Chapter 2
Transporters: Importance in Drug Absorption, Distribution, and Removal

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Abstract There is an increasing appreciation of the role that transport proteins play in the absorption, distribution, and elimination of a wide variety of drugs in clinical use. These transporters can be divided into efflux transporters belonging to the ATP-binding cassette (ABC) family and solute carrier (SLC) family members that mediate the influx or bidirectional movement of drugs across the cell membrane. Their coordinated expression and activities at the basolateral and apical side of transporting epithelia are significant determinants of drug disposition, drug–drug interactions, and variability in drug response and toxicity. This chapter focuses on the major SLC and ABC drug transporters expressed in intestine, liver, and kidney, with special emphasis on their distribution, mode of action, and drug substrate specificity.

2.1 Introduction

During the last 20 years, a large number of membrane transport proteins have been identified. These transporters are important determinants in the absorption, distribution, and elimination of drugs. The involvement of carrier-mediated processes in drug excretion was already appreciated long before the first transporters were cloned, and it has become increasingly apparent that transporters also play a critical role in drug absorption and tissue uptake.

Drug transport proteins can be grouped into two major classes, the solute carriers (SLC) and ATP-binding cassette (ABC) transporters. Over 380 unique SLC sequences have been obtained from the human genome, which can be divided into 48 subfamilies (Fredriksson et al., 2008). The transport activities for xenobiotics for

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approximately 19 of these gene families have been described. These transporters include the organic anion transporting polypeptide (*SLCO*), the oligopeptide transporter (*SLC15*), the organic anion/cation/zwitterion transporter (*SLC22*), and the organic cation transporter (*SLC47*) families. Seven subfamilies of ABC transporter genes have been identified, encoding for 49 different proteins (Dean and Allikmets, 2001; Sheps and Ling, 2007). A number of them have specificities for drugs, in particular transporters belonging to the *ABCB*, *ABCC*, and *ABCG* subfamilies (Szakacs et al., 2008).

SLC and ABC transporters share a wide distribution in the body and are involved in the transport of a broad range of substrates. Many of them can potentially contribute to the permeability of drugs into cells and the processes by which drugs gain access to their pharmacological and toxicological targets. There is growing evidence to suggest that it is the rule rather than the exception that a given drug will interact with a set of membrane transporters at some point of its disposition in the body (Dobson and Kell, 2008). Since the intestine, liver, and kidney are the principal organs that determine the absorption, distribution, and elimination of drugs, this chapter focuses on the general characteristics of the major human drug transporters expressed in these organs. The SLC and ABC transporters listed in Tables 2.1 and 2.2 are currently considered to exert the greatest impact on overall drug disposition, pharmacokinetic variability, and drug–drug interactions (Mizuno and Sugiyama, 2002; Tsuji, 2002; Ho and Kim, 2005; Ito et al., 2005; Shitara et al., 2005; Endres et al., 2006; Zair et al., 2008).

Depending on the direction in which carrier proteins translocate the substrate across the cell membrane, they can be categorized as influx or efflux transporters. ABC transporters are by definition efflux transporters because they use energy derived from ATP hydrolysis to mediate the primary active export of drugs from the intracellular to the extracellular milieu, often against a steep diffusion gradient. Many of the SLC family members facilitate the cellular uptake or influx of substrates, either by facilitated diffusion down the electrochemical gradient acting as a channel or uniporter or by secondary active transport against a diffusion gradient coupled to the symport or antiport of inorganic or small organic ions to provide the driving force. Certain SLC transporters exhibit efflux properties or are bidirectional, depending on the concentration gradients of substrate and coupled ion across the membrane.

It is important to understand that the interplay between transporters located on apical and basolateral membranes in epithelial cells is critical in determining the extent and direction of drug movement in organs such as the intestine, liver, and kidney (Figs. 2.1, 2.2, and 2.3). Transport across each of these epithelia may be impeded or facilitated by the asymmetrical membrane distribution of influx and efflux transporters and ultimately contributes to the pharmacokinetic profile of a drug substrate in the body. In this respect, transporters that mediate vectorial drug transfer into the systemic circulation are named as absorptive transporters, regardless of whether they are influx or efflux transporters. In contrast, secretory transporters are involved in the excretion of substrates from the circulation into bile, urine, or gut lumen.

Table 2.1 Major human SLC drug transporters expressed in small intestine, liver, and kidney

Gene	Protein	Mechanism	Tissue distribution	Membrane localization	Examples of drug substrates	References
SLC15 family						
SLC15A1	PEPT1	H^+/peptide symporter	Intestine Kidney	BBM	Ampicillin, amoxicillin, bestatin, cefaclor, cefadroxil, cefixime, enalapril, temocapril, temocaprilat, midodrine, valacyclovir, valganciclovir	Russel et al. (2002), Brandsch et al. (2008), Dobson and Kell (2008), and Rubio-Aliaga and Daniel (2008)
SLC15A2	PEPT2	H^+/peptide symporter	Kidney	BBM	Amoxicillin, bestatin, cefaclor, cefadroxil, valganciclovir	Russel et al. (2002), Brandsch et al. (2008), Dobson and Kell (2008), and Rubio-Aliaga and Daniel (2008)
SLC22 family						
SLC22A1	OCT1	OC uniporter	Intestine Liver	BLM SM	Acyclovir, ganciclovir, metformin, cimetidine, quinine, quinidine, zidovudine	Koepsell et al. (2007), Ciarimboli (2008), and Dobson and Kell (2008)
SLC22A2	OCT2	OC uniporter	Kidney	BLM	Mepiperphenidol, memantine, cimetidine, famotidine, ranitidine, metformin, propranolol, pancuronium, quinine, zidovudine, cisplatin	Koepsell et al. (2007), Ciarimboli (2008), and Dobson and Kell (2008)
SLC22A4	OCTN1	H^+ or OC antiporter	Intestine Kidney	BBM	Mepyramine, quinidine, verapamil, ergothioneine, gabapentin	Koepsell et al. (2007), Dobson and Kell (2008), and Urban et al. (2008)
SLC22A5	OCTN2	OC antiporter Na^+ symporter (carnitine)	Intestine Kidney	BBM	Mepyramine, quinidine, verapamil, valproate, cephaloridine, emetine	Koepsell et al. (2007), Dobson and Kell (2008)

Table 2.1 (continued)

