inducers [160]. Human PK interaction studies have shown that CYP3A4 inhibitors clarithromycin and itraconazole modestly increase the area under the plasma AUC of repaglinide to ~40% [166]. However, the AUC of repaglinide increased 8.1-fold when coadministered with gemfibrozil [166]. The large drug

interaction with gemfibrozil and its glucuronide metabolite is attributed to inhibition of OATP1B1 and CYP2C8 [166]. Further, OATP1B1-mediated hepatic uptake of repaglinide is important for its elimination by CYP-mediated reactions as evidenced by elevation of repaglinide plasma levels in subjects with the OATP1B1 (521CC) genotype.

In comparison to other statins, pravastatin has greater hydrophilicity that limits its penetration into the intracellular space of nonhepatic tissues, which is consistent with its poor penetration through the BBB [180]. Pravastatin CL is equivalent between renal and nonrenal routes (i.e., biliary excretion and biotransformation), and therefore, dose adjustments are generally not required during hepatic or renal impairment. Furthermore, as metabolism by CYPs is minimal, this reduces the extent of drug interactions for pravastatin when compared to other statins that undergo extensive metabolism. Despite pravastatin having minimal CYP metabolism, drug interactions can be pronounced, for example, a 10-fold change in pravastatin AUC is observed when it is coadministered with cyclosporine (Table 6.3).

### 6.9.2 OATs

It is well documented that renal tubular secretion is inhibited by the coadministration of probenecid, which increases the circulating levels of penicillin and cephalosporin antibiotics [181,182]. Probenecid inhibits active renal transport processes via inhibition of OAT1 and OAT3 [183]. As shown in Table 6.4, probenecid alters the exposure of several antibiotics, pravastatin, fexofenadine, and some antiviral drugs, with a change in AUC from 11% to 260%.

### 6.9.3 OCTs

The number of significant drug interactions associated with OCT is limited. Apricitabine is a deoxycytidine analog nucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus-1 infection. Increased apricitabine drug exposure (AUC) of 66% and 42% was observed with concomitant administration of cotrimoxazole (trimethoprim/sulfamethoxazole) and tipranavir/ritonavir combination, respectively [201,202]. The extent of drug interaction observed for cimetidine and metformin was modest as the AUC of metformin increased by 54% (Table 6.3). Genetic polymorphism of OCT2 was evaluated in the Chinese population, and the 808G>T polymorphism was attributed to a reduced metformin renal tubular CL. Furthermore, this mutation correlated with the extent of cimetidine-mediated inhibition of metformin renal tubular secretion [203].

### 6.9.4 NTCP Transporter

To date, there are no published clinically relevant drug–transporter interactions with human NTCP. However, *in vitro* studies utilizing HeLa-NTCP cell lines and human hepatocytes have demonstrated that rosuvastatin uptake is mediated by this transporter, accounting for ~35% of its uptake into the liver [204]. Moreover, cyclosporine and gemfibrozil can inhibit the uptake of rosuvastatin with $IC_{50}$ values of 0.4 and 23 μM, respectively. Polymorphisms related to NTCP have shown that the NTCP*2 variant,

