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Martin F. Fromm
Richard B. Kim
Editors

Drug Transporters

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Drug Transporters

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Preface

In the past 15 years, there has been a dramatic increase in the extent of our knowledge regarding the importance of transporter proteins that govern drug disposition and response. For example, the human multidrug and toxin extrusion protein 1, which is important for the elimination of organic cations and drugs, was cloned and functionally characterized in 2005. Regarding their function, transporters can be classified as uptake or efflux transporters and mediate the uptake of endogenous compounds and drugs into or out of the cells, respectively. This book focuses on transporters of the solute carrier family (SLCs; e.g., organic anion transporting polypeptides, OATPs; organic anion transporters, OATs; organic cation transporters, OCTs; multidrug and toxin extrusion proteins, MATEs; apical sodium-dependent bile acid cotransporter, ASBT; sodium taurocholate transporting polypeptide, NTCP) and the ATP-binding cassette (ABC) transporters (e.g., bile salt export pump, BSEP; P-glycoprotein; multidrug resistance proteins, MRPs; breast cancer resistance protein, BCRP). Each tissue has a distinct pattern of expression for uptake and efflux transporters.

The main focus of this book is transporters expressed in the intestine, liver, and kidney of relevance to drug response and toxicity. Increasing intensity of research in this area has resulted in a much better understanding of the localization of transporters in cell membranes and their role for polarized drug transport. For example, drugs are delivered via the portal venous blood to the basolateral membrane of hepatocytes and taken up by distinct transporters localized in the basolateral membrane into hepatocytes with subsequent intracellular phase I and II metabolism and excretion of parent compounds or of metabolites via other transporters localized to the canalicular membrane into bile. Individual chapters in this book will also address the role of transporters located in tissues other than intestine, liver, and kidney to the local accumulation and effect of drugs at the site of action (e.g., CNS accumulation of HIV protease inhibitors and P-glycoprotein in the blood–brain barrier).

As highlighted in this book, transporters are also important to our understanding of (patho-)physiological processes as well as drug disposition and effects. For

example, the chapter on intestinal bile acid transporters highlights not only our current understanding of the absorption of bile acids from the intestinal lumen, but also shows how this knowledge is currently used for development of new hypocholesterolemic or hepatoprotective drugs.

Interindividual variability in drug response is a major problem for optimal drug therapy. The transporter field has contributed substantially to a better understanding of the determinants that account for intersubject differences in drug disposition and effects. Genetic polymorphisms in transporters can cause certain diseases, for example the Dubin-Johnson syndrome, in patients with certain mutations in the *ABCC2* gene encoding MRP2. Moreover, genetic polymorphisms in genes encoding uptake and efflux transporters have been identified as determinants of drug disposition. The knowledge summarized in this book on substrate specificity of individual transporters as well as the potential of drugs for inhibiting specific transporters has helped improve our understanding of mechanisms for drug–drug interactions. For example, increased plasma concentrations and toxicity of the cardiac glycoside digoxin with coadministration of multiple drugs (e.g., quinidine, verapamil) have been observed dating back to the 1970s, but the mechanism underlying these drug–drug interactions remained unclear for a long time. This changed when digoxin was identified as substrate of the efflux pump P-glycoprotein and when comedications such as quinidine and verapamil were identified as potent inhibitors of P-glycoprotein function.

For these reasons, regulatory agencies are increasingly asking pharmaceutical companies for detailed information on whether transporters are involved in disposition of a new drug entity and whether the new drug entity itself might cause undesired drug–drug interactions due to inhibition of specific drug transporters. This process is supported by the International Transporter Consortium, which recently published recommendations for investigations on Membrane Transporters in Drug Development (International Transporter Consortium 2010). However, it should be noted that our knowledge of the role of transporters in the disposition and effects of older, marketed drugs is far from complete and clearly requires further investigation.

We would like to thank all the authors for their outstanding contributions to this book. We would also like to acknowledge and thank Susanne Dathe from Springer for her thoughtful and constant support for this project.

Erlangen, Germany — Martin F. Fromm
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Uptake Transporters of the Human OATP Family

Molecular Characteristics, Substrates, Their Role in Drug–Drug Interactions, and Functional Consequences of Polymorphisms

Jörg König

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Abstract Organic anion transporting polypeptides (OATPs, gene family: *SLC21*/*SLCO*) mediate the uptake of a broad range of substrates including several widely prescribed drugs into cells. Drug substrates for members of the human OATP family include HMG-CoA-reductase inhibitors (statins), antibiotics, anticancer agents, and cardiac glycosides. OATPs are expressed in a variety of different tissues including brain, intestine, liver, and kidney, suggesting that these uptake transporters are important for drug absorption, distribution, and excretion. Because of their wide tissue distribution and broad substrate spectrum, altered transport kinetics, for example, due to drug–drug interactions or due to the functional consequences of genetic variations (polymorphisms), can contribute to the interindividual variability of drug effects. Therefore, the molecular characteristics of human OATP family members, the role of human OATPs in drug–drug interactions, and the in vitro analysis of the functional consequences of genetic variations in *SLCO* genes encoding OATP proteins are the focus of this chapter.

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Keywords Organic anion transporting polypeptide · Human OATP family · OATP1B1 · OATP1B3 · Drug transport · Pharmacogenomics · Polymorphisms · Drug–drug interactions

Abbreviations

BSP	Bromosulfophthalein
K_m	Kinetic constant (Michaelis–Menten constant)
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
SLCO	Gene of the SLCO family encoding OATP uptake transporters
V_{max}	Maximal transport velocity

1 Introduction

Transport proteins located in plasma membranes of cells are important for the absorption, distribution, and excretion of endogenous compounds and drugs. Principally, they can be subdivided into two major groups: uptake transporters mediating the uptake of substances from the extracellular space into cells, and export pumps actively secreting substances out of cells. Most of these export pumps belong to the superfamily of ABC (ATP-binding cassette) transporters (Fromm 2003; Keppler et al. 2001; König et al. 1999; Kruh and Belinsky 2003) energized by the hydrolysis of ATP and secreting substances (e.g., drugs or drug conjugates) against a concentration gradient [see respective chapters about P-glycoprotein, multidrug resistance proteins (MRPs), or the breast cancer resistance protein (BCRP)]. Uptake transporters mostly belong to the superfamily of solute carriers [SLC superfamily (Hediger et al. 2004)]. Currently, this superfamily is comprised of 48 SLC families (SLC1–SLC48) with more than 360 identified members (see: www.bioparadigms.org/slc/intro). Genes encoding the organic anion transporting polypeptide (OATP in humans and Oatp in rodents) are classified as the SLCO family (formerly termed SLC21 family). Today, the human OATP family consists of 11 members (Hagenbuch and Meier 2003; Mikkaichi et al. 2004b) including 10 OATPs and the prostaglandin transporter OATP2A1 [formerly termed PGT (Lu et al. 1996)]. Because trivial names for individual proteins do not correspond to the continuous numbering based on the chronology of protein identification and because some rodent Oatp proteins have no direct human orthologue, Hagenbuch and Meier (2004) introduced a new nomenclature for the entire OATP family. In this chapter I will follow this new nomenclature and designate all human proteins in capitals (e.g., OATP1B1), all rodent proteins as, for example, Oatp1a1, and all

genes encoding OATP proteins according to the SLCO gene nomenclature in italics (e.g., *SLCO1B1* encoding the human hepatocellular uptake transporter OATP1B1).

OATPs are a group of membrane carriers with a wide spectrum of amphipathic transport substrates (König et al. 2006; Kullak-Ublick et al. 2000, 2001; Meier et al. 1997). Although some members in rodents and humans are predominantly, if not exclusively expressed in liver (Hsiang et al. 1999; König et al. 2000a, b), most OATP/Oatp family members are expressed in multiple tissues including brain (Kusuhara and Sugiyama 2005), kidney (van Montfoort et al. 2003), and intestine (Kunta and Sinko 2004; Zair et al. 2008). As characterized so far, OATPs have a wide substrate spectrum transporting several endogenously synthesized compounds (e.g., bile salts, hormones steroid conjugates) as well as several xenobiotics and widely prescribed drugs like HMG-CoA-reductase inhibitors (statins), anticancer agents, and antibiotics [see reviews (Hagenbuch and Gui 2008; König et al. 2006; Kullak-Ublick et al. 2001; Niemi 2007)].

Because of their wide substrate spectrum and their expression in several tissues important for the absorption, distribution, and excretion of drugs, altered transport kinetics of these uptake transporters may contribute to the interindividual variability in drug response. Two major molecular mechanisms may account for altered uptake transporter kinetics. First, the functional consequences of genetic variations (so-called polymorphisms) in transporter genes resulting in amino acid exchanges in the transport protein can influence the expression, localization or transport kinetics of the uptake transporter. If, for example, a mutation in the hepatocyte-specific uptake transporter OATP1B1 leads to reduced hepatic uptake of drug substrates, the plasma concentration of these drugs increases due to decreased transport of the drug from blood into hepatocytes compared with individuals carrying the wild-type protein. A second possibility for altered uptake kinetics of one drug is the coadministration of a second drug, which is also a substrate of the drug transporter. This so-called transporter-mediated drug–drug interaction is another reason for adverse drug reactions as the inhibition or stimulation of drug uptake into cells may, as described already in detail for the inhibition of metabolizing enzymes, alter the pharmacokinetics of one or both drug substrates.

Therefore, members of the human OATP family of uptake transporters, their molecular features, their role in transporter-mediated drug–drug interactions, and the in vitro analysis of the functional consequences of polymorphisms in transporter genes encoding mutated uptake transporter proteins are the focus of this chapter.

2 The Human OATP Family

2.1 Molecular Characteristics of Human OATP Family Members

Rat Oatp1a1 (formerly termed oatp or Oatp1) was the first member of the OATP/Oatp family identified in 1994 by expression cloning (Jacquemin et al. 1994). It was demonstrated that this transport protein mediates the uptake of several organic

anions including conjugated and unconjugated bile salts (Kullak-Ublick et al. 1994). Based on this sequence information several Oatp1a1-related transport proteins could be identified and characterized in the following years, and today more than 40 different OATP/Oatp family members from rat, mouse, and human are known (Hagenbuch and Meier 2003; Mikkaichi et al. 2004b). The phylogenetic classification of human and rodent (mouse and rat) members of the OATP/Oatp family demonstrates that most of the human OATP family members have direct orthologous proteins in both rodent species as shown by related rat and mouse sequences (Fig. 1). Interestingly, the founding member of the human OATP family, OATP1A2, and both highly-in-hepatocytes-expressed family members OATP1B1 and OATP1B3, have no direct rodent orthologues. For OATP1A2 several rat and mouse Oatps could be identified as closely related proteins, and OATP1B1 and OATP1B3 have the highest sequence homology to each other, with mouse or rat Oatp1b2 being a more remote relative (Fig. 1). Orthologous proteins in rat and/or mouse showing the same tissue expression pattern and a comparable substrate spectrum to the respective human family member are the prerequisite for using animals as model systems for the functional characterization of the human proteins. Because both hepatocyte-specific OATPs OATP1B1 and OATP1B3 have no

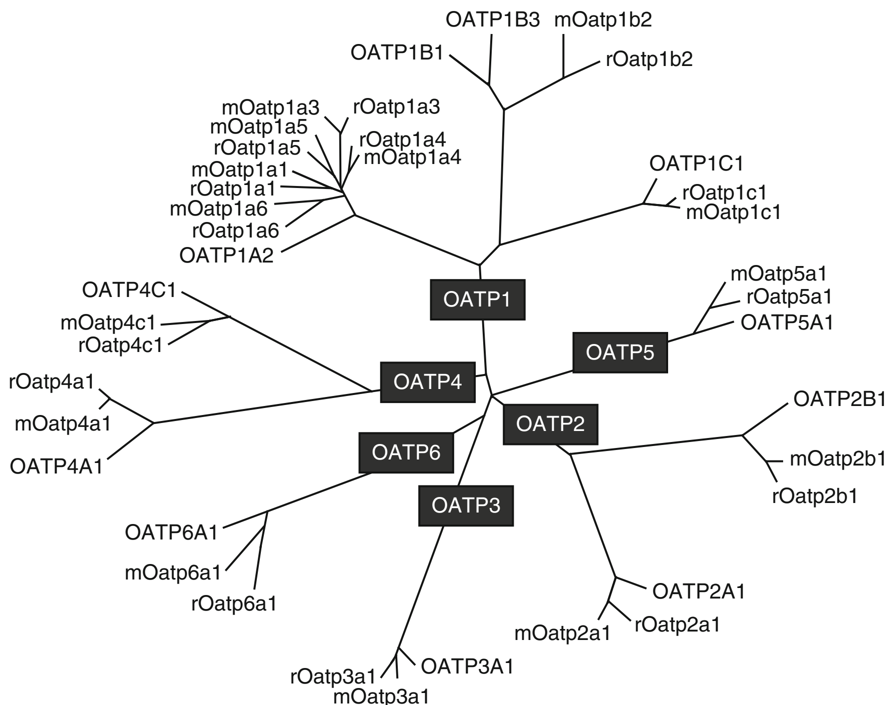


Fig. 1 Phylogenetic tree of human and rodent OATP/Oatp family members. The OATP subfamilies (OATP1–OATP6) are depicted in boxes. rOatp stands for the rat family member, mOatp for the respective mouse Oatp family member

orthologous proteins in other species, it has become evident that the transferability of animal data (e.g., analyzing the hepatobiliary elimination of endogenous substances or drugs) to the human situation at least regarding the function of the hepatic OATP proteins is limited. Therefore, the availability of genetically engineered cell models stably expressing the respective human OATP family members is important for the functional analysis of these uptake transporters.

Interestingly, the four human OATPs of the OATP1 subfamily (OATP1A1, OATP1B1, OATP1B3, and OATP1C1) are all localized on the short arm of chromosome 12 (chromosome 12p12), whereas the genes of all other members of the OATP family are distributed over the whole genome (Table 1). The proteins have a medial length of 710 amino acids with OATP2A1 being the shortest family member (643 amino acids) and OATP5A1 the longest (848 amino acids).

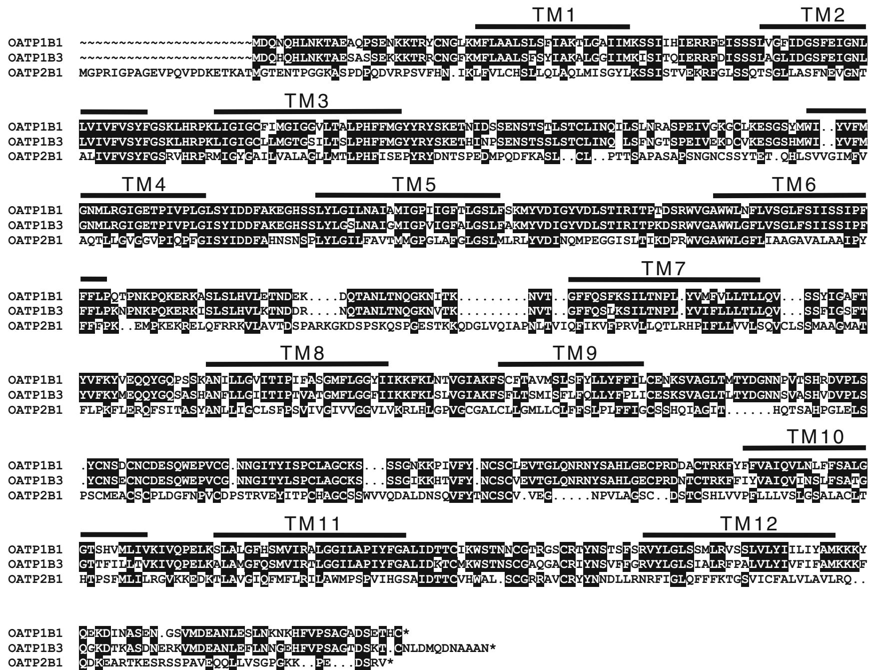
All OATP/Oatp family members share a very similar topology (Fig. 2). Based on computational analyses they consist of 12 transmembrane domains [so-called TMs; (Fig. 2)] and a large fifth extracellular loop between TMs 9 and 10. This loop contains many conserved cysteine residues. N-glycosylation sites are in the extracelluar loops two and five and the OATP family signature D-X-RW-(I,V)-GAWW-X-G-(F,L)-L is located at the border between extracellular loop 3 and TM 6 (Hagenbuch and Meier 2003). Based on expressed sequence tags (EST) and genomic database entries OATP-related proteins were identified in many other species including nematodes (*Caenorhabditis elegans*), zebrafish (*Danion rerio*), the frog *Xenopus laevis*, chicken (*Gallus gallus*), fruitfly (*Drosophila melanogaster*), pig (*Sus scrofa*), and cow (*Bos taurus*). Interestingly, although studied intensively, no sequence similarities were found in the genomes of bacteria and yeast.

Although most of the human OATP family members are expressed in multiple tissues, OATP1B1 and OATP1B3 are predominantly if not exclusively expressed in liver (Hsiang et al. 1999; König et al. 2000a, b). Despite the fact that some studies identified *SLCO1B1* (expressing OATP1B1) and *SLCO1B3* (expressing OATP1B3)

Table 1 Characteristics of human OATP family members

Protein name	Gene symbol	Sequence accession ID	Chromosomal localization	Amino acids	Tissue distribution
OATP1A2	*SLCO1A2*	NM_021094	12p12	670	Brain, Kidney
OATP1B1	*SLCO1B1*	NM_006446	12p	691	Liver
OATP1B3	*SLCO1B3*	NM_019844	12p12	702	Liver
OATP1C1	*SLCO1C1*	NM_017435	12p12.2	712	Brain, Testis
OATP2A1	*SLCO2A1*	NM_005630	3q21	643	Ubiquitous
OATP2B1	SLCO2B1	NM_007256	11q13	709	Ubiquitous
OATP3A1	*SLCO3A1*	NM_013272	15q26	710	Ubiquitous
OATP4A1	*SLCO4A1*	NM_016354	20q13.33	722	Ubiquitous
OATP4C1	*SLCO4C1*	NM_180991	5q21.2	724	Kidney
OATP5A1	*SLCO5A1*	NM_030958	8q13.3	848	?
OATP6A1	*SLCO6A1*	NM_173488	5q21.1	719	Testis(?)

Data compiled from NCBI (www.ncbi.nml.nih.gov) and SLC tables (www.bioparadigms.org/slc) databases



	OATP1A2	OATP1B1	OATP1B3	OATP1C1	OATP2B1	OATP3A1	OATP4A1	OATP4C1	OATP5A1	OATP6A1
OATP1A2	100	43,8	42,2	47,5	33,9	35,9	32,4	32,5	34,5	24,6
OATP1B1		100	43,8	46,6	34,7	37,3	31,4	34,4	35,9	27,5
OATP1B3			100	48,3	35,6	36,0	33,6	34,9	36,9	26,1
OATP1C1				100	34,1	37,3	33,8	32,9	33,8	25,4
OATP2B1					100	36,0	33,8	34,8	36,2	27,1
OATP3A1						100	38,3	39,0	38,8	28,6
OATP4A1							100	46,1	41,5	35,3
OATP4C1								100	37,4	44,8
OATP5A1									100	28,9
OATP6A1										100

Fig. 2 Box alignment of the hepatic OATP family members OATP1B1, OATP1B3, and OATP2B1 and amino acid identities of the human OATP family members. Predicted transmembrane domains are shown above the alignment (TM1–TM12)

mRNA expression in the intestine (Glaeser et al. 2007) or (in the case of OATP1B3) in colorectal carcinoma (Abe et al. 2001; Lee et al. 2008) no protein data have been published so far demonstrating the occurrence of both proteins in other tissues than human liver. Although identified and cloned from human liver (Kullak-Ublick et al. 1995), OATP1A2 shows a very low expression there, but it is highly expressed in brain and testis (Kullak-Ublick et al. 1995). OATP2B1, OATP3A1, and OATP4A1 seem to be ubiquitously expressed in all tissues investigated so far, whereas

OATP4C1 is highly expressed in kidney (Mikkaichi et al. 2004a). No protein expression data have been published from human OATP5A1 and OATP6A1, in rats Oatp6a1 is expressed at the blood–testis barrier (Augustine et al. 2005). Almost all OATP family members are localized to the basolateral membrane of polarized cells. OATP1B1, OATP1B3, and OATP2B1 are localized to the basolateral membrane of human hepatocytes (Hsiang et al. 1999; König et al. 2000a, b), whereas OATP1A2 has been localized to the basolateral membrane of brain epithelial cells (Lee et al. 2005) and to the basolateral membrane of proximal kidney tubule cells (Kullak-Ublick et al. 1995). Interestingly, in addition to the basolateral localization of OATP1A2 and OATP2B1 both proteins have been detected in the apical membrane of enterocytes (Glaeser et al. 2007; Kobayashi et al. 2003). OATP4A1 is predominantly localized at the apical surface of the syncytiotrophoblast in placenta (Sato et al. 2003) and, together with OATP3A1 and OATP1C1 to the basolateral membrane of the nonpigmented human ciliary body epithelium (Gao et al. 2005). OATP4C1 has been found to be highly expressed at mRNA level in human kidney, and so far only rat Oatp4c1 has been localized to the basolateral membrane of kidney proximal tubular cells (Mikkaichi et al. 2004a). The expression and subcellular localization of human OATP5A1 and OATP6A1 remains to be analyzed.

Despite the fact that all human OATP proteins share a similar membrane topology the knowledge on protein regions or structures involved in the transport process is limited. Some indirect evidence identifying amino acid residues important for substrate recognition or transport resulted from the characterization of the functional consequences of polymorphisms. Analyzing the effect of mutations in the second extracellular loop of the OATP1B1 protein showed that some residues located there are important for substrate recognition (Michalski et al. 2002), whereas several in vitro and in vivo studies have demonstrated that the exchange OATP1B1p.V174A (allelic nomenclature: OATP1B1*5) is critical for transport kinetics [for review see: (König et al. 2006; Seithel et al. 2008)]. Only a few studies directly used side-directed mutagenesis to identify structural domains and/or residues important for substrate selectivity or transport. One study identified three key residues located in the transmembrane domain 10 (TM10) of the OATP1B3 protein (Gui and Hagenbuch 2008), being important for the transport of the OATP1B3 substrate choleocystokinin-8 (CCK-8). In detail, mutations of the residues Tyr537, Ser545, and Thr550 alone or in combination alter the kinetic constant (K_m value) as well as the maximal transport velocity (V_{max} value) of the OATP1B3 protein, suggesting that TM10 is important for the transport process. A second study published by Miyagawa et al. (2009) demonstrated that alterations in the eighth and ninth transmembrane domain of the OATP1B1 protein resulted in a change of the transport kinetics of the mutated protein. A detailed study analyzing conserved lysine and arginine in transmembrane helices demonstrated that the amino acid residues Lys41 (in TM1) and Arg580 (in TM11) are pivotal for the transport activity of the OATP1B3 protein (Glaeser et al. 2009). These few studies demonstrated that several regions in the protein should be important for substrate recognition and transport, and it would be important to have data based on the crystal

structure of an OATP/Oatp protein to gain more insight into the molecular nature of the translocation of substrates from one site of the membrane to the other.

2.2 Substrate Spectrum of Human OATP Family Members

OATP1A2 (formerly termed OATP or OATP-A) was cloned based on its homology to rat Oatp1a1 [rat Oatp1 (Jacquemin et al. 1994)] as a sodium-independent uptake transporter for the organic anion bromosulfophthalein and bile salts in human liver (Kullak-Ublick et al. 1995). Using *Xenopus laevis* oocytes it was shown that OATP1A2 transports the bile acids cholate, taurocholate, glycocholate, taurochenodeoxycholate, and tauroursodeoxycholate (Kullak-Ublick et al. 1995). Further studies investigating the substrate spectrum of OATP1A2 in more detail demonstrated the capability of OATP1A2 to transport a wide range of amphipathic organic anions including bile salts, steroid hormones, and their conjugates (Bossuyt et al. 1996), thyroid hormones (Friesema et al. 1999) as well as some organic cations like *N*-methyl-quinidine (van Montfoort et al. 1999).

Interestingly, studies determining the substrate spectrum of other human OATP family members demonstrated that most of them have a similarly broad substrate spectrum at least partially overlapping with the substrate spectrum of OATP1A2. Best-characterized members of the human OATP family with respect to the substrate spectrum are the hepatocyte-specific OATPs OATP1B1 and OATP1B3. For these uptake transporters several endogenously synthesized substances have been identified as substrates including bile salts like taurocholate (Abe et al. 1999, 2001; Cui et al. 2001; Hsiang et al. 1999; Letschert et al. 2004), conjugated steroids like estradiol-17ß-glucuronide (König et al. 2000a, b), or estrone-3-sulfate (Nozawa et al. 2004c; Tamai et al. 2000) and hormones like tyroxine (Abe et al. 1999; Kullak-Ublick et al. 2001; Omote et al. 2006). The so-called model substrate for these OATPs and for OATP1A2 is the organic anion bromosulfophthalein (BSP), which is transported with very low K_m values of 20 μM, 0.1 μM, and 3.3 μM for OATP1A2, OATP1B1, and OATP1B3, respectively (Cui et al. 2001; König et al. 2000a; Kullak-Ublick et al. 2001). Interestingly, for other human OATPs the substrate spectrum has not been characterized in that detail, and only a few substances have been identified as substrates for OATP2B1, OATP1C1, OATP3A1, OATP4A1, and OATP4C1, whereas to this day no substrates have been identified for OATP5A1 and OATP6A1. A substrate common for all OATPs (except OATP4C1) is estrone-3-sulfate, whereas BSP is transported in addition by OATP1C1 and OATP2B1 (Kullak-Ublick et al. 2001; Pizzagalli et al. 2002).

Beside endogenously synthesized substances, several drugs have been identified as substrates for human OATP family members (Table 2). Especially the hepatocyte-specific family member OATP1B1 seems to be important in drug transport mediating the uptake of a variety of different drug substrates from blood into hepatocytes. Drug substrates of OATP1B1 include HMG-CoA-reductase inhibitors (statins) like

Table 2 Selected drug substrates for human OATP family members

Substrate	K_m value (μM)	References
OATP1A2		
BQ123		Kullak-Ublick et al. (2001)
Chloambuciltaurocholate		Kullak-Ublick et al. (1997)
Bamet-R2	24	Briz et al. (2002)
Bamet-UD2	14	Briz et al. (2002)
Deltorphin II	330	Gao et al. (2000)
DPDPE	202	Gao et al. (2000)
Fexofenadine	6	Cvetkovic et al. (1999)
GD-B20790	92	Pascolo et al. (1999)
N-Methyl-quinidine	26	van Montfoort et al. (1999)
Methotrexate	457	Badagnani et al. (2006)
Ouabain	5.5	Bossuyt et al. (1996)
Rocuronium		van Montfoort et al. (1999)
Tauroursodeoxycholate	19	Kullak-Ublick et al. (1995)
OATP1B1		
Atorvastatin	12	Kameyama et al. (2005)
Atrasentan		Katz et al. (2006)
Benzylpenicillin		Tamai et al. (2000)
Bamet-R2	10	Briz et al. (2002)
Bamet-UD2	10	Briz et al. (2002)
Bosentan	44	Treiber et al. (2007)
BQ-123		Kullak-Ublick et al. (2001)
Caspofungin		Sandhu et al. (2005)
Cerivastatin		Shitara et al. (2004b)
DADLE		Nozawa et al. (2003)
DPDPE		Kullak-Ublick et al. (2001)
Enalapril	260	Liu et al. (2006)
Fluvastatin	3.5	Kopplow et al. (2005)
Glycoursodeoxycholate	5	Maeda et al. (2006b)
Methotrexate		Abe et al. (2001)
Olmesartan	13	Yamada et al. (2007)
Pitavastatin	3	Hirano et al. (2004)
Pravastatin	35	Hsiang et al. (1999)
Rifampin	2	Tirona et al. (2003)
Rosuvastatin	8	Schneck et al. (2004)
SN-38		Nozawa et al. (2005)
Tauroursodeoxycholate	8	Maeda et al. (2006b)
Temocaprilat		Maeda et al. (2006a)
Troglitazone sulphate		Nozawa et al. (2004b)
TR-14035	7.5	Tsuda-Tsukimoto et al. (2006)
Valsartan	1.4	Yamashiro et al. (2006)
OATP1B3		
Atrasentan		Katz et al. (2006)
Bosentan	141	Treiber et al. (2007)
BQ-123		Kullak-Ublick et al. (2001)
Deltorphin II		Kullak-Ublick et al. (2001)
Digoxin		Kullak-Ublick et al. (2001)
Docetaxel		Smith et al. (2005)
DPDPE		Abe et al. (2001)
Enalapril		Liu et al. (2006)
Fexofenadine	108	Shimizu et al. (2005)

(*continued*)

Table 2 (continued)

Substrate	K_m value (μM)	References
Fluvastatin		Kopplow et al. (2005)
Glycoursodeoxycholate	25	Maeda et al. (2006b)
Methotrexate	25	Abe et al. (2001)
Olmesartan	44	Yamada et al. (2007)
Ouabain		Kullak-Ublick et al. (2001)
Paclitaxel	7	Smith et al. (2005)
Pitavastatin	3	Hirano et al. (2004)
Pravastatin		Seithel et al. (2007)
Rifampin	2	Vavricka et al. (2002)
Tauroursodeoxycholate	16	Maeda et al. (2006b)
Telmisartan	0.8	Ishiguro et al. (2006)
TR-14035	5.3	Tsuda-Tsukimoto et al. (2006)
Valsartan	18	Yamashiro et al. (2006)
OATP2B1		
Atorvastatin	0.2	Grube et al. (2006)
Benzylpenicillin		Tamai et al. (2000)
Bosentan	202	Treiber et al. (2007)
CP-671.305	4	Kalgutkar et al. (2007)
Fexofenadine		Nozawa et al. (2004a)
Fluvastatin	0.8	Kopplow et al. (2005)
Glibenclamide	6.3	Satoh et al. (2005)
M17055	4.5	Nishimura et al. (2007)
OATP3A1		
Benzylpenicillin		Tamai et al. (2000)
OATP4A1		
Benzylpenicillin		Tamai et al. (2000)
OATP4C1		
Digoxin	8	Mikkaichi et al. (2004a)
Methotrexate		Mikkaichi et al. (2004a)
Ouabain	0.4	Mikkaichi et al. (2004a)
OATP5A1 and OATP6A1		
Neither endogenous substrates nor drugs as substrates identified yet		

Bamet-R2: [*cis*-diammine-chloro-cholylglycinate-platinum(II)], Bamet-UD2: [*cis*-diammine-bisursodeoxycholate-platinum(II)], DADLE: [D-Ala(2), D-Leu(5)]enkephalin, DPDPE: [D-penicillamine-2,5]enkephalin, M17055: 7-chloro-2,3-dihydro-1-(2-methylbenzoyl)-4(1H)-quinolinone 4-oxime-O-sulfonic acid potassium salt, TR-14035: *N*-(2,6-dichlorobenzoyl)-4-(2′,6′-bismethoy-phenyl)phenylamine

pravastatin (Hsiang et al. 1999), rosuvastatin (Schneck et al. 2004), atorvastatin (Kameyama et al. 2005), and pitavastatin (Hirano et al. 2004). Other drugs transported by OATP1B1 include antibiotics [benzylpenicillin, rifampicin (Tamai et al. 2000; Tirona et al. 2003)], antineoplastic agents like methotrexate (Abe et al. 1999), the endothelin receptor antagonist bosentan (Treiber et al. 2007), and the angiotensin II receptor antagonist valsartan (Yamashiro et al. 2006). Interestingly, for some drugs it has been shown by in vivo studies that genetic variations in the *SLCO1B1* gene encoding OATP1B1 are associated with altered pharmacokinetics of this drug, but direct evidence that this drug is also a transport substrate for OATP1B1 is lacking. So it has been demonstrated that the genetic variation *SLCO1B1c.521T>C*

(resulting in the protein OATP1B1p.Val174Ala; OATP1B1*5) is associated with an increase in the area under the concentration time curve (AUC) for the oral antidiabetic drug repaglinide (Niemi et al. 2005), clearly showing that this genetic variation is a major determinant in the interindividual variability of repaglinide pharmacokinetics. In vitro studies using stably transfected HEK293 cells confirmed these results showing that repaglinide inhibits OATP1B1-mediated BSP and pravastatin uptake (Bachmakov et al. 2008), but studies demonstrating that repaglinide is an OATP1B1 substrate have not been published to date. On the other hand it has been demonstrated that this genetic variation did not affect the pharmacokinetic of rosiglitazone, a second oral antidiabetic drug (Kalliokoski et al. 2007), whereas in vitro studies have demonstrated that rosiglitazone affects OATP1B1-mediated uptake (Bachmakov et al. 2008). Like shown for the organic cation transporters (OCTs, gene family *SLC22*) it seems that substances may interact with OATPs by influencing transport kinetics without being transported themselves.