Gene	Protein	Mechanism	Tissue distribution	Membrane localization	Examples of drug substrates	References
SLC22A6	OAT1	DC/OA antiporter	Kidney	BLM	Adefovir, cidofovir, acyclovir, didanosine, stavudine, trifluridine, ganciclovir, PMEG, PMEDAP, tenofovir, zalcitabine, zidovudine, tetracycline, methotrexate, bumetanide, furosemide, ibuprofen, indomethacin, ketoprofen, PAH, cimetidine, ranitidine	Russel et al. (2002), Rizwan and Burckhardt (2007), Dobson and Kell (2008), and Srimaroeng et al. (2008)
SLC22A7	OAT2	OA antiporter	Liver	SM	Erythromycin, cimetidine, ranitidine, zidovudine, 5-fluorouracil, methotrexate, taxol, bumetanide, allopurinol, salicylate, PAH, theophylline	Russel et al. (2002), Rizwan and Burckhardt (2007), Dobson and Kell (2008), and Srimaroeng et al. (2008)
SLC22A8	OAT3	DC/OA antiporter	Kidney	BLM	Benzylpenicillin, tetracycline, valacyclovir, zidovudine, cimetidine, ranitidine, methotrexate, furosemide, ibuprofen, indomethacin, ketoprofen, salicylate, PAH, pravastatin, olmesartan	Russel et al. (2002), Rizwan and Burckhardt (2007), Dobson and Kell (2008), Srimaroeng et al. (2008), and Kusuhara and Sugiyama (2009)
SLC22A9	OAT4	Cl^-/OA antiporter	Kidney	BBM	Tetracycline, zidovudine, methotrexate, bumetanide, ketoprofen, salicylate, PAH	Russel et al. (2002), Rizwan and Burckhardt (2007), Dobson and Kell (2008), and Srimaroeng et al. (2008)
SLC47 family						
SLC47A1	MATE1	H^+/OC antiporter	Liver Kidney (PT, DT)	CM BBM	Cimetidine, procainamide, metformin, cephalexin, cephadrine, fexofenadine	Koepsell et al. (2007), Tanihara et al. (2007), Moriyama et al. (2008), Terada and Inui (2008), and Matsushima et al. (2009)

Table 2.1 (continued)

Gene	Protein	Mechanism	Tissue distribution	Membrane localization	Examples of drug substrates	References
SLC47A2	MATE2-K	H^+/OC antiporter	Kidney	BBM	Cimetidine, procainamide, metformin, fexofenadine, oxaliplatin	Ho and Kim (2005), Koepsell et al. (2007), Moriyama et al. (2008), Terada and Inui (2008), and Matsushima et al. (2009)
SLCO family						
SLCO1A2	OATP1A2	OA antiporter	Intestine Kidney (DT)	BBM BBM	Fexofenadine, indomethacin, ouabain, rocuronium, enalapril, temocaprilat, rosuvastatin, pitavastatin, levofloxacin, methotrexate, imatinib, saquinavir	Dobson and Kell (2008), Hagenbuch and Gui (2008), and Hu et al. (2008)
SLCO1B1	OATP1B1	OA antiporter	Liver	BBM	Benzylpenicillin, rifampicin, atorvastatin, pravastatin, cerivastatin, fluvastatin, pitavastatin, rosuvastatin, simvastatin, valsartan, olmesartan, troglitazone, bosentan, enalapril, caspofungin, fexofenadine, SN-38	Noe et al. (2007), Dobson and Kell (2008), Hagenbuch and Gui (2008), Hu et al. (2008), and Nies et al. (2008)
SLCO1B3	OATP1B3	OA antiporter	Liver (around central vein)	BLM	Digoxin,ouabain, rifampicin, bosentan, enalapril, fluvastatin, pitavastatin, rosuvastatin, telmisartan, valsartan, fexofenadine, methotrexate, paclitaxel	Dobson and Kell (2008), Hagenbuch and Gui (2008), Hu et al. (2008), and Nies et al. (2008)
SLCO2B1	OATP2B1	OA antiporter	Liver Intestine	SM BBM	Benzylpenicillin, bosentan, atorvastatin, pravastatin, pitavastatin, fluvastatin, rosuvastatin, glibenclamide	Dobson and Kell (2008), Hagenbuch and Gui (2008), Hu et al. (2008), and Nies et al. (2008)
SLCO4C1	OATP4C1	ND	Kidney	BLM	Digoxin, ouabain, methotrexate	Dobson and Kell (2008), Hagenbuch and Gui (2008), and Hu et al. (2008)

Abbreviations: PMEG = 9-(2-phosphonylmethoxyethyl)guanine; PMEDAP = 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine; BBM = brush border membrane; BLM = basolateral membrane; SM = sinusoidal membrane; CM = canalicular membrane; PT = proximal tubule; DT = distal tubule; DC = dicarboxylate; PAH = *p*-aminohippuric acid; SN-38 = active metabolite of irinotecan; ND = not determined

Table 2.2 Major human ABC drug transporters expressed in small intestine, liver, and kidney

Gene	Protein	Mechanism	Tissue distribution	Membrane localization	Examples of drug substrates	References
ABCB family						
ABCB1	MDR1/ P-glyco-protein	Primary active	Intestine Liver Kidney	BBM CM BBM	Vinblastine, vincristine, daunorubicin, doxorubicin, colchicine, docetaxel, paclitaxel, ortataxel, etoposide, imatinib, methotrexate, bisantrene, mitoxantrone, paclitaxel, topotecan, digoxin, digitoxin, celiprolol, talinolol, indinavir, nelfinavir, ritonavir, saquinavir, levofloxacin, grepafloxacin, sparfloxacin, erythromycin, ivermectin, chloroquine, amiodarone, lidocaine, losartan, lovastatin, mibefradil, fexofenadine, terfenadine, carbamazepine, desipramine, loperamide, methadone, morphine, sumatriptan, vecuronium, cyclosporin A, tacrolimus, sirolimus	Russel et al. (2002), Dietrich et al. (2003), Sarkadi et al. (2006), Hu et al. (2008), Murakami and Takano (2008), Zhou (2008), and Oostendorp et al. (2009)
ABCC family						
ABCC2	MRP2	Primary active	Intestine Liver Kidney	BBM CM BBM	Vinblastine, vincristine, doxorubicin, etoposide, cisplatin, methotrexate, indinavir, ritonavir, saquinavir, grepafloxacin, glutathione conjugates, PAH	Russel et al. (2002), van de Water et al. (2005), Nies and Keppler (2007), and Zhou et al. (2008)
ABCC3	MRP3	Primary active	Intestine Liver Kidney	BLM SM BLM (CCD)	Glucuronide conjugates (morphine, acetaminophen, etoposide, ethinylestradiol), methotrexate	Russel et al. (2002), van de Water et al. (2005), Borst et al. (2007), and Zhou et al. (2008)

Table 2.2 (continued)