**TABLE 6.4 Summary of OAT-Mediated Clinical Drug Interactions**

| Precipitant | Object | Object Therapeutic Class | Percentage Change in AUC | References |
|---|---|---|---|---|
| Probenecid | Cephradine | Antibiotics | 263.2 | 182 |
| | Furosemide | Diuretics | 167.9 | 184 |
| | Cephradine | Antibiotics | 141.6 | 185 |
| | Cephradine | Antibiotics | 138.8 | 186 |
| | Cefoxitin | Antibiotics | 136.5 | 187 |
| | Cefaclor | Antibiotics | 114 | 185 |
| | Cefonicid | Antibiotics | 109.2 | 188 |
| | Famotidine | $H_2$ receptor antagonists | 81.1 | 189 |
| | Ciprofloxacin | Antibiotics | 74.7 | 190 |
| | Fexofenadine (terfenadine carboxylate) | $H_1$ receptor antagonists | 69.1 | 191 |
| | Dicloxacillin | Antibiotics | 66.8 | 192 |
| | Cefmetazole | Antibiotics | 58 | 193 |
| | Cefoxitin | Antibiotics | 45.3 | 187 |
| | Acyclovir | Antivirals | 40 | 194 |
| | Ceftriaxone | Antibiotics | 34 | 195 |
| Rifampin | Pravastatin | HMG-CoA reductase inhibitors (statins) | 126.6 | 196 |
| Trimethoprim | Zidovudine | NRTIs | 30.5 | 197 |
| Disufenton sodium | Cefuroxime | Antibiotics | 26.6 | 198 |
| Pravastatin | Olmesartan | Angiotensin II inhibitors (ARBs) | 21.1 | 199 |
| Cotrimoxazole (trimethoprim/ sulfamethoxazole) | Zidovudine | NRTIs | 17.9 | 197 |
| Furosemide | Lomefloxacin | Antibiotics | 11.9 | 200 |

*Abbreviations*: ARBs, angiotensin receptor blockers; NRTIS, nucleoside reverse transcriptase inhibitors.
*Source*: The data for this table was obtained from the University of Washington Metabolism and Transporter Drug Interaction Database.

which is known to have a near-complete loss of function for bile acids, exhibits elevated activity toward rosuvastatin uptake *in vitro*. Therefore, *in vitro* data suggest that NTCP may contribute toward the disposition of rosuvastatin, and consequently, inhibitors of this transporter may have the potential to cause a clinical drug interaction; however, the extent of the interaction in humans is not known.

Circulating levels of bile acid are dependent on the interplay between several transporters such as NTCP, BSEP, and MRP2/3/4 [205,206]. Animal models have shown that reduced expression of Bsep and inhibition of this transporter may explain impaired bile excretion leading to cholestasis [207]. Caution must be applied when extrapolating animal data to humans, as bosentan is a more potent inhibitor of rat Ntcp than human NTCP [208]. While NTCP may contribute toward the regulation of bile acids, there is no clinical evidence directly linking this transporter to cholestasis.

## 6.10 NUCLEAR RECEPTOR-MEDIATED SLC TRANSPORTER REGULATION

Similar to drug-metabolizing enzymes, hepatic transcriptional factors mediate the regulation of drug transporters. The major transcriptional factors involved in transporter-mediated regulation are AhR; CAR; PXR; FXR; PPARα (peroxisome proliferator-activated receptor α); Nrf2; hepatocyte nuclear factors HNF1α, HNF3β, and HNF4α; LXRα; and retinoid X receptor/retinoic acid receptor dimer (RXRα/RARα).

### 6.10.1 OATP Transporters

The regulation of these uptake transporters has been reported with AhR, PXR, CAR, and PPAR, on the basis of rodent induction studies [209]. Hepatic mRNA expression was evaluated after treatment with AhR ligands (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, polychlorinated biphenyl 126, and α-naphthoflavone) and Oatp2b1 was increased, and PXR activators (pregnenolone-16α-carbonitrile and spironolactone) increased Oatp1a4, while CAR activators (phenobarbital, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene and diallyl sulfide) decreased Oatp1a1 mRNA expression. PXR activators and PPARα ligands (clofibrate, ciprofibrate, and diethylhexyl phthalate) decrease Oatp1a1, 1b2, 2a1, and 2b1 mRNA expression in liver, and Nrf2 activators (oltipraz, ethoxyquin, and butylated hydroxyanisole) downregulate Oatp1a1 and upregulate Oatp2b1 mRNA expression. In rodents, it appears that only a few transcriptional factors increase Oatp expression, while multiple factors decrease Oatp expression [209].