Because OATP1B1 and OATP1B3 share a nearly identical substrate spectrum, most of the drugs identified as substrates for OATP1B1 are also transported by OATP1B3 (Table 2). These OATP1B3 drug substrates include statins [pravastatin, fluvastatin, pitavastatin (Hirano et al. 2004; Kopplow et al. 2005; Seithel et al. 2007)], as well as antibiotics [rifampicin (Vavricka et al. 2002)] and antineoplastic agents [methotrexate (Abe et al. 2001)]. In addition, the antihistaminic drug fexofenadine (Shimizu et al. 2005) and the chemotherapeutic agent paclitaxel (Smith et al. 2005) have been identified as substrates for OATP1B3. Fexofenadine has also been shown to be a substrate for OATP1A2 (Cvetkovic et al. 1999), which in addition also transports the muscle relaxant rocuronium (van Montfoort et al. 1999) and the cardiac glycoside ouabain (Bossuyt et al. 1996). The knowledge on drugs as substrates for other human OATP family members is limited. Some drugs have been identified as substrates for OATP2B1 including statins [atorvastatin, fluvastatin (Grube et al. 2006; Kopplow et al. 2005), fexofenadine (Shimizu et al. 2005), and the antidiabetic drug glibenclamide (Satoh et al. 2005)]. Selected drug substrates for human OATP family members are presented in Table 2.

2.3 Hepatic OATPs and Drug–Drug Interactions

Besides the impact of polymorphisms in *SLCO/SLC21* genes encoding human OATPs on the pharmacokinetics of drug substrates, a second possibility influencing drug plasma concentrations are transporter-mediated drug–drug interactions. Because several widely prescribed drugs (e.g., statins) are substrates of hepatic OATP proteins, concomitantly administered drugs, which are also substrates for these uptake transporters, may lead to elevated plasma levels of statins due to their reduced hepatic uptake. Statins are used as inhibitors of the de novo synthesis of cholesterol in the liver and are widely used to treat dyslipidaemia. OATP-mediated uptake of statins into hepatocytes is a prerequisite for the subsequent intracellular inhibition of the HMG-CoA-reductase, and reduced hepatic uptake of statins due to

coadministered OATP drug substrates may cause severe side effects such as myopathy and rhabdomyolysis (Bruno-Joyce et al. 2001; East et al. 1988).

Macrolides are known to cause severe drug–drug interactions and it has been demonstrated that some of these interactions are due to the inhibition of metabolizing enzymes. Some macrolides have been identified as potent inhibitors of the phase I enzyme CYP3A4, and this macrolides-induced inhibition may increase the plasma concentrations of coadministered drugs that are also CYP3A4 substrates (Ito et al. 2003; Polasek and Miners 2006). Clarithromycin, for example, increases the plasma concentrations of the concomitantly administered statins atorvastatin and simvastatin that are both metabolized by CYP3A4. Interestingly, clarithromycin also increases the plasma concentration of coadministered pravastatin, which is not metabolized by cytochromes and excreted almost unchanged into bile (Jacobson 2004), and the observed interaction cannot be due to the inhibition of metabolizing enzymes. Therefore, Seithel et al. analyzed whether macrolides inhibit OATP1B1- and OATP1B3-mediated uptake of pravastatin in stably transfected HEK cells recombinantly expressing these uptake transporters (Seithel et al. 2007). They found that not only clarithromycin but also erythromycin and roxithromycin inhibited OATP1B1- and OATP1B3-mediated pravastatin uptake (Fig. 3). Interestingly, this uptake inhibition by clarithromycin and roxithromycin was significant at the tested low macrolides concentration of 10 μM, which is below the calculated macrolides concentration in the portal venous blood after oral administration. These in vitro studies demonstrated that besides the inhibition of metabolizing enzymes by macrolides the inhibition of uptake transport proteins could be responsible for macrolides-induced alterations in the plasma concentrations of concomitantly administered drugs.

In addition to oral antidiabetic drugs (metformin, glitazones, or repaglinide), most patients with type 2 diabetes are concomitantly treated with cardiovascular drugs (e.g., statins or ACE inhibitors). Niemi and coworkers have demonstrated that the genetic variation *SLCO1B1c.521T>C* encoding the OATP1B1*5 variant is associated with altered plasma concentrations of repaglinide (Niemi et al. 2005), whereas glitazones are known substrates for OATP1B1 (Nozawa et al. 2004b). Interestingly, although the OATP1B1*5 variant leads to altered plasma concentrations of repaglinide it has obviously no effect on the pharmacokinetics of rosiglitazone (Kalliokoski et al. 2007). Based on these data Bachmakov and coworkers investigated whether the oral antidiabetic drugs metformin, repaglinide, and rosiglitazone affect OATP1B1- and OATP1B3-mediated pravastatin uptake (Bachmakov et al. 2008). Using HEK293 cells stably expressing human OATP1B1 or OATP1B3 they demonstrated that repaglinide inhibited OATP1B1- and OATP1B3-mediated pravastatin uptake at a low concentration of 10 μM. Interestingly, rosiglitazone at the same low concentration stimulated pravastatin uptake into OATP1B1- and OATP1B3-expressing cells to 170% and 400%, respectively, and inhibited pravastatin uptake only at a high concentration of 100 μM (Bachmakov et al. 2008). These results suggest that not only inhibition of uptake transporter function but also the stimulation of uptake rates have to be considered as possible molecular mechanisms for altering drug plasma concentrations.

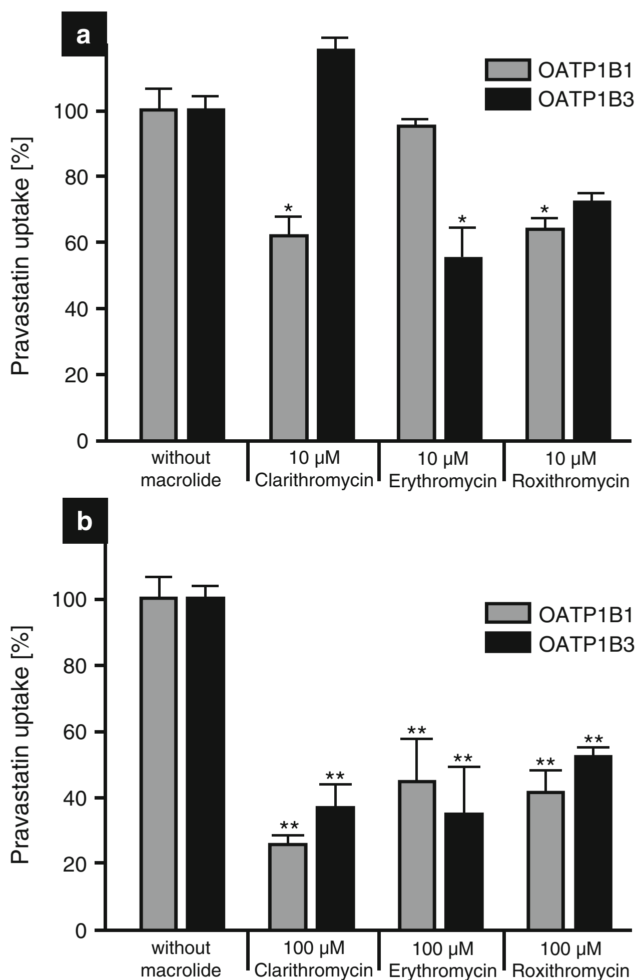


Fig. 3 OATP1B1- and OATP1B3-mediated drug–drug interactions using pravastatin as drug substrate and macrolides antibiotics as potential uptake inhibitors. Uptake of pravastatin was measured in HEK293 cells stably expressing the respective uptake transporter. (**a**) Macrolides (clarithromycin, erythromycin, and roxithromycin) were added in a concentration of 10 µM into the uptake solution. (**b**) Macrolides were added in a concentration of 100 µM. Pravastatin uptake without added macrolides was set to 100%

Interactions of statins with coadministered drugs have been reported earlier with cerivastatin given together with the immunosuppressant drug cyclosporin A (Shitara et al. 2003) or the fibrate gemfibrozil (Shitara et al. 2004b). Kidney transplant recipients treated simultaneously with cerivastatin and cyclosporin A showed increased plasma concentrations of cerivastatin (Muck et al. 1999). This interaction was studied using human liver microsomes and it was demonstrated that

cyclosporin A inhibits both the metabolism and the hepatic uptake of cerivastatin mediated by OATP1B1 (Shitara et al. 2004a). Interestingly, uptake inhibition was observed at lower cyclosporin A concentrations than the inhibition of metabolizing enzymes, suggesting that uptake inhibition is also an important mechanism in vivo. In the case of gemfibrozil it has been reported that coadministration together with cerivastatin leads to a sixfold increase in cerivastatin AUC (Backman et al. 2002). Several studies described potential molecular mechanisms of this interaction. Possible molecular targets could be the phase I enzyme CYP2C8 and the phase II enzymes UGT1A1 and UGT1A3 (Prueksaritanont et al. 2002). Both enzymes are important for the metabolism of cerivastatin and both could be inhibited by gemfibrozil. Furthermore, it has been reported using MDCK (Madin-Darby canine kidney cells) recombinantly expressing human OATP1B1 that gemfibrozil and its metabolite gemfibrozil 1-O-ß-glucuronide inhibit OATP1B1-mediated cerivastatin uptake (Shitara et al. 2004b). Therefore, this uptake inhibition may lead to elevated cerivastatin plasma levels with an increased risk for statin-induced myopathy. All three observed interactions may contribute to these severe drug–drug interactions, and consequently cerivastatin was withdrawn from the market.

Another example for transporter-mediated drug–drug interactions has been observed with the endothelin receptor antagonist bosentan. Bosentan is metabolized in hepatocytes mainly by CYP2C9 and CYP3A4. Several studies have demonstrated that simultaneously administered drugs can increase the plasma concentration of bosentan. It has been reported, for example, for coadministered ketoconazol, cyclosporin A, rifampicin, and sildenafil that all of these drugs increased the plasma concentration of bosentan (Treiber et al. 2007; van Giersbergen et al. 2002). Interestingly, cyclosporin A led to a 30-fold increase of bosentan concentrations, whereas ketoconazol, a potent CYP3A4 inhibitor, led only to a twofold increase in bosentan plasma concentrations suggesting that other molecular mechanisms besides the inhibition of metabolizing enzymes occur. Recently, it has been demonstrated that bosentan is a substrate for OATP1B1 and OATP1B3 (Treiber et al. 2007). Using CHO (Chinese hamster ovary) cells stably expressing OATP1B1 or OATP1B3 it has been shown that bosentan uptake is inhibited by cyclosporine A, rifampicin, and sildenafil. Furthermore, also the transporter-mediated uptake of the bosentan metabolite Ro 48-5033 is inhibited. Kinetic analysis of the inhibitory effect revealed that rifampicin inhibited bosentan uptake with IC_{50} values of 0.3 μM and 0.8 μM for OATP1B1 and OATP1B3, respectively, whereas rifampicin inhibited OATP1B1 with an IC_{50} value of 3.2 μM and OATP1B3 with an IC_{50} value of 1.6 μM (Treiber et al. 2007). Because cyclosporin A could reach plasma concentrations of 1.3 μM and rifampicin of 15 μM a combined effect of uptake inhibition and of the inhibition of metabolizing enzymes may be responsible for the in vivo observed increase in bosentan plasma concentrations in the presence of both drugs. For sildenafil IC_{50} values of 1.5 μM (for OATP1B1) and 0.8 μM (for OATP1B3) were determined whereas plasma concentrations of 1.2 μM could be reached. Because sildenafil is not an inhibitor of CYP3A4 or CYP2C9, inhibition of uptake transporters may be the major determinant of this drug–drug interaction.

Taken together, the presented in vitro studies demonstrated that inhibition of uptake transporter-mediated drug transport by concomitantly administered drugs could be an additional important mechanism underlying previously observed drug–drug interactions.

2.4 Functional Consequences of Genetic Variations in Transporter Genes

2.4.1 Pharmacogenomics of OATP1B1

Besides transporter-mediated drug–drug interactions the functional consequences of frequent genetic variations, so-called polymorphisms, in transporter genes play an important role in the interindividual variability of drug disposition and drug response. Considerable effort has been made in recent years to identify single nucleotide polymorphisms (SNPs) or haplotypes to determine their frequency in different ethnic populations and to establish the functional consequences on protein expression, localization, or transport function. A summary of reported sequence variations in the *SLCO1B1* gene, encoding human OATP1B1, and of variations in the genes *SLCO1A2*, *SLCO1B3*, and *SLCO2B1*, encoding human OATP1A2, OATP1B3, and OATP2B1, is depicted in Fig. 4. Functional relevant polymorphisms in other human OATP family members have not been identified so far. A recently published study investigating thyroid hormone transport mediated by human OATP1C1 described two frequent polymorphisms in the *SLCO1C1* gene that both had no effect on the function of the mutated OATP1C1 protein (van der Deure et al. 2008).

Tirona and coworkers have published the first detailed in vitro analysis of genetic variations in the *SLCO1B1* gene encoding the hepatocellular uptake transporter OATP1B1 (Tirona et al. 2001). They investigated 14 nonsynonymous polymorphisms identified in a population of European and African Americans and found that some polymorphisms of haplotypes affect protein localization or transport function of the mutated OATP1B1 proteins. In general, they found that the genotypic frequencies were dependent on race and that amino acid exchanges located within the transmembrane-spanning domains and in the extracellular fifth loop were associated with alteration in transport kinetics. In detail, the variant OATP1B1*2 (*SLCO1B1c.217T>C*) alone or together with the exchange *SLCO1B1c.1964A>G* (OATP1B1*12) increased the K_m value for estrone-3-sulfate from 0.54 to 5.9 μM and 8.1 μM, respectively. A significant increase in the K_m value (2.2 μM) could also be observed analyzing the haplotype OATP1B1*13 (*SLCO1B1c.245T>C/c.467A>G/c.2000A>G*). Both variants also reduced transport compared to transport mediated by the OATP1B1*1a allele. Reduced transport of the substrates estrone-3-sulfate and estradiol-17ß-glucuronide was also observed for the variants OATP1B1*3 (*SLCO1B1c.245T>C/c.467A>G*, OATP1B1p.V82A/p.Q156G), OATP1B1*5 (*SLCO1B1c.521T>C*, OATP1B1p.V174A), OATP1B1*6

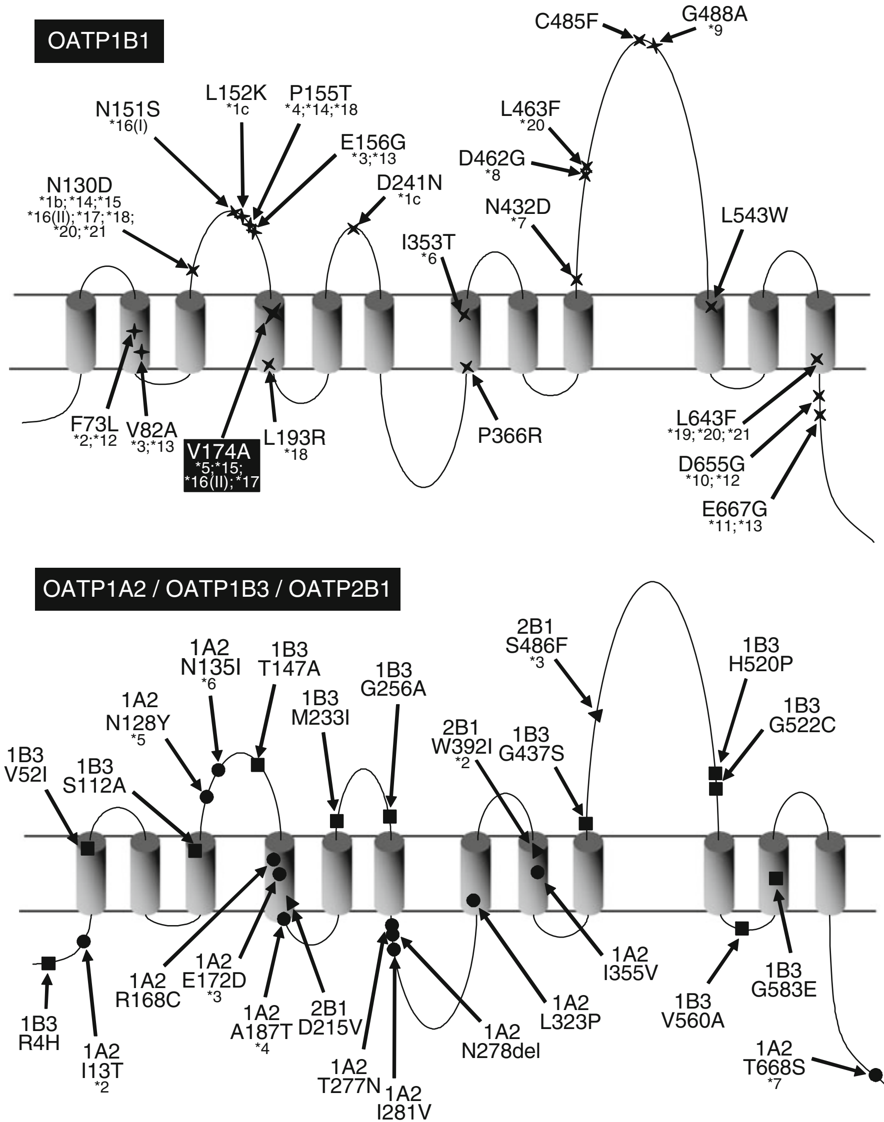


Fig. 4 Schematic two-dimensional model of human OATP1B1 (*above*) and OATP1A2/OATP1B3/OATP2B1 (*below*). For OATP1B1 the localization of genetic variations (polymorphisms) after translation is indicated as *stars*, for OATP1A2 as *circles*, for OATP1B3 as *boxes*, and for OATP2B1 as *triangles*. If valid, the respective allele and haplotype nomenclature of the variation is written (*1–*21). For OATP1B1, the important allele OATP1B1p.V174A (contained in the haplotypes *5, *15, *16(II), and *17) is highlighted

(*SLCO1B1c.1058T>C*, OATP1B1p.I353T), and OATP1B1*9 (*SLCO1B1c.1463G>C*, OATP1B1p.G488A). As mentioned previously, most of these variations are located within transmembrane-spanning regions or in the second or fifth extracellular loop (Fig. 4) suggesting that these protein regions are important for substrate recognition and/or transport (Tirona et al. 2001).

Michalski et al. (2002) have published the first naturally occurring mutation within the *SLCO1B1* gene together with the detailed analysis of the functional consequences of two frequent polymorphisms. They analyzed 81 human liver samples originating from Caucasians and identified one sample showing reduced OATP1B1 protein amount compared to the protein amount in all other liver samples. Analyzing the sequence of the *SLCO1B1* gene from this sample identified one haplotype containing two synonymous and three nonsynonymous base pair exchanges. Two of them corresponded to the recently before identified frequent polymorphisms OATP1B1*1b and OATP1B1*4 (OATP1B1p.P155T), whereas the third nonsynonymous exchange (OATP1B1*18, OATP1B1p.L193R) could be analyzed as the first naturally occurring mutation in the *SLCO1B1* gene with a frequency below 0.3%. For the analysis of the functional consequences of these variations, the authors established stably transfected MDCKII cells recombinantly expressing single-mutated OATP1B1 proteins for the analysis of each polymorphism and one MDCKII cell line expressing the mutated OATP1B1 protein encoded by the haplotype gene. Analyzing the protein localization in these OATP1B1-expressing cell lines it was demonstrated that both frequent variants were localized like the OATP1B1*1a protein in the lateral membrane of MDCKII cells, whereas a pronounced change in localization was observed for the OATP1B1p. L193R protein, which was hardly detectable in the lateral plasma membrane. Using bromosulfophthalein (BSP), estradiol-17ß-glucuronide ($E_2$17ßG), and taurocholate as substrates they found that BSP was transported by the OATP1B1*1b and by the *4 variant according to transport rates determined for the OATP1B1*1a allele; transport of $E_2$17ßG was significantly reduced by the OATP1B1*4 variant. Using taurocholate as substrate, the transport by the OATP1B1*1b variant was reduced and totally abolished by the OATP1B1*4 protein. None of the tested substrates was transported by the OATP1B1 protein carrying the single mutation L193R or by the protein encoded by the haplotype gene (Michalski et al. 2002). These analyses demonstrated that alterations in the second extracellular loop, where both frequent analyzed variations were located, could influence the substrate spectrum of the OATP1B1 protein, whereas the mutation OATP1B1p.L193R totally abolishes the transport function and influences in addition the localization of the protein.

Nozawa et al. (2002) have published a detailed investigation of polymorphisms in the Japanese population. They found that the previously identified OATP1B1*1c allele could not be detected in 267 Japanese subjects, whereas the OATP1B1*1b and *5 alleles were present with 54% and 0.7% in the Japanese population, respectively. Furthermore, they identified the novel haplotype OATP1B1*15, containing the two mutations *SLCO1B1c.388A>G* and *SLCO1B1c.521T>C*, which has an allelic frequency of 3.0% in the investigated population. Using transfected HEK293 cells expressing the different mutant OATP1B1 proteins they investigated

the consequences of the variations on OATP1B1-mediated transport. They found no significant changes in V_{max} or K_m values for estrone-3-sulfate uptake. Using estradiol-17ß-glucuronide as substrate, these results were confirmed in a second study analyzing the same genetic variations (Iwai et al. 2004). In this study the authors found no changes in K_m values, whereas the V_{max} value was slightly decreased for the haplotype OATP1B1*15. In a second study analyzing the functional consequences of the OATP1B1*15 haplotype Nozawa and coworkers demonstrated that the *15 allele shows decreased transport activities for the HMG-CoA-reductase inhibitor pravastatin and for SN-38, the active metabolite of the topoisomerase inhibitor irinotecan, suggesting that this haplotype contributes to the interindividual variability in drugs that are substrates for this hepatic uptake transporter (Nozawa et al. 2005). Kameyama and colleagues systematically investigated the effect of the OATP1B1*1a, *1b, *5, and *15 alleles alone and in combination with the polymorphism *SLCO1B1c.1007C>G* on OATP1B1-mediated transport of several statins using stably transfected HEK293 cells recombinantly expressing the different OATP1B1 proteins (Kameyama et al. 2005). For atorvastatin and pravastatin, the maximum transport velocities (V_{max} values) and the intrinsic clearance (V_{max}/K_m) were significantly reduced for the alleles OATP1B1*5, *15, and *15 + 1007C>G compared to the transport mediated by the wild-type protein (*1a allele). No differences in transport could be observed in HEK cells expressing the OATP1B1*1b allele. Interestingly, all alleles affecting transport have the polymorphism *SLCO1B1c.521T>C* (OATP1B1*5) in common, demonstrating that this polymorphism is responsible for the reduced transport activity of the OATP1B1 protein.

The functional consequences of the polymorphisms OATP1B1*1b and OATP1B1*5 on the transport of ezetimibe have been investigated using stably transfected HEK293 cells expressing the mutated OATP1B1 proteins (Oswald et al. 2008). In this study, the authors compared in vivo data obtained by studies with healthy participants with in vitro transport data and found that the ezetimibe metabolite ezetimibe glucuronide inhibited OATP1B1-, OATP1B3- and OATP2B1-mediated bromosulfophthalein (BSP) uptake with very low IC_{50} values of 0.15 μM, 0.26 μM, and 0.14 μM, respectively. Analyzing the functional consequences of the genetic variations they showed that uptake of ezetimibe glucuronide was significantly reduced for the OATP1B1*1b and OATP1B1*5 variant. Together with their in vivo data, these results demonstrated that the pharmacokinetics of ezetimibe is influenced by OATP1B1 polymorphisms (Oswald et al. 2008). Recently, the OATP1B1*5 variant was found to be associated with higher lopinavir plasma concentrations (Hartkoorn et al. 2010), also suggesting decreased hepatic uptake due to reduced OATP1B1-mediated transport function. In summary, it can be stated that several polymorphisms in the *SLCO1B1* gene leading to amino acid exchanges in the OATP1B1 protein have been characterized with respect to their impact of drug transport. The variant *SLCO1B1c.512T>C* [OATP1B1*5, also present in the haplotypes *15, *16(II), and *17 (see Fig. 4)], is associated with reduced transport activity of the OATP1B1 protein leading in vivo to altered pharmacokinetics of drugs that are substrates of this uptake transporter. The impact

of this polymorphism has been demonstrated recently in a genomewide association study demonstrating that this variant is strongly associated with an increased risk of statin-induced myopathy (Link et al. 2008).

2.4.2 Pharmacogenomics of Other Human OATP Family Members

In contrast to polymorphisms in the *SLCO1B1* gene investigated in detail, genetic variations in other *SLCO* genes have not been investigated with such detail. The following chapter will briefly summarize reported findings on the functional consequences of genetic variations in the *SLCO1B3*, *SLCO2B1,* and *SLCO1A2* genes.

Like OATP1B1, OATP1B3 (gene symbol *SLCO1B3*) is localized in the basolateral membrane of human hepatocytes (König et al. 2000a). It has 80% amino acid identity to OATP1B1 (Fig. 2), and both have an overlapping, nearly identical substrate spectrum with differences in the transport kinetics of the substrates. Like OATP1B1, OATP1B3 transports endogenous substances as well as several highly prescribed drugs like statins and antibiotics (see Table 2). Interestingly, literature analyzing functional consequences in the *SLCO1B3* gene is relatively sparse. Iida et al. have described genetic variations in the *SLCO1B3* gene (Iida et al. 2001) without the analysis of the functional consequences of the identified variations. Letschert et al. (2004) identified additional mutations in the Caucasian population and investigated their functional impact on protein localization and transport activity of the mutated OATP1B3 proteins. In their study they identified the common variants *SLCO1B3c.334T>G* (OATP1B3p.S112A) and *c.699G>A* (p.M233I) with allelic frequencies of 74% and 71%, respectively. These frequencies were confirmed in a study by Smith et al. (2007) where they also demonstrated that these two variants exist in complete linkage disequilibrium. Furthermore, using stably transfected HEK293 and MDCKII cells expressing the mutated proteins, Letschert and coworkers analyzed the impact of the polymorphisms *SLCO1B3c.334T>G* (OATP1B3p.S112A), *c.699A>G* (p.M233I) and *c.1564G>T* (p.G522C) and of the artificial mutation *c.1748G>A* (p.G583E) on the cellular localization and the transport function. These results demonstrated that effects of the mutations on the cellular localization and on the transport function were substrate and cell line dependent (Letschert et al. 2004). The frequent polymorphisms *SLCO1B3c.334T>G* and c.*699G>A* did not lead to significant changes in protein expression or transport activity of the OATP1B3 substrates bromosulfophthalein (BSP), taurocholate, or estrone-3-sulfate. For these two polymorphisms, the results were confirmed in *Xenopus laevis* oocytes using the chemotherapeutic drug paclitaxel as substrate for OATP1B3 (Smith et al. 2007). Interestingly, in vivo both variations seem to have an impact on the pharmacokinetics of mycophenolate mofetil (MPA) and its glucuronide [MPAG (Picard et al. 2010)] when studied in 70 renal transplant patients receiving combination treatment of MMF with either tacrolimus or sirolimus. In contrast to the data for the frequent variations, the infrequent variant *SLCO1B3c.1564G>T* (allelic frequency in the

Caucasian population = 1.9%) revealed substrate and cell line-dependent changes in transport activity. For BSP and taurocholate this variant showed significantly reduced transport activity in both cell lines, whereas for estradiol-17ß-glucuronide the transport was almost totally abolished in the HEK293 cells compared with the transport measured in the MDCKII cells expressing this mutant. The transport of cholecystokinin-8 was significantly reduced in both cell lines, whereas the transport of estrone-3-sulfate was totally repressed in HEK293 cells but not in MDCKII cells. Furthermore, increased intracellular retention and a decreased amount of protein could be detected in both cell lines, suggesting that for the analysis of the functional consequences of polymorphisms several prototypic substrates of one transport protein have to be tested because mutations may alter the substrate specificity instead of changing the transport kinetics of one given substrate.