Gene	Protein	Mechanism	Tissue distribution	Membrane localization	Examples of drug substrates	References
ABCC4	MRP4	Primary active	Intestine Liver Kidney	BBM? SM BBM	Methotrexate, leucovorin, topotecan, 6-mercaptopurine, 6-thioguanine, adefovir, tenofovir, ceftizoxime, cefazolin, cefotaxime, cefmetazole, hydrochlorothiazide, furosemide, olmesartan, edaravone glucuronide, PAH	Russel et al. (2002), van de Water et al. (2005), Nies and Keppler (2007), Russel et al. (2008), and Zhou et al. (2008)
ABCG family						
ABCG2	BCRP	Primary active	Intestine Liver Kidney	BLM CM BBM	Mitoxantrone, flavopiridol, topotecan, SN-38, camptothecin, methotrexate, imatinib, gefitinib, erlotinib, abacavir, lamivudine, zidovudine, nelfinavir, cerivastatin, pitavastatin, rosuvastatin, glibenclamide, olmesartan, dipyridamole, cimetidine, edaravone sulfate, albendazole sulfoxide, oxfendazole, ciprofloxacin, norfloxacin, ofloxacin, sulfasalazine, nitrofurantoin,	Sarkadi et al. (2006), van Herwaarden and Schinkel (2006), Cusatis and Sparreboom (2008), and Robey et al. (2009)

Abbreviations: BBM = brush border membrane; BLM = basolateral membrane; SM = sinusoidal membrane; CM = canalicular membrane; CCD = cortical collecting duct; PAH = *p*-aminohippuric acid; SN-38 = active metabolite of irinotecan

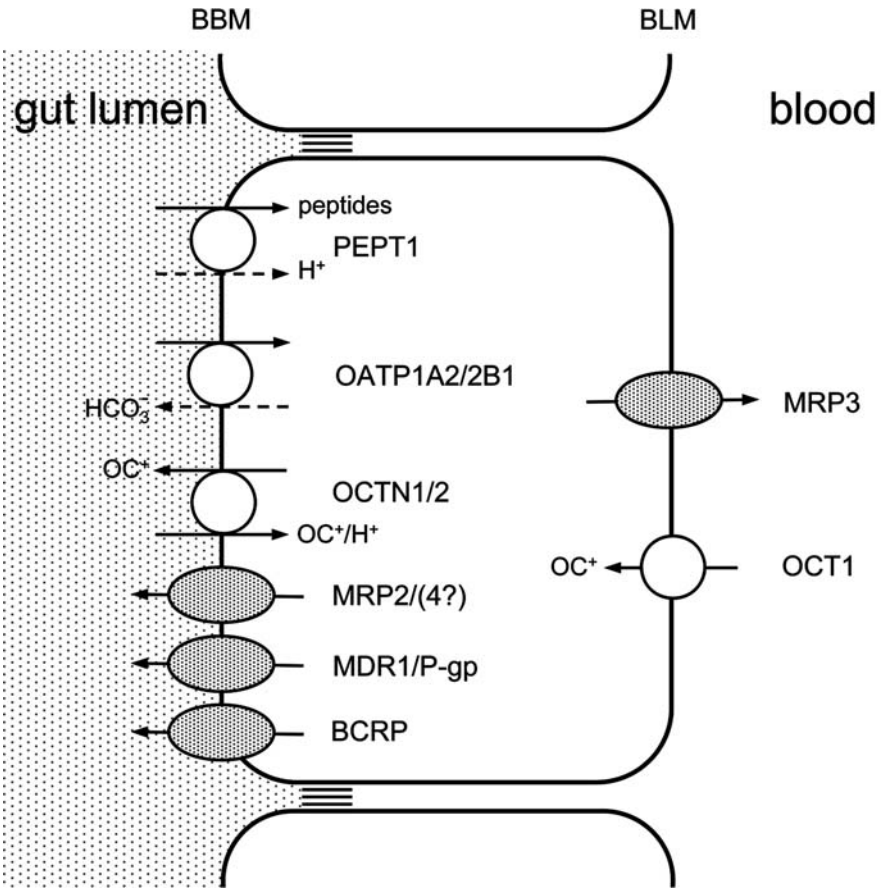

Fig. 2.1 Schematic model of the major drug transporters in enterocytes of human small intestine. SLC transporters are depicted by *open circles* and ABC transporters by *shaded ovals*. *Solid arrows* indicate the direction of drug transport. *Dashed arrows* depict the movement of driving ions. OCT1 is an electrogenic uniporter that transports organic cations (OC^+) from blood into the cell driven by the inside-negative membrane potential. OCTN1 mediates OC^+ uptake from gut lumen as a H^+/OC^+ antiporter or can operate like OCTN2 as a bidirectional cation exchanger, mediating influx or efflux. Peptidomimetic drugs are taken up by a H^+/peptide symporter (PEPT1). Amphipathic drugs are transported into the cell by the organic anion/HCO_3^- antiporters OATP1A2 and OATP2B1 and are extruded as parent compound or metabolites back to the lumen or into blood by the primary active ABC transporters MDR1/P-gp, MRP2, MRP3, MRP4, and BCRP

2.2 SLC Drug Transporters

The SLC subfamilies *SLC15*, *SLC22*, and *SLCO* are considered to have a major role in drug uptake into intestine, liver, and kidney, whereas *SLC47* members mediate drug efflux into bile and urine (Table 2.1, Figs. 2.1, 2.2, and 2.3). Most of these transporters have a similar protein structure in that they consist of 12 putative