In humans, FXR, HNF1α, HNF3β, and HNF4α transcriptionally regulate OATP1B1, OATP1B3, and OATP2B1 [210–214] by binding to the promoter region [212]. Site-directed mutagenesis revealed consensus binding sites in the 5′-flanking region from −38 to −24 bp for FXR, HNF1α, and HNF3β that are to be important for transcriptional activity of OATP1B3, as mutations in this region resulted in significantly decreased luciferase activity. Additionally, a vector containing the double mutant of HNF1α and HNF3β at the binding sites (−38 to −24 bp region) had significantly decreased luciferase activity as compared to the single transcriptional factor mutation. Using a different model, Kamiyama *et al*., established an adenovirus vector expressing human HNF4α-(hHNF4α) -siRNA that selectively suppressed HNF4α expression in human hepatocytes [212]. Data from this model suggests that OATP1B1 is directly regulated by hHNF4α, unlike the CYP450 enzymes, which are indirectly regulated by HNF4α via the upregulation of PXR and CAR expression in hepatocytes. Studies with the bile acid chenodeoxycholate (CDCA), the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA), endogenous ligands, and effective activators of FXR reveal a significant increase in OATP1B1 expression when FXR is activated [214].

### 6.10.2 OATs

Saji *et al*., showed that deletion of Hnf1α in mice (Hnf1α null mice) resulted in a significant reduction of renal Oat1 mRNA levels. This was followed with the observation that promoter activity of Oat1 was stimulated by cotransfection of HNF1α alone or the combination of HNF1α and HNF1β; however, HNF1β alone showed no effect [213].

For human OAT1, using the luciferase reporter gene assay, a series of 5′-truncated-promoter constructs of human OAT1 with or without the mutation in the HNF1 motif showed that HNF1α alone, or HNF1α and HNF1β, bound to the promoter region within 111 bp upstream of the transcriptional start site significantly increased luciferase activity, confirming binding to the HNF1 motif [213]. HNF1α/HNF1α or HNF1α/HNF1β dimers were found to bind to the HNF1 motif in the OAT1 promoter [213]. Similarly, HNF4α was shown to activate the OAT1 promoter in the regions spanning −1191 to −700 bp and −140 to −79 bp [215]. Kikuchi *et al*. showed that human OAT3 expression was transactivated by HNF1α and HNF1β. Similar to OAT1, there is a synergistic action of the HNF1α/HNF1α or HNF1α/HNF1β dimers toward transactivation and HNF1β alone, and this does not activate expression of OAT3. Moreover, it was found that OAT3 promoter activity was repressed by DNA methylation [216].

### 6.10.3 OCTs

OCT1 is transcriptionally activated by HNF1α, and two adjacent putative DNA-response elements interact with HNF1α binding in the promoter region [212,217]. A luciferase reporter construct containing the OCT1 promoter was successfully activated by HNF4α in transiently transfected Huh7 cells. Saborowski *et al*. showed that site-directed mutagenesis of either DR-2 element alone or in combination severely decreased the HNF4α-mediated activation of the OCT1 promoter, indicating that both elements are functionally important [217]. In addition to HNF1/4α, upstream stimulating factor (USF) 1 and 2 bind to the E-box regulatory element (CACGTG) of the proximal promoter, as deletion analysis suggested that the region spanning −141 to −69 bp was essential for the basal core transcriptional activity of OCT1 [218]. It appears there is coordination as the transactivation effect of USFs on the OCT1 promoter is further stimulated by HNF4α binding.

In the case of OCT2, USF-1 binds to the E-box in the proximal promoter region, as a mutation of the E-box resulted in decreased OCT2 promoter activity, while the overexpression of USF-1 transcriptional factor enhanced OCT2 activity in a dose-dependent manner [219]. In addition, *in vitro* studies showed that methylation of the OCT2 proximal promoter resulted in significant reduction of transcriptional activity of USF-1 [220].