OATP2B1 (gene symbol *SLCO2B1*) is the third hepatic OATP family member but in contrast to the more liver-specific OATPs OATP1B1 and OATP1B3, this uptake transporter is expressed in addition in several other tissues (Grube et al. 2006, 2007). To date, only three variants were characterized with respect to their functional impact on OATP2B1-mediated transport. Tamai et al. (2000) identified two variations and subsequently the allelic frequencies and the functional consequences of these polymorphisms were investigated in detail (Nozawa et al. 2002). The most common variant *SLCO2B1c.1457C>T* (OATP2B1p.S486F; OATP2B1*3) occurred with an allelic frequency of 30.9% in the Japanese population and showed a reduced maximal transport velocity (V_{max}) for estrone-3-sulfate compared to the transport mediated by the wild-type protein. The variant *SLCO2B1c.1175C>T* (OATP2B1p.W392I; OATP2B1*2) was not found in the investigated Japanese population and the occurrence of this polymorphism needs to be verified in other ethnicities. Mougey et al. (2009) analyzed the impact of a third variant (*SLCO2B1c.935G>A*; OATP2B1p.Arg312Gln) and found that this variant is associated with reduced plasma concentrations of the leukotriene receptor antagonist Montelukast.

OATP1A2 (formerly termed OATP-A or OATP, gene symbol *SLCO1A2*) was the first human OATP family member to be cloned (Kullak-Ublick et al. 1995) and functionally characterized. *SLCO1A2* mRNA expression has been detected in various tissues with a high expression in brain (Gao et al. 2000). There, the protein has been localized to the capillary endothelium suggesting that OATP1A2 plays a role in the constitution of the blood–brain barrier. Given its tissue distribution and its ability to transport drug substrates it is reasonable to assume that polymorphisms in the *SLCO1A2* gene may also have an impact on drug disposition. Several studies have identified genetic variations within this gene, and some of them studied the functional consequences of the resulting mutated OATP1A2 protein. Iida and colleagues identified several polymorphisms within the 5′-regulatory region of the *SLCO1A2* gene without further analyzing their consequences on gene regulation (Iida et al. 2001). A detailed analysis published by Lee et al. identified six variations in exonic regions from people of a mixed ethnic background with three polymorphisms having functional consequences (Lee et al. 2005). When analyzed in transfected HeLa cells the variants *SLCO1A2c.404A>T* (OATP1A2p.N135I,

OATP1A2*6) and *SLCO1A2c.516A>C* (OATP1A2p.E172D; OATP1A2*3) showed reduced uptake of estrone-3-sulfate, deltorphin II, and DPDPE (D-Pen2, D-Pen5)-enkephalin, whereas only the uptake of deltorphin II was reduced for the variant *SLCO1Ac.2559G>A* (OATP1A2p.A187T; OATP1A2*4). A subsequent study identified 11 polymorphisms in a mixed ethnic population and used *Xenopus laevis* oocytes for the analysis of the functional consequences of these genetic variations (Badagnani et al. 2006). In this study the authors found that the variant *SLCO1A2c38T>C* (OATP1A2p.I13T, OATP1A2*2) increased the uptake of estrone-3-sulfate and methotrexate, whereas the results for the variant OATP1A2*3 with reduced uptake were confirmed. An additional variant (*SLCO1A2c.502C>T*, OATP1A2p.R168C) also displayed decreased uptake of the substrates estrone-3-sulfate and methotrexate. Despite the fact that there is some overlap in the functional consequences of polymorphisms analyzed by these two studies, their studies resulted in some differences for three of the variations studied by both groups. For example, the variant OATP1A2*2 had no effect on the uptake of estrone-3-sulfate in the study by Lee et al. but showed a significant increase in uptake in the study published by Badagnani et al. One possible explanation for these discrepancies could be that both studies have been performed in different in vitro models. Nevertheless, these analyses demonstrated that genetic variations in the *SLCO1A2* gene encoding the uptake transporter OATP1A2 can lead to altered transport kinetics and it must be studied in the future whether these effects also have an impact on the pharmacokinetics of drugs that are substrates for OATP1A2.

3 Conclusions

Because it has been demonstrated that uptake transporters of the human OATP family are important for the absorption, distribution, and excretion of drugs it becomes evident that alterations in uptake transporter function may have an impact on the pharmacokinetics of a given drug substrate. Furthermore, because drug-metabolizing enzymes are located intracellularly, uptake of drugs into cells is a prerequisite for subsequent metabolism, and therefore uptake transporter function could be an important determinant of drug metabolism. Two major molecular mechanisms may lead to altered uptake transporter rates: transporter-mediated drug–drug interactions and the functional consequences of genetic variations (polymorphisms).

Transporter-mediated pharmacokinetic interactions between two (or more) simultaneously administered drugs may occur when they address the same transport protein. Several in vitro as well as in vivo analyses have demonstrated that observed drug–drug interactions could be explained by alteration in uptake transporter function. Because several widely prescribed drugs (e.g., statins) are substrates for human OATP proteins and as administration of more than one drug gets more common in drug therapy, these uptake transporters are important molecular targets of this mechanism of drug–drug interactions. However, even when only one drug is

administered transport kinetics of a drug uptake transporter can be altered due to the functional consequences of genetic variations (so-called polymorphisms). In the past years, in vitro as well as in vivo studies have demonstrated that polymorphisms in transporter genes may influence the expression, localization, or transport kinetics of the respective transport protein resulting in altered plasma concentrations of a given drug substrate. So it has been shown in vitro and in vivo that the frequent polymorphism OATP1B1*5 is associated with a reduced uptake of statins in hepatocytes resulting in an increase in statin plasma concentrations with a reduced lipid-lowering effect and an elevated risk for adverse side effects such as myopathies. This has been confirmed by a genomewide associations study where it has been demonstrated that the *5 allele is strongly associated with an increased risk for statin-induced myopathy.

Taken together, uptake transporters of the OATP family are increasingly recognized as important determinants for the pharmacokinetics of drugs that are substrates for these transporters; modification of uptake rates by drug competition or due to the functional consequences of polymorphisms are additional molecular mechanisms important for the interindividual variability in drug response. Continued investigations in this area are likely to have a considerable impact on the attempts to further individualize and optimize treatment regiments and for avoiding or predicting adverse drug reactions.

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In Vitro and In Vivo Evidence of the Importance of Organic Anion Transporters (OATs) in Drug Therapy

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Abstract Organic anion transporters 1-10 (OAT1-10) and the urate transporter 1 (URAT1) belong to the SLC22A gene family and accept a huge variety of chemically unrelated endogenous and exogenous organic anions including many frequently described drugs. OAT1 and OAT3 are located in the basolateral membrane of

renal proximal tubule cells and are responsible for drug uptake from the blood into the cells. OAT4 in the apical membrane of human proximal tubule cells is related to drug exit into the lumen and to uptake of estrone sulfate and urate from the lumen into the cell. URAT1 is the major urate-absorbing transporter in the apical membrane and is a target for uricosuric drugs. OAT10, also located in the luminal membrane, transports nicotinate with high affinity and interacts with drugs. Major extrarenal locations of OATs include the blood–brain barrier for OAT3, the placenta for OAT4, the nasal epithelium for OAT6, and the liver for OAT2 and OAT7. For all transporters we provide information on cloning, tissue distribution, factors influencing OAT abundance, interaction with endogenous compounds and different drug classes, drug/drug interactions and, if known, single nucleotide polymorphisms.

Keywords Organic anion transporter · Drug/drug interaction · Kidney · Proximal tubule · Single nucleotide polymorphisms · Species differences · Gender differences · Tissue localization · ACE inhibitors · Antibiotics · Antidiabetics · Antineoplastics · Antivirals · AT1 receptor blockers · Diuretics · Histamine receptor antagonists · NSAIDs · Statins

1 Organic Anion Transporters Within the SLC22A Gene Family

The human SCL22 family of transporters encompasses at least 22 members with an additional six structurally related but not yet assigned genes. The respective Slc22 families of rats and mice contain 21 and 23 members, respectively, and each six unassigned genes (Jacobsson et al. 2007). Substrates of the transporters encoded by these genes are organic cations, organic anions, and/or carnitine. For at least half of the gene products, transport function and substrate specificity have not been determined so far. Table 1 shows those SLC22 family members that have been functionally proven as organic anion transporters in humans, rats, and mice. Transporters for organic cations (SLC22A1-3), organic cations, and carnitine (SLC22A4, 5, and 16) as well as orphan transporters (SLC22A10, 13-15, 17-25, and unassigned gene products) are not included.

In this review, we restrict ourselves to organic anion transporters that have been functionally characterized, including human OAT1-4, OAT7, OAT10, and URAT1 (transporter symbols in capital letters for primates) as well as Oat1-3, Oat5-6, Oat8-9, and Rst/Urat1 from rodents (transporter symbols in lowercase for nonprimates). Human OAT1 to OAT4, URAT1, and OAT10 have been identified, at least at the mRNA level, in various organs and cells (Table 2). Of these transporters, OAT1, OAT3, OAT4, URAT1, and OAT10 are predominantly expressed in kidneys (Nishimura and Naito 2005; Bahn et al. 2008). There are contradictory results with respect to the presence and absence of mRNA expression of OATs in the following

Table 1 Functionally characterized organic anion transporters in humans, rats, and mice

Gene names	Chromosomal position			Protein names, aliases
	Man	Rat	Mouse	Human/rodents
SLC22A6/Slc22a6	11q12.3	1q43	19qA	OAT1/Oat1
SLC22A7/Slc22a7	6p21.1	9q12	17qB3	OAT2/Oat2, Nlt
SLC22A8/Slc22a8	11q12.3	1q43	19qA	OAT3/Oat3, Roct
SLC22A9/Slc22a9	11q12.3	1q43	–	OAT7, UST3/Oat8, Ust1
SLC22A11/–	11q13.1	–	–	OAT4/–
SLC22A12/Slc22a12	11q13.1	1q43	19qA	URAT1/Rst
SLC22A13	3p21.2	8q32	9qF3	OAT10/ORCTL3/Octl1
–/Slc22a19	–	1q43	19qA	–/Oat5
–/Slc22a20	–	1q43	19qA	–/Oat6

Data are adapted from Jacobsson et al. (2007)

human tissues: adrenal gland, epididymis, heart, liver, lung, skeletal muscle, stomach, testis, and thyroid gland. The reported discrepancies might be the result of different methods for mRNA preparation, the quality of the mRNA, the usage of different primer sets, and other experimental procedures that determine the detection limit of mRNA expression.

Within the kidneys of various species, organic anion transporters are expressed in proximal tubules and, for rodent Oat3, also in connecting tubules and collecting ducts (see Fig. 1). In proximal tubules of all species, OAT1/Oat1 and OAT3/Oat3 are localized in the basolateral membrane and take up organic anions and anionic drugs from the blood into the tubule cell. OAT2 is present in the basolateral membrane in humans, but in the apical membrane in rodents. The transport mode of OAT2/Oat2 is still unclear. The transporters OAT4, OAT10, and URAT1/Urat1 are located in the apical membrane of proximal tubules. OAT4 operates in uptake and release mode for organic anions. URAT1 is involved in urate absorption from the filtrate, and OAT10 in the absorption of nicotinate. Also indicated in Fig. 1 are sex differences occurring in rats and mice, but potentially not in other species. Gender and species differences cloud a straightforward cross-species comparison of renal handling of organic anions and drugs.

2 Organic Anion Transporter 1 (OAT1/Oat1; Gene name SLC22A6/Slc22a6)

2.1 Cloning, Structure

The organic anion transporter 1 was cloned from man (Reid et al. 1998; Cihlar et al. 1999; Hosoyamada et al. 1999; Lu et al. 1999; Race et al. 1999), monkey (Tahara et al. 2005b), pig (Hagos et al. 2002), rabbit (Bahn et al. 2002), rat (Sekine et al. 1997; Sweet et al. 1997), and mouse (Lopez-Nieto et al. 1997). Initially used aliases were PAHT for human (Lu et al. 1999), ROAT1 for human and rat (Sweet et al.

Table 2 Expression of human organic anion transporter mRNAs in various tissues

Organ/tissue	OAT1	OAT2	OAT3	OAT4	URAT1	OAT10
Adipose	+	−	−		+	
Adrenal gland	−, +	+	−	−	−, +	+
Bladder	+	+	−	−	−	
Bone marrow	−	+	−	−	−	+
Brain (total)	+	+	+	−	−	+
Cerebellum	+	+	+	−	+	
Hippocampus	+	+	+	−	+	
Hypothalamus	+	+	+	−	−	
Pons	+	+	+	−	−	
Temporal cortex	−	+	−	−	−	
Cervix	−	+	−	−	−	
Choroid plexus	+					
Colon	−	−, +	−, +	−	−	+
Duodenum	−	+	−	−	−	
Epididymis	+	+				
Heart	−, +	+	−	−	−, +	+
Ileum	−	+	−	−	+	
Jeunum	−	+	−	−	−, +	+
Kidney	+	+	+	+	+	+
Liver	−, +	+	−, +	−, +	−	+
Lung	−, +	+	−	−	−, +	+
Mammary gland	+	+	+	−	−	
Ovary	−	+	−	−	−	
Pancreas	−	+	−	−	−	+
Peripheral leukocytes	−	+	−	−	−, +	
Pituitary	+	+	−	−	+	
Placenta	−	+	−	+	−, +	+
Prostate	−	+	−	−	−, +	+
Retina (eye)	+	+	+	−	−	
Salivary gland	+	+	−	−	−, +	+
Sertoli cells	−	+	−			
Skeletal muscle	−, +	+	−	−	−, +	−, +
Skin	−	+	−	−	−	
Spinal cord	+	+	+	−	−	+
Spleen	+	−	+	−	−	+
Stomach	−, +	+	−	−	−	+
Testis	−, +	+	−	−	+	+
Thymus	+	+	−	−	−	+
Thyroid gland	−,+	+	−	−	−	+
Trachea	+	+	+	−	−	+
Uterus	+	+	+	−	−	+

+, mRNA was found in the respective tissue; −, no mRNA was found or amount of mRNA was below detection limit; blank, not tested for mRNA expression. Data are derived from: Lopez-Nieto et al. (1997), Race et al. (1999), Hosoyamada et al. (1999), Lu et al. (1999), Cihlar et al. (1999), Sun et al. (2001), Alebouyeh et al. (2003), Nishimura and Naito (2005), Bleasby et al. (2006) and Hilgendorf et al. (2007)

1997; Reid et al. 1998), and NKT for mouse (Lopez-Nieto et al. 1997) organic anion transporter 1, respectively. Transporters cloned from winter flounder (fROAT; Wolff et al. 1997) and *Caenorhabditis elegans* (ceOAT1; George et al.

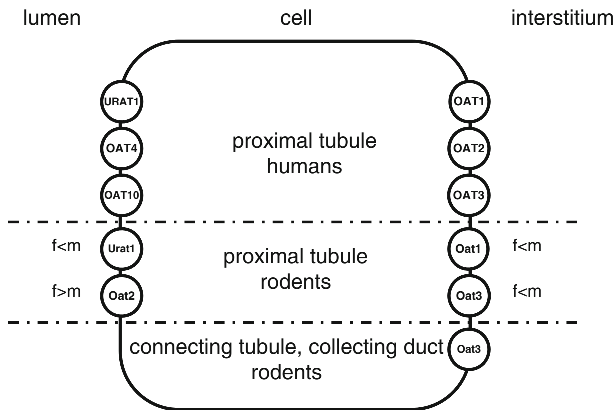

Fig. 1 Cellular localization of organic anion transporters in renal tubule cells and species and gender differences. f > m, transporter expression in female rodents is higher than in male rodents; f < m, transporter expression in female rodents is lower than in male rodents

1999) may not be homologues of OAT1, and are not discussed further in this review.

The human OAT1 protein is composed of 563 (long isoform; OAT1-1) or 550 (predominantly expressed shorter isoform; OAT1-2) amino acids (Hosoyamada et al. 1999). Two further splice variants, OAT1-3 and OAT1-4, were found to be nonfunctional (Bahn et al. 2000, 2004). Rat and mouse Oat1 have 551 and 546 amino acids, respectively (Sweet et al. 1997; Lopez-Nieto et al. 1997). Secondary structure prediction revealed twelve putative transmembrane helices with intracellularly located *N*- and *C*-termini, a large extracellular loop between helices 1 and 2, and a large intracellular loop between helices 6 and 7 (overview in: Burckhardt and Wolff 2000; Koepsell and Endou 2004). This predicted topology was verified for the hOAT1 (Hong et al. 2007). Four of the five potential *N*-glycosylation sites within the large extracellular loop of human and mouse OAT1 are glycosylated for proper targeting of OAT1 to the plasma membrane and its function (Tanaka et al. 2004). The in vivo use of predicted phosphorylation sites for protein kinases A and C, casein kinase II, and tyrosine kinase (overview in Burckhardt and Wolff 2000) in the regulation of OAT1 activity is not yet clear.

2.2 *Tissue Distribution of mRNA*

OAT1/Oat1 transcripts are expressed abundantly in the kidneys of humans (Lopez-Nieto et al. 1997; Cihlar et al. 1999; Hosoyamada et al. 1999; Lu et al. 1999; Race et al. 1999; Sun et al. 2001; Nishimura and Naito 2005; Bleasby et al. 2006), monkeys (Tahara et al. 2005b; Bleasby et al. 2006), sheep (Wood et al. 2005), pigs (Hagos et al. 2002), dogs (Bleasby et al. 2006), rabbits (Bahn et al. 2002), rats

(Sekine et al. 1997; Sweet et al. 1997; Bleasby et al. 2006), and mice (Lopez-Nieto et al. 1997; Bleasby et al. 2006). In human (Motohashi et al. 2002) and rat (Leazer and Klaassen 2003; Augustine et al. 2005) renal cortex, the mRNA for OAT1 was second most abundant, following that of OAT3. In other studies on human kidney, OAT1 mRNA was equal to or slightly higher than that of OAT3 (Sakurai et al. 2004; Hilgendorf et al. 2007).

Table 3 shows that messenger RNA for OAT1/Oat1 was also detected in brain (human: Cihlar et al. 1999; Race et al. 1999; rat: Sekine et al. 1997; mouse: Lopez-Nieto et al. 1997), including mouse choroid plexus (Sweet et al. 2002). Microarray analyses on human brain tissues revealed a low mRNA expression in temporal cortex, hypothalamus, hippocampus, and cerebellum, as well as in retina (Bleasby et al. 2006). Further organs and tissues expressing low levels of OAT1/Oat1 mRNA included liver, stomach, pancreas, salivary glands, urinary bladder, skeletal muscle, and mammary glands; also some blood cells, that is, leukocytes, neutrophils, and mononuclear cells expressed OAT1 mRNA to a limited extent (Bleasby et al. 2006; for an overview see Table 2). In general, OAT1/Oat1 expression was much higher

Table 3 Cross-species mapping of OAT1/Oat1 with respect to mRNA and protein expression

OAT1/Oat1	Human		Rat		Mouse	
Organ/tissue	mRNA	Protein	mRNA	Protein	mRNA	Protein
Adrenal gland	−, +			Zona fasc.		
Brain (total)	+		−		+	Neurons
Brain cerebellum	+				+	
Brain hippocampus	+	+			+	
Brain hypothalamus	+					
Brain pons	+					
Brain temporal cortex	−					
Choroid plexus	+	Membrane cytoplasm	+		+	
Heart	−, +		−		−	
Ileum	−		−			
Jeunum	−		−			
Kidney	+	S2/S3 bl	+	S2 bl	+	S1/S2 bl
Liver	−, +		−		−	
Lung	−, +		−		−	
Placenta	−, +				−	
Retina (eye)	+		−			
Sertoli cells	−		−			
Skeletal muscle	−, +		−			
Spleen	−		−		−	
Stomach	−, +				−	
Testis	−, +		+			

+, mRNA was found in the respective tissue; −, no mRNA was found or the amount of mRNA was below detection limit; blank, not tested. bl, basolateral membrane; S1, S2, S3, segments of proximal tubules; zona fasc., zona fasciculata. Data are collected from: Lopez-Nieto et al. (1997), Sekine et al. (1997), Hosoyamada et al. (1999), Tojo et al. (1999), Kojima et al. (2002), Motohashi et al. (2002), Sweet et al. (2002), Alebouyeh et al. (2003), Beéry et al. (2003), Bahn et al. (2005), Nishimura and Naito (2005) and Tahara et al. (2005b)

in the kidneys than in other organs (Lopez-Nieto et al. 1997; Sekine et al. 1997; Cihlar et al. 1999; Hosoyamada et al. 1999; Lu et al. 1999; Race et al. 1999; Sun et al. 2001; Buist et al. 2002; Buist and Klaassen 2004; Nishimura and Naito 2005; Bleasby et al. 2006). OAT1/Oat1 may thus be regarded as a transporter specific to kidneys.

2.3 Immunolocalization of OAT1/Oat1 Protein

Immunocytochemistry revealed that human and monkey OAT1 as well as rat and mouse Oat1 are located at the basolateral membrane of renal proximal tubular cells (Hosoyamada et al. 1999; Tojo et al. 1999; Nakajima et al. 2000; Enomoto et al. 2002c; Kojima et al. 2002; Motohashi et al. 2002; Ljubojevic et al. 2004; Bahn et al. 2005; Tahara et al. 2005b; Villar et al. 2005; Brandoni et al. 2006a, b; Kwon et al. 2007; Di Giusto et al. 2008, 2009). OAT1/Oat1 is restricted to proximal tubules, in contrast to OAT3/Oat3, which is located in almost all nephron segments (see Sect. 4.3).

In the brain, OAT1 was immunostained in the cytoplasm and the cell membranes of human choroid plexus cells (Alebouyeh et al. 2003). A GFP-Oat1 fusion protein was directed to the apical side of rat choroid plexus cells (Pritchard et al. 1999). Using antibodies, however, Nagata et al. (2002) found Oat3, but not Oat1, in rat choroid plexus. Mouse Oat1, but not Oat3, was found in cortex cerebri and hippocampus where the antibodies stained neurons and their axons (Bahn et al. 2005). In rat adrenal gland, Oat1 was localized to the outer zona fasciculata (Beéry et al. 2003). Taken together, data on immunolocalization of OAT1 in brain and other tissues are scarce and require further experimentation.

2.4 Species Differences, Age and Gender Dependence of Expression

Human OAT1 was found all along the proximal tubule (Motohashi et al. 2002). Rat Oat1 was much higher in the S2 segment than in S1 and S3 segments (Tojo et al. 1999; Enomoto et al. 2002c; Kojima et al. 2002; Ljubojevic et al. 2004; Di Giusto et al. 2008). Mouse Oat1 was high in segments S1 and S2 and very low in S3 (Bahn et al. 2005). Thus, OAT1/Oat1 expression along the proximal tubule exhibited species differences.

Renal Oat1 transcripts appeared at midgestation (Pavlova et al. 2000; Nakajima et al. 2000; Wood et al. 2005; Sweet et al. 2006; Shah et al. 2009), coinciding with proximal tubule differentiation, and gradually increased during nephron maturation. Controversial observations were made with regard to Oat1 protein expression. Whereas Nakajima et al. (2000) observed Oat1 protein only in Western blots, but

not in kidney sections of rat embryonic day 20, Sweet et al. (2006) observed in a rat kidney culture model Oat1 staining at comparable embryonic days. Using fetal rat kidney slices of embryonic day 20, probenecid-sensitive PAH or fluorescein uptake was noted (Nakajima et al. 2000). Independent of the species tested, Oat1 increased after birth. Immature rabbits showed less Oat1 mRNA expression than mature animals (Groves et al. 2006). In rats, Oat1 mRNA expression started to rise at day 10 after birth, and reached a maximum in adult animals (Buist et al. 2002). In mice, Oat1 mRNA stayed low and constant until day 25 after birth, and rose thereafter up to day 40 (Buist and Klaassen 2004). These observations indicate an immature excretory capacity of the fetal and neonatal kidney due to low expression of Oat1. In addition, Oat1 mRNA was observed in the fetal brain of rats and man (Pavlova et al. 2000; Bleasby et al. 2006), but not in rat and human fetal liver and lung (Bleasby et al. 2006).

Gender differences in Oat1 *mRNA* expression were observed in adult rats and mice. From approximately day 30 after birth on, mRNA for rat Oat1 fell slightly in female rats, whereas it further increased in male animals (Buist et al. 2002). Gonadectomy in male rats abolished the gender difference in Oat1 expression, suggesting a regulatory function of testosterone (Buist et al. 2003). Other investigators did not find an influence of testosterone on Oat1 mRNA expression in rats (Urakami et al. 1999; Ji et al. 2002; Kobayashi et al. 2002a). The reason for these discrepancies is not known. In mice, Oat1 mRNA rose from day 25 on, but much more so in male than in female animals (Buist and Klaassen 2004).

Gender-dependent *protein* expression was also tested by immunocytochemistry and Western blots. In prepubertal rats, Oat1 protein expression was weak and gender-independent. In adult female rats, however, Oat1 protein was only 20% that of male rats in Western blots. Staining for OAT1 in the basolateral membranes of proximal tubules was clearly less intense in kidneys from adult female rats as compared to male rats (Ljubojevic et al. 2004). In the same study, testosterone strongly and progesterone slightly increased Oat1 expression, whereas estrogens decreased it. Thus, Oat1 is under positive control of testosterone, leading to a considerably higher expression in adult male rats as compared to adult female rats. Consequently, the renal clearance of Oat1-specific drugs should be higher in male than in female rats. In rabbits, Oat1 expression did not show gender differences (Groves et al. 2006). In pigs and man, gender differences are also absent (Sabolic and coworkers; preliminary results), suggesting that the influence of sex hormones on Oat1 expression is species-dependent.

2.5 Factors Influencing Activity and Abundance of OAT1/Oat1

The expression of the human OAT1 is under positive control of hepatocyte nuclear factors (HNFs). Transfection of HNF-1α alone or of HNF-1α and HNF-1ß into HEK293 cells led to the expression of human OAT1 (Saji et al. 2008). HNF-1α knockout mice exhibited a considerably decreased amount of renal Oat1 (Maher

et al. 2006; Saji et al. 2008). HNF-4α increased the expression of a reporter construct of the human OAT1 gene promoter (Ogasawara et al. 2007). In this work, HNF-4α was considered essential for OAT1 expression, but not important for tissue distribution, because HNF-4α is present in proximal tubules and hepatocytes, whereas OAT1 is not detectable in the liver.

Because proximal tubule cells are endowed with hormone receptors signaling through protein kinases it is important to consider posttranslational modification of OAT1/Oat1. Activation of the conventional protein kinase C (PKC), happening in vivo through receptors for epinephrin and angiotensin II, decreased OAT1/Oat1-mediated transport in model cells (Uwai et al. 1998; Lu et al. 1999; You et al. 2000; Wolff et al. 2003). Transport inhibition was caused by endocytic retrieval of OAT1 from the membrane by a dynamin-dependent process (Wolff et al. 2003; Zhang et al. 2008). Mutation of all potential protein kinase C sites of human OAT1 did not prevent PKC-induced reduction in transport, suggesting that a direct phosphorylation is not important (Wolff et al. 2003). Indeed, PKC activation did not lead to a phosphorylation of OAT1 (You et al. 2000). Because OAT1 interacted with caveolin-2 (Kwak et al. 2005b), caveolin itself or caveolin-associated proteins may be phosphorylated by PKC, leading to endocytosis of OAT1.

Activated through insulin, the nonconventional PKCζ increased the transport of an Oat1 substrate in rat kidney cortex slices (Barros et al. 2009). Epidermal growth factor increased Oat1-mediated transport activity through a complicated signal cascade involving mitogen-activated kinases, phospholipase A_2-induced release of arachidonic acid, cyclooxygenase 1-dependent production of prostaglandin E_2, binding of released PGE_2 to EP_4 receptors, intracellular elevation of cAMP and, finally, activation of protein kinase A (Sauvant et al. 2002, 2003, 2004).

Recent studies revealed that the expression of OATs is affected in several diseases. These diseases can be, at least in part, simulated by animal models that provided further insight into the regulation of OATs. During the progression of renal insufficiency, various uremic toxins derived from dietary proteins accumulate in the plasma of the patients (for reviews see: Vanholder et al. 2003; Enomoto and Niwa 2007) and are, if not removed by adequate dialysis, risk factors for cardiovascular and further renal diseases (Enomoto and Endou 2005; Obermayr et al. 2008; Saito 2010), as well as for neuropathies and myopathies. In addition, drug metabolism and drug transport can be impaired (Dreisbach and Lertora 2008; Dreisbach 2009), not only in the kidneys but also in the intestinal tract (Naud et al. 2007).

Several groups (Motojima et al. 2002; Deguchi et al. 2004) showed that the uremic toxins indoxyl sulfate, indole acetate, and hippurate are substrates of OAT1/Oat1. Two other compounds, aristolochic acid and ochratoxin A (OTA), induce interstitial nephritis (Pfohl-Leszkowics and Manderville 2007; Debelle et al. 2008) and are substrates of Oat1 (Bakhiya et al. 2009; Jung et al. 2001; Tsuda et al. 1999). Low concentrations of OTA increased while high concentrations of OTA decreased the abundance of Oat1 in rat kidney, respectively (Zlender et al. 2009). Hyperuricemia induced by feeding rats for several days with a chow containing uric and oxonic acid (Habu et al. 2005) decreased the expression of Oat1.

OAT expression in various kidney diseases, such as renal insufficiency and nephritic syndrome, was analyzed by real-time PCR (Sakurai et al. 2004). This study showed that the level of human OAT1 mRNA was significantly lower in the kidney of patients with renal diseases than in normal controls. These findings were confirmed in the renal dysfunction models of ischemia/reperfusion (Kwon et al. 2007; Matsuzaki et al. 2007; Schneider et al. 2007; Di Giusto et al. 2008; Schneider et al. 2009) and ureter obstruction (Villar et al. 2005, 2008) where decreased Oat1 mRNA and protein levels were observed. As opposed to renal disease models, Oat1 expression was increased by mimicking hepatic diseases through acute biliary obstruction (Brandoni et al. 2003, 2006a; Torres 2008). After 3 days of bile duct ligation, however, the amount of Oat1 had normalized, but most of the protein was found in intracellular vesicles rather than in the basolateral membrane (Brandoni et al. 2006b).

Induction of fever led to increased concentrations of PGE_2 that not only down-regulated time-dependently the uptake of PAH but also diminished rat Oat1 protein (Sauvant et al. 2006). These observations led to the hypothesis that the increased plasma PGE_2 concentrations observed during fever and inflammation were due to reduced PGE_2 secretory capacity of the kidneys. In line with this hypothesis, COX-2 inhibition attenuated endotoxin-induced downregulation of organic anion transporters in rat renal cortex (Höcherl et al. 2009).