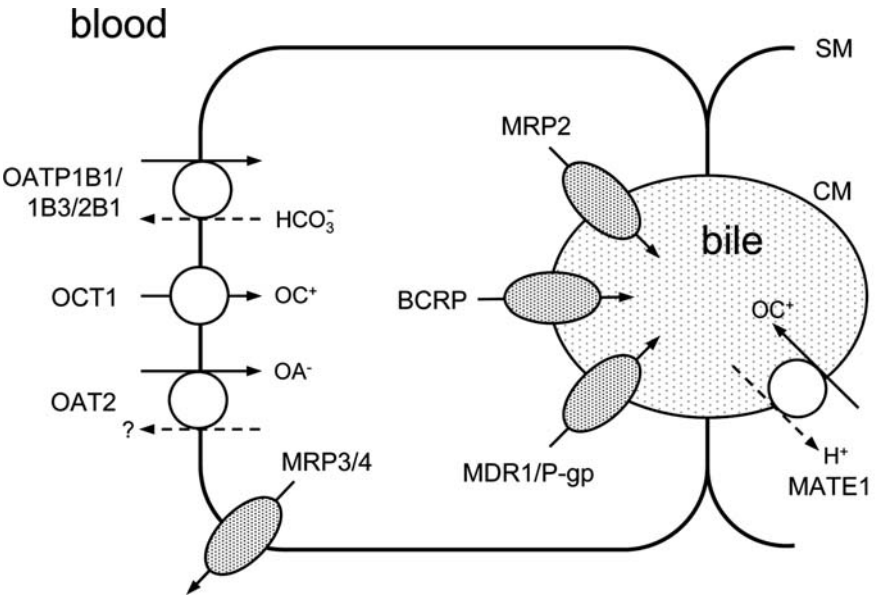

Fig. 2.2 Schematic model of the major drug transporters in human hepatocytes. SLC transporters are depicted by *open circles* and ABC transporters by *shaded ovals*. *Solid arrows* indicate the direction of drug transport. *Dashed arrows* depict the movement of driving ions. OCT1 is an electrogenic uniporter that transports organic cations (OC^+) from blood into the cell driven by the inside negative membrane potential. MATE1 is a biliary OC^+ efflux transporter that operates as a H^+ antiporter. Organic anions (OA^-) are taken up by OAT2, an antiporter for which the driving ion is unknown. Amphipathic drugs are transported into the cell by the organic anion/HCO_3^- antiporters OATP1B1, OATP1B3, and OATP2B1 and are extruded as parent compound or metabolites into bile or blood by the primary active ABC transporters MDR1/P-gp, MRP2, MRP4, and BCRP

membrane-spanning domains and their molecular mass is approximately between 50 and 100 kDa.

Oligopeptide transporters (*SLC15*) operate as H^+-coupled symporters of di- and tripeptides and have the ability to transport peptidomimetics and other drug substrates. PEPT1 is the small intestinal low-affinity, high-capacity peptide transporter, which is also expressed at low levels in the kidney. PEPT2 is the predominant high-affinity, low-capacity renal peptide transporter, although expression has also been observed in other organs (Brandsch et al., 2008; Kamal et al., 2008; Rubio-Aliaga and Daniel, 2008). Typical PEPT1 substrates have amino and carboxylic groups that can be mapped onto a di- or tripeptide skeleton, which allows for rational prodrug design to improve oral drug availability upon increased intestinal absorption (Bailey et al., 2006). PEPT2 reabsorbs renally filtered drugs from tubular fluid into proximal tubules, thereby affecting the systemic pharmacokinetics of some drugs. PEPT1/2 substrates include many important drug classes, including antivirals, β-lactam antibiotics, and angiotensin-converting enzyme (ACE) inhibitors (Table 2.1).

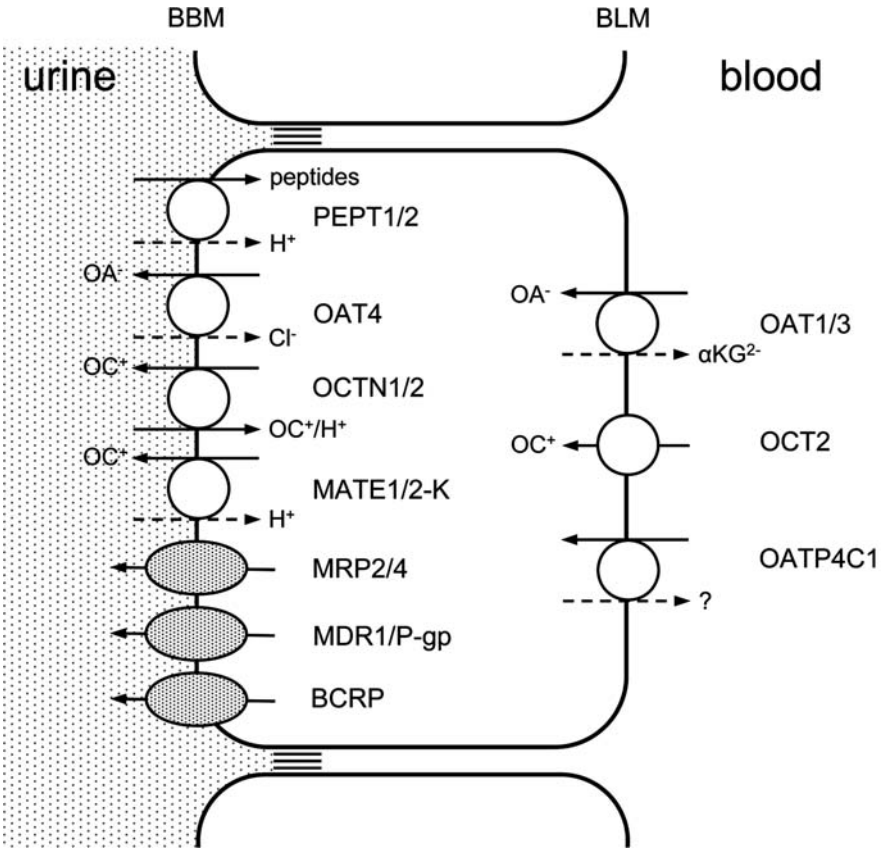

Fig. 2.3 Schematic model of the major drug transporters in human renal proximal tubular cells. SLC transporters are depicted by *open circles* and ABC transporters by *shaded ovals*. *Solid arrows* indicate the direction of drug transport. *Dashed arrows* depict the movement of driving ions. OCT2 is an electrogenic uniporter that transports organic cations (OC^+) from blood into the cell driven by the inside-negative membrane potential. OCTN1 mediates luminal OC^+ uptake as a H^+/OC^+ antiporter or can operate like OCTN2 as a bidirectional cation exchanger, mediating influx or efflux. MATE1 is a urinary OC^+ efflux transporter that operates as a H^+ antiporter. Peptidomimetic drugs are taken up by the H^+/peptide symporters PEPT1 and PEPT2. Organic anions (OA^-) are taken up by the antiporters OAT1 and OAT3, which are driven by the exchange with α-ketoglutarate (α-KG^{2-}), and released by OAT4 in exchange for Cl^-. A few amphipathic drugs are transported into the cell by the organic anion antiporter OATP4C1, for which the driving ion is unknown. The primary active ABC transporters MDR1/P-gp, MRP2, MRP4, and BCRP drive the efflux of a wide variety of amphipathic drugs and metabolites into urine

Organic cation/anion/zwitterion transporters (SLC22) are characterized by a remarkably broad substrate specificity and wide tissue distribution. The major drug transporting members of this family can be found in the liver, kidney, and intestine. The organic anion transporters (OATs) are mainly located in the kidney, but some

also occur in the liver, placenta, and brain. They function as antiporters, by coupling the cellular influx of an organic anion to the exchange with dicarboxylates or other organic anions from the cell. The existing inside-out concentration gradient of these anions provides the driving force for the active uptake of anionic drugs against the inside-negative membrane potential. OATs are part of the excretory systems for anionic drug substrates and they typically interact with hydrophilic, small molecular weight (MW < 400–500 Da) substrates, like *p*-aminohippuric acid (PAH), which are categorized as type I organic anions (Table 2.1).