### 6.10.4 NTCP Transporter

The hepatocyte nuclear factors HNF1α and 4α and RXRα/RARα bind and transactivate the rat but not the human or mouse NTCP/Ntcp promoters [221]. A 4-bp insertion in the HNF1α recognition site located within the transcription start site of human and mouse NTCP/Ntcp gene resulted in a disrupted HNF1α binding site and the transactivation of a consensus motif for the CCAAT/enhancer binding protein-β (CEBP-β) [221]. HNF3β was found to be the only transcriptional factor with conserved binding sites in the 5′-regulatory region of the human, mouse, and rat NTCP/Ntcp genes. HNF3β was found to repress NTCP activity by forming a specific DNA–protein complex, as assessed by electrophoretic mobility shift assays [221]. For RXRα/RARα, Jung *et al*. suggested that a putative binding site for HNF4α is localized with the RXRα/RARα sequence based on coexpressed studies, in which rat Ntcp promoter activity increased two-fold [221].

On the basis of mutagenesis studies, a functional glucocorticoid receptor (GR) response element was identified in the promoter region of NTCP [222]. Dexamethasone increased reporter-linked NTCP promoter activity. Eloranta *et al*. showed that a DNA element arranged as a direct repeat of hexamers separated by nine nucleotides (DR-9) at nucleoside −32/−12 of the NTCP promoter mediates the glucocorticoid response and that GR directly interacts with this motif. The authors indicate that upstream and downstream elements are also required for full NTCP promoter transactivation activity by GR/dexamethasone. Adding more complexity, the induction of NTCP gene by GR in the presence of glucocorticoids can be enhanced by coexpression of the transcriptional coactivator, PPARα (PGC-1α) [222].

Bile acids may regulate NTCP/Ntcp indirectly by modulating the capacity of nuclear factors to activate gene expression [221]. When bile acid levels are elevated, the uptake of bile acids into hepatocytes from sinusoidal blood is reduced because of the reduced expression of NTCP/Ntcp in both rodents and humans [206]. In rats, bile acid-mediated suppression involves inhibition of the activity of the RXRα/RARα heterodimer binding to the Ntcp promoter [221]. CDCA can cause reverse transcriptional activation of NTCP gene by GR/dexamethasone, and it has been proposed that GR is a target for negative feedback regulation of NTCP expression by bile acids in humans [222].

## 6.11 GENETIC POLYMORPHISMS OF SLC TRANSPORTERS AND CLINICAL IMPLICATIONS

Extensive human gene variation was reported after the near-complete sequencing of the human genome, with ∼12 million single nucleotide polymorphisms (SNPs) identified [223]. These polymorphisms can occur in the regions coding for proteins (nonsynonymous polymorphisms) to give rise to amino acid changes or in noncoding regions of the genome (synonymous polymorphisms) to regulate the expression and function of proteins. Genetic polymorphisms could affect protein expression or functional activity via alteration of substrate affinity. Information from gene polymorphisms has advanced our understanding of the contribution of population PK variations to tolerability and safety profiles of therapeutics. Genetic variants may be associated with susceptibility to disease or important differences in PK/PD drug parameters. The PK and distribution of substrate drugs could be altered by the changes of transport capability caused by genetic variants. The variants might also affect the apparent activity of the transporter by lowering the affinity for substrates ($K_m$), and in some cases, they cause loss of function of the transporter.

Since membrane transporters handle many drugs that are prescribed clinically and regulate the ADME, these proteins play a critical role in pharmacological and toxicological processes. Genotypic and phenotypic polymorphisms in the SLC family contribute to interindividual, interethnic, and gender-based variability in PK and drug distribution. Transporter polymorphisms are associated with changes in efficacy, drug interactions, and safety concerns, including idiosyncratic hepatic toxicity. On the basis of the US Food and Drug Administration (FDA) *Guidance for Industry on Pharmacogenomics Data Submissions*, pharmacogenomics data is required for all known or probable valid biomarkers. The FDA also encourages submission of data for those genes (including

ADME genes) for which there is limited knowledge about the associated clinical variants. This pharmacogenetic information is needed to support scientific arguments and it could be included in the drug label.