Some drugs caused a decrease in renal OAT1/Oat1 abundance. Cisplatin treatment of mice resulted in tubular damage and decreased Oat1 expression (Aleksunes et al. 2008). Gentamycin, possibly through generation of H_2O_2, decreased human OAT1 expressed in mouse proximal tubule cells (Takeda et al. 2000a). A single dose of methotrexate decreased renal abundance of Oat1 in rats (Shibayama et al. 2006). On the other hand, chronic (7 days) treatment of rats with furosemide or hydrochlorothiazide increased the abundance of Oat1 in Western blots (Kim et al. 2003).

2.6 Substrates

Under physiologic conditions, OAT1/Oat1 most probably operates as an organic anion/α-ketoglutarate exchanger. The dicarboxylate α-ketoglutarate is a metabolite of the citric acid cycle and, in addition, is taken up into proximal tubule cells at the basolateral cell side via a sodium coupled system, NaDC3. The sodium concentration difference driving α-ketoglutarate through NaDC3 into the cell is maintained by the Na^+, K^+-ATPase in the basolateral membrane (Burckhardt and Burckhardt 2003; Wright and Dantzler 2004). By coupling the efflux of α-ketoglutarate to the uptake of various organic anions from the blood OAT1/Oat1 constitutes the first step in organic anion secretion (the second step being the release of organic anions across the apical cell membrane).

OAT1/Oat1 is well known for its very broad substrate specificity: following expression in *Xenopus laevis* oocytes and various cell lines (see VanWert et al.

2010) it interacted with several endogenous and a multitude of exogenous compounds/drugs/toxins of various chemical structures (since 2000 reviewed in: van Aubel et al. 2000; Sekine et al. 2000; Dresser et al. 2001; Burckhardt and Burckhardt 2003; van Montfoort et al. 2003; Lee and Kim 2004; Ho and Kim 2005; Rizwan and Burckhardt 2007; Srimaroeng et al. 2008; vanWert et al. 2010). For most compounds, inhibition of OAT1/Oat1-mediated transport was determined. Inhibition of transport indicates an interaction of the test substance with OAT1/Oat1, but does not prove its translocation by this transporter.

Radiolabeled *p*-aminohippurate (PAH) is the prototypical test anion for OAT1/Oat1. Using various expression systems, K_m values for human OAT1 were found between 3.1 and 112.7 μM with a mean of 28.5 μM (Cihlar et al. 1999; Hosoyamada et al. 1999; Cihlar and Ho 2000; Takeda et al. 2000a, 2001; Islinger et al. 2001; Ichida et al. 2003; Hong et al. 2004; Sakurai et al. 2004; Bleasby et al. 2005; Fujita et al. 2005; Srimaroeng et al. 2005a, b; Tahara et al. 2005b; Perry et al. 2006; Xu et al. 2006b; Kimura et al. 2007; Rizwan et al. 2007; Ueo et al. 2005, 2007; Uwai et al. 2007b; Windass et al. 2007; Zhang et al. 2008). The K_m values for monkey (10.1 μM; Tahara et al. 2005b), rabbit (15.5 μM; Bahn et al. 2002), rat (11–85.1 μM; mean 41.6 μM from 12 publications; Sekine et al. 1997; Uwai et al. 1998, 2000a; Cihlar et al. 1999; Takeda et al. 1999; Pombrio et al. 2001; Sugiyama et al. 2001; Hasegawa et al. 2002; Nagata et al. 2002; Deguchi et al. 2004; Keller et al. 2008; Minematsu et al. 2008), and mouse Oat1 (37 and 162 μM; Kuze et al. 1999; You et al. 2000) have also been determined.

2.6.1 Endogenous Substrates of OAT1/Oat1

Second messengers. cAMP and cGMP were transported by rat Oat1 (Sekine et al. 1997), and cGMP by human OAT1 (Cropp et al. 2008).

Citric acid cycle intermediates, dicarboxylates. Citrate weakly inhibited OAT1 (Sugawara et al. 2005). The dicarboxylate α-ketoglutarate was transported by human and rat OAT1/Oat1 (Sekine et al. 1997; Lu et al. 1999) and inhibited transport by human OAT1 (Cihlar et al. 1999; Hosoyamada et al. 1999; Lu et al. 1999; Race et al. 1999; Motojima et al. 2002; Ichida et al. 2003; Hagos et al. 2008), monkey OAT1 (Tahara et al. 2005b), rabbit Oat1 (Bahn et al. 2002), rat Oat1 (Sekine et al. 1997; Sweet et al. 1997; Uwai et al. 1998; Nakakariya et al. 2009), and mouse Oat1 (Kuze et al. 1999). Maleate inhibited rat (Shikano et al. 2004) and mouse Oat1 (Kaler et al. 2007a).

The interaction of OAT1/Oat1 with dicarboxylates depended on the length of the carbon chain separating the two negatively charged carboxyl groups with a maximum inhibition by dicarboxylates with five or six carbons (Uwai et al. 1998). Affinities, that is K_m or K_i values, have not been reported for any of the dicarboxylates. For preloading cells, α-ketoglutarate is replaced by the nonmetabolizable glutarate. This five-carbon dicarboxylate was transported by human OAT1 (K_m 6.8 or 10.7 μM; Cihlar and Ho 2000; Rizwan et al. 2007) and mouse Oat1 (Bahn et al. 2005), and inhibited human (IC_{50} 4.9 or 38.3 μM; Cihlar and Ho 2000; Kimura et al.

2007), rat (Uwai et al. 1998) and mouse Oat1 (IC_{50} 6.7 μM; Kaler et al. 2007a). Preloading of cells with glutarate *trans*-stimulated OAT1-mediated uptake of test anions (Ichida et al. 2003; Bakhiya et al. 2007).

Monocarboxylates, short chain fatty acids. Hexanoate and heptanoate inhibited mouse Oat1 with much higher affinity (IC_{50} 38 and 16.7 μM, respectively) than propionate (IC_{50} 8.18 mM), butyrate (IC_{50} 3.5 mM), and pyruvate (IC_{50} 11.9 mM; Kaler et al. 2007a). Octanoate inhibited human (IC_{50} 5.41 μM; Jung et al. 2001) and rat Oat1 (Tsuda et al. 1999). Lactate did not inhibit at all human OAT1 (Ichida et al. 2003). Taken together, OAT1/Oat1 has a very low or no affinity for monocarboxylates with three or four carbons, but a very high affinity for monocarboxylates beyond a chain length of six carbons.

Bile salts. Cholate inhibited mouse Oat1 (Kaler et al. 2007a), and deoxycholate inhibited rat Oat1 (Chen et al. 2008). For taurocholate either a weak inhibition (Sugiyama et al. 2001; Islinger et al. 2001; Mori et al. 2004) or no inhibition (Sekine et al. 1997; Uwai et al. 1998; Chen et al. 2008) of human and rat OAT1/Oat1 was reported. Transport of taurocholate by human and rat OAT1/Oat1 was not observed (Sekine et al. 1997; Chen et al. 2008). Thereby, OAT1/Oat1 differs from OAT3/Oat3, which transports taurocholate (see Sect. 4.6.1).

Hormones, *hormone derivatives*. Corticosterone inhibited rat Oat1 (Beéry et al. 2003). Dehydroepiandrosterone sulfate inhibited rat Oat1, but was not transported (IC_{50} 80.9 μM; Hasegawa et al. 2003). No transport was also found for human OAT1 (Ueo et al. 2005; Nozaki et al. 2007a). With regard to estrone-3-sulfate (ES), differing results were reported. For human OAT1, either no uptake of ES (Aslamkhan et al. 2006; Ueo et al. 2007; Chen et al. 2008) or a weak uptake (Ueo et al. 2005; Uwai et al. 2007a) was found. In addition, OAT1 was (Srimaroeng et al. 2005b) or was not (Srimaroeng et al. 2005a) inhibited by ES. Monkey OAT1 did not transport ES (Tahara et al. 2005b). Data for rat Oat1 are again ambiguous: ES was not (Sweet et al. 2003; Aslamkhan et al. 2006) or weakly transported (Hasegawa et al. 2003). Rat (IC_{50} 50.1 μM; Hasegawa et al. 2003) and mouse Oat1 (IC_{50} 203 μM; Kaler et al. 2007a) were inhibited by ES. The ambiguous handling of ES distinguishes OAT1/Oat1 from OAT3/Oat3 and many other OATs that clearly transport estrone sulfate (see later).

Local hormones. Prostaglandin E_2 was transported by human OAT1 (K_m 0.97 μM; Kimura et al. 2002), as well as by rat and mouse Oat1 (Sekine et al. 1997; Kaler et al. 2007a). In one study, no transport of PGE_2 by human OAT1 was found (Lu et al. 1999). Prostaglandin $F_{2\alpha}$ was transported by human OAT1 (K_m 0.58 μM; Kimura et al. 2002); at odds, no inhibition of OAT1 by $PGF_{2\alpha}$ was found in another study (Lu et al. 1999). Most data suggest that OAT1/Oat1 has a very high affinity for these prostaglandins and is able to translocate them.

Purine metabolites, urate. Hypoxanthine and xanthine inhibited OAT1 (K_i 243.9 μM for xanthine; Sugawara et al. 2005). For urate uptake by human OAT1, two differing K_m values, 197.6 μM (Sato et al. 2008) and 943 μM (Ichida et al. 2003) were reported. In another study (Race et al. 1999), no urate transport could be detected. With exception of a single study (Race et al. 1999), an inhibition of human OAT1 by urate was reported with IC_{50} values between 46 and 440 μM (mean out of

four publications: 295.9 μM; Cihlar et al. 1999; Hosoyamada et al. 1999; Motojima et al. 2002; Bahn et al. 2004; Sugawara et al. 2005). Rabbit Oat1 was inhibited by urate (Bahn et al. 2002). Rat Oat1 transported urate and was weakly or not inhibited by urate (Sekine et al. 1997; Sweet et al. 1997). Taken together, OAT1/Oat1 interacts with urate and may be involved in proximal tubular urate secretion or absorption (Hediger et al. 2005).

Acidic neurotransmitter metabolites. Several anionic neurotransmitter metabolites including 3,4-dihydroxymandelate, 3,4-dihydroxyphenylacetate, homovanillate, and 5-hydroxyindole acetate and many others inhibited human OAT1 (Alebouyeh et al. 2003) and mouse Oat1 (Bahn et al. 2005).

Mercapturic acid derivatives. The *N*-acetyl-*S*-cysteine conjugates are formed in the liver and renally excreted. *N*-acetyl-L-cysteine (NAC) itself only weakly interacted with rat Oat1, but some conjugates showed μmolar affinities, for example *N*-acetyl-S-2,4-dinitrophenyl-L-cysteine (DNP-NAC; IC_{50} 1.9 μM; K_m for uptake 2 μM; Pombrio et al. 2001). Among the many conjugates tested as inhibitors of Oat1 (Pombrio et al. 2001), NAC conjugates of mercury were found to be transported. Radiolabeled NAC–Hg (K_m 44 μM) and NAC–Hg–NAC (K_m 144 μM) were taken up into cells expressing human OAT1 (Aslamkhan et al. 2003; Zalups and Ahmad 2005). Because OAT1/Oat1 takes up mercury conjugates from the blood it contributes to the proximal tubular damage observed after mercury poisoning.

Further compounds. Oat1 knockout mice exhibited a decreased renal organic anion secretion in line with increased plasma concentrations of endogenous organic anions such as 3-hydroxyisobutyrate, 3-hydroxybutyrate, benzoate and others, indicating that these compounds are endogenous substrates of Oat1 (Eraly et al. 2006).

2.6.2 Drugs

OAT1/Oat1 was shown to interact with numerous, frequently prescribed drugs (see Table 4). For reviews see Burckhardt and Burckhardt (2003), Rizwan and Burckhardt (2007), and VanWert et al. (2010).

ACE inhibitors. Transport by human OAT1 was shown for captopril and quinaprilat (Ueo et al. 2005, 2007; Yuan et al. 2009). With rat Oat1, a K_m of 0.56 μM was determined for temocaprilat uptake (Hasegawa et al. 2003). There is, however, no systematic study on transport of ACE inhibitors in any species. Thus, the role of OAT1/Oat1 in ACE inhibitor excretion remains to be studied further.

Angiotensin II receptor 1 blockers (ARB). A complete list of inhibitory constants is available for human OAT1. Olmesartan (K_m 68.3 nM; IC_{50} 280 nM; Yamada et al. 2007; Sato et al. 2008) and telmisartan (IC_{50} 460 nM; Sato et al. 2008) exhibited very high affinities for OAT1, followed by losartan (IC_{50} 12 μM) and pratosartan (IC_{50} 12 μM), valsartan (IC_{50} 16 μM) and candesartan (IC_{50} 17 μM; Sato et al. 2008). For rat Oat1, a few, and for mice no IC_{50} values are available, respectively (see Table 4).

Table 4 Drugs interacting with OAT1/Oat1

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
ACE inhibitors						
Captopril	+	(+)				(+)
Enalaprilat						+
Lisinoprilat						–
Quinaprilat	+					
Temocaprilat			0.56			
Angiotensin II receptor blockers						
Candesartan		17		–		
Losartan		12				
Olmesartan	0.068	0.28				
Pratosartan		12		6.47		
Telmisartan		0.46		0.316		
Valsartan		16				
Diuretics						
Acetazolamide	I: 75		+	1,100		
Amiloride	no I					
Bendroflumethazide						8
Bumetanide	+	+ or –; 7.6	+	5.5		
Chlorothiazide		3.78		+		
Cyclothiazide		84.3		+		
Ethacrynate		29.6		+		
Ethoxzolamide				+		
Furosemide	+	14–20	+	9.5		8.1
Hydrochlorothiazide		67.3 or –		150		
Methazolamide		438				
Torasemide		55.2				
Trichlormethiazide		19.2				
Statins/fibrates						
Atorvastatin		–				
Bezafibrate		+				
Fluvastatin		26.3				
Pravastatin	–	408 or –	–	1,150–1,620		
Rosuvastatin	–	–				
Simvastatin		73.6				
Antibiotics						
Amoxicillin				+		
Ampicillin						
Benzylpenicillin		+ or –	+ or –	418–2,763		328
Carbenicillin				500		1,280
Cefaclor		1,096				
Cefadroxil		6,140		+		+ or –
Cefamandol		30	+ or –; 450			
Cefazolin	–	100–180	+	72–560		
Cefdinir	(+)	692				
Cefoperazone		210	–	298–480		
Cefoselis	–	2,601				
Cefotaxime		3,130		+ or –		
Cefotiam	–	640	+ or –	2,718		

(*continued*)

Table 4 (continued)

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
Cefsulodin				+		
Ceftazidime				+		
Ceftibuten	+	563				
Ceftizoxime	+	3,599				
Ceftriaxone		230		840		
Cephalexin			+	2,310–6,010		
Cephaloridine	(+)	740–2,470	+	1,320–2,330		
Cephalothin		220		290–530		
Cephradine		1,600				
Cinoxacin		+		+		
Ciprofloxacin	–	–			–	–
Chloramphenicol				(+)		
Cloxacillin				+		
Doxycyclin		+				
Erythromycin				–		
Gentamycin				–		
Grepofloxacin			–	(+)		
Levofloxacin		–	–	(+)		
Minocyclin		+				
Nafcillin				+		
Nalidixate		+		+		
Norfloxacin				–		
Ofloxacin				–		
Oxytetracycline		+				
Piperacillin				+		
Streptomycin				–		
Tetracycline	(+)	+				
Ticarcillin						530
Vancomycin				–		
Antivirals						
Acyclovir	342	+	242	981		209
Adefovir	17.2–30.0	28–65	270			36
Amantadine				(+)		
Cidofovir	30–63	60	238			25.7
Cidofovir prodrug	309	1,100				
Didanosine			+	(+)		600
Foscarnet			–	–		
Ganciclovir	896	(+)				
Lamivudine			+			104.3
PMEDAP	+					
PMEG	+					
Stavudine			+	(+)		628
Tenofovir	22.3					81
Trifluridine			+	+		
Vidarabine				(+)		
Zalcitabine	+	1,230	+	(+)		1,479
Zidovudine	45.9	+	41.5–68			78.3

(*continued*)

Table 4 (continued)

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
Antineoplastics						
Azathioprine		–				
Cisplatin						–
Imatinib	–					
6-Mercaptopurine			98			
Methotrexate	724 or –	–	0.87–14.9	+	+	901
Immune suppressants						
Cyclosporin A		–		–		
Mycophenolate	–	1.52–10.0				
Tacrolimus		–				
Antidiabetics						
Chlorpropamide				39.5		
Glibenclamide				1.6		
Metformin	–					
Nateglinide				9.2		
Sitagliptin	–	–				
Tolbutamide				55.5		
Histamine receptor 2 blockers/antacidic drugs						
Cimetidine	+	492	+ or –	+ or –	–	1,038
Famotidine	–	–				
Omeprazole		+				
Ranitidine	+					
NSAIDs						
Acetaminophen		639				
Acetylsalicylate	–	769		428		687
Aminopyrine				+		
Antipyrin		(+)				
Benzydamine				+		
Carprofen						+
Diclofenac		4.0–6.07		1.52–4.56		
Diflusinal		0.85		+		
Etodolac		50–103		–		
Flufenamate		+		+		
Flurbiprofen		1.5		+		
Ibuprofen	+ or –	1.38–8.0		3.5–4.33		4.7
Indomethacin	+	3.0–6.72		4.2–10.0		+
Ketoprofen	+ or –	0.98–4.43		0.5–6.11		
Loxoprofen		27.1				
Meclofenamate				+		
Mefenamate		0.14–0.83				
Naproxen		1.18–5.8		2.0–5.54		
Oxiphenbutazone				32		
Paracetamol				2,099		
Phenacetin		200–275		488		
Phenylbutazone		71.6		47.9		
Piroxicam		19.8–62.8		52		
Salicylate	+	280–1.573	+	341–2,110		145
Salicylurate				11		
Sulfinpyrazone				+		

(*continued*)

Table 4 (continued)

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
Sulindac		36.2–77.8		99.9		+
Tolmetin		5.08		15.4		
Antihistaminic						
Fexofenadine	–					
Antiepileptic						
Valproate		+		+		
Uricosurics/antihyperuricemia drugs						
Allopurinol		+				
Benzbromarone		4.6				
Probenecid		3.9–12.5		1.44–31		6.4

+, transport of inhibition was observed without determination of K_m, K_i, or IC_{50}; −, no transport or no inhibition was observed; (+), weak transport or inhibition; blank, not determined; numbers, reported K_m, K_i, or IC_{50} values in micromoles/liter (μM); abbreviations: PMEDAP, 9-(2-phosphonyl-methoxyethyl)-diaminopurine; PMEG, 9-(2-phosphonyl-methoxyethyl)-guanidine. For references see text in Sect. 2.6.2

Diuretics. Table 4 shows *carbonic anhydrase blockers* (acetazolamide, ethoxzolamide, methazolamide), *loop diuretics* (bumetanide, ethacrynate, furosemide, torasemide), and *thiazide diuretics* (bendroflumethiazide, chlorothiazide, cyclothiazide, hydrochlorothiazide, trichloromethiazide). Loop and thiazide diuretics have to reach their downstream target salt transporters from the lumen and thus must be secreted in the proximal tubules. Human OAT1 may be involved in the secretion, because it interacted with many diuretics with high affinity (IC_{50} between 1 and 10 μM: bumetanide, chlorothiazide; Hasannejad et al. 2003) or intermediate affinity (IC_{50} between 10 and 100 μM: furosemide, torasemide, ethacrynate, trichloromethiazide, hydrochlorothiazide, cyclothiazide, acetazolamide; Hasannejad et al. 2003; Bahn et al. 2004; Hagos et al. 2007a). Few quantitative data are available for the interaction of rat and mouse Oat1 with diuretics (see Table 4; Sekine et al. 1997; Uwai et al. 2000b; Shikano et al. 2004; Vallon et al. 2008b). In comparison, OAT1 appears to have a higher affinity for most of the thiazide diuretics whereas loop diuretics exhibit higher affinities for OAT3 (see later). Translocation of diuretics was shown for acetazolamide by rat Oat1 (Uwai et al. 2000b), and for bumetanide and furosemide by human and rat OAT1/Oat1 (Hasannejad et al. 2003; Uwai et al. 2000b). It is likely that other diuretics are transported as well, but direct experimental evidence for transport is still lacking. In Oat1 knockout mice, renal furosemide excretion and, hence, its diuretic action was decreased (Eraly et al. 2006). Furosemide and bendroflumethiazide excretion was not only decreased in Oat1 knockouts, but also in Oat3 knockout mice, indicating that both Oat1 and Oat3 contribute to proximal tubular secretion of diuretics and their targeting to the salt transporters in the distal tubule (Vallon et al. 2008b).

Statins. Fluvastatin (IC_{50} 26.3 μM) and simvastatin (IC_{50} 73.6 μM), but not atorvastatin and rosuvastatin, inhibited human OAT1 (Takeda et al. 2004; Windass et al. 2007). For pravastatin, mixed results for the inhibition of human OAT1 were

obtained (inhibition: Enomoto et al. 2003; Khamdang et al. 2004; Sugawara et al. 2005; no inhibition: Windass et al. 2007). Monkey OAT1 (Tahara et al. 2005b) and rat Oat1 were inhibited by pravastatin (Hasegawa et al. 2002; Khamdang et al. 2004). Pravastatin and rosuvastatin were not transported by any OAT1 (Hasegawa et al. 2002; Takeda et al. 2004).

Antibiotics. The antibiotics tested with OAT1/Oat1 include *penicillines* (amoxicillin, ampicillin, benzylpenicillin, carbenicillin, cloxacillin, nafcillin, piperacillin, ticarcillin), *cephalosporines* (cefaclor, cefadroxil, cefamandol, cefazolin, cefdinir, cefoperazone, cefoselis, cefotaxime, cefotiam, cefsulodin, ceftazidime, ceftibuten, ceftixozime, ceftriaxone, cephalexin, cephaloridine, cephalotin, cephradine), *tetracyclines* (doxycyclin, minocyclin, oxytetracycline, tetracycline), *quinolones* (cinoxacin, ciprofloxacin, grepofloxacin, levofloxacin, nalidixate, norfloxacin, ofloxacin), *aminoglycoside antibiotics* (gentamycin, streptomycin), *macrolide antibiotics* (erythromycin, vancomycin) and others (chloramphenicol).

With exception of benzylpenicillin (Table 4, mixed results; Hosoyamada et al. 1999; Lu et al. 1999) no other penicillins were tested with human OAT1. With rat Oat1, most penicillins inhibited transport. When determined, the affinity of rat Oat1 to benzylpenicillin and carbenicillin and of mouse Oat1 to ticarcillin was low (IC_{50} values greater than 100 μM; Jariyawat et al. 1999; Nagata et al. 2002; Alebouyeh et al. 2003; Hasegawa et al. 2002, 2003; Deguchi et al. 2004; Kaler et al. 2007a). As shown in Table 4, IC_{50} values are available for the interaction of most cephalosporines with human OAT1. With the exception of cefamandol, the tested compounds inhibited OAT1-mediated transport with low affinities, ranging from 100 μM to 6.14 mM (Cihlar and Ho 2000; Takeda et al. 2002a; Ueo et al. 2005, 2007). Generally speaking, the affinities of OAT1/Oat1 to penicillins tended to be lower, and to cephalosporines higher than those of OAT3/oat3 to these antibiotics (IC_{50} values compared in Rizwan and Burckhardt 2007).

Tetracyclines inhibited human OAT1, and tetracycline was weakly transported (Babu et al. 2002a). IC_{50} values were not determined, leaving open whether OAT1 contributes to the renal secretion of tetracycline. The quinolones showed weak or no interaction with human, rat, and mouse OAT1/Oat1 (Sekine et al. 1997; Uwai et al. 1998; Jariyawat et al. 1999; Sugawara et al. 2005; VanWert et al. 2008). Erythromycin, gentamycin, streptomycin, and vancomycin did not interact with rat Oat1, and chloramphenicol showed only a weak inhibition of Oat1-mediated transport (Jariyawat et al. 1999).

Antivirals. Due to their nephrotoxicity, antivirals were amply tested on OAT1/Oat1-mediated transport. Antiviral drugs can be structurally classified into *acyclic nucleoside analogues* (acyclovir, ganciclovir), *nucleoside analogues* (didanosine, lamivudine, stavudine, trifluridine, vidarabine, zalcitabine, zidovudine), *nucleotide analogues* (adefovir, cidofovir, tenofovir), and others (amantadine, foscarnet). Although they do not bear a negative charge, the acyclic nucleoside analogues acyclovir (K_m 342 μM) and gancyclovir (K_m 896 μM) were transported by human OAT1 with low affinity (Takeda et al. 2002b). Rat Oat1 also transported acyclovir with low affinity (K_m 242 μM; Wada et al. 2000; Hasegawa et al. 2003), and mouse Oat1 was inhibited by acyclovir with low affinity (IC_{50} 209 μM; Truong et al.

2008). Among the nucleoside analogues, transport was demonstrated for didanosine, lamivudine, stavudine and trifluridine (rat Oat1; Wada et al. 2000), as well as for zalcitabine and zidovudine (human and rat OAT1; Wada et al. 2000; Takeda et al. 2002b; Hasegawa et al. 2003; Jin and Han 2006). For mouse Oat1, IC_{50} values were determined for didanosine, lamivudine, stavudine, zalcitabine and zidovudine (see Table 4; Truong et al. 2008). The lowest K_m values (highest affinities for transport) were observed with the negatively charged nucleotide analogues adefovir (K_m between 17.2 and 30 μM), cidofovir (K_m between 30 and 63.4 μM), and tenfovir (K_m 22.3 μM) at human OAT1 (Cihlar et al. 1999; Cihlar and Ho 2000; Ho et al. 2000; Bleasby et al. 2005; Aslamkhan et al. 2006; Perry et al. 2006; Chu et al. 2007). As compared to human OAT1, rat Oat1 transported adefovir and cidofovir with lower affinity (Table 4; Cihlar et al. 1999). Mouse Oat1 was inhibited with high affinity by adefovir, cidofovir, and tenofovir (Table 4; Truong et al. 2008). Amantadine and foscarnet showed low or no interaction with rat Oat1 (Lu et al. 1999; Wada et al. 2000). Taken together, OAT1/Oat1 is involved in uptake of most antivirals from the blood into proximal tubule cells. Within the cells, antivirals may be nephrotoxic, an effect that is mediated by OAT1 (Ho et al. 2000), and coadministration with probenecid or NSAIDs attenuated cytotoxicity (Mulato et al. 2000).

Antineoplastics. Methotrexate was transported by rat Oat1 with high affinity (Table 4; Nozaki et al. 2004; inhibition without evaluation of affinity: Sekine et al. 1997; Uwai et al. 1998). Transport of methotrexate was reported also for human OAT1 (K_m 724 μM; Uwai et al. 2004; other reports: Ueo et al. 2005, 2007) and mouse Oat1 (Kaler et al. 2007a). No transport by human and monkey OAT1 (Lu et al. 1999; Tahara et al. 2005b), and no inhibition of human OAT1 by methotrexate (Lu et al. 1999; Srimaroeng et al. 2005a) were found in other investigations, leaving a somewhat mixed picture of the involvement of OAT1/Oat1 in renal methotrexate handling. 6-mercaptopurine was transported with intermediate affinity by rat Oat1 (Motohashi et al. 2004); azathioprine (Uwai et al. 2007b), cisplatin (Kuze et al. 1999) and imatinib (Hu et al. 2008) did not interact with OAT1/Oat1 (see Table 4).

Immune suppressants. Cyclosporin A and tacrolimus did not interact with human and rat OAT1/Oat1 (Sweet et al. 1997; Cihlar et al. 1999; Uwai et al. 2007b). For mycophenolate, no transport by human OAT1 was found; this compound and its glucuronide metabolites, however, potently inhibited this transporter (Uwai et al. 2007b; Wolff et al. 2007).

Antidiabetics. Glibenclamide (IC_{50} 1.6 μM) and nateglinide (IC_{50} 9.2 μM) inhibited rat Oat1 with high, and chlorpropamide (IC_{50} 39.5 μM) and tolbutamide (IC_{50} 55.5 μM) with intermediate affinities (Uwai et al. 2000a). No transport and no inhibition were observed for metformin and sitagliptin at human OAT1 (Kimura et al. 2005; Chu et al. 2007).

Histamine receptor 2 (HR2) blockers. Cimetidine and ranitidine, but not famotidine were found to be transported by human OAT1 (Burckhardt et al. 2003; Motohashi et al. 2004; Tahara et al. 2005a; Ueo et al. 2005, 2007), monkey OAT1 (Tahara et al. 2006a), rabbit (Zhang et al. 2004), and rat Oat1 (Nagata et al. 2002). No transport and no inhibition were found in another study on rat

Oat1 (Prueksaritanont et al. 2004). Taken together, most studies indicate the transport of cimetidine by OAT1 (and preferably by OAT3, see Sect. 4.6.2).

Nonsteroidal anti-inflammatory drugs (NSAIDs). Numerous NSAIDs were tested on OAT1 as shown in Table 4. Diclofenac (IC_{50} values in μM in brackets) (between 4 and 6.07), diflusinal (0.85), flurbiprofen (1.5), ibuprofen (1.38; 8.0; 55.6), indomethacin (between 3.0 and 6.72), ketoprofen (between 0.89 and 4.43), mefenamate (0.14; 0.83), naproxen (between 1.18 and 5.8), and tolmetin (5.08) inhibited human OAT1 with IC_{50} values below 10 μM, that is, with high affinity (Cihlar and Ho 2000; Mulato et al. 2000; Khamdang et al. 2002; Kimura et al. 2007; Nozaki et al. 2007b). Inhibition of OAT1 with intermediate affinity (IC_{50} between 10 and 100 μM) was observed with etodolac, loxoprofen, phenylbutazon, piroxicam, and sulindac (see Table 4; Mulato et al. 2000; Jung et al. 2001; Khamdang et al. 2002; Uwai et al. 2004; Nozaki et al. 2007b), and with low affinity with acetaminophen, acetylsalicylate, phenacetin, and salicylate (see Table 4; Cihlar and Ho 2000; Mulato et al. 2000; Khamdang et al. 2002; Ichida et al. 2003; Nozaki et al. 2007b). Data on translocation by OAT1 are conflicting for ibuprofen and ketoprofen (Mulato et al. 2000; Khamdang et al. 2002); no transport was found for acetylsalicylate, but was demonstrated for salicylate (Khamdang et al. 2002). Interaction with rat Oat1 occurred with high affinity (IC_{50} below 10 μM) for diclofenac, ibuprofen, indomethacin, ketoprofen, and naproxen (Apiwattanakul et al. 1999; Uwai et al. 2000c; Nozaki et al. 2004, 2007b). Oxyphenbutazone, phenylbutazone, piroxicam, salicylurate, sulindac, and tometin inhibited rat Oat1 with intermediate affinity (IC_{50} between 10 and 100 μM), and acetylsalicylate, paracetamol, phenacetin, and salicylate with low affinity (see Table 4; Apiwattanakul et al. 1999; Uwai et al. 2000c; Hasegawa et al. 2003; Mori et al. 2004; Nozaki et al. 2004). For mouse Oat1, a few IC_{50} values are available, that is, for acetylsalicylate, ibuprofen, and salicylate (Kaler et al. 2007a). Transport was demonstrated only for salicylate by rat Oat1 (Apiwattanakul et al. 1999; Hasegawa et al. 2003).