SLC22 subfamily members that specialize in the translocation of cationic drugs consist of the organic cation transporters (OCT1/2) that operate as uniporters, the organic cation and carnitine transporter (OCTN1) that could be a proton antiporter, and the Na^+-carnitine symporter (OCTN2) that also acts as a Na^+-independent organic cation antiporter. The tissue distribution of these transporters differs among species; in human, OCT1 is widely expressed, most strongly in liver, to a lesser extent in intestine and even lower in kidney. OCT2 is present abundantly in the kidney, whereas the expression is low in the small intestine and absent in the liver. OCTN1 is strongly expressed in kidney, skeletal muscle, bone marrow, and trachea, and weaker in intestine and liver. OCTN2 is distributed ubiquitously, with strongest expression in kidney, skeletal muscle, heart, placenta, and liver (Koepsell et al., 2007). Both OCT1 and OCT2 mediate cellular uptake by facilitated diffusion down the inside-negative electrochemical gradient of comparatively small monovalent, type I (MW<400 Da) organic cations (Table 2.1). For OCT2, recent quantitative structure–activity relationship models have emphasized the importance of hydrophobicity, molecular size, and shape as important determinants in defining the binding of drug substrates and may prove useful in the prediction of unwanted drug interactions (Suhre et al., 2005). OCTN1 and OCTN2 exhibit a similar though somewhat less extensive substrate specificity (Koepsell et al., 2007).

The *SLCO* subfamily consists of organic anion transporting polypeptides (OATPs) that are involved in the cellular uptake of more bulky (MW>450 Da) and relatively hydrophobic organic anions that are classified as type II (Table 2.1). In addition, the substrate specificity of the OATPs covers a wide range of amphipathic organic compounds, including bile salts, steroid conjugates, thyroid hormones, and various drugs (Hagenbuch and Gui, 2008). Current evidence suggests that these transporters act as organic anion exchangers, although the precise mechanism remains ambiguous. In rodents, substrate exchange for glutathione was observed but for the human transporters there is evidence to support a role for bicarbonate as a counterion (Li et al., 1998; Mahagita et al., 2007; Leuthold et al., 2009). OATPs seem to operate as pH-dependent bidirectional antiporters, stimulated by an acidic extracellular environment (Hagenbuch and Gui, 2008). The tissue distribution of OATPs differs for each of the isoforms, some are expressed ubiquitously, while for others, the expression is restricted to a single organ. OATP1A2 is expressed in kidney, small intestine, and bile duct, OATP1B1 and OATP1B3 have only been detected in liver, whereas OATP2B1 has a wide tissue distribution, and OATP4C1 is considered to be kidney specific (Hagenbuch and Gui, 2008). The multitude of structurally diverse compounds that are recognized by OATPs strongly suggests

the presence of multiple substrate binding sites, and evidence for this is emerging (Hagenbuch and Gui, 2008).

Recently, two human orthologs of the multidrug and toxin extrusion (MATE) family of bacteria have been identified as H^+/organic cation antiport systems and assigned as members of the *SLC47* subfamily (Table 2.1). MATE1 is primarily expressed in the kidney, but also exists in the liver, adrenal gland, testis, and skeletal muscle, whereas MATE2-K has been found exclusively in the kidney (Tanihara et al., 2007; Terada and Inui, 2008) MATE1 and MATE2-K are efflux transporters energized by an inwardly directed proton gradient to overcome the outside positive membrane potential (Moriyama et al., 2008). They typically transport type I organic cations and with a few exceptions their substrate specificity is similar and much like that of the OCT and OCTN transporters (Tanihara et al., 2007; Terada and Inui, 2008).

2.3 ABC Drug Transporters

ABC transporters that are involved in drug transport can be found in the *ABCB*, *ABCC*, and *ABCG* families (Table 2.2). Because of their expression in transporting epithelia, including the intestine, liver, and kidney, the ABC transporters play an important role in the absorption, distribution, and removal of drugs (Figs. 2.1, 2.2, and 2.3). Many of them are also associated with multidrug resistance (MDR) of tumor cells causing treatment failure in cancer. The minimal functional configuration of an ABC transporter has a molecular weight of 150–200 kDa and is made of two transmembrane domains, each consisting of six transmembrane helices and two cytoplasmic ATP-binding domains (Locher, 2009). They bind and hydrolyze ATP to drive primary active drug efflux, which is directly linked to their ATPase activity.

The MDR1/P-glycoprotein (P-gp) is encoded by the *MDR1/ABCB1* gene and has an unusually broad substrate specificity, recognizing hundreds of compounds ranging from small molecules of 350 Da up to polypeptides of 4000 Da. A large number of P-gp substrates fall in the category of bulky, often polyvalent, organic cations (generally >500 Da), which are classified as type II organic cations (Zhou, 2008). Very recently, crystal structures of mammalian P-gp were reported, showing distinct partially overlapping drug-binding sites in the internal cavity of the protein, which provides the first molecular basis for its multispecificity (Aller et al., 2009). P-gp is expressed in many tissues and is located on the apical side of intestine, liver, and kidney epithelia where it reduces systemic drug exposure by limiting oral absorption and promoting urinary and biliary excretion.

A close relative to P-gp is the bile salt export pump, BSEP/ABCB11, which is exclusively expressed in the liver where it is localized at the apical membrane (Stieger et al., 2007). BSEP is predominantly responsible for the excretion of monovalent bile acids into bile and constitutes the major driving force for the generation of bile flow. Its substrate specificity is narrow and largely restricted to bile acids, but a poor rate of transport for taxol, vinblastine, and pravastatin has been reported (Alrefai and Gill, 2007). However, the relevance of BSEP in the overall biliary

excretion of these drugs is uncertain. On the other hand, BSEP appears to be a key target of drug-induced cholestasis. Drugs such as glibenclamide, rifampicin, bosentan, and troglitazone can inhibit BSEP, which leads to intracellular accumulation of bile salts, decreased bile flow, and ultimately liver injury (Stieger et al., 2007).