### 6.11.1 OATP Transporters

Several genetic variants, including 388A>G (130Asn>Asp, rs2306283) and 521T>C (174Val>Ala, rs4149056), of the *SLCO1B1* gene that codes for OATP1B1 protein have been identified. The polymorphisms of OATP1B1 and 1B3 have been characterized to occur at different frequencies in various racial and ethnic populations. For example, the frequency of OATP1B1 c388G allele (*1b) in Caucasians, Asians, and African-Americans is about 40%, 60%, and 75%, respectively, and the 521T>C in codon 174 is ~15%, 15%, and 2%, respectively [6]. The polymorphisms have different activities that can affect PK and result in deleterious side effects. For example, individuals with the OATP1B1*5 or OATP1B1*15 haplotypes have increased exposure to the substrates including pravastatin, simvastatin acid, atorvastatin, and rosuvastatin, and this may result in myopathy or increased risk of hepatotoxicity. These variants also have a significant impact on the PK/PD profiles of substrate drugs. When OATP1B1 polymorphisms were mapped in individuals taking simvastatin, a significantly increased risk for myopathy correlated with increased tissue exposure and Val174Ala polymorphism [224]. Cerivastatin was withdrawn from the market because of a serious adverse effect rhabdomyolysis, which has been related to the variants in SLCO1B1 with an odds ratio of 1.89, but not the variants of CYP2C8 or UGTs that metabolize cerivastatin [225]. When transfected with the variant SLCO1B1*15 gene (rs2306283+rs4149056), HEK cells uptake activity with cerivastatin is reduced ~40% compared with the cells expressing the SLCO1B1 wild-type gene [225]. The reduced transport capability is associated with decreased liver uptake of cerivastatin, leading to increased systemic exposure and risk of rhabdomyolysis. Pravastatin is an OATP1B1 substrate that is minimally metabolized and mainly excreted into bile unchanged. There is an ~10-fold variation in the AUC between individuals mainly because of the genetic polymorphisms in OATP1B1 that reduce elimination, thereby causing a significant increase in plasma concentrations of pravastatin. [226].

Genetic polymorphisms in regulatory regions of SLCO genes regulate the expression level of OATPs [227]. For example, OATP1A2 is expressed in brain capillary endothelial cells and contains one genetic variant located in a putative HNF1α binding site [228].

### 6.11.2 OCTs

Western blotting and mRNA quantification in human liver samples revealed that expression of OCT1 and OCT3 was highly variable between individuals. A reduced expression associated with nonsynonymous coding variants of OCT1 (rs12208357) or OCT3 (rs2292344) was reported [229]. OCT transports metformin into hepatocytes, and the functional loss of variants of OCT1 is linked to the reduced hepatic uptake of metformin and subsequently, its PD effect [230]. Individuals carrying reduced function OCT1 alleles, including SNPs in positions 420, 401, and 465, appear to have reduced metformin CL based on the observed higher AUC and $C_{max}$ [230].

Doors are opening to a new paradigm in drug development and therapy through pharmacogenetic research with human drug transporter proteins, which could lead to a clearer understanding of the individual susceptibility to drug toxicity and efficacy.

## 6.12 REGULATORY GUIDANCE FOR SLC TRANSPORTERS

The European Medicines Agency (EMA) issued a draft guideline on the investigation of drug interactions that included a section on drug transporters, in April 2010 and the Food and Drug Administration (FDA) issued a draft guidance for drug interaction studies in February 2012 that provides a more detailed information on drug interaction studies, including decision trees for the SLC (and ABC) transporters.