Miscellaneous. The antihistaminic fexofenadine was not transported by OAT1 (Tahara et al. 2006a). The antiepileptic drug valproate (valproic acid) inhibited human and rat OAT1/Oat1, but it is not known whether this organic anion is translocated (Sekine et al. 1997; Mori et al. 2004; Sugawara et al. 2005). The uricosuric drug, benzbromarone, inhibited with high affinity human OAT1 (Ichida et al. 2003). For probenecid, see Sect. 2.7.

2.7 Inhibitors

The prototypical, though not OAT1-specific inhibitor is *probenecid* (*p*-(dipropylsulfamoyl) benzoate). This drug was initially developed to decrease the renal excretion of penicillin (Beyer et al. 1951). Following oral administration, absorption is complete involving an unknown uptake system in the intestine. The half-life of probenecid in man is 4–12 h. Probenecid and its metabolites are mainly renally excreted (reviewed in: Cunningham et al. 1981). Meanwhile it is known that

probenecid inhibits several renal organic anion transporters, explaining its action on the excretion of various anionic drugs. Using different expression systems and test anions, human OAT1 was inhibited by probenecid with IC_{50} or K_i values between 4.29 and 12.5 μM (Cihlar and Ho 2000; Ho et al. 2000; Mulato et al. 2000; Jung et al. 2001; Takeda et al. 2001; Ichida et al. 2003; Hashimoto et al. 2004; Khamdang et al. 2004; Chu et al. 2007). A single study reported a higher IC_{50} (45.7 μM; Kimura et al. 2007). Most of the reported IC_{50} values are below the therapeutic free plasma concentration of probenecid (18.7 μM; Nozaki et al. 2007b). Rat Oat1 was also inhibited by probenecid with IC_{50} values ranging between 1.44 and 31 μM (Uwai et al. 2000c; Sugiyama et al. 2001; Khamdang et al. 2004; Minematsu et al. 2008). For mouse Oat1, a K_i of 5.2 μM was reported (Kaler et al. 2007a). Monkey and rabbit OAT1/Oat1 were inhibited by probenecid (no IC_{50} or K_i available; Bahn et al. 2002; Tahara et al. 2005b). Probenecid was not transported by rat Oat1 (Uwai et al. 1998). Thus, probenecid binds to OAT1/Oat1 with high to intermediate affinity, but may not be translocated, thereby blocking transport of other organic anions.

2.8 Drug/Drug Interactions

Since OAT1/Oat1 handles numerous drugs, interactions are very likely to occur whenever two or more anionic drugs are coadministered and inhibit each other during renal proximal tubular secretion (for reviews see Eraly et al. 2003a; Mizuno et al. 2003; Shitara et al. 2005; Sweet 2005; Li et al. 2006). Here, we mention few examples of drug/drug interaction that may relate to the action of OAT1.

Probenecid/β-lactam antibiotics interaction. As mentioned previously, a classic example of (desired) drug/drug interaction is the decrease of renal penicillin excretion by probenecid in man (Beyer et al. 1951; Overbosch et al. 1988). Probenecid also inhibited the uptake of benzylpenicillin into rat kidney (Tsuji et al. 1990). Nowadays, it appears that the probenecid-*penicillin* interaction takes place mainly at OAT3, the main transporter for penicillin excretion (see Sect. 4.6.2). Probenecid also decreased the renal excretion of various cephalosporins (Brown 1993), a drug/drug interaction that may well take place at OAT1.

Probenecid/methotrexate interaction. The reported interaction between probenecid and *methotrexate* in man (Aherne et al. 1978) may not only relate to drug/drug interaction at OAT1 because OAT3 has a much higher affinity for methotrexate than does OAT1 (Takeda et al. 2002c). Nevertheless, probenecid inhibited competitively the uptake of methotrexate by rat Oat1 with a K_i of 15.8 μM (Uwai et al. 2000c), that is, in the range of the free plasma concentration of probenecid (18.7 μM; Nozaki et al. 2007b).

Probenecid/furosemide interaction. The renal clearance of furosemide (frusemide) was decreased by coapplication of probenecid in man (Homeida et al. 1977; Honari et al. 1977; Vree et al. 1995; for further literature see Uwai et al. 2000b). The interaction may take place at OAT1 and OAT3, the latter having a slightly higher affinity for the diuretic (see Rizwan and Burckhardt 2007). It is highly likely

that probenecid also decreases renal excretion of other loop and thiazide diuretics and thus prolongs their action.

Probenecid/antiviral drug interaction. Oral probenecid decreased the renal excretion of cidofovir in man and attenuated the nephrotoxic effect of this antiviral drug (Cundy et al. 1995; Lalezari et al. 1995). Also in rabbits and cynomolgus monkeys, probenecid decreased cidofovir renal accumulation and toxicity (Cundy et al. 1996; Lacy et al. 1998). Thus, probenecid can be used in antiviral therapy to prevent kidney damage.

NSAID/antiviral drug interaction. Nonsteroidal anti-inflammatory drugs potently inhibited OAT1, the transporter involved in renal excretion of, and in nephrotoxicity caused by, antiviral drugs. Indeed, NSAIDs decreased the adefovir-mediated cytotoxicity in OAT1-expressing cells (Mulato et al. 2000). Thus, NSAIDs may be used clinically to overcome the nephrotoxic effects of antiviral drugs.

NSAID/methotrexate interaction. The coadministration of NSAIDs with methotrexate may cause a life-threatening suppression of hematopoiesis due to the accumulation of methotrexate (e.g., Thyss et al. 1986; Frenia and Long 1992). The affinity of OAT1 for methotrexate is low as compared to OAT3 (Takeda et al. 2002c). Hence, at lower dosages, NSAID/methotrexate interaction may preferably take place at OAT3; at higher dosages of methotrexate, however, OAT1 may become an additional site of drug interaction. As indicated previously and shown in Table 4, NSAIDs inhibited OAT1/Oat1 with affinities ranging between 0.14 and 2,110 μM. Importantly, both affinity and free plasma concentration determine the inhibition of OAT1/Oat1 and, hence, the degree of interaction of NSAIDs with methotrexate. For practically all NSAIDs the plasma concentrations are considerably lower than their IC_{50} values at rat Oat1, suggesting that NSAID/methotrexate interaction does not take place at Oat1 (Nozaki et al. 2004). With human OAT1, salicylate may well be involved in NSAID/methotrexate interaction because its free plasma concentration is 431 μM and its IC_{50} was 84.5 μM (Takeda et al. 2002c).

2.9 Pharmacogenomics

The amino acid diversity (nonsynonymous single nucleotide polymorphisms) in human OAT1 is less frequent than average diversity, suggesting a lower mutability or relatively high selection pressure against mutated OAT1 (Fujita et al. 2005; Urban et al. 2006). Within the promoter region, no polymorphism was found in 63 human nephrectomy specimens (Ogasawara et al. 2008). In another study (Bhatnagar et al. 2006), an A → G exchange was found 3,655 bp upstream from the starting point in a subject of Icelandic descent. In noncoding or intronic regions, five SNPs in genomic DNA samples from 92 humans (Bleasby et al. 2005), and 19 SNPs in 276 DNA probes (Fujita et al. 2005) were reported. The functional consequence of these SNPs is not known. In coding regions (exons), nine SNPs were found, seven of which were synonymous and two nonsynonymous, leading to the amino acid substitutions R50H and K525I (Bleasby et al. 2005). Fujita et al.

(2005) detected 12 SNPs in coding regions, six of them being nonsynonymous, leading to the amino acid replacements R50H, P104L, I226T, A256W, R293W, and R454Q. Xu et al. (2005) found two nonsynonymous SNPs, causing the amino acid replacements L7P and R50H (for an overview see Srimaroeng et al. 2008). The mutant R50H found in probes from subjects of African descent (frequency: 0.032 in 160 probes) and in Mexican-Americans (frequency 0.01 in 100 probes), exhibited an unaltered affinity for *p*-aminohippurate, ochratoxin A, and methotrexate (Fujita et al. 2005). The affinities for adefovir, cidofovir, and tenofovir, however, were significantly higher for R50H than for the wild type, suggesting that subjects carrying this SNP may be more prone to toxic side effects of antiviral drugs on the kidneys (Bleasby et al. 2005). The R454Q replacement leads to a nonfunctional transporter (Fujita et al. 2005). Three members of an African family carrying this SNP did not show any change in adefovir clearance, an unexpected finding given the importance of OAT1 for the renal secretion of antivirals. Possibly, another step besides uptake by OAT1 is rate-limiting in the secretion of antivirals.

3 Organic Anion Transporter 2 (OAT2/Oat2, Gene Name SLC22A7/Slc22a7)

3.1 Cloning, Structure

OAT2/Oat2 was cloned from man (Sun et al. 2001), rat ("novel liver transporter NLT": Simonson et al. 1994; recloned by: Sekine et al. 1998), and mouse (Kobayashi et al. 2002b). The gene coding for human OAT2 is located on chromosome 6p21.1, and is not paired with any other SLC22 gene (Eraly et al. 2003b). The next relative to OAT2 is OAT3 with 37% amino acid identity (Jacobsson et al. 2007). OAT2/oat2 proteins consist of 535–548 amino acids, arranged in 12 putative transmembrane helices with intracellularly located *N*- and *C*-termini (topology not yet experimentally proven). For rat Oat2, two *N*-glycosylation sites, and each two putative phosphorylation sites for protein kinase A and C were described (Simonson et al. 1994). Mouse Oat2 contains three potential *N*-glycosylation and six putative protein kinase C phosphorylation sites (Kobayashi et al. 2002b). The functional importance of these sites is unknown.

3.2 Tissue Distribution of mRNA

Northern blot analysis indicated that hOAT2 and rOat2 mRNA are expressed predominantly in the liver, with lower levels in the kidney and other tissues including choroid plexus (Simonson et al. 1994; Sekine et al. 1998; Sun et al. 2001; Buist et al. 2002; Kobayashi et al. 2002b; Kojima et al. 2002; Sweet et al. 2002; Augustine et al. 2005; Nishimura and Naito 2005; Hilgendorf et al. 2007).

On a quantitative basis, OAT2/Oat2 mRNA was higher in liver, but considerably lower than mRNAs for OAT1 and OAT3 in kidneys (Buist et al. 2002; Hilgendorf et al. 2007).

3.3 Immunolocalization of OAT2/Oat2 Protein

Whereas human OAT2 was documented in the basolateral membrane of proximal tubules (Enomoto et al. 2002b; Kojima et al. 2002), the mouse and rat orthologs were localized to the luminal membrane in the late S3 segment or even the connecting duct (Kojima et al. 2002; Ljubojevic et al. 2007; Zlender et al. 2009). Though it is assumed that OAT2/Oat2 is located in the sinusoidal membrane of hepatocytes, an immunolocalization of Oat2 has not yet been performed to substantiate this hypothesis.

3.4 Species Differences, Age and Gender Dependence of Expression

As indicated previously, the subcellular localization of OAT2/Oat2 in kidneys differs between species. A basolateral localization in the proximal tubules of human kidneys suggests a role of OAT2 in uptake of organic anions from the blood as the first step in secretion. The apical localization in rodent kidneys indicates a different, as yet undefined role of Oat2, for example, the release of organic anions into the urine or the uptake of organic anions from the filtrate.

In rats, there are clear cut gender differences in Oat2 expression. Male rats showed greater mRNA abundance in liver than in kidneys, whereas in adult female rats, Oat2 mRNA in kidneys was higher than in the liver (Buist et al. 2002, 2003; Kato et al. 2002; Kobayashi et al. 2002a; Ljubojevic et al. 2007). In male rats, mRNA expression in rat kidneys remained low, whereas in female rats, mRNA rose sharply after postnatal day 30 (Buist et al. 2002). Gonadectomy decreased renal Oat2 expression, and growth hormone increased it in female rats (Buist et al. 2003). The gender differences were visible also at the level of the protein: female rats showed more immunoreactive protein in the brush-border membrane of S3 cells than male animals; testosterone decreased expression and estrogens and progesterone slightly elevated it (Ljubojevic et al. 2007). In mice, renal Oat2 mRNA expression was also low until day 25 and rose thereafter, however, with no obvious gender differences (Buist and Klaassen 2004; Cheng et al. 2008). However, immunohistological data suggested the presence of gender differences in mice similar to those in the rat (Ljubojevic et al. 2007). An increasing Oat2 expression with no gender differences was observed for rabbit kidneys (Groves et al. 2006). For man, no information is available on gender

differences. Taken together, Oat2 expression in the kidneys is highly age-dependent and, at least in rats, gender-dependent.

3.5 Factors Influencing Activity and Abundance of OAT2/Oat2

Oat2 was much less expressed in kidneys and liver of HNF-1α knockout mice, suggesting an important positive influence of this transcription factor on both renal and hepatic expression (Maher et al. 2006). HNF-4α stimulated the expression of a human OAT2/reporter gene construct (Popowski et al. 2005). Interestingly, HNF-4α is downregulated by endogenous and exogenous compounds interacting with the hepatocyte farnesoid receptor (FXR); chenodeoxycholate, for instance, attenuated both HNF-4α and OAT2 expression (Popowski et al. 2005).

A nitric oxide (NO) donor decreased Oat2 mRNA expression in rat liver slices (Aoki et al. 2008), and an inhibitor of iNOS prevented the decrease of expression following intraperitoneal injection of lipopolysaccharide (LPS) in rats (Cha et al. 2002), suggesting that NO leads to a downregulation of Oat2 expression in hepatocytes. In another study, LPS had no effect on Oat2 expression in rats (Cherrington et al. 2004). Phenobarbital strongly, and rifampicin slightly suppressed hepatic Oat2 expression, indicating that interaction of drugs with nuclear receptors (CAR, CXR, and others) has an impact on Oat2 (Jigorel et al. 2006). In the kidneys, Oat mRNA fell in diabetic rats (Manautou et al. 2008) as well as in cisplatin-treated mice (Aleksunes et al. 2008). Methotrexate treatment, which reduced Oat1 and Oat3, did not change Oat2 expression in rats (Shibayama et al. 2006). The influence of renal disorders on Oat2 has, to our knowledge, not been studied.

3.6 Substrates

The mode of operation of OAT2/Oat2 is, to our opinion, not clear. Conflicting results have been published with respect to the interaction with dicarboxylates of 4 and 5 carbons lengths. α-Ketoglutarate (5 carbons), for instance, was transported by human OAT2, but did not inhibit OAT2-mediated transport (Sun et al. 2001) nor did it *trans*-stimulate uptake of estrone-3-sulfate (Kobayashi et al. 2005). Rat Oat2 was reported to either transport α-ketoglutarate (Sekine et al. 1998) or not (Morita et al. 2001). Glutarate (C5) was transported by human OAT2, but did not exert a *trans*-stimulation (Kobayashi et al. 2005). On the other hand, fumarate and succinate (both 4 carbons) *trans*-stimulated OAT2-mediated estrone-3-sulfate uptake (Kobayashi et al. 2005), suggesting that OAT2 may be an organic anion/dicarboxylate exchanger with preference for C4 dicarboxylates.

In most studies OAT2/Oat2 was probed with radiolabeled salicylate, *p*-aminohippurate, and prostaglandin $F_{2\alpha}$. Especially $PGF_{2\alpha}$ appears to be well suited because of a high affinity for OAT2/Oat2 and low background uptake in nonexpressing control cells (Enomoto et al. 2002b).

3.6.1 Endogenous Substrates

Citric acid cycle intermediates. As mentioned previously, α-ketoglutarate may or may not be a substrate of OAT2. Fumarate and succinate *trans*-stimulated OAT2-mediated transport of estrone sulfate and therefore acted as counter anions for exchange (Kobayashi et al. 2005). A systematic survey on citric acid cycle intermediates is, however, missing.

Nucleobases, nucleosides, nucleotides. Adenine, adenosine, cytidine, guanidine, guanosine, GMP, GDP, GTP, and inosine, but not cytosine, thymine, and thymidine were found to be transported by human OAT2 (Cropp et al. 2008).

Cyclic nucleotides. cAMP and cGMP were translocated by OAT2, the latter with a K_m of 88 μM (Sun et al. 2001; Cropp et al. 2008).

Bile acids. Cholate and taurocholate were not transported themselves by rat Oat2 (Sekine et al. 1998), but taurocholate inhibited Oat2 (Morita et al. 2001).

Local hormones. Prostaglandin E_2 (PGE_2) was transported by human OAT2 with an exquisite affinity (K_m 0.71 μM; Kimura et al. 2002). Rat Oat2 also transported PGE_2, but with lower affinity (K_m 38.5 μM; Morita et al. 2001). The K_m values for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) for human and rat OAT2/Oat2 were even lower (0.425 and 0.414 μM, respectively; Kimura et al. 2002; Khamdang et al. 2003).

Sulfated steroid hormones. Estrone-3-sulfate was taken up by human OAT2 (Kobayashi et al. 2005), but not by rat Oat2 (Sekine et al. 1998). Dehydroepiandrosterone sulfate was transported by human OAT2 in one study (Kobayashi et al. 2005), but not in another (Sun et al. 2001).

3.6.2 Drugs

ACE inhibitors. Enalaprilat did not interact with rat Oat2 (Sekine et al. 1998).

Diuretics. As shown in Table 5, loop and thiazide diuretics have been tested with human OAT2, all showing inhibition of OAT2-mediated transport with IC_{50} values ranging from 39.2 μM (cyclothiazide) to 2.2 mM (chlorothiazide) (Hasannejad et al. 2003; Kobayashi et al. 2005). Bumetanide was transported with high affinity in one study (Kobayashi et al. 2005), but not in another (Hasannejad et al. 2003). The carboanhydrase inhibitors acetazolamide and methozolamide did not inhibit transport by OAT2 (Hasannejad et al. 2003). Rat Oat2 was inhibited by bumetanide (Sekine et al. 1998), and mouse Oat2 transported labeled bumetanide with high affinity (Kobayashi et al. 2005). As compared to OAT1/Oat1, the affinities for most diuretics for OAT2/Oat2 are smaller (higher IC_{50} values).

Statins. Pravastatin inhibited OAT2 and rat Oat2 with low affinity (IC_{50} greater than 100 μM; Khamdang et al. 2004)

Antibiotics. Table 5 shows the antibiotics tested with human OAT2. For most of the cephalosporins the IC_{50} values were in the millimolar range, indicating a low affinity of OAT2 toward these antibiotics (Khamdang et al. 2003). In general, OAT1 had a higher affinity for the same cephalosporins (cf. Table 4). Erythromycin was transported with good affinity by human OAT2 (K_m 18.5 μM; Kobayashi et al.

Table 5 Drugs interacting with OAT2/Oat2

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
ACE inhibitor						
Enalaprilat				–		
Diuretics						
Bumetanide	7.52 or –	77.5		+		9.12
Chlorothiazide		2,205				
Cyclothiazide		39.2				
Ethacrynate		121				
Furosemide	–	603				
Hydrochlorothiazide		1,023				
Methazolamide		–				
Trichlormethiazide		1,220				
Statin						
Pravastatin		352		449		
Antibiotics						
Benzylpenicillin				+		
Cefadroxil		6,410				
Cefamandol		430				
Cefazolin		5,090				
Cefoperazone		1,140				
Cefotaxime		5,210				
Ceftriaxone		6,760				
Cephaloridine		2,090				
Cephalothin		1,040				
Chloramphenicol		+				
Doxycyclin		(+)				
Erythromycin	18.5	+		–		
Minocyclin		(+)				
Oxytetracycline		(+)				
Rifampicin				+		
Tetracycline	440	+				
Antivirals						
Acyclovir	–	–				
2′,3′-Dideoxycytidine			3,080			
Ganciclovir	–	(+)				
Valacyclovir	–					
Zidovudine	26.8	81	26			
Antineoplastics						
6-Fluorouracil	0.054					
Imatinib	–					
6-Mercaptopurine	–					
Methotrexate	+	8.9	+ or –	(+)		
Taxol	0.142					
Histamine receptor 2 blockers						
Cimetidine	+	–		+ or –		
Famotidine	–					
Ranitidine	+					

(*continued*)

Table 5 (continued)

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
NSAIDs						
Acetaminophen		+ or −				
Acetylsalicylate	−	−	(+)			
Diclofenac		14.3–18.7		49.3		
Ibuprofen		692		155		
Indomethacin	−	49.5–64.1	0.37			
Ketoprofen	−	272.8–400		1.84		
Mefenamate		20.6–21.7				
Naproxen		486				
Phenacetin		1,878				
Piroxicam		70.3				
Salicylate	+ or −	−	81.2–122			
Sulindac		440				
Tolmetin						

+, transport of inhibition was observed without determination of K_m, K_i, or IC_{50}; −, no transport or no inhibition was observed; (+), weak transport or inhibition; blank, not determined; numbers, reported K_m, K_i, or IC_{50} values in micromoles/liter (μM). For references see text in Sect. 3.6.2

2005), and tetracycline with low affinity (K_m 439.9 μM; Babu et al. 2002a). Other tetracyclines showed weak inhibition. Chloramphenicol inhibited OAT2 (Kobayashi et al. 2005). Benzylpenicillin was only tested with rat Oat2 and inhibited transport (Morita et al. 2001) as did rifampicin (Sekine et al. 1998; Morita et al. 2001), but not erythromycin (Sekine et al. 1998).

Antivirals. With exception of zidovudine no other antiviral drugs were transported by OAT2 (Takeda et al. 2002b). Rat Oat1 transported 2′,3′-dideoxycytidine and zidovudine (Morita et al. 2001).

Antineoplastic drugs. Human OAT2 was found to have a very high affinity for 5-fluorouracil and for taxol with half-maximal transport rates at 0.054 μM and 0.142 μM, respectively (Kobayashi et al. 2005). Methotrexate was transported by OAT2 and inhibited transport with an IC_{50} of 8.9 μM (Sun et al. 2001; Kimura et al. 2007). Imatinib and 6-mercaptopurine were not translocated by OAT2 (Mori et al. 2004; Hu et al. 2008). Rat Oat1 did or did not transport methotrexate (Sekine et al. 1998; Morita et al. 2001).

Histamine receptor 2 blockers. Cimetidine and ranitidine, but not famotidine were translocated by human OAT2 (Tahara et al. 2005a). At odds, cimetidine did not inhibit OAT2-mediated $PGF_{2\alpha}$ transport (Khamdang et al. 2004). Weak or no inhibition at all by cimetidine were reported for rat Oat2 (Morita et al. 2001; Khamdang et al. 2004; Minematsu et al. 2008)

NSAIDs. As shown in Table 5, a number of NSAIDs inhibited human OAT2 with IC_{50} values ranging from 14.3 μM to 1.8 mM. Diclofenac, indomethacin, mefenamate, and piroxicam showed an intermediate affinity (IC_{50} between 10 and 100 μM); ibuprofen, ketoprofen, naproxen, and sulindac had a low affinity, and phenacetin a very low affinity for OAT2 (Khamdang et al. 2002; Kimura et al. 2007). Acetylsalicylate, indomethacin, and ketoprofen were not transported by

OAT2; and for salicylate, mixed results have been reported (Sun et al. 2001; Khamdang et al. 2002; Kobayashi et al. 2005). Rat Oat2 did transport indomethacin and salicylate and, to some extent, acetylsalicylate (Sekine et al. 1998; Morita et al. 2001; Minematsu et al. 2008), and was inhibited by diclofenac, ibuprofen, and ketoprofen (Morita et al. 2001). In general, the affinity of OAT2/Oat2 toward NSAIDs is considerably lower than that of OAT1/Oat1.

3.7 Inhibitors

No specific inhibitor is present for OAT2/Oat2. The general inhibitor, probenecid, has only a low affinity for OAT2/Oat2 with IC_{50} values ranging between 410 and 977 μM (Enomoto et al. 2002b; Khamdang et al. 2004; Kimura et al. 2007).

3.8 Drug/Drug Interactions

To our knowledge, no drug/drug interactions have been reported for OAT2.

3.9 Pharmacogenomics

In 63 probes from human kidneys, no single nucleotide polymorphisms were detected in the promoter region (Ogasawara et al. 2008). In the coding region, one synonymous SNP and three nonsynonymous SNPs leading to the amino acid changes T110I, V192I, and G507D were reported (Xu et al. 2005). The functional consequences of these changes have not been tested.

4 Organic Anion Transporter 3 (OAT3/Oat3, Gene Name SLC22A8/Slc22a8)

4.1 Cloning, Structure

The organic anion transporter has been cloned from man (Race et al. 1999; Cha et al. 2001), monkey (Tahara et al. 2005b), pig (Hagos et al. 2005), rabbit (Zhang et al. 2004), rat (Kusuhara et al. 1999), and mouse (alias: roct for reduced in osteosclerosis; Brady et al. 1999). As shown in Table 1, the gene SLC22A8 is located on chromosome 11q12.3, and is paired with SLC22A6, the gene for OAT1 (Eraly et al. 2003b). The close pairing suggests a common ancestor and concerted

regulation of expression of OAT1 and OAT3. Functionally, the flounder organic anion transporter shares substrates with OAT1 and OAT3 and may be related to this presumed common ancestor that is possibly found also in killifish and zebrafish (Aslamkhan et al. 2006). A concerted regulation of expression, however, may not take place, because OAT3 is found also at places where no OAT1 is expressed, for example, the distal tubule or in the liver (see the following). Mammalian OAT3/Oat3 proteins are composed of 536–542 amino acids; secondary structure predictions suggested 12 transmembrane helices with intracellularly located *N*- and *C*-termini. The large extracellular loop between helices 1 and 2 carries potential *N*-glycosylation sites, the large intracellular loop between helices 6 and 7 potential phosphorylation sites for regulation by protein kinases (Cha et al. 2001; reviewed in: Burckhardt and Wolff 2000). In rat Oat3, amino acid residues in transmembrane helix 7, 8, and 11 have been identified by directed mutagenesis to be involved in substrate binding (Feng et al. 2001, 2002).

4.2 Tissue Distribution of mRNA

Northern blot analyses have detected OAT3/Oat3 mRNA in the kidneys of humans (Cha et al. 2001; Sun et al. 2001; Nishimura and Naito 2005; Bleasby et al. 2006), monkeys (Tahara et al. 2005b; Bleasby et al. 2006), dogs (Bleasby et al. 2006), pigs (Hagos et al. 2005), rats (Kusuhara et al. 1999) and mice (Kobayashi et al. 2004). OAT3/Oat3 mRNA was also found in brain (Kusuhara et al. 1999; Cha et al. 2001; Buist et al. 2002; Choudhuri et al. 2003), brain capillaries (Ohtsuki et al. 2002; Kikuchi et al. 2003), liver (Kusuhara et al. 1999; Buist et al. 2002, 2003), skeletal muscle (Cha et al. 2001), and adrenal gland (Asif et al. 2005; Table 2). In all species, OAT3 mRNA expression was highest in the kidneys (Nishimura and Naito 2005; Bleasby et al. 2006). In human kidneys, the mRNA for OAT3 was expressed to a considerably higher extent than that for OAT1 (Motohashi et al. 2002)

4.3 Immunolocalization of OAT3/Oat3 Protein

In the kidneys, OAT3/Oat3 is localized in the basolateral membrane of proximal tubular cells (Cha et al. 2001; Hasegawa et al. 2002; Motohashi et al. 2002). Whereas, in rat kidneys, OAT1 was highest in the S2 segment, OAT3 was present in all proximal tubule segments (S1–S3; Kojima et al. 2002; Ljubojevic et al. 2004). The basolateral localization fits with the assumption that OAT3/Oat3 is involved in the uptake of organic anions from the blood into proximal tubule cells. Later on, Oat3 immunoreactivity was found at the basolateral cell side also in distal tubule segments (thick ascending limb, connecting tubule, collecting duct; Kojima et al. 2002; Ljubojevic et al. 2004; Di Giusto et al. 2008). The function of OAT3/Oat3 in these nephron segments is unclear. At the blood–brain barrier, Oat3 was detected in

the basal membrane of endothelial cells (Kikuchi et al. 2003; Mori et al. 2003; Ohtsuki et al. 2004). Furthermore, Oat3 was immunolocalized to the choroid plexus (Alebouyeh et al. 2003). It is assumed that Oat3 is involved in the efflux of, for example, penicillin from cerebrospinal fluid and brain tissue into the blood.

4.4 Species Differences, Age and Gender Dependence of Expression

During rat embryogenesis, Oat3 alias Roct appeared in liver and brain, and vanished toward birth (Pavlova et al. 2000). After birth, renal Oat3 mRNA expression in rats rose steadily to reach a plateau around day 30 with no gender difference (Buist et al. 2002). Also in rabbits, Oat3 message increased with age and did not exhibit gender differences (Groves et al. 2006). In immunohistological studies on rat renal Oat3, a higher protein expression was found for proximal tubules, whereas for the distal tubule and collecting duct, no gender differences were seen (Ljubojevic et al. 2004). In contrast, clear gender differences were seen in the liver: male rats had much more Oat3 mRNA than female animals (Buist et al. 2003). In mice, however, the male dominant expression was much less accentuated than in rats (Buist and Klaassen 2004).

4.5 Factors Influencing Activity and Abundance of OAT3/Oat3

Transfection of hepatocyte nuclear factor (HNF)-1α and HNF-1β induced the expression of OAT3 that is otherwise not existent in human embryonic kidney (HEK) 293 cells (Kikuchi et al. 2006). HNF-1α knockouts had a diminshed renal expression of Oat3, but a largely increased expression in the duodenum (Maher et al. 2006). Inhibition of promoter methylation increased Oat3 expression, suggesting that an epigenetic regulation takes place (Kikuchi et al. 2006).