Within the *ABCC* subfamily, nine full multidrug resistance (associated) protein (MRP) members have been identified, among which MRP2/ABCC2, MRP3/ABCC3, and MRP4/ABCC4 are the most important drug transporters (Table 2.2). The MRPs mediate the transport of organic anionic (generally type II) compounds, including glucuronide, glutathione, and sulfate conjugates, but also uncharged amphipathic, and even some cationic substrates in the presence of reduced glutathione (Deeley et al., 2006). MRPs are expressed in intestine, liver, and kidney, and other tissues with a barrier function, such as placenta and brain capillaries (van de Water et al., 2005; Zhou et al., 2008). MRP2 is located at the apical membrane of polarized cells, emphasizing its important function in the terminal excretion of anionic drugs and conjugates (Nies and Keppler, 2007). Because of its basolateral localization, MRP3 mediates the cellular efflux of mainly glucuronidated drug conjugates from the intestine and liver into the blood (Borst et al., 2007). A remarkable feature of MRP4 is its dual membrane localization. In hepatocytes the transporter is localized at the basolateral membrane, whereas it is expressed at the apical membrane of renal proximal tubule cells (van Aubel et al., 2002; Rius et al., 2003). In the intestine, the subcellular distribution of MRP4 has not been established yet, and localization to both the apical and the basolateral membrane was found in a colonic epithelial cell line, with a higher apical abundancy (Li et al., 2007; Russel et al., 2008).

The *ABCG2* gene product, breast cancer resistance protein (BCRP), is the only member of this subfamily that is involved in drug transport. Like other G-subfamily members, BCRP is comprised of one ATP-binding site and one transmembrane region, a structure half the size of a functional ABC transporter. These half transporters are generally thought to homo- or heterodimerize to create the active transporter. BCRP was initially discovered in drug-resistant cancer cell lines and therefore many anticancer drugs are among the first reported substrates. To date, a large number of hydrophobic drug substrates have been described, and although a clear structure–transport relationship has not been identified, it should be noted that many of them are also transported by P-gp (Table 2.2). Similar to P-gp, BCRP is expressed in the apical membrane of intestine, liver, kidney, placenta, and brain capillaries, and often in stem cell populations, where it is thought to play a role in differentiation and protection against xenobiotics (Huls et al., 2009).

2.4 Transporters for Intestinal Drug Absorption

The absorption of drugs from the gastrointestinal tract is a critical factor in determining oral bioavailability. Enterocytes of the small intestine are equipped with an array of influx transporters at the luminal membrane for the absorption of

food components and drugs. Although much less is known about the subsequent transport of substrates into the blood stream, ABC transporters in the basolateral membrane could facilitate this step. However, as a first barrier against xenobiotics, the intestine also has a high expression of ABC transporters in the brush border membrane that can effectively pump drugs back into the intestinal lumen, thereby limiting the extent of substrate drug absorption (Fig. 2.1, Tables 2.1 and 2.2).

A number of SLC drug transporting proteins have been described at the brush border membrane of human enterocytes, including PEPT1, OATP1A2, OATP2B1, OCTN1, and OCTN2. Expression levels of some of these transporters appear to vary along the gastrointestinal tract, but results from different studies on mRNA and protein expression do not concord, except for PEPT1, which is predominantly expressed in the small intestine (Glaeser et al., 2007; Hilgendorf et al., 2007; Meier et al., 2007; Oostendorp et al., 2009). PEPT1 recognizes various peptide-like drugs and targeting this transporter has been used to improve the oral bioavailability of poorly absorbed drugs such as nucleoside analogs (Brandsch et al., 2008; Rubio-Aliaga and Daniel, 2008). An example is acyclovir, the bioavailability of which was enhanced by a factor of 2–3 via the oral administration of its valine ester (valacyclovir), which is a PEPT1 substrate. Another promising but yet to be established target for prodrug design is the apical sodium-dependent bile acid transporter (ASBT/SLC10A2) (Balakrishnan and Polli, 2006).

Uptake of cationic drugs from the gut lumen is mediated by OCTN1 and OCTN2, which are energized by electroneutral cation–cation exchange (Koepsell et al., 2007). Mutations in the genes encoding for these transporters have been associated with inflammatory bowel disease and polymorphisms could be of influence on cationic drug absorption (Koepsell et al., 2007; Zair et al., 2008). OATP1A2 and OATP2B1 are responsible for the uptake of a broad range of amphipathic drugs (Hagenbuch and Gui, 2008). While there is quite some overlap in specificity, some substrates are preferentially or exclusively transported by one of them. For example, only OATP1A2 is able to mediate fexofenadine uptake and the likely target of inhibition by grapefruit juice (Glaeser et al., 2007).

The first step in secretion of cationic drugs from blood to gut lumen is mediated by OCT1 in the basolateral membrane, followed by the action of efflux transporters in the brush border membrane (Fig. 2.1). These are the OCTNs that can also operate as secretory transporters by exchanging luminal organic cations against a higher concentration of intracellular cationic drugs. In addition, MDR1/P-gp pumps positively charged hydrophobic drugs back into the lumen, which could have entered the cells by passive diffusion. The ABC transporters P-gp, MRP2, and BCRP are all expressed in the brush border membrane where they have an important role as gatekeeper in the gut, limiting the oral bioavailability of many drug substrates. Modulation of their activity with selective inhibitors could be a useful strategy to increase the oral bioavailability of substrate drugs. Examples of drug inhibitors of P-gp and BCRP are HIV protease inhibitors (ritonavir, indinavir, saquinavir, nelfinavir) and benzimidazole proton pump inhibitors (omeprazole, pantoprazole, lansoprazole, and rabeprazole) (Oostendorp et al., 2009). The exact subcellular

distribution of MRP4 needs to be clarified, as this relates to its importance in intestinal drug transport.

The interplay between ABC transporters and drug-metabolizing enzymes makes this barrier even more effective. P-gp and the Phase I enzyme cytochrome P450 (CYP)3A4 appear to be functionally linked as they share the same substrates, while MRP2 and BCRP accept anionic drug conjugates formed by Phase II-metabolizing enzymes, including UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), and glutathione *S*-transferases (GSTs) (Kivisto et al., 2004; Nies et al., 2008). At the basolateral membrane of enterocytes, MRP3 mediates transport into the blood of non-conjugated and mostly conjugated organic anions, with a preference for glucuronidated compounds (Borst et al., 2007). The expression of MRP1 and MRP5 at the basolateral membrane has been described, but their role in facilitating intestinal drug absorption needs further investigation.