### 6.12.1 FDA Drug Interaction Guidance

As per the FDA guidance, all investigational drugs should be evaluated *in vitro*, using transfected cell lines and empty transfected cells to subtract out the background by endogenous transporters. Inhibition studies should be conducted in vitro for the hepatic transporters OATP1B1 and OATP1B3 and the renal transporters OAT1, OAT3 and OCT2, as well as the ABC transporters MDR1 and BCRP. Substrate assays for the hepatic transporters OATP1B1 and OATP1B3 are required when hepatic clearance is more than or equal to 25% of the total clearance. In vitro substrate assays for the three renal transporters are required when their renal active secretion is more than or equal to 25% of total clearance. The EMA includes the above transporters as well as OCT1 and BSEP, the latter being important for safety testing. An interaction is considered significant if uptake into the transfected cells is $\geq$2 fold of that in the empty vector transfected cells and can be inhibited by $\geq$50% by a known inhibitor. The FDA guidance suggests that when evaluating drug candidates, selection of the interacting drugs should be based on established, relevant and strong inhibitors of the transporter pathways under investigation. Additional transporters: MRPs, MATE and BSEP are mentioned and it is suggested that these be considered when appropriate.

For clinical studies, the FDA advises that the choice of substrate for a particular transport pathway be based on the therapeutic area and most likely to be co-administered drugs, whose PK is markedly altered by known specific inhibitors of the transporter pathway, so as to enable the largest impact of the interacting investigational drug. The FDA acknowledges that the observed clinical interactions may be a result of inhibition of multiple pathways, if the investigational drug is also an inhibitor for multiple pathways.

### 6.12.2 EMA Drug Interaction Guidance

The EMA suggests that *in vitro* studies be conducted to assess substrate and inhibition potential of new drug candidates with OATP1B1, OATP1B3, OCT2, OCT1, OAT1, OAT3 and the efflux transporters P-gp and BCRP and also BSEP for pharmacodynamic and safety monitoring. *In vitro* inhibition assays are suggested for all the transporters. To assess the clinical substrate potential of an investigational drug for a particular transporter, the EMA suggests the transporter be evaluated if it contributes to the absorption, distribution or elimination of a compound. For distribution, evaluating *in*

*vitro* data along with tissue specific expression of the transporter, data on distribution in preclinical species, clinical safety data in patients with altered transport due to genetic polymorphism and expected clinical consequences of altered distribution should be considered.

The EMA guideline recommends that if renal and biliary secretion account for $\geq$25% of systemic clearance, the transporter(s) involved in the active secretion should be identified. Potential OATP uptake should be investigated *in vitro* for non-cationic drugs with $\geq$25% hepatic elimination. The contribution of biliary secretion should be based on available mass balance and interaction data, pharmacogenetic information and hepatic impairment, if any. Renal secretion should be estimated by comparing total renal clearance to renal filtration clearance of the unbound drug, similar to the FDA guideline.

The recommended time line for *in vitro* evaluations is before Phase II clinical trials; unless all concomitant drug treatments are not expected to have interactions and *in vivo* interaction studies should be conducted prior to Phase III. The EMA also suggests that the possible effects of transporter-enzyme interplay, such as between Pgp and CYP3A, should be discussed, and if required, clinical studies considered.

The major differences between the two guidelines are the inclusion of OCT1 and BSEP as major transporters suggested by the EMA, while this is not a part of the FDA guidelines. In addition, while the FDA guidance allow for $IC_{50}$ values to be calculated, the EMA recommends $K_i$ values.

### 6.12.3 Induction

The FDA and EMA guidance also discuss induction of transporters. Since expression levels of these transporters are regulated in coordination with metabolizing enzymes by activation of the same nuclear receptors, induction of CYP1A2, CYP2B6 and CYP3A4 can be used as the initial evaluation of the induction potential of an investigational drug on transporters. The definitive studies continue to be clinical induction studies. For example, induction of CYP3A4 by the drug candidate will serve as a trigger for a clinical P-gp induction study, using digoxin as the probe substrate.