OAT3 is subject to regulation by various factors. Activation of the conventional protein kinase C by phorbol esters downregulated the activity of rat Oat3 expressed in mouse proximal tubules (Takeda et al. 2000b) and of Oat3-mediated estrone-3-sulfate uptake into isolated rabbit renal proximal tubules (Soodvilai et al. 2004). Thus, ligands working through PKC inhibit tubular secretion of organic anions (Terlouw et al. 2003). On the other hand, insulin and epidermal growth factor (EGF) increased Oat3 activity in rat kidney, involving the atypical isoform PKCζ (Barros et al. 2009). EGF also stimulated Oat3-mediated transport in rabbit kidney proximal tubules (Soodvilai et al. 2004). A complex signal cascade (Sauvant et al. 2002, 2003, 2004) involving MAP kinases, phospholipase A_2, cyclooxygenase (COX) 1, prostaglandin E_2 release and PGE_2 receptor-mediated intracellular cAMP elevation finally leads to Oat3 activation; indeed, dbcAMP directly stimulated Oat3 in rabbit tubules (Soodvilai et al. 2004, 2005). In addition, cAMP increased the promoter activity of human OAT3 (Ogasawara et al. 2006).

Although PGE_2 acutely stimulated Oat3 activity, long-term exposure to PGE_2 resulted rather in a decrease in Oat3 abundance (Sauvant et al. 2006). A COX2-inhibitor, parecoxib, prevented the LPS-induced decrease of Oat3 in rat kidneys, suggesting that prostaglandins were involved (Höcherl et al. 2009).

The following maneuvers led to a reduction in OAT3 (and OAT1) protein abundance in homogenates, in basolateral plasma membranes, and/or in reduced uptake of the prototypical substrates: ischemia and reperfusion of the kidneys (Matsuzaki et al. 2007; Schneider et al. 2007; Di Giusto et al. 2008; Schneider et al. 2009), bilateral ureteral obstruction (Villar et al. 2005, 2008), several kidney diseases (Sakurai et al. 2004), and inflammation and fever (Sauvant et al. 2006). In contrast, experimental simulation of hepatic failure by biliary obstruction increased Oat3 abundance, both in the cytoplasm and, after three days, also in the basolateral membrane (Brandoni et al. 2006a, b; Torres 2008). In a rat model of hyperuricemia, the decrease in Oat3 was only transient and Oat3 function was completely restored within 14 days upon stopping the diet, which induced hyperuricemia (Habu et al. 2005). In rats devoid of the multidrug resistance-associated protein 2, Eisai hyperbilirubinemic rats, protein expression of Oat3 was significantly increased as compared to unaffected wild-type animals (Chen et al. 2008), and no change in Oat1 protein abundance was observed. 5/6-nephrectomized rats showed a higher Oat3 protein expression and an increased uptake of indoxyl sulfate (Enomoto et al. 2002c), whereas Oat1 was only slightly affected.

Methotrexate treatment of rats decreased renal Oat3 abundance (Shibayama et al. 2006). A decrease was also observed after treatment of rats with cisplatin and with tripterygium glycosides from Chinese herbs (Huang et al. 2001; Dan et al. 2008).

An Oat3 knockout mouse has been developed (Sweet et al. 2002). These mice are normal, but show a decreased renal excretion of diuretics (Vallon et al. 2008b), benzylpenicillin (Van Wert et al. 2007), quinolones (VanWert et al. 2008), and methotrexate (VanWert and Sweet 2007). In the brain, the choroid plexus showed a reduced accumulation of the organic anion fluorescein (Sweet et al. 2002).

4.6 Substrates

OAT3/Oat3 most likely operates as an organic anion/α-ketoglutarate exchanger (Bakhiya et al. 2003; Sweet et al. 2003). Thereby, uptake of organic anions from the blood into the cell is coupled to and energized by the release of α-ketoglutarate into the blood. Thus, cellular localization and mode of operation of OAT1 and OAT3 are the same. The substrate specificities of OAT1 and OAT3 overlap but are not identical. In general, OAT3 handles bulkier and more lipohilic organic anions than does OAT1.

The usual test substrate for OAT3/Oat3 is estrone-3-sulfate (ES), because the uptake is easily detectable (low background in nonexpressing cells) and the affinity of OAT3/Oat3 to ES is high. Expressed in various cells, the K_m of human OAT3 for

ES varied between 2.2 and 21.2 μM in 12 publications with a mean of 8.8 ± 5.3 μM (Takeda et al. 2000a, 2001; Cha et al. 2001; Feng et al. 2002; Sakurai et al. 2004; Erdman et al. 2005; Srimaroeng et al. 2005a; Tahara et al. 2005b; Ueo et al. 2005, 2007; Uwai et al. 2007b; Windass et al. 2007). ES was transported also by monkey (K_m 10.6 μM; Tahara et al. 2005b), rabbit (4.5 μM; Zhang et al. 2004), rat (2.34–7.13 μM; Kusuhara et al. 1999; Takeda et al. 2000b; Feng et al. 2001; Hasegawa et al. 2003; Minematsu et al. 2008) and mouse Oat3 (5.5–12.4 μM; Ohtsuki et al. 2004; VanWert et al. 2008).

4.6.1 Endogenous Substrates

Second messengers. Human OAT3 transported cAMP (Cha et al. 2001) and cGMP (Cropp et al. 2008).

Citric acid cycle intermediates. Citrate did not inhibit human OAT3 and slightly decreased the transport by rabbit Oat3 (Bakhiya et al. 2003; Zhang et al. 2004). α-Ketoglutarate transport was, surprisingly, not proven experimentally, but inhibition of OAT3 activity was demonstrated for human (Bakhiya et al. 2003; Hagos et al. 2008), monkey (Tahara et al. 2005b), and rabbit (Zhang et al. 2004); for the latter species, an IC_{50} of 50.3 μM was determined. Fumarate and succinate, but not oxaloacetate, slightly inhibited OAT3 (Bakhiya et al. 2003). Succinate was not transported by human OAT3 (Cha et al. 2001) and did not inhibit rabbit and rat Oat3 (Zhang et al. 2004; Anzai et al. 2005); malate and oxaloacetate did not inhibit rabbit Oat3 (Zhang et al. 2004). It appears that OAT3/Oat3 interacts with 5-carbon, but not with 4-carbon dicarboxylates. Accordingly, the nonphysiologic C5 dicarboxylate, glutarate, was transported by human, rat, and mouse OAT3/Oat3 (Bakhiya et al. 2003; Ohtsuki et al. 2004; Bahn et al. 2005; Nilwarangkoon et al. 2007).

Vitamins. Folate inhibited mouse Oat3 (VanWert and Sweet 2007). Nicotinate did not inhibit rabbit Oat3 (Zhang et al. 2004).

Bile salts. Several bile salts interacted with OAT3. The bile salts, cholate and taurocholate, were taken up into cells expressing the human and rat OAT3/Oat3 (Cha et al. 2001; Chen et al. 2008), and they inhibited transport mediated by these transporters with IC_{50} values between 230 and 554 μM for cholate and between 790 and 2,360 μM for taurocholate (Sugiyama et al. 2001; Chen et al. 2008). Among the other bile salts, transport was demonstrated only for glycocholate by rat Oat3, but not by human OAT3 (Chen et al. 2008). K_i values for inhibition of human and rat OAT3/Oat3 have been published for chenodeoxycholate, deoxycholate, glycochenodeoxycholate, glycocholate, and taurochenodeoxycholate (Chen et al. 2008). In kidney cortex slices obtained from Oat3 knockout mice, the accumulation of taurocholate was defective, proving bile salt transport by Oat3 (Sweet et al. 2002). In the same study, liver slices did not show abnormalities, suggesting that Oat3 does not play a role in the uptake of bile salts into hepatocytes.

Hormones and sulfated hormones. Human OAT3 transported radiolabeled cortisol with a K_m of 2.4 μM (Asif et al. 2005) and was inhibited by unlabeled corticosterone (Cha et al. 2001). Oat3 knockout mice, however, responded normally to ACTH

infusion, suggesting that Oat3 does not play a role in cortisol release from adrenal cells (Vallon et al. 2008a). Furthermore, OAT3 transported dehydroepiandrosterone sulfate (Cha et al. 2001; Ueo et al. 2005; Nozaki et al. 2007a) as well as the prototypical substrate, estrone-3-sulfate (for K_m values and literature on human, monkey, rabbit, rat, and mouse OAT3/Oat3 see previous). Mouse Oat3 was inhibited by aldosterone (IC_{50} 12 μM), corticosterone (10 μM), desoxycorticosterone (9 μM), and progesterone (29 μM) as well as by androsterone sulfate, estradiol disulfate, estradiol-17ß-glucuronide, 17ß-estradiol-3-sulfate, estrone-3-ßD-glucuronide, and estrone-3-sulfate (Vallon et al. 2008a). Taken together, OAT3/Oat3 interacts with numerous steroid hormones and their sulfated or glucuronidated derivatives.

Local hormones. Prostaglandin E_2 was transported by human (K_m 0.35 μM; Kimura et al. 2002), rat (K_m 1.4 μM; Nilwarangkoon et al. 2007), and mouse OAT3/Oat3 (Kobayashi et al. 2004), and prostaglandin $F_{2\alpha}$ by human (K_m 1.1 μM; Kimura et al. 2002) and mouse OAT3/Oat3 (Kobayashi et al. 2004). Thus, this transporter has a very high affinity for these prostaglandins.

Nucleobases, purine metabolites, urate. Among the tested bases, only adenine slightly inhibited rat Oat3 (Mori et al. 2004). The nucleoside thymidine was transported by mouse Oat3 and inhibited its function with an IC_{50} of 384 μM (Vallon et al. 2008a). Thymidine was found to decrease blood pressure in mice. For this reason, Oat3 knockout mice or mice treated with inhibitors of Oat3 such as eosin Y or probenecid showed a lower blood pressure due to impaired thymidine excretion (Vallon et al. 2008a).

The purine metabolite, urate, was transported by human and rabbit OAT3 (Cha et al. 2001; Zhang et al. 2004) and inhibited human, rabbit, and mouse Oat3 (Bakhiya et al. 2003; Zhang et al. 2004; Ohtsuki et al. 2004; VanWert and Sweet 2007). The K_m for urate uptake by human OAT3 was 380 μM (Sato et al. 2008); IC_{50} values for inhibiting human and rabbit OAT3 were 255 μM and 733 μM, respectively (Bakhiya et al. 2003; Zhang et al. 2004). Thereby, the affinity of OAT3 toward urate is higher than that of OAT1.

Neurotransmitters and their metabolites. Dopamine, glutamate, histamine, and serotonin did not interact with rat Oat3 (Mori et al. 2003). Melatonin inhibited human and rat OAT3 (Kusuhara et al. 1999; Alebouyeh et al. 2003). A large number of acidic neurotransmitter metabolites were tested with human (h), rat (r), and mouse (m) transporters: 3,4-dihydroxymandelate (h: no inhibition, r: inhibition), 3,4-dihydroxyphenlacetate (h: IC_{50} 990 μM; r: inhibition), homovanillate (h: no transport but inhibition with IC_{50} 760 μM; r: transport with K_m 274 μM; m: transport and inhibition), 5-hydroxyindole-3-actetate (h: IC_{50} 910 μM; r and m: inhibition), 4-hydroxy-3-methoxymandelate, 4-hydroxy-3-methoxyphenylacetate, 4-hydroxy-3-methoxyphenylglycol, imidazol-4-acetate (r: inhibition), 5-methoxyindole -3-acetate (h: IC_{50} 70 μM; r: inhibition), 5-methoxytryptamine (h: IC_{50} 610 μM), 5-methoxytryptophol (h: IC_{50} 490 μM; r: inhibition), 1-methyl-4-imidazolate (r: inhibition; Alebouyeh et al. 2003; Bahn et al. 2005; Kusuhara et al. 1999; Mori et al. 2003; Ohtsuki et al. 2004; VanWert and Sweet 2007). Neuroactive metabolites of the tryptophan metabolism inhibited mouse Oat3: 3-hydroxykynurenate,

kynurenate (IC_{50} 8 μM), picolinate, and xanthurenate (IC_{50} 11.5 μM; Bahn et al. 2005). In summary, OAT3/Oat3 located at the blood–brain barrier and the choroid plexus is probably involved in the efflux of acidic metabolites from brain tissue.

4.6.2 Drugs

ACE inhibitors. Quinapril was transported by human (K_m 13.4 μM) and rat OAT3/Oat3 (Yuan et al. 2009); inhibition of OAT3 by quinapril occurred with an IC_{50} of 6.2 μM (Chu et al. 2007). Captopril was transported by human OAT3 (Ueo et al. 2005), and enalapril inhibited human and mouse OAT3/Oat3 (Chu et al. 2007; Kobayashi et al. 2004). Temocaprilat was taken up into rat Oat3-expressing cells with high affinity (K_m 1.4 μM; Hasegawa et al. 2003).

Angiotensin II receptor 1 blockers. Candesartan, losartan, olmesartan, pratosartan, telmisartan and valsartan inhibited human OAT3 with very high affinities (Table 6; IC_{50} values between 0.027 and 1.6 μM; Sato et al. 2008). For olmesartan, transport by OAT3 was demonstrated (Yamada et al. 2007). Inhibition of rat Oat3 with high affinity was found for pratosartan and telmisartan (Li et al. 2008).

Diuretics. As shown in Table 6, for human OAT3 a near-complete set of IC_{50} values is available for carboanhydrase inhibitors (acetazolamide, methazolamide), loop diuretics (bumetanide, ethacrynate, furosemide, torasemide), and thiazide diuretics (chlorothiazide, cyclothiazide, hydrochlorothiazide, trichlormethiazide). The highest affinities were observed for bumetanide, ethacrynate and furosemide (IC_{50} values between 0.58 and 7.31 μM; Hasannejad et al. 2003; Chu et al. 2007), intermediate ones for torasemide and most thiazides (IC_{50} values ranging between 27.9 and 97.5 μM; Hasannejad et al. 2003; Hagos et al. 2007a), and low affinities for acetazolamide and hydrochlorothiazide (Hasannejad et al. 2003). Transport by human OAT3 was demonstrated for bumetanide and furosemide (Hasannejad et al. 2003). Bumetanide was also transported by mouse Oat3 with very high affinity (K_m 1.1 μM; Kobayashi et al. 2005). Inhibition of monkey OAT3 by bumetanide and furosemide (Tahara et al. 2005b), rat Oat3 by bumetanide and furosemide (Kusuhara et al. 1999; Erdman et al. 2005), and of mouse Oat3 by bendroflumethiazide and furosemide (Kusuhara et al. 1999; Feng et al. 2001; Vallon et al. 2008b) were also reported. From these data it is likely that OAT3/Oat3 is, together with OAT1/Oat1, involved in proximal tubular secretion of diuretics. Indeed, renal excretion of furosemide and bendroflumethiazide was decreased in both Oat1 and Oat3 knockout mice (Vallon et al. 2008b).

Statins. Pravastatin and rosuvastatin were translocated by human and rat OAT3/Oat3 with high to moderate affinities (Table 6: K_m values between 4.7 and 13.4 μM; Hasegawa et al. 2002; Takeda et al. 2004, Windass et al. 2007). Atorvastatin, fluvastatin, pravastatin, rosuvastatin and simvastatin inhibited human OAT3 with IC_{50} values between 5.79 and 96.5 μM, that is, with high to moderate affinities (Khamdang et al. 2004; Takeda et al. 2004; Windass et al. 2007). Inhibitions of

Table 6 Drugs interacting with OAT3/Oat3

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
ACE inhibitors						
Captoprilat	+					
Enalaprilat		+ or −				+
Quinaprilat	13.4	6.2	+			
Angiotensin II receptor blockers						
Candesartan		0.3				
Losartan		1.6				
Olmesartan	0.12	0.027				
Pratosartan		0.095		1.285		
Telmisartan		1.6		0.723		
Valsartan		0.2				
Diuretics						
Acetazolamide		816				
Bendroflumethiazide						21.3
Bumetanide	1.59	0.75		+	1.01	
Chlorothiazide		65.3				
Cyclothiazide		27.9				
Ethacrynate		0.58				
Furosemide	+	1.7–7.31		+		2.8
Hydrochlorothiazide		942				
Methazolamide		97.5				
Torasemide		89.9				
Trichlormethiazide		71.2				
Statins						
Atorvastatin		13.1				
Fluvastatin		5.79				
Pravastatin	+	13.7–96.5	13.4	15.6		+
Rosuvastatin	7.4	25.7	4.7			
Simvastatin		32.3–48.1				
Antibiotics						
Benzylpenicillin	52.1	+	82.6–85.1	52.8–132	40	+
Cefaclor	+	120.2				
Cefadroxil		8,620		1,780		
Cefamandol		46		90		
Cefazolin	+	116.6–550		780		
Cefdinir	+	271.5				
Cefoperazone		1,890		670		
Cefoselis	+	2,925				
Cefotaxime		290		800		
Cefotiam	+	212.6	+			
Ceftibuten	+	247.3				
Ceftizoxime	+	956.7				
Ceftriaxone		4,390		−		
Cephalexin				630		
Cephaloridine	+	626.4	+	1,140		
Cephalothin		40		48		
Ciprofloxacin		+			69.8	198
Doxycyclin		−				

(*continued*)

Table 6 (continued)

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
Gatifloxacin		+				941
Minocyclin		–				
Norfloxacin		–				558
Ofloxacin		–				745
Oxytetracycline		–				
Tetracycline	566.2	–				
Antivirals						
Acyclovir	–	+	(+)	1,460		729
Adefovir	(+) or –		–			
Amantadine						–
Cidofovir	(+)					+ or –
Didanosine						136.9
Lamivudine						140
Stavudine						2,113
Tenofovir	(+)					384
Valacyclovir	+					
Zalcitabine					125.9	203
Zidovudine	145.1	(+) or –	(+)	143		38.5
Antineoplastics						
5-Fluorouracil					0.054	
6-Mercaptopurine			50.5	+	4.01	
Methotrexate	10.9	+	+	28	60.6	+
6-Thioguanine				172		
Topotecan	56.5		21.9			
Immune suppressants						
Azathioprine		–		15.7		
Cyclosporin A		–				
Mycophenolate	–	0.52–1.5				
Tacrolimus		–				
Histamine receptor 2 blockers						
Cimetidine	40.0–174	42.9–92.4	40.0–90.7	8.74–166	105	85.0
Famotidine	124	179	345			+
Ranitidine	234		155			
NSAIDs						
Acetaminophen		–				
Acetylsalicylate		717				
Anthranilate						+
Diclofenac		6.57–7.78		3.17		–
Etodolac		12.0		9.98		
Flufenamate		+				
Ibuprofen	+ or –	3.7–6.0		3.57		+
Indomethacin	(+)	0.61–0.979		1.29	+	+
Ketoprofen	+	5.04–5.98		4.31		
Loxoprofen		8.7				
Mefenamate		0.78				
Naproxen		4.67–7.15		19.1		
Phenacetin		19.4				
Phenylbutazone		6.82		8.48		

(*continued*)

Table 6 (continued)

	Human		Rat		Mouse	
	Transport	Inhibition	Transport	Inhibition	Transport	Inhibition
Piroxicam		4.83–4.88		4.19		
Salicylate	+	50.0–111	–	511–519	+	+
Sulfinpyrazone				+		
Sulindac		3.62–6.89		7.72		
Tolmetin						

+, transport of inhibition was observed without determination of K_m, K_i, or IC_{50}; −, no transport or no inhibition was observed; (+), weak transport or inhibition; blank, not determined; numbers, reported K_m, K_i, or IC_{50} values in micromoles/liter (μM). For references see text in Sect. 4.6.2

monkey (Tahara et al. 2005b), rat (Khamdang et al. 2004), and mouse Oat3 (Ohtsuki et al. 2004) by pravastatin have also been reported.

Antibiotics. Benzylpenicillin was transported by human, monkey, rat, and mouse OAT3/Oat3 with K_m values between 40 and 85.1 μM (Hasegawa et al. 2002; Nagata et al. 2002; Ohtsuki et al. 2004; Tahara et al. 2005b). Other studies have shown that benzylpenicillin inhibits human, rat, and mouse OAT3/Oat3 (Kusuhara et al. 1999; Cha et al. 2001; Hasegawa et al. 2002; Mori et al. 2003; Deguchi et al. 2004; Minematsu et al. 2008; VanWert and Sweet 2007; VanWert et al. 2008). Because OAT3 has a higher affinity for benzylpenicillin than OAT1, it is believed that OAT3 is mainly involved in renal penicillin secretion. Indeed, Oat3 knockout mice showed a decreased renal excretion of penicillin, but not of PAH, a substrate of Oat1 (VanWert et al. 2007).

A number of *cephalosporins* were tested on human OAT3 (see Table 6). Transport was demonstrated for cefaclor, cefazolin, cefdinir, cefoselis, cefotiam, ceftibuten, ceftizoxime, and cephaloridine (Ueo et al. 2005). The IC_{50} values for inhibition of OAT3-mediated transport ranged between 40 μM and 8.6 mM indicating a wide range from intermediate (cefamandol, cephalothin) to very low affinities (cefadroxil, ceftriaxone, cefoselis, cefoperazone; Jung et al. 2002; Takeda et al. 2002a; Ueo et al. 2005). Rat Oat3 transported cefotiam and cephaloridine, and was inhibited by several cephalosporins shown in Table 6 (Jung et al. 2002; Chen et al. 2008). Also for rat Oat3, IC_{50} values have been determined (data shown in Table 6 are taken from Jung et al. 2002).

The *quinolones* ciprofloxacin, gatifloxacin, norfloxacin, and ofloxacin inhibited mouse Oat3 with low affinity (IC_{50} values between 198 and 941 μM), and ciprofloxacin was transported with a K_m of 69.8 μM (VanWert et al. 2008). In the same study, ciprofloxacin and gatifloxacin, but not norfloxacin and ofloxacin, inhibited human OAT3. In Oat3 knockout mice, the AUC of quinolones was increased, proving an important role of Oat3 in the renal excretion of these compounds (VanWert et al. 2008). *Tetracyclines* did not inhibit human OAT3, although uptake of tetracycline with a K_m of 566 μM was reported (Babu et al. 2002a).

Antivirals. A weak transport by human OAT3 was shown for cidofovir, tenofovir, and valacyclovir; for zidovudine uptake a K_m of 145.1 μM was determined (Takeda et al. 2002b; Uwai et al. 2007a). Acyclovir was not transported, but inhibited OAT3 (Cha et al. 2001; Takeda et al. 2002b). For adefovir (weak

transport: Uwai et al. 2007a; no transport: Aslamkhan et al. 2006) and zidovudine (weak inhibition: Takeda et al. 2002b; no inhibition: Cha et al. 2001), conflicting data are found in the literature. Rat Oat3 transported weakly acyclovir and zidovudine, and was inhibited by these antivirals (Kusuhara et al. 1999; Hasegawa et al. 2003); adefovir was not transported (Aslamkhan et al. 2003). A more complete data set is available for the inhibition of mouse Oat3 by antivirals (see Table 6). The IC_{50} values range beween 38.5 μM (zidovudine) and 2.1 mM (stavudine; Truong et al. 2008). No inhibition was found for amantadine (Kobayashi et al. 2004) and either inhibition (Vallon et al. 2008a) or no inhibition (Truong et al. 2008) was reported for cidofovir. It appears that antivirals are primarily transported rather by OAT1/Oat1 than by OAT3/Oat3.

Antineoplastics. Methotrexate transport occurred with a K_m of 10.9 μM by human OAT3 (Cha et al. 2001), monkey OAT3 (Tahara et al. 2005b), rat (Nozaki et al. 2004), and mouse Oat3 (K_m 60.6 μM; VanWert and Sweet 2007). Unlabeled methotrexate inhibited human (Srimaroeng et al. 2005a, b), rat (IC_{50} 28 μM; Mori et al. 2004), and mouse Oat3 (Ohtsuki et al. 2004; Vallon et al. 2008a). Thus, it is obvious that OAT3/Oat3 transports the antifolate methotrexate. In support of this conclusion, Oat3 knockout mice had a lower renal methotrexate excretion (VanWert and Sweet 2007). Mouse Oat3 transported 5-fluorouracil (K_m 0.054 μM) and 6-mercaptopurine (K_m 4.01 μM) with very high affinities (Kobayashi et al. 2004); rat Oat3 showed a higher K_m for 6-mercaptopurine (50.5 μM; Mori et al. 2004). 6-thioguanine inhibited rat Oat3 (IC_{50} 172 μM; Mori et al. 2004). Topotecan was transported by human (K_m 56.5 μM) and rat (K_m 21.9 μM) OAT3/Oat3 (Matsumoto et al. 2007).

Immune suppressants. Mycophenolate competitively inhibited human OAT3 with high affinity (IC_{50} 0.52–1.5 μM), but was not transported (Uwai et al. 2007b; Wolff et al. 2007). A competitive inhibition was also found for the glucuronide and the acyl-glucuronide of mycophenolate (Wolff et al. 2007). Azathioprine, cyclosporin A, and tacrolimus did not interact with OAT3 (Uwai et al. 2007b). Azathioprine, however, inhibited rat Oat3 with an IC_{50} of 15.7 μM (Mori et al. 2004).

Antidiabetics. Metformin was not transported by OAT3 (Kimura et al. 2005).

Histamine receptor 2 blockers. Cimetidine was transported by human (K_m values between 40 and 174 μM; Feng et al. 2001; Erdman et al. 2005; Tahara et al. 2005a, b; Chu et al. 2007) and monkey OAT3 (K_m 68.5 or 70.9 μM; Tahara et al. 2005a, 2006b), as well as by rabbit (K_m 89 μM; Zhang et al. 2004), rat (K_m between 40 and 90.7 μM; Feng et al. 2001; Tahara et al. 2005a), and mouse Oat3 (K_m 105 μM, Ahn et al. 2009). The IC_{50} values reported for human (Hashimoto et al. 2004; Khamdang et al. 2004; Motohashi et al. 2004; Chu et al. 2007), rabbit (Zhang et al. 2004), rat (Nagata et al. 2002; Khamdang et al. 2004; Minematsu et al. 2008) and mouse (Ahn et al. 2009) were in the same range as the K_m values for uptake (see also Table 6). Another H2 blocker, famotidine, was transported by human (K_m 124 μM; Tahara et al. 2005a), monkey (K_m 154 μM; Tahara et al. 2006a), and rat OAT3 (K_m 345 μM Tahara et al. 2005a), ranitidine by human (K_m 234 μM), monkey (K_m 125 μM), and rat Oat3 (K_m 155 μM; references as for famotidine). Taken together it is clear that OAT3/Oat3 translocates H2 blockers, although they are not organic anions but rather organic cations at physiological pH.

Nonsteroidal anti-inflammatory drugs (NSAIDs). Transport by human OAT3 was shown for indomethacin, ketoprofen, salicylate, and, with mixed results, for ibuprofen (Cha et al. 2001; Khamdang et al. 2002). For most NSAIDs inhibitory constants are available for their interaction with human OAT3 (see Table 6). Interaction with very high and high affinities (IC_{50} values below 10 μM) occurred with diclofenac, ibuprofen, indomethacin, ketoprofen, loxoprofen, mefenamate, naproxen, phenylbutazone, piroxicam, and sulindac, with intermediate affinity (IC_{50} below 100 μM) with etodolac, phenacetin, salicylate, and with low affinity ($IC_{50} > 100$ μM) with acetylsalicylate (Jung et al. 2001; Khamdang et al. 2002: Srimaroeng et al. 2005b; Chu et al. 2007; Nozaki et al. 2007b; Uwai et al. 2004). Flufenamate inhibited human OAT3, but no IC_{50} was determined (Uwai et al. 2004), and acetaminophen did not inhibit OAT3-mediated transport (Khamdang et al. 2002). A series of IC_{50} values is available also for the inhibition by NSAIDs by rat Oat3 (see Table 6). Most NSAIDs showed IC_{50} values below 10 μM; the only exceptions being naproxen and salicylate (Hasegawa et al. 2003; Nozaki et al. 2004). For sulfinpyrazone, inhibition of rat Oat3 has been shown (Mori et al. 2004). Mouse Oat3 transported indomethacin and salicylate, and was inhibited by anthranilate, ibuprofen, indomethacin, and salicylate (Kobayashi et al. 2004; Ohtsuki et al. 2004; Bahn et al. 2005; VanWert and Sweet 2007).

4.7 Inhibitors

Probenecid is a competitive inhibitor of human OAT3 (Hashimoto et al. 2004). The reported K_i or IC_{50} values range between 1.3 and 9 μM with a mean of 4.13 μM (Jung et al. 2001; Takeda et al. 2001; Hashimoto et al. 2004; Tahara et al. 2005a, 2006b; Chu et al. 2007). These values are below the therapeutical plasma concentration (18.7 μM; Nozaki et al. 2007b). The IC_{50} values for monkey (2.97–5.67 μM; Tahara et al. 2006a) and rat (1.13–9.0 μM; Jung et al. 2001; Takeda et al. 2001; Khamdang et al. 2004; Tahara et al. 2005a; Minematsu et al. 2008) Oat3 are within the same range. Hence, OAT1 and OAT3 are similarly sensitive to probenecid.

4.8 Drug/Drug Interactions

OAT3/Oat3 interacts with numerous drugs. It is highly likely that drug–drug interaction takes place during drug uptake from the blood into renal proximal tubule cells by competition for OAT3/Oat3. The interactions mentioned in Sect. 2.8 for OAT1/Oat1 may well hold also for OAT3/Oat3 because of the overlapping substrate specificities.

Probenecid/benzylpenicillin interaction (for literature see Sect. 2.8) is likely to primarily occur at OAT3 rather than at OAT1, because (1) OAT3/Oat3 has a higher affinity to this ß-lactam antibiotic (IC_{50} for rat Oat1: 418–2,763 μM; Jariyawat et al. 1999; Hasegawa et al. 2002, 2003; Nagata et al. 2002; Deguchi et al. 2004; IC_{50} for

rat Oat3: 52.8–132 μM, Hasegawa et al. 2002; Deguchi et al. 2004), (2) OAT3/Oat3 clearly transported benzylpenicillin (K_m 40–85.1 μM; Hasegawa et al. 2002; Nagata et al. 2002; Ohtsuki et al. 2004; Tahara et al. 2005b), and (3) OAT3/Oat3 is inhibited by probenecid at therapeutic plasma concentrations (see previous). The affinities of OAT1 and OAT3 for many cephalosporins (cefadroxil, cefamandol, cefazolin, cefdinir, cefoselis, ceftibuten, cephaloridine) are comparable, that is, IC_{50} values differ by a factor of less than three, indicating that *probenecid/cephalosporin* interaction (Brown 1993) may take place at both transporters to the same extent.