The same applies to the recently identified basolateral bile acid carrier, the heteromeric organic solute, and steroid transporter OSTα/OSTβ (Ballatori, 2005). The genes encoding these transporters are unique in the human and mouse genome and do not belong to the SLC or ABC transporter families. OSTα/OSTβ is expressed at relatively high levels in the small intestine and substrate specificity is mainly restricted to steroid-derived molecules, including the cardiac glycoside digoxin (Ballatori et al., 2005).

Expression of MDR1/P-gp varies over the total length of the gastrointestinal tract, gradually increasing from the stomach and duodenum to highest levels in colon. *MRP2* and *BCRP* transcript levels are highest in duodenum, even higher than that of *MDR1*, and they decrease in the direction of colon. *MPR3* expression is higher in duodenum, ileum, and colon as compared to jejunum (Dietrich et al., 2003; Murakami and Takano, 2008; Oostendorp et al., 2009). Regional distribution of these transporters along the gastrointestinal tract could be of influence on the site of drug absorption. In addition, genetic polymorphisms of particularly MDR1/P-gp and BCRP affecting expression level and transporter function have been shown to impact oral drug availability of a number of substrates (Maeda and Sugiyama, 2008; Nakamura et al., 2008).

2.5 Transporters for Hepatic Drug Elimination

The liver has a remarkable ability to efficiently extract drugs with high protein binding from the blood circulation. The hepatic uptake of drugs is frequently followed by Phase I and Phase II biotransformation and efflux of the metabolite(s) into bile and contributes to the hepatic first-pass effect. Influx and efflux transporters expressed at the sinusoidal (basolateral) and canalicular (apical) membrane of hepatocytes have been recognized as critical determinants in drug elimination (Fig. 2.2, Tables 2.1 and 2.2).

Drug influx transporters expressed at the sinusoidal membrane include OATP1B1, OATP1B3, OATP2B1, OAT2, and OCT1. In particular OATP1B1 is

recognized as an important uptake transporter for many clinically relevant drugs, such as macrolide antibiotics, statins (HMG-CoA-reductase inhibitors), glitazones (thiazolidinediones), sartans (angiotensin II receptor antagonists), and angiotensin-converting enzyme (ACE) inhibitors (Table 2.1). Clinically relevant drug–drug interactions have been described for OATP1B1-mediated statin transport with the immunosuppressant drug cyclosporine A (Endres et al., 2006). The homolog OATP1B3 has similar substrate specificity, but its expression is more confined to the hepatocytes surrounding the central vein. Because the activity of these transporters is often the rate-limiting step in hepatobiliary elimination, their inhibition and genetic variability are critical factors in the interindividual variation in drug disposition and exposure. A recent genomewide study emphasized the strong association of *SLCO1B1* variants with an increased risk of simvastatin-induced myopathy (Link et al., 2008). These genotypes are known to be associated with higher statin blood concentrations, although surprisingly in vitro studies in cells expressing *SLCO1B1* and its variants were inconsistent in identifying simvastatin as an OATP1B1 substrate (Kameyama et al., 2005; Noe et al., 2007).

OAT2 is moderately expressed and could be involved in the hepatic uptake of type I organic anions, such as salicylate and indomethacin (Rizwan and Burckhardt, 2007). Although OAT2 likely functions as an antiporter, its transport mode and particularly the identity of the intracellular counterions have not yet been resolved. OCT1 mediates the influx of type I organic cations into hepatocytes; however, as a bidirectional electrogenic uniporter it can also facilitate the efflux of cationic drugs back into the blood, depending on the electrochemical gradient (Koepsell et al., 2007). A clinically important substrate is metformin, which is among the most widely prescribed drugs for the treatment of type 2 diabetes. Its antidiabetic action is dependent on uptake into hepatocytes and certain loss of function variants of the transporter could be associated with reduced therapeutic efficacy, although recent clinical studies have not confirmed this supposition (Shikata et al., 2007; Shu et al., 2007; Zhou et al., 2009).

Efflux transporters expressed in the canalicular membrane represent the final step in the vectorial transport of drugs and drug metabolites from blood into bile. Excretion of type I and II cationic drugs across the canalicular membrane is mediated by MATE1 and MDR1/P-gp, respectively (Tables 2.1 and 2.2). Metformin is a good substrate of MATE1, although the drug is mainly excreted into urine by active tubular secretion. MRP2 and BCRP are primarily responsible for the canalicular efflux of unconjugated and conjugated anionic drugs, including glucuronide-, sulfo-, and glutathione conjugates (Table 2.2) (Nies and Keppler, 2007; Robey et al., 2009). Not all Phase II drug metabolites formed in the hepatocyte are transferred to bile. The localization of MRP3 and MRP4 at the sinusoidal membrane indicates that these conjugates are also transported back into the circulation so that they can undergo renal elimination. Interindividual variation in MRP3 protein levels is about 80-fold, whereas MRP4 abundance is very low under normal conditions (Lang et al., 2004; Nies et al., 2008). Under cholestatic conditions, protein levels of both transporters are increased and are able to mediate the efflux of bile salts since these are substrates of both MRP3 and MRP4 (Nies et al., 2008). Whether MRP3 and MRP4

have an impact on the overall hepatic clearance depends on the kinetic properties of the drug. Their contribution will be negligible if uptake is the rate-limiting step in elimination (Kusuhara and Sugiyama, 2009).

2.6 Transporters for Renal Drug Elimination

The renal handling of drugs involves passive processes, including glomerular filtration and back diffusion along the nephron, and carrier-mediated secretion and reabsorption that are mainly located in the proximal tubule. For most drugs that undergo carrier-mediated transport in the kidney, renal secretion can be considered as a vectorial process involving the uptake of substances from the blood across the basolateral membrane of proximal tubular cells, followed by their efflux across the brush border membrane into urine. At the basolateral membrane, separate influx transporters exist for the uptake of mainly type I organic anions and cations, which are notable for their high clearance capacity, wide variety of substrates accepted, and involvement in drug–drug interactions (Masereeuw and Russel, 2001). Because of efficient uptake, many drugs tend to accumulate in the cell sometimes causing nephrotoxicity. The large number of efflux transporters expressed at the brush border membrane emphasizes the importance to ensure rapid efflux of potentially toxic compounds into urine (Fig. 2.3, Table 2.1 and 2.2).