## 6.13 SUMMARY

While earlier studies with transporters were conducted primarily with the ABC family, particularly MDR1, the SLC transporters are now considered equally important in the disposition of xenobiotics within the body and continue to be extensively evaluated. There is a significant amount of work being conducted that this review has not discussed. For example, we were not able to cover all transporters involved in drug disposition, for example, amino acid transporters such as System L (LAT) are not included. We have mentioned but not discussed the differences in transporter expression, regulation, and activity across ethnic groups and between different age groups mainly because there have been very few studies on this so far and we have limited knowledge of the effect of these on ADME. Another gap in this review and also in current knowledge is information on the number of binding domains in individual SLC transporters, similar to what has been reported for ABC transporters such as MRP2. Megaraj *et al*. developed transporter cells lines with MRP2 and various SNPs and

found that SNPs located in the MRP2 nucleotide-binding domains variably decreased the transport of all substrates studied; an SNP in the membrane spanning domain 1 selectively decreased the apparent affinity for glutathione and glucuronide conjugated substrates, and an SNP in the carboxyl terminus altered only bile acid transport [231]. The presence of multiple binding sites further complicates transporter evaluations, and these need to be identified for the SLC transporters as well. Most importantly, we still do not have adequate models to extrapolate *in vitro* transporter data to *in vivo*, and the different models currently available can result in very different outcomes. For example, fluvastatin uptake by OATP1B1, 1B3, and 2B1 is inhibited 60–90% by gemfibrozil in the transfected cell lines but only by 27% in the more intact primary hepatocytes, indicating that there is more to fluvastatin transport than uptake by these three transporters [161]. It is clear that we still have a long way to go in understanding the SLC transporters and their significance in ADME, and this story will evolve as the data unfolds.

## ABBREVIATIONS

### Transporters

| | |
|---|---|
| ABC | ATP-Binding Cassette |
| ASBT | Sodium-Dependent Bile Salt Transporter |
| BSEP | Bile Salt Export Pump |
| CNT | Concentrative Nucleoside Transporter |
| CT | Na+/Carnitine Cotransporter |
| ENT | Equilibrative Nucleoside Transporter |
| LAT | System L Transporter |
| MATE | Multidrug and Toxic Compound Extrusion Transporter |
| MDR1 | Multidrug Resistance Protein 1 |
| NTCP | Sodium Taurocholate-Cotransporting Polypeptide |
| OAT | Organic Anion Transporter |
| OATP | Organic Anion-Transporting Polypeptide |
| OCT | Organic Cation Transporter |
| OCTN | Organic Cation Transporter Novel |
| SLC | Solute Carrier |
| UGT | UDP-Glucuronosyltransferase |
| URAT | Urate Transporter |

### Nuclear Receptors

| | |
|---|---|
| AhR | Aryl Hydrocarbon Receptor |
| CAR | Constitutive Androstane Receptor |
| FXR | Farnesoid X Receptor |
| GR | Glucocorticoid Receptor |
| HNF | Hepatocyte Nuclear Factor |
| Nrf2 | Nuclear Erythroid 2-Related Factor 2 |
| PPAR | Peroxisome Proliferator-Activated Receptor |

| | |
|---|---|
| PXR | Pregnane X Receptor |
| RXR | Retinoid X Receptor |
| SNP | Single Nucleotide Polymorphism |

### ADME Terms

| | |
|---|---|
| AUC | Area Under the Plasma Concentration Time Curve |
| BBB | Blood-Brain Barrier |
| CL | Clearance |
| DDI | Drug–Drug Interaction |
| GFR | Glomerular Filtration Rate |
| PK | Pharmacokinetics |

### Chemicals

| | |
|---|---|
| 6-CFL | 6-Carboxyfluorescein |
| $MPP^+$ | 1-Methyl-4-Phenylpyridinium |
| PAH | *para*-Aminohippuric Acid |
| TEA | Tetraethylammonium |

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