Probenecid/methotrexate interaction (Aherne et al. 1978) most probably occurs at OAT3, because this transporter has a much higher affinity (K_m 10.9 μM; Cha et al. 2001) than OAT1 (K_m 724 μM; Uwai et al. 2004) for this antineoplastic drug.

The *probenecid/furosemide* interaction (for literature see Sect. 2.8) may take place on both OAT1 (IC_{50} 14–20 μM; Hasannejad et al. 2003; Bahn et al. 2004) and OAT3 (IC_{50} 1.7–7.31 μM; Hasannejad et al. 2003; Chu et al. 2007) due to comparable affinities for this diuretic.

Because OAT1 readily transported several antiviral drugs, it is believed that *probenecid/antiviral drug* interaction takes place at OAT1 rather than at OAT3. Unfortunately, IC_{50} determinations for human OAT3 are scarce precluding a comparison of affinities. For mouse Oat1 and Oat3, comparable IC_{50} values have been found for a number of antivirals, however, data on transport are lacking. Hence, a final conclusion as to whether OAT1, OAT3 or both are involved in probenecid/antiviral interaction remains open. The same holds true for *NSAID/antiviral drug* interaction.

The *probenecid/fexofenadine* interaction is likely to take place at OAT3. The antihistaminic fexofenadine was taken up by OAT3-, but not by OAT1-expressing cells and probenecid inhibited uptake with a K_i of 1.3 μM (Tahara et al. 2005a).

As mentioned earlier, *NSAID/methotrexate* interaction can lead to life-threatening side effects. OAT3 has a higher affinity for methotrexate (K_m 10.9 μM; Cha et al. 2001) than OAT1 (K_m 724 μM; Uwai et al. 2004) and must thus be regarded as the main transporter for renal methotrexate excretion. The affinity of OAT3 for most NSAIDs is similar to that of OAT1. Exceptions are phenacetin, phenylbutazone, piroxicam, salicylate, and sulindac, for which OAT3 has a considerably higher affinity than OAT1. More important is to consider the IC_{50} values for NSAIDs in relation to their free plasma concentrations (Takeda et al. 2002c; Nozaki et al. 2004, 2007b). Only salicylate (IC_{50} at OAT3 50–111 μM; Khamdang et al. 2002; Nozaki et al. 2007b; free plasma concentration: 55–440 μM), phenylbutazone (IC_{50} 6.82 μM; free plasma concentration 6.3–19 μM; Nozaki et al. 2007b), indomethacin (IC_{50} 0.61–0.98 μM; Khamdang et al. 2002; Nozaki et al. 2007b; free plasma concentration 0.084–8.4 μM), and loxoprofen (IC_{50} 12.2 μM; free plasma concentration 20 μM; Uwai et al. 2004) can inhibit OAT3 in vivo in order to decrease renal methotrexate excretion (Takeda et al. 2002c). Why other NSAIDs are also causing side effects is not clear. Possibly this interaction takes place at other transporters, for example, MRP4 (Nozaki et al. 2007b).

Gemfibrozil/pravastatin interaction: the administration of the lipid lowering drug gemfibrozil decreased the renal clearance of pravastatin. Because OAT3, but not OAT1, transported labeled pravastatin and pravastatin was inhibited

by gemfibrozil, it is likely that OAT3 is involved in this drug–drug interaction (Nakagomi-Hagihara et al. 2007)

Gemcabene/quinaprilat interaction: the lipid lowering drug gemcabene increases the antihypertensive action of quinaprilat. Human and rat OAT3/Oat3 transport quinaprilat much more rapidly than OAT1/Oat1; gemcabene and its glucuronide inhibited quinaprilat transport, suggesting that drug interaction takes place at OAT3 (Yuan et al. 2009).

4.9 Pharmacogenomics

The overall mutation rate for OAT3 was with 0.74×10^{-4} lower than the average rate for the whole genome (2×10^{-4}; Urban et al. 2006). Within the 5′-untranslated region Bhatnagar et al. (2006) described seven polymorphisms found in probes of 96 persons. Of these, two were quite frequent (G → C at −1.882; $f = 0.47$; G → A at −1.851; $f = 0.2$) whereas the reminder showed fequencies of 0.0005 (Urban et al. 2006). Ogasawara et al. (2008) described five single nucleotide polymorphisms in the 5′ untranslated region. None of them had an influence on the expression as tested with luciferase constructs. In 120 healthy Japanese, Nishizato et al. (2003) found five SNPs, one being nonsynonymous leading to the amino acid exchange A389V (frequency 0.017). None of the SNPs had an influence on the kinetics of pravastatin transport. Additional nonsynonymous SNPs were reported by Erdman et al. (2005) and Urban et al. (2006). These correspond to the following amino acid replacements: F129L, R149S, N239X, I260R, R277W, V281A, I305F, A399S, and V488I. Q239X and I260R occurred only in Asian-Americans ($f = 0.002$), and R277W in African-Americans ($f = 0.0002$); R149S was found in Europeans and Asian-Americans ($f = 0.004$), I305F in Asian-Americans ($f = 0.035$) and Mexican-Americans ($f = 0.011$). Following expression, the mutants F149S, Q239X, and I260R did not function at all, and the mutants R277W and I305F showed a slower transport of estrone-3-sulfate with a slight preference for cimetidine as compared to the wild type (Nishizato et al. 2003; Urban et al. 2006). Thus, three loss-of-function mutations (R149S, Q239X, I260R) occur in Asian-Americans with possible impact on their drug excretion.

5 Organic Anion Transporter 4 (OAT4, Gene Name SLC22A11)

5.1 Cloning, Structure

The organic anion transporter 4 (OAT4) is human-specific, that is, there is no known ortholog in rodents or other species. The OAT4 protein consists of 550 amino acids arranged in twelve putative transmembrane helices, and five potential

N-glycosylation sites and nine putative protein kinase C phosphorylation sites were reported (Cha et al. 2000). The glycosylation was required for targeting of OAT4 to the membrane and had an influence on the affinity toward estrone-3-sulfate (Zhou et al. 2005). The *C*-terminal three amino acids, threonine, serine, leucine (TSL), constitute a PDZ binding motif. Indeed, OAT4 was found to interact with the scaffolding proteins PDZK1 and NHERF1 (Kato et al. 2004; Miyazaki et al. 2005). In LLC-PK1 cells, coexpression of PDZK1 or NHERF1 increased the surface expression of OAT4, and truncation of the last three amino acids abolished the effect (Zhou et al. 2007b). Likewise, coexpression in HEK293 cells of PDZK1 or NHERF1 with OAT4 increased maximal transport rate without affecting the affinity (Miyazaki et al. 2005), indicating that the interaction of the PDZ domain of OAT4 is important for proper targeting to, and maintenance of the transporter in the apical cell membrane of renal cells. Mutational analysis on OAT4 revealed the importance for the glycine residues G241 and G400 as well as of several histidine residues for targeting and substrate affinity (Zhou et al. 2004a, b).

The gene for OAT4, SLC22A11, is located on chromosome 11q13.1 and is paired with the gene SLC22A12 coding for URAT1 (Eraly et al. 2003b). It should be noted that another transporter, cloned by Sun et al. (2001) and also named OAT4 (renamed OAT7; gene SLC22A9), is not identical to the OAT4 described here (see Sect. 10.1).

5.2 Tissue Distribution of mRNA

OAT4 mRNA transcripts were only found in kidney, placenta (Cha et al. 2000; Nishimura and Naito 2005; Bleasby et al. 2006), and adrenal gland (Asif et al. 2005). Adipose tissue, bladder, different regions of the brain, cervix, colon, duodenum, epididymis, heart, ileum, jejunum, liver, lung, lymph node, mammary gland, olfactory mucosa, ovary, pancreas, peripheral leukocytes, pituitary, prostate, retina, salivary gland, skeletal muscle, skin, spinal cord, stomach, testis, thymus, thyroid gland, trachea, and uterus were all negative (see Table 2; Nishimura and Naito 2005; Bleasby et al. 2006).

5.3 Immunolocalization of OAT4 Protein

Immunohistochemical staining of hOAT4 was found in the luminal membrane of proximal tubule cells (Babu et al. 2002b; Ekaratanawong et al. 2004). Thereby, OAT4 and the interacting scaffolding proteins PDZK1 and NHERF1 are colocalized at the apical cell pole (Miyazaki et al. 2005). It is clear from these findings that OAT4 is involved in the release of organic anions into the urine and/or in the uptake of organic anions from the primary filtrate into the proximal tubule cells. In the placenta, hOAT4 appears at the basal (fetal) side of the syncytiotrophoblast where it

takes up sulfated C19-steroid precursers for placental estrogen synthesis (Ugele et al. 2003, 2008).

5.4 Species Differences, Age and Gender Dependence of Expression

OAT4 is expressed only in humans. Presently, it is unclear which transporter in other species takes over the task of human OAT4. Data on age- and gender-dependent expression of OAT4 are not available.

5.5 Factors Influencing Activity and Abundance of OAT4

Because OAT4 is only expressed in humans, animal disease models cannot be used to study the influence of pathophysiological states on OAT4 expression.

5.6 Substrates

Similar to other OATs is OAT4 able to operate as an organic anion/dicarboxylate exchanger (Ekaratanawong et al. 2004). Later on, it became clear that OAT4 has several modes of operation. In the influx (absorption) mode it couples the uptake of estrone-3-sulfate or urate to the release of dicarboxylates (α-ketoglutarate); in the efflux (secretion) mode, organic anions such as *p*-aminohippurate and drugs can be exchanged against extracellular chloride (Hagos et al. 2007b). In addition, OAT4 is able to perform organic anion/hydroxyl ion exchange and can thus be functionally coupled to the Na^+/H^+ exchanger NHE3 in the brush-border membrane (Hagos et al. 2007b). Hence, OAT4 accepts inorganic (hydroxyl, chloride) and organic anions.

The prototypical organic anion to study OAT4 is estrone-3-sulfate (ES); the reported K_m values for ES uptake range between 1.01 and 21.7 µM (mean 9.89 µM in six determinations; Cha et al. 2000; Yamashita et al. 2006; Zhou et al. 2005, 2006, 2007a; Ugele et al. 2008). As a nonradioactive substrate, 6-carboxyfluorescein uptake into OAT4-expressing cells can be measured fluorimetrically (K_m for uptake: 108 µM; Hagos et al. 2007b).

5.6.1 Endogeneous Substrates

Second messengers. *cGMP* was not transported (Cropp et al. 2008); cAMP was not tested.

Citric acid cycle intermediates, other dicarboxylates. Citrate, succinate, fumarate, and oxaloacetate, but not malate inhibited OAT4 (Hagos et al. 2007b). For α-ketoglutarate and its nonphysiologic derivative glutarate, contradictory results were obtained (inhibition: Ekaratanawong et al. 2004; Anzai et al. 2005; Yamashita et al. 2006; Hagos et al. 2007b; no inhibition: Cha et al. 2000; Hagos et al. 2008). Also for glutarate uptake by OAT4, mixed results were reported (uptake, Ekaratanawong et al. 2004; no uptake, Hagos et al. 2007b). However, *trans*-stimulation of OAT-mediated transport by intracellular glutarate was observed in both studies. The differing results probably reflect the asymmetry of transport: (α-keto-)glutarate is better accepted from the cytosolic side for *trans*-stimulation, and less so from the extracellular side, explaining mixed effects in *cis*-inhibition experiments.

Monocarboxylates. Lactate and pyruvate did not inhibit OAT4 (Hagos et al. 2007b). The short chain fatty acid octanoate, however, inhibited the transporter (Babu et al. 2002b).

Bile salts. Cholate and taurocholate inhibited OAT4 (Cha et al. 2000; Yamashita et al. 2006). Taurochenodesoxycholate did not interact with OAT4 (Yamashita et al. 2006).

Hormones and hormone derivatives. Transport was shown for estrone-3-sulfate (Cha et al. 2000; Zhou et al. 2004a, b, 2005; Miyazaki et al. 2005; Yamashita et al. 2006; Zhou et al. 2006, 2007a; Ugele et al. 2008). Based on the K_m values for uptake, OAT4 has a high affinity for ES (mean K_m at 9.89 μM; see previous). Transport was also demonstrated for dehydroepiandrosterone sulfate (DHEAS; K_m between 0.63 and 29.2 μM; Cha et al. 2000; Ugele et al. 2008). Corticosterone inhibited OAT4, but was not transported (Cha et al. 2000; Asif et al. 2005). Inhibitions were demonstrated for estrone, 17ß-estradiol-3-sulfate, ß-estradiol-3-sulfate, ß-estradiol-3,7-disulfate, but not for ß-estradiol, ß-estradiol-3ßD-glucuronide, and progesterone (Cha et al. 2000; Yamashita et al. 2006; Zhou et al. 2005, 2006)

Local hormones. Prostaglandin E_2 (K_m 0.154 μM) and prostaglandin $F_{2\alpha}$ (K_m 0.692 μM) were transported by OAT4 with very high affinities (Kimura et al. 2002).

Purine metabolite. *Urate* was transported by OAT4 and inhibited its function, indicating that urate is a substrate (Iwanaga et al. 2005; Hagos et al. 2007b; Sato et al. 2008).

5.6.2 Drugs

ACE inhibitors. The only substance tested, captopril, inhibited OAT4 (Zhou et al. 2005).

Angiotensin II receptor 1 blockers. Table 7 shows the IC_{50} values for a series of *sartanes*. OAT4 exhibited a high affinity for olmesartan and telmisartan, an intermediate one for candesartan, losartan, pratosartan, and valsartan, and a low affinity for candesartan cilexitil (Yamashita et al. 2006; Sato et al. 2008).

Diuretics. OAT4 transported the loop diuretic bumetanide with high affinity (K_m 0.31 μM) although it inhibited OAT4-mediated ES transport with an IC_{50} of

Table 7 Drugs interacting with OAT4

	Human	
	Transport	Inhibition
ACE inhibitor		
Captopril		+
Angiotensin II receptor blockers		
Candesartan		60–88.9
Candesartan cilexetil		135.2
Losartan		18.0–24.8
Olmesartan		4.4
Pratosartan		31
Telmisartan		1.2
Valsartan		19.9–26.0
Diuretics		
Acetazolamide		415
Bumetanide	0.31	348
Chlorothiazide		2,632
Cyclothiazide		–
Ethacrynate		8.76
Furosemide	–	44.5
Hydrochlorothiazide	+*	+ or –
Methazolamide		–
Torasemide	+*	47.0
Trichlormethiazide		1,505
Statin		
Pravastatin		+
Antibiotics		
Benzylpenicillin		+
Cefadroxil		+
Cefamandol		1,140
Cefazolin		1,740
Cefoperazone		2,800
Cefotaxime		6,150
Ceftriaxone		2,380
Cephaloridine		3,630
Cephalothin		200
Clarithromycin		–
Doxycyclin		–
Enoxacin		–
Erythromycin		–
Levofloxacin		–
Minocyclin		–
Oxytetracycline		–
Tetracycline	122.7	+
Antivirals		
Acyclovir	–	–
Ganciclovir	–	–
Tenofovir		
Valacyclovir	–	
Zidovudine	151.8	+ or –

(*continued*)

Table 7 (continued)

	Human	
	Transport	Inhibition
Antineoplastics		
Methotrexate	17.8	
Mitoxantrone		–
Immune suppressant		
Cyclosporin A		–
Histamine receptor 2 blocker		
Cimetidine		–
NSAIDs		
Acetaminophen		–
Acetylsalicylate	–	–
Diclofenac		34.5
Diflusinal		+
Ibuprofen	–	103
Indomethacin		10.1
Ketoprofen	(+)	70.3
Mefenamate		61.7
Naproxen		85.4
Phenacetin		–
Phenylbutazone		+
Piroxicam		84.9–107.8
Salicylate	(+)	+ or –
Sulfinpyrazone		+
Sulindac		617
Uricosurics		
Benzbromarone		+ or –
Probenecid		44.4–67.7

+, transport of inhibition was observed without determination of K_m, K_i, or IC_{50}; +*, trans-stimulation of uptake of a test anion; –, no transport or no inhibition was observed; (+), weak transport or inhibition; blank, not determined; numbers, reported K_m, K_i, or IC_{50} values in micromoles/liter (μM). For references see text in Sect. 5.6.2

348 μM (Hasannejad et al. 2003). Torasemide and two of its metabolites, M1 and M3, *trans*-stimulated ES uptake, indicating that OAT4 translocates also this loop diuretic (Hagos et al. 2007a). No transport could be demonstrated for furosemide (Hasannejad et al. 2003). The IC_{50} values for ethacrynate, furosemide, and torasemide were below 50 μM, suggesting that OAT4 has a high to intermediate affinity for these loop diuretics (Hasannejad et al. 2003; Hagos et al. 2007a). The thiazide diuretics showed very low (chlorothiazide, trichlormethiazide) or no (cyclothiazide, hydrochlorothiazide) affinity (Hasannejad et al. 2003). In another study (Hagos et al. 2007b), hydrochlorothiazide *trans*-stimulated ES uptake, suggesting that this diuretic is better bound at the cytosolic side of OAT4 and effluxed than it interacts with the extracellular side to be taken up. Among the carboanhydrase blockers, acetazolamide showed a low affinity, and methazolamide no affinity for OAT4 (Hasannejad et al. 2003).

Statins. Pravastatin inhibited OAT4 (Enomoto et al. 2003). Data on other statins are not available.

Antibiotics. Benzylpenicillin inhibited OAT4, but an IC_{50} has not been reported (Cha et al. 2000; Babu et al. 2002b; Takeda et al. 2002c; Yamashita et al. 2006). With exception of cefadroxil, IC_{50} determinations have been performed for a number of cephalosporines, most of which have a very low affinity for OAT4 (see Table 7; Takeda et al. 2002a). Tetracycline was transported by OAT4 with a K_m of 122.7 μM; all other tetracyclines (doxycycline, minocycline, oxytetracycline) did not show an interaction with OAT4 (Babu et al. 2002a). Other antiobiotics (clarithromycin, enoxacin, erythromycin, levofloxacin) did also not inhibit OAT4 (Yamashita et al. 2006). Taken together, OAT4 has a very limited capacity to interact with antibiotics, at least from the extracellular side.

Antivirals. Acyclovir, ganciclovir, and valacyclovir were not transported by OAT4, and acyclovir, ganciclovir did not inhibit OAT4; only zidovudine was translocated (K_m 151.8 μM) and inhibited OAT4-mediated transport (Takeda et al. 2002b). These data suggest that OAT4 is not primarily involved in transport of antiviral drugs.

Antineoplastics. The folate antagonist methotrexate was transported by OAT4 with appreciable affinity (K_m 17.8 μM; Takeda et al. 2002c); mitoxanthrone did not interact with OAT4 (Yamashita et al. 2006).

Immune suppressants. Cyclosporin A did not interact with OAT4 (Yamashita et al. 2006).

Histamine receptor 2 blockers. Cimetidine showed no inhibition of OAT4 (Babu et al. 2002b; Hashimoto et al. 2004; Khamdang et al. 2004; Yamashita et al. 2006).

Nonsteroidal anti-inflammatory drugs (NSAIDs). A weak uptake of ketoprofen and salicylate was shown whereas acetylsalicylate and ibuprofen were not transported by OAT4 (Khamdang et al. 2002). An inhibition of OAT4 was reported for diclofenac, diflusinal, ibuprofen, indomethacin, ketoprofen, mefenamate, naproxen, piroxicam, sulfinpyrazone, and sulindac (for IC_{50} values see Table 7). No inhibition was found for acetaminophen, acetylsalicylate, phenacetin, and mixed results are available for salicylate and ibuprofen (Cha et al. 2000; Babu et al. 2002b; Khamdang et al. 2002; Takeda et al. 2002c; Yamashita et al. 2006; Zhou et al. 2005). Taken together, OAT4 does interact with most NSAIDs with intermediate affinity, that is, NSAIDs can inhibit OAT4 without being appreciably transported.

Uricosurics. For benzbromarone, either no inhibition (Iwanaga et al. 2005) or a strong inhibition of OAT4 (Hagos et al. 2007b) was found. Probenecid inhibited OAT4 in several studies (Cha et al. 2000; Babu et al. 2002b; Enomoto et al. 2002b, 2003; Takeda et al. 2002c; Hashimoto et al. 2004; Yamashita et al. 2006; Hagos et al. 2007b). The determined K_i values fall into the narrow range between 44.4 and 67.7 μM (mean: 56.2 μM; Babu et al. 2002b; Enomoto et al. 2002b; Hashimoto et al. 2004).

Miscellaneous. The antiepileptic, valproate, was not transported, but inhibited OAT4; a tranquilizer, carbamazepine, and a cardiotonic, digoxin, did not inhibit OAT4 (Yamashita et al. 2006).

5.7 Inhibitors

As indicated previously (Sect. 5.6.2; uricosurics), probenecid is a competitive inhibitor of OAT4 with reasonable affinity (mean K_i 56.2 μM out of three publications).

5.8 Drug/Drug Interactions

Methotrexate/NSAID interaction was tested with human OAT1-4, but only OAT3 showed a high enough affinity for NSAIDs to be appreciably inhibited at pharmacologically meaningful free plasma concentrations (Takeda et al. 2002c). Because OAT4 is involved in the uptake of urate from the primary filtrate, the efflux of torasemide and its metabolites, M1 and M3, and of hydrochlorothiazide causes an increased renal urate reabsorption and consequently an increase in plasma urate (Hagos et al. 2007a, b).

5.9 Pharmacogenomics

In the promoter region, an SNP was found that, however, did not cause a change in gene expression (Ogasawara et al. 2008). In the coding region, three synonymous and eight nonsynonymous SNPs were reported. The latter ones gave rise to the amino acid exchanges V13M, R48X, T62R, V155M, A244V, E278K, V339M, and T392I (Xu et al. 2005). The deleterious R48X mutation was found in one probe out of 18 specimens of Northern Europeans. Ashkenazi-Jewish people did not show any SNPs; the other described SNPs occurred in Chinese, Mexican-Americans, and Sahara-Africans. The functional consequences of the mutations are unknown. In comparison to OAT1 and OAT3, much more nucleotide variations were found for OAT4 (and URAT1; Xu et al. 2005).

6 Urate Transporter 1 (URAT1; Urat1/Rst, Gene Name SLC22A12/Slc22a12)

6.1 Cloning, Structure

Initially, this transporter was cloned as “renal specific transporter” (Rst) from mouse kidney (Mori et al. 1997). The protein consisted of 553 amino acids arranged in twelve transmembrane helices. *N*-glycosylation sites in the large extracellular

loop between helices 1 and 2 as well as several potential phosphorylation sites for protein kinases A and C were found, but function was not demonstrated. The first functional cloning of the human ortholog revealed its involvement in renal urate reabsorption, and the name URAT1 was proposed without reference to RST and to the OAT family (Enomoto et al. 2002a). The human URAT1 protein consisted of 555 amino acids and showed 42% identity with OAT4. A functional clone of mouse Rst/Urat1 was also obtained (Hosoyamada et al. 2004; Imaoka et al. 2004). The gene, SLC22A12, for human URAT1 is located on chromosome 11q13.1, being paired with the gene SLC22A11 for OAT4 (Eraly et al. 2003b). A promoter fragment of the human URAT1 was cloned containing 1,863 bp of the 5′-untranslated region (Li et al. 2004). The 5′-UTR regions from human, rat, and mouse were 80% identical containing the same transcription factor binding sites including HNF1-, CEBP-, AP1-, and GATA1 sites.

6.2 Tissue Distribution of mRNA

URAT1/Rst expression has been detected in human and mouse kidneys. In situ hybridization localized Rst mRNA to the proximal tubules (Mori et al. 1997). Human URAT1 mRNA and protein were also detected in human vascular smooth muscle cells by RT-PCR and Western blot analysis (Price et al. 2006). Except for some expression in testis, all other human tissue samples were negative for URAT1 (Nishimura and Naito 2005). Bleasby et al. (2006), however, found URAT1 mRNA also in samples from human adrenal gland, brain, colon, heart, ileum, jejunum, liver, lung, pancreas, placenta, peripheral leukocytes, pituitary, prostate, salivary gland, skeletal muscle, stomach, and testis. Mouse Urat1/Rst was mainly found in the kidneys and additionally at the blood–brain barrier and the choroid plexus (Imaoka et al. 2004).

6.3 Immunolocalization of URAT1/Urat1/Rst Protein

Human and mouse URAT1/Rst are localized at the apical (brush-border) membrane of proximal tubule cells (Anzai et al. 2004; Hosoyamada et al. 2004; Xu et al. 2006a). Thereby, URAT1 interacts through its C-terminal amino acids STQF with the PDZ domains 1, 3, and 4 of the scaffolding protein PDZK1. Coexpression of PDZK1 with URAT1 increased surface expression and maximal transport velocity (Anzai et al. 2004). In addition, mouse Urat1/Rst interacted with NHERF1; mice deficient in NHERF1 showed less Urat1/Rst protein in the apical membrane, more protein in the cytoplasm, and a higher renal urate excretion (Cunningham et al. 2007).

6.4 Species Differences, Age and Gender Dependence of Expression

Species differences and age dependence of URAT1/Rst expression are unknown. However, in mice there are clear gender differences in Rst expression: male mice showed 2.3-fold more Urat1/Rst protein than female mice (Hosoyamada et al. 2004), indicating a higher urate reabsorption capacity in males. It appears likely that a similar gender difference is present in humans, because men have higher urate levels than women (Hediger et al. 2005).

6.5 Factors Influencing Activity and Abundance of URAT1/Urat1/Rst

HNF-1α and HNF-1β increased the expression of reporter constructs with human or mouse URAT1/Urat1 and bound to the respective HNF sites at the promoters (Kikuchi et al. 2007). Likewise, Urat1/Rst expression was diminished in HNF-1α knockout mice. The Urat1/Rst promoter was found to be relatively hypomethylated in mouse kidney, suggesting that a potentially tissue-specific epigenetic control over Urat1/rst expression takes place (Kikuchi et al. 2007).

URAT1 transport activity depends on its proper localization in the apical plasma membrane. As for another transporter in the apical membrane, OAT4, binding to the apically located scaffolding proteins (PDZK1 and NHERF1) ensures insertion into and maintance within the brush-border membrane (see previous). Factors influencing this process or triggering endocytotic retrieval are not known.

6.6 Substrates

Similar to the other family members, URAT1 operates as an anion exchanger. The predominant mode is most probably urate uptake from the filtrate into the cell in exchange for intracellular lactate being released into the filtrate (Enomoto et al. 2002a; Hosoyamada et al. 2004). Lactate, in turn, is taken back up into the cell via a sodium-coupled lactate transporter, SMCT (SLC5A8; Gopal et al. 2004). Thus, urate absorption is tertiary active with the Na^+, K^+-ATPase in the basolateral membrane being the primary active system, the SMCT the secondary active system utilizing the Na^+ gradient for intracellular lactate accumulation, and URAT1/Rst the tertiary system being driven by the lactate gradient. URAT1 also transports chloride (Enomoto et al. 2002a; Hosoyamada et al. 2004). Given the higher chloride concentration in the tubule lumen, chloride influx could drive the efflux of lactate or other organic anions. The physiological importance of this transport mode is not clear. Uptake of organic anions by mouse Urat1/Rst was accelerated by an inside

positive membrane potential (Hosoyamada et al. 2004). It is not clear whether potential-dependent uniport is another operation mode of the transporter otherwise working as an electroneutral anion exchanger.

The prototypic test anion for URAT1/Rst is urate, which is transported with low affinity (human: K_m between 198.7 and 371 μM; Enomoto et al. 2002a; Anzai et al. 2004; Iwanaga et al. 2007; mouse: K_m 1,213 μM; Hosoyamada et al. 2004).

6.6.1 Endogenous Substrates

Inorganic anions. Extracellular chloride *cis*-inhibited urate uptake and intracellular chloride *trans*-stimulated urate efflux. Similar effects were seen with bromide, iodide, nitrate, but not fluoride (Enomoto et al. 2002a; Hosoyamada et al. 2004). Thus URAT1/Rst can perform the exchange between an inorganic anion (physiologically chloride) and an organic anion, for example, urate.

Monocarboxylates. Extracellular lactate *cis*-inhibited and intracellular lactate *trans*-stimulated urate uptake (Enomoto et al. 2002a). Similar effects were seen with nicotinate, and a number of drugs discussed in the following. For extracellular acetoacetate and ß-hydroxybutyrate (10 mM each), an inhibition of URAT1 was shown, suggesting that these organic anions may interact with URAT1, at least at high concentrations (Enomoto et al. 2002a).

Dicarboxylates. Inhibition was found for α-ketoglutarate and succinate, again at high concentrations (Enomoto et al. 2002a). Whether URAT1 can perform organic anion/dicarboxylate exchange is unknown.

Purine metabolism. As already discussed, urate is the prototypic anion transported by URAT1/Rst. Orotate inhibited URAT1, but xanthine did not inhibit human and mouse URAT1/Rst (Enomoto et al. 2002a; Hosoyamada et al. 2004).

Hormones, hormone derivatives. Estrone-3-sulfate did not interact with human and mouse URAT1/rst (Enomoto et al. 2002a; Hosoyamada et al. 2004). To our knowledge, only OAT1 and URAT1 do not transport ES. Dehydroepiandrosterone sulfate inhibited mouse Urat1/Rst; uptake of DHEAS was stimulated by an inside positive membrane potential (Hosoyamada et al. 2004).

Anionic neurotransmitter metabolites. 3,4-dihydroxymandelate, 3-methoxy-4-hydroxymandelate, 5-hydroxyindole acetate, 3-methoxy-4-hydroxyphenylacetate, and 5-methoxyindoleacetate inhibited mouse Urat1/Rst (Imaoka et al. 2004). The importance of URAT1 in removing these metabolites from the brain across the blood–brain barrier and in secreting them into the urine remains to be elucidated.

6.6.2 Drugs

Not tested were ACE inhibitors, statins, antiviral drugs, immune suppressants, antidiabetics, and histamine receptor blockers.

Angiotensin II receptor 1 blockers. Losartan, which is uricosuric, inhibited human URAT1 with extremely high affinity (K_i 0.0077 μM; Iwanaga et al. 2007).

Pratosartan (K_i 0.0067 μM) and telmisartan (K_i 0.0182 μM) were equally effective inhibitors. At 0.01 μM, candesartan and olmesartan did not inhibit URAT1. But, following intracellular preloading, candesartan, losartan, olmesartan, and pratosartan, but not telmisartan, *trans*-stimulated urate uptake, indicating that angiotensin receptor blockers are accepted from the cytosolic side and serve as counter anions for urate uptake (Iwanaga et al. 2007). Thus, all these drugs should accelerate urate absorption with exception of losartan and pratosartan that both are filtered at concentrations sufficient to effectively compete with urate for URAT1.