The uptake of anionic drugs at the basolateral membrane of renal proximal tubule is regulated by OAT1 and OAT3. Both transporters have overlapping substrate specificities and share the same mode of transport driven by the exchange of organic anions with dicarboxylates (Rizwan and Burckhardt, 2007). OAT1 has a higher affinity for hydrophilic organic anions with small molecular weights (type I), like PAH, adefovir, cidofovir, and tenofovir (Table 2.1). OAT3 also transports some amphipathic organic anions (type II) that are liver OATP substrates, including benzylpenicillin, pravastatin, and olmesartan, and even some cationic drugs, such as cimetidine and ranitidine (Table 2.1). The broader specificity, as well as the relatively higher renal expression levels of OAT3 compared to OAT1, suggests a more pronounced role of OAT3 in human renal organic anion transport (Masereeuw and Russel, 2001; El-Sheikh et al., 2008). Severe drug–drug interactions have been reported between methotrexate and NSAIDs due to competition for OAT1- and OAT3-mediated uptake, although the interaction at the level of the apical efflux transporters MRP2 and MRP4 probably also contributes to this mechanism (El-Sheikh et al., 2007).

The first step in tubular secretion of cationic drugs is mediated by OCT2, the predominant organic cation uniporter in the basolateral membrane. A splice variant of OCT2 (OCT2A), which shares 81% identity, exhibits some transport activity for cationic drugs with different affinity (Urakami et al., 2002). Metformin is also transported by OCT2, even with a higher affinity than by OCT1. Several studies have identified race-specific OCT2 variants, but little is known regarding their effects on pharmacokinetic variability (Zair et al., 2008). Coadministration of

cimetidine with metformin has been shown to reduce the renal clearance of metformin, leading to a clinically relevant increase in plasma concentration (Wang et al., 2008).

In human kidney, only OATP4C1 is expressed in the basolateral membrane of proximal tubular cells, and a remarkable species difference exist with rodents with regard to the renal expression of OATPs. Except for the ortholog Oatp4c1, at least three Oatps, absent in humans, are located in the brush border membrane of the rodent kidney (Sekine et al., 2006). The substrate specificity of OATP4C1 is restricted to only a few drugs that are mainly excreted by the kidney. Important examples are methotrexate, the cardiac glycosides, digoxin, and ouabain, as well as thyroid hormones (Table 2.1). The transport mechanism of OATP4C1 and the counter ion it exchanges its substrates for are not yet identified.

At the proximal tubular brush border membrane, a team of four ABC transporters mediate the primary active efflux of drugs, viz., MDR1/P-gp, MRP2, MRP4, and BCRP. P-gp likely provides the efflux pathway for digoxin and a number of hydrophobic cationic drugs (Masereeuw and Russel, 2001; Zhou, 2008). MRP2 and MRP4 are involved in the efflux of anionic drugs and drug conjugates that have been either formed in the proximal tubular cell or released from the liver and taken up from the circulation (van de Water et al., 2005). MRP4 appears to have a higher affinity for type I organic anions and its protein expression is approximately fivefold higher (Smeets et al., 2004; Russel et al., 2008). BCRP has recently been localized to the proximal tubule brush border membrane, suggesting its potential involvement in renal drug excretion (Huls et al., 2008).

The SLC organic cation transporters MATE1, MATE2-K, OCTN1, and OCTN2 mediate the secondary active efflux of cationic drugs across the brush border membrane. The H^+/organic cation or organic cation/organic cation antiport used by these transporters helps to overcome the outside positive membrane potential. Because of their bidirectionality, OCTNs could be also involved in organic cation reabsorption. The outside-in H^+ gradient across the brush border membrane provides the driving force for the MATE transporters. Several genetic variants of MATE1 and MATE2-K with decreased activity have been recently described that could contribute to the variability in renal handling of various cationic drugs such as metformin and lead to accumulation of oxaliplatin, causing drug-induced nephrotoxicity (Kajiwara et al., 2009).

The H^+/peptide symporters, PEPT1 and PEPT2, are both expressed in a sequential order along the renal proximal tubule (Brandsch et al., 2008). However, PEPT2, the high affinity, low capacity transporter appears to be the major player in the renal reabsorption of peptide-like drugs (Kamal et al., 2008).

OAT4 exhibits characteristics of an asymmetric antiporter mediating efflux of anionic drugs into urine, perhaps in exchange of Cl^-, and reabsorption of endogenous substrates like urate and estrone sulfate into the proximal tubular cell. The substrate specificity of OAT4 has not been fully explored, but the number of drugs accepted seems smaller than for OAT1 and OAT3 (Rizwan and Burckhardt, 2007).

2.7 Conclusions

SLC and ABC transporters expressed in the intestine, liver, and kidney are increasingly being recognized as significant determinants of drug disposition and drug–drug interactions. Many examples have emerged in the literature describing the impact of transport proteins on the pharmacokinetics of established drugs and new chemical entities and in recent years pharmaceutical companies and drug regulatory authorities have realized the need for including transporter studies in the early stages of drug development, in particular, for predicting the impact of transporter-based drug interactions and genetic polymorphisms. Since there exists a considerable functional redundancy in transporters, especially for apical efflux at the intestine, liver, and kidney membranes, clinically relevant drug–drug interactions and genetic variability are difficult to predict from in vitro experiments. An important step is to elucidate the relative contribution of the target transporter to the overall membrane transport of a specific drug (Endres et al., 2006; Kusuhara and Sugiyama, 2009). Though not addressed in this chapter, the creation of a variety of transporter knockout mouse models has contributed greatly to our understanding of the pharmacological and toxicological roles of transporter proteins, despite their species differences.

A wealth of information has been accumulated about the structure and function of transporter proteins, but much remains unresolved regarding their molecular mechanisms, structure–function relationships, and regulation. Many of the necessary tools are now available to gain a greater insight into each of these fundamental questions. A next challenge is to study the coordinated action of influx and efflux transporters, drug-metabolizing enzymes, and their coregulation by nuclear receptors as an integrated system (see Chapter 17). Such a system that includes quantitative information on distribution, kinetics, genetic variation, and abundance of transport proteins throughout the body, as already available for many metabolizing enzymes, will eventually lead to an in silico human model which has the ability to predict and simulate effectively the entire disposition and exposure of drugs in terms of pharmacological networks. As genetic variability in pharmacokinetics is concerned, it seems an oversimplification to suggest that one gene or single nucleotide polymorphism really matters. Rather, it is more likely that genes combine to create interindividual variability (Maeda and Sugiyama, 2008; Robey et al., 2009). One example discussed in this chapter is the interaction of metformin with different organic cation transporters. To reach the goal of true individualized therapy, a systems pharmacology approach would likely prove to be essential. A similar case can be made for studying drug–drug and drug–nutrient interactions in a comprehensive model.

This chapter aims to give a concise overview of the importance of major human transporters involved in the handling of clinically relevant drugs by intestine, liver, and kidney and therefore has only briefly touched upon the manifold aspects regarding their significance for drug–drug interactions, pharmacokinetic variability, and drug-induced toxicity. Many of them will be discussed in more depth in the chapters to follow.

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