Diuretics. Furosemide inhibited URAT1 (Enomoto et al. 2002a). Other diuretics were not tested with exception of torasemide that did not inhibit URAT1 (Hagos et al. 2007a).

Antibiotics. The only antibiotic tested was benzylpenicillin that inhibited mouse Urat1/Rst and showed an uptake stimulated by an inside positive potential (Hosoyamada et al. 2004).

Antineoplastics. Methotrexate did not inhibit URAT1 (Enomoto et al. 2002a).

Nonsteroidal anti-inflammatory drugs. Indomethacin, phenylbutazone, salicylate, and sulfinpyrazone inhibited human URAT1 (Enomoto et al. 2002a).

Uricosurics. The increased renal excretion of urate could be due to the inhibition of the dominant reabsorptive transporter, URAT1, by uricosurics. Indeed, benzbromarone and probenecid inhibited human and mouse URAT1/Rst (Enomoto et al. 2002a; Hosoyamada et al. 2004; Imaoka et al. 2004; Iwanaga et al. 2005; Hagos et al. 2007a). IC_{50} values are, however, not available.

Antiuricosuric. Pyrazinoate is known for a long time to increase urate reabsorption. It has been shown for human and mouse URAT1/Rst that intracellular pyrazinoate exchanges readily with extracellular urate, thus driving urate absorption in proximal tubules (Enomoto et al. 2002a; Hosoyamada et al. 2004; Iwanaga et al. 2007). Pyrazinoate may be taken back up into proximal tubule cells by the Na^+-driven lactate transporter (SMCT; SLC5A8; Gopal et al. 2004).

6.7 Inhibitors

Special inhibitors are not known. We could recommend losartan as an inhibitor with very high affinity (Iwanaga et al. 2007).

6.8 Drug/Drug Interactions

Drug–drug interactions are not known. Clinically relevant are the drug/urate interactions, which either lead to a higher (losartan) or lower (pyrazine as precursor of pyrazinoate) renal urate excretion.

6.9 Pharmacogenomics

URAT1 is the only transporter of the SLC22 family for which mutations have been clearly related to a disease. Loss-of-function mutations cause the familial idiopathic hypouricemia (lowered plasma levels of urate), a disease detected in the Japanese and Korean populations. The first mutation to be reported was a truncation mutation, W258X (Enomoto et al. 2002a). Most of the hypouricemic patients (74.1%) carry this mutation (Enomoto and Endou 2005). Further mutations detected in the patients were R90H, V138M, G164S, T217M, Q382L, G412A, M430T, R477H, G490A (Ichida et al. 2004; Komoda et al. 2004; Cheong et al. 2005). In healthy persons, six synonymous single nucleotide polymorphisms were found (Xu et al. 2005).

7 Organic Anion Transporter 10 (OAT10/ORCTL3, Gene Name SLC22A13)

7.1 Cloning, Structure

The organic cation transporter like 3 (ORCTL3) and ORCTL4 were cloned as a byproduct in the search for tumor-associated genes on chromosome 3 (Nishiwaki et al. 1998). From the structure of ORCTL3 and ORCTL4, the authors concluded that the gene products must be organic cation transporters. A functional investigation was not performed at that time. Bahn et al. (2008) expressed human ORCTL3 and found it to transport organic anions, but not organic cations. Therefore, they proposed to rename ORCTL3 as OAT10. The gene for OAT10 is located on chromosome 3p21.2, shows 10 exons, and is paired with that of ORCTL4 (Nishiwaki et al. 1998). The predicted OAT10 protein has 551 amino acids. Evolutionarily speaking, OAT10 constitutes its own branch. BLAST searches revealed rat and mouse orthologs that have not been functionally tested so far (Bahn et al. 2008).

7.2 Tissue Distribution of mRNA

ORCTL3 was found to be ubiquitously distributed (Nishiwaki et al. 1998). Full-length OAT10, however, was expressed predominantly in the kidneys with weaker signals in brain, heart, and colon. Splice variants without exon 3 or/and exon 4 were expressed in many organs (Bahn et al. 2008). The model cell line for the investigation of intestinal absorption, Caco-2, expressed the full-length OAT10 (Bahn et al. 2008). Within the kidneys, mRNA for OAT10 was mainly expressed in proximal tubules and – to a lesser extent – in cortical collecting ducts (Bahn et al. 2008).

7.3 Immunolocalization of OAT10 Protein, Gender Differences

Western blots showed a 55 kDa band in brush-border, but not in basolateral membranes, suggesting a localization in the apical membrane of tubule cells. The band was stronger in brush-border membranes from female rats than from male rats, indicating a female-dominant expression (Bahn et al. 2008).

7.4 Substrates

Similar to other members of the SLC22 family, OAT10 can operate as an anion exchanger. Similar to other OATs located in the apical membrane, the 5-carbon dicarboxylate glutarate is not suited as exchange partner, but rather the 4-carbon succinate. Moreover, lactate and nicotinate can exchange for *p*-aminohippurate or urate. The pH dependence of OAT10 further suggests that an exchange against hydroxyl ions may be a mode of transport. The physiologically most important substrate is nicotinate; OAT10 has a high affinity for this vitamin and is responsible for its uptake from the diet in the intestine and for reabsorption in proximal tubules to avoid its loss with the urine. Next important is the ability of OAT10 to transport urate. Besides URAT1, this transporter should be involved in renal urate reabsorption. The driving force for urate uptake, that is, exchange of urate in the tubule lumen for intracellular lactate, also resembles URAT1, and couples OAT10 functionally to the Na^+-driven lactate transporter SMCT (Bahn et al. 2008).

As a model test anion we suggest nicotinate, the uptake of which by OAT10 is high above background (nonexpressing cells). The K_m is 22 μM (Bahn et al. 2008).

7.4.1 Endogenous Substrates (Bahn et al. 2008)

Monocarboxylates. Extracellular lactate *cis*-inhibited OAT10-mediated urate uptake and intracellular lactate *trans*-stimulated it, indicating that OAT10 transports lactate and can perform urate/lactate exchange. The affinity of OAT10 for lactate has not been determined. *cis*-inhibition and *trans*-stimulation have also been observed for nicotinate, for which a K_m of 22 μM was determined.

Dicarboxylates. Succinate *cis*-inhibited and *trans*-stimulated OAT10-mediated urate transport, whereas the nonphysiologic 5-carbon glutarate showed *cis*-inhibition, but no *trans*-stimulation, suggesting a preference for C4 dicarboxylates.

Glutathione trans-stimulated urate uptake, suggesting that this tripeptide is transported by OAT10, at least from the intra- to the extracellular compartment.

7.4.2 Drugs (Bahn et al. 2008)

Diuretics. Furosemide and hydrochlorothiazide inhibited OAT10. Further diuretics were not tested. Hydrochlorothiazide was not able to *trans*-stimulate urate uptake, leaving open whether this thiazide can be transported by OAT10.

Immune suppressants. Cyclosporin A *cis*-inhibited OAT10-mediated nicotinate uptake and *trans*-stimulated urate uptake, suggesting that this compound is indeed translocated by OAT10. It is open whether OAT10 is involved in cyclosporin A-induced damage of proximal tubule cells.

Nonsteroidal anti-inflammatory drugs. Only sulfinpyrazone was tested that inhibited OAT10-mediated nicotinate transport.

7.5 Inhibitors, Drug/Drug Interactions, Pharmacogenomics

Probenecid showed a weak inhibition. An IC_{50} value has not been determined. At present, a good and specific inhibitor is not at hand. There are no reports on drug/drug interactions and single nucleotide polymorphisms.

8 Organic Anion Transporter 5 (Oat5, Gene Name Slc22a19)

8.1 Cloning, Structure

Oat5 was cloned from rat (Anzai et al. 2005) and mouse (Youngblood and Sweet 2004). A human orthologue is not existent, the human OAT5 cloned by Sun et al. (2001) being not related to rat and mouse Oat5. Rat Oat5 has 551 amino acids, twelve putative transmembrane helices, four *N*-glycosylation, and five putative protein kinase C phosphorylation sites.

8.2 Tissue Distribution of mRNA, Immunolocalization, Gender Differences

Rat and mouse Oat5 are restricted to the kidney with no gender difference (Youngblood and Sweet 2004). With antibodies a localization at the apical (brush-border) membrane has been shown in the late segments ($S2 < S3$) of the proximal tubule (Anzai et al. 2005; Kwak et al. 2005a). More recently, a female-dominant Oat5 protein expression was found in rats (Sabolic and collaborators, unpublished results).

8.3 Substrates

Oat5 did not interact with glutarate, a 5-carbon dicarboxylate, but – in one study (Anzai et al. 2005) – readily with succinate, a 4-carbon dicarboxylate. Because intracellular succinate *trans*-stimulated, Oat5 may operate as an organic anion/dicarboxylate exchanger similar to OAT1-4. Unlike the other OATs, however, C5 dicarboxylates may not be the preferred counter anion. In another study (Youngblood and Sweet 2004), intracellular succinate was unable to *trans*-stimulate Oat5. Thus, more work is needed to finally clarify the mode of transport.

In most tests, estrone-3-sulfate has been used as a test anion. The K_m of rat Oat5 for ES was 18.9 µM (Anzai et al. 2005), whereas the K_m of mouse Oat5 was 2.2 µM (Kwak et al. 2005a). Other researchers used ochratoxin A (K_m 0.34 µM and 2.0 µM for rat and mouse Oat5, respectively) as test organic anion (Youngblood and Sweet 2004). *p*-aminohippurate, the prototypic test anion for OAT1/Oat1, was not transported (Youngblood and Sweet 2004; Anzai et al. 2005).

8.3.1 Endogenous Substrates

Dicarboxylates. As already mentioned, mixed results have been published for the interaction of Oat5 with succinate. Malate, malonate, and oxalate did not interact with Oat5, but the nonphysiological longer dicarboxylates suberate, pimelate, and azelate inhibited (Youngblood and Sweet 2004; Anzai et al. 2005).

Hormones, hormone derivatives. ES (see previous) and DHEAS (K_m 2.3 µM for rat, and 3.8 µM for mouse Oat5; Anzai et al. 2005; Kwak et al. 2005a) were transported with high affinity. ß-Estradiol sulfate, but not ß-estradiol-3ß-D-glucuronide, inhibited rat Oat5 (Anzai et al. 2005). Rat Oat5 did not transport prostaglandins E_2 and $F_{2\alpha}$ (Anzai et al. 2005).

Urate was not transported (Anzai et al. 2005).

8.3.2 Drugs (Rat Oat5: Anzai et al. 2005; Mouse Oat5: Youngblood and Sweet 2004)

Diuretics. Furosemide inhibited rat Oat5.

Antibiotics. Benzylpenicillin inhibited rat Oat5.

Nonsteroidal anti-inflammatory drugs. Diclofenac and ibuprofen inhibited rat Oat5, and salicylate both rat and mouse Oat5.

8.4 Inhibitors, Drug/Drug Interactions, Pharmacogenomics

Probenecid inhibited rat and mouse Oat5, but there were no IC_{50} reported in the literature (Youngblood and Sweet 2004; Anzai et al. 2005). Drug/drug interactions

are not known. Single nucleotide polymorphisms have, to our knowledge, not been reported.

9 Organic Anion Transporter 6 (Oat6, Gene Name Slc22a20)

9.1 Cloning, Structure, Tissue Distribution

Oat6 was cloned from mouse; similar to the other members of the SLC22 family, the gene was organized in 10 exons and 9 introns (Monte et al. 2004). Unlike the other members, Oat6 was expressed in the nasal epithelium, but not in kidneys and liver. In testis, a low expression of Oat6 mRNA was detected. The exact localization of Oat6 in the nasal epithelium is unknown. Nevertheless it has been postulated that Oat6 is involved in odorant detection, a task for which it must be expressed in olfactory sensory cells. Due to its specificity partially overlapping with that of Oat it was hypothesized that mice can smell with Oat6 what has been excreted by Oat1 with the urine (Kaler et al. 2007b).

9.2 Species Differences, Age and Gender Dependence of Expression; Abundance

The message for Oat6 is detectable on embryonic day e7, that is, before the mRNA for other Oats appears (Monte et al. 2004). Further data are not available.

9.3 Substrates

Uptake of estrone-3-sulfate was *trans*-stimulated by preloading Oat6-expressing oocytes or CHO cells with glutarate, suggesting that this transporter, similar to Oat1 and Oat3, can perform organic anion/α-ketoglutarate exchange (Schnabolk et al. 2006). Whether a Na^+-coupled dicarboxylate transporter that fuels Oat1 and Oat3 in proximal tubules is present also in the nasal epithelium is unknown.

Estrone-3-sulfate appears to be a good test anion for Oat6. K_m values of 45 and 110 μM have been reported (Schnabolk et al. 2006).

9.3.1 Endogenous Substrates

Monocarboxylates. The short chain fatty acids, propionate, butyrate, hexanoate, and heptanoate inhibited Oat6. The respective IC_{50} values (in μM) were 279 for

propionate, 82 for butyrate, 9.0 for hexanoate, and 8.2 for heptanoate (Kaler et al. 2007a), indicating that affinity increased with increasing lipophilicity. The hydrophilic pyruvate inhibited with an IC_{50} of 271 μM; hydroxylated or methylated derivatives of lactate and butuyrate also inhibited Oat6 (Kaler et al. 2007a).

Dicarboxylates. Fumarate and maleate did not inhibit Oat6. The nonphysiologic dicarboxylate glutarate showed a low affinity (IC_{50} 5.51 mM) but *trans*-stimulated ES uptake (Kaler et al. 2007a).

Bile salts. Cholate and taurocholate did not inhibit Oat6 (Kaler et al. 2007a).

Hormones, hormone derivatives. Estrone-3-sulfate inhibited uptake with an IC_{50} of 58 μM and was transported with K_m between 45 and 110 μM (Schnabolk et al. 2006; Kaler et al. 2007a). Estradiol disulfate had a much smaller affinity than ES (IC_{50} of 7.2 mM; Kaler et al. 2007a). Prostaglandin E_2, however, had a high affinity for Oat6 (IC_{50} 18 μM; Kaler et al. 2007a).

9.3.2 Drugs

Antibiotics. Benzylpenicillin (IC_{50} 452 μM or 1.45 mM), carbenicillin (IC_{50} 1.33 mM), and ticarcillin (IC_{50} 533 μM) inhibited Oat6 (Schnabolk et al. 2006; Kaler et al. 2007a).

Antiviral drugs. Stavudine (IC_{50} 1.67 mM), zalzitabine (IC_{50} 729 μM), and zidovudine (IC_{50} 218 μM) inhibited Oat6 whereas acyclovir, adefovir, cidofovir, didanosine, lamivudine, tenofovir did not (Truong et al. 2008).

Antineoplastic drugs. Methotrexate inhibited with an IC_{50} of 597 μM (Kaler et al. 2007a).

Histamine receptor blockers. Cimetidine and histamine itself did not interact with Oat6 (Ahn et al. 2009).

Nonsteroidal anti-inflammatory drugs. Acetylsalicylate (IC_{50} 101 μM), ibuprofen (1.1 μM), and salicylate (IC_{50} 44 or 49 μM) inhibited Oat6 (Schnabolk et al. 2006; Kaler et al. 2007a).

Miscellaneous cationic drugs. Buspirone, clonidine, metoclopramide, nicotine, procainamide, quinidine, and verapamil did not inhibit Oat6 (Ahn et al. 2009).

9.4 Inhibitors

Probenecid had a quite high affinity for Oat6 (IC_{50} 8.3 or 8.4 μM; Schnabolk et al. 2006; Kaler et al. 2007a), suggesting that this uricosuric can be used to block Oat6 efficiently.

9.5 Drug/Drug Interactions: Pharmacogenomics

No data available.

10 Organic Anion Transporter 7 (OAT7, Gene Name SLC22A9)

10.1 Cloning, Structure, Tissue Distribution, Localization

OAT7 was cloned from a human liver library (Shin et al. 2007) and was previously known as human UST3 or OAT4 (Sun et al. 2001). The gene is located on chromosome 11q12.3 (Table 1), and the protein has 554 amino acids. OAT7 is only expressed in the liver where the protein has been detected at the sinusoidal membrane. Developmental changes and gender differences are unknown.

10.2 Substrates (Shin et al. 2007)

OAT7 transported estrone-3-sulfate (K_m 8.7 μM) and dehydroepiandrosterone sulfate (K_m 2.2 μM) with high affinity. A series of other sulfated compounds including ß-estradiol-sulfate, 4-methylumbeliferyl sulfate, α-naphthylsulfate, and minodixil sulfate inhibited OAT7. Unlike many other OATs OAT7 was not inhibited by *p*-aminohippurate and probenecid. Also unlike OAT1 and OAT3, no interaction with α-ketoglutarate and glutarate was found, but a series of monocarboxylates/short chain fatty acids (nicotinate, lactate, acetate, propionate, butyrate, valerate, caproate) inhibited OAT7, and propionate, butyrate, valerate, and caproate *trans*-stimulated estrone sulfate transport. The authors postulated that OAT7 releases estrone sulfate from hepatocytes in exchange for butyrate. OAT7 does not play a role in uptake of bile acids into hepatocytes, because cholate and taurocholate did not interact. Among drugs, salicylate was not transported, and indomethacin and benzylpenicillin did not inhibit.

11 Organic Anion Transporter 8 (Oat8, Gene Name Slc22a9)

11.1 Cloning, Structure, Tissue Distribution, Localization

This transporter was previously cloned from a rat kidney library as unknown solute transporter 1, Ust1 (Schömig et al. 1998). Meanwhile, functional expression revealed transport of organic anions, and Ust1 was renamed as rat Oat8 (Yokoyama et al. 2008), although the human homologue was named OAT7 by the same group (Shin et al. 2007). Message was found in proximal tubules and collecting ducts of rat kidney, and nowhere else. With antibodies, a colocalization with the V-type H^+-ATPase in intercalated cells was shown. In type A cells, immunoreactivity was found at the apical pole (apical membrane and subapical vesicles), in type B

intercalated cells at the basal cell pole, and in nonA, nonB cells distributed throughout the cell. The physiological role of Oat8 remains to be determined.

11.2 Substrates (Yokoyama et al. 2008)

Oat8 transported estrone-3-sulfate (K_m 3.1 μM) and dehydroepiandrosterone sulfate (K_m 2.1 μM), but not *p*-aminohippurate, urate, prostaglandin E_2 and prostaglandin $F_{2\alpha}$. The dicarboxylates succinate, glutarate, suberate, and azelate, but not malonate, adipate, and pimelate inhibited Oat8, and glutarate *trans*-stimulated Oat8-mediated ES uptake, suggesting that Oat8 functions as an organic anion/dicarboxlyate exchanger. Taurocholate inhibited transport, suggesting an interaction of bile salts with Oat8. The diuretic furosemide, but not bumetanide, the NSAID salicylate, but not ibuprofen, inhibited Oat8. No inhibition was seen with penicillin G. The cytostatic methotrexate inhibited Oat8.

12 Organic Anion Transporter 9 (Oat9, Gene Name Unknown)

12.1 Cloning, Tissue Distribution, Substrates

Oat9 was cloned from mouse and is expressed in kidneys and brain (Anzai et al. 2006). Transported endogenous substrates were nicotinate and prostaglandin E_2. Among drugs, only salicylate was tested and found to be transported (Anzai et al. 2006).

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Organic Cation Transporters (OCTs, MATEs), In Vitro and In Vivo Evidence for the Importance in Drug Therapy

Anne T. Nies, Hermann Koepsell, Katja Damme, and Matthias Schwab

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Abstract Organic cation transporters (OCTs) of the solute carrier family (SLC) 22 and multidrug and toxin extrusion (MATE) transporters of the SLC47 family have been identified as uptake and efflux transporters, respectively, for xenobiotics including several clinically used drugs such as the antidiabetic agent metformin, the antiviral agent lamivudine, and the anticancer drug oxaliplatin. Expression of human OCT1 (SLC22A1) and OCT2 (SLC22A2) is highly restricted to the liver and kidney, respectively. By contrast, OCT3 (SLC22A3) is more widely distributed. MATEs (SLC47A1, SLC47A2) are predominantly expressed in human kidney. Data on in vitro studies reporting a large number of substrates and inhibitors of OCTs and MATEs are systematically summarized. Several genetic variants of human OCTs and in part of MATE1 have been reported, and some of them result in reduced in vitro transport activity corroborating data from studies with knockout mice. A comprehensive overview is given on currently known genotype–phenotype correlations for variants in OCTs and MATE1 related to protein expression, pharmacokinetics/-dynamics of transporter substrates, treatment outcome, and disease susceptibility.

Keywords Drug transporters · Organic cation transport · Excretion · OCT1 · OCT2 · OCT3 · MATE1 · MATE2-K · Liver · Kidney · tissue distribution · Knockout mice · Pharmacogenomics · Genotype–phenotype correlation · Metformin · Single nucleotide polymorphisms · Drug response · Interindividual variability · Drug–drug interaction · Pharmacokinetics

1 Introduction

A large number of clinically used drugs are administered orally, from which approximately 40% are cations or weak bases at physiological pH (Neuhoff et al. 2003). For absorption, distribution, metabolism, and elimination (ADME), they need to be taken up into and effluxed from various cell types in the body. Several families of membrane transporters have been recognized to play a role in the transport of organic cations across the plasma membrane. These include members of the solute carrier (SLC) family 22 (organic cation transporters, OCTs) and of the SLC family 47 (multidrug and toxin extrusion, MATEs) (Koepsell et al. 2007). The

human SLC22 family can be divided into several subgroups according to substrates and transport mechanisms (Koepsell and Endou 2004) (Fig. 1). One subgroup comprises OCT1, OCT2, and OCT3, which translocate organic cations and weak bases in an electrogenic manner. Human MATE transporters have only recently been identified as proton/cation antiporters participating in the excretion of organic cations in the liver and kidney (Otsuka et al. 2005; Masuda et al. 2006). Alterations in the expression and function of these transporters may significantly contribute to drug pharmacokinetics and the interindividual variability of drug response. This review summarizes current knowledge about the molecular characteristics, tissue

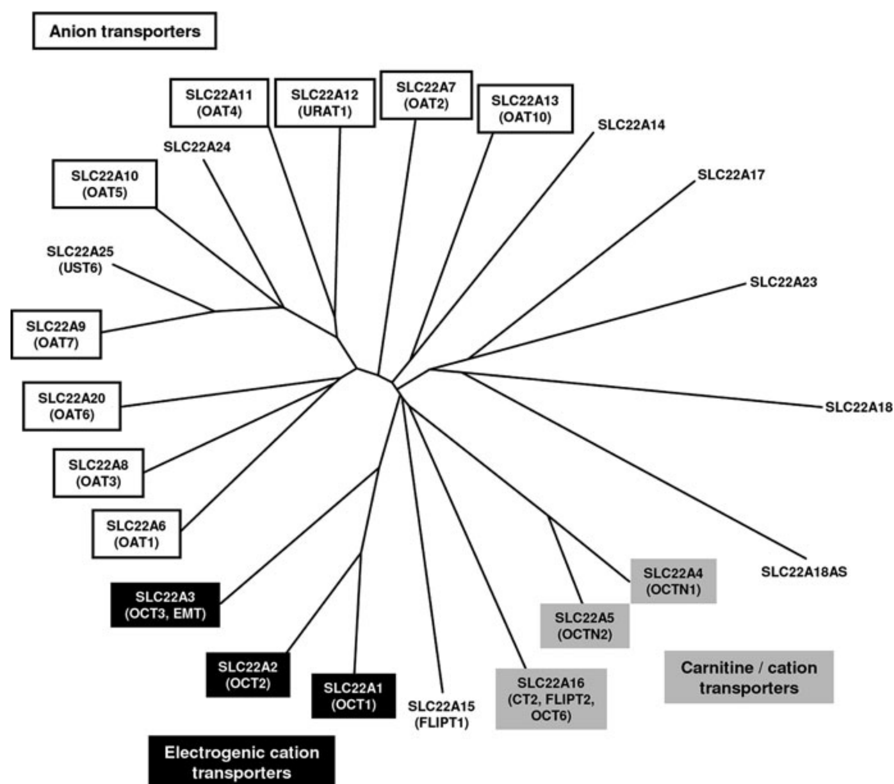

Fig. 1 Phylogenetic tree of the 23 transporters of the human SLC22 family. Protein sequences were downloaded from the NCBI gene database and aligned with the ClustalX2 program (Larkin et al. 2007). The tree was drawn with the "drawtree" program of the PHYLIP3.67 program package (http://evolution.genetics.washington.edu/phylip.html). The distance along the branches is inversely correlated to the degree of sequence identity. For example, the amino acid sequence identity of OCT1 and OCT2 is 70% and that of OCT1 and URAT1 31%. Electrogenic cation transporters are marked by *black boxes*, transporters for organic cations and carnitine by *gray boxes*, and transporters for organic anions by *white boxes*. Transporters whose function is as yet unknown are unmarked

distribution, (drug) substrates and inhibitors, drug–drug interactions, and the fast-growing field of pharmacogenomics of human OCT and MATE transporters.

2 Cloning and Molecular Characterization of OCT and MATE Transporters

A large number of physiological and biochemical studies had suggested the presence of different carrier systems mediating the transport of organic solutes in hepatocytes and renal proximal tubule cells (Giacomini et al. 1988; Boyer et al. 1992). However, molecular identification of these transporters succeeded not until molecular biology techniques became available in the late 1980s. The first member of the electrogenic OCT family was isolated from rat kidney by expression cloning (Gründemann et al. 1994). It took another 11 years until Otsuka et al. identified in 2005 human orthologs of the bacterial MATE family as proton/organic cation exchangers responsible for the electroneutral transport of organic cations into bile and urine.

2.1 OCT Transporters

The genes encoding human OCT1 (gene symbol: SLC22A1), OCT2 (SLC22A2), and OCT3 (SLC22A3) are located in a cluster on chromosome 6q26–q27 and have a common structure of 11 coding exons and 10 introns (Koehler et al. 1997; Gründemann et al. 1998; Hayer et al. 1999; Verhaagh et al. 1999; Gründemann and Schömig 2000). The amino acid sequence identity of OCT1 and OCT2 is 70%, and 50% for both OCT1/OCT3 and OCT2/OCT3. OCT orthologs have been cloned from other mammalian species as well (Koepsell et al. 2007) (Fig. 2).

Based on sequence and hydropathy analyses, OCTs have a predicted topology comprising 12 transmembrane helices, an intracellular amino and carboxyl terminus, and a large glycosylated extracellular loop between the first two transmembrane helices (Fig. 3a). The large intracellular loop between transmembrane helix 6 and 7 carries several putative phosphorylation sites that are used for short-term modulation of OCT activity (Koepsell et al. 2007; Ciarimboli 2008). Employing detailed mutagenesis and modeling of the tertiary structure in analogy to the crystallized structure of lactose permease from *Escherichia coli* (Abramson et al. 2003), several amino acids in the 4th, 10th, and 11th transmembrane helix of rat Oct1 were identified that are involved in substrate and/or inhibitor binding (Gorboulev et al. 1999, 2005; Popp et al. 2005; Sturm et al. 2007; Volk et al. 2009). These amino acids are localized within the center of a large cleft that may exist in an outward- or inward-facing conformation. The cleft contains high- and low-affinity substrate and/or inhibitor binding sites (Popp et al. 2005; Gorbunov

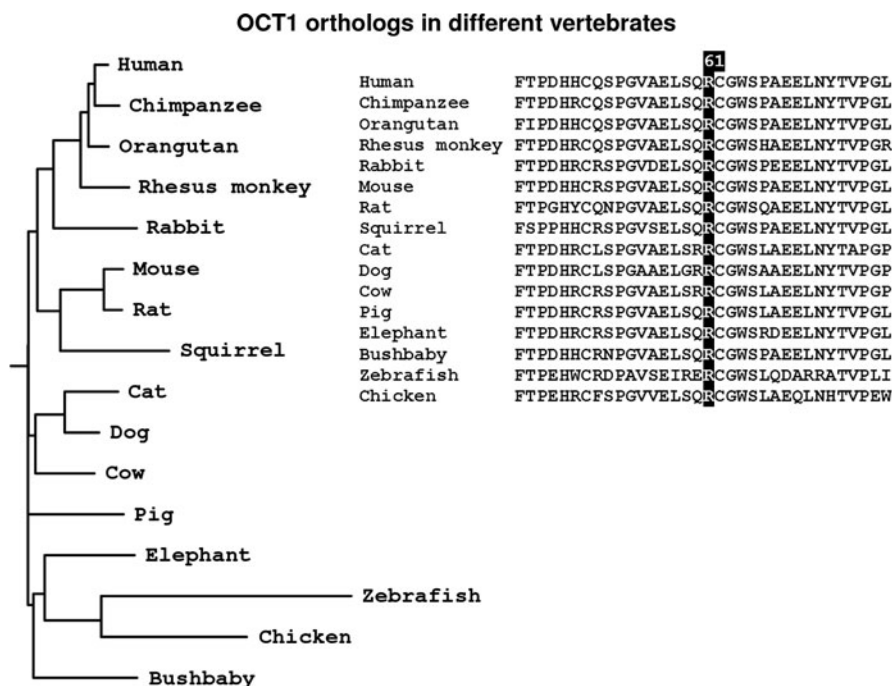

Fig. 2 OCT1 orthologs in different vertebrates. The phylogenetic tree on the left was constructed from OCT1/Oct1 protein sequences aligned using the ClustalX2 program (Larkin et al. 2007) and drawn with the "drawgram" program of the PHYLIP3.67 program package (http://evolution.genetics.washington.edu/phylip.html). The sequence comparison on the right shows the aligned sequences in the vicinity of amino acid arginine 61, which is highly conserved among species. A genetic variant was identified in human OCT1 that leads to a nonsynonymous exchange of arginine 61 to a cysteine (Kerb et al. 2002; Shu et al. 2003). OCT1-Cys61 shows a reduced in vitro transport function (Kerb et al. 2002; Shu et al. 2003, 2007), is associated with a significant decrease of hepatic OCT1 protein levels (Nies et al. 2009), and affects metformin pharmacokinetics in humans (Shu et al. 2008). For further details see Tables 4–12. The following protein sequences were used for alignments: human NP_003048; orangutan ENSPPYP00000019207; chimpanzee XP_527554; rhesus monkey ENSMMUP00000020546; dog XP_850971; mouse NP_033228; rat NP_036829; cow NP_001094568; pig NP_999154; elephant ENSLAFP00000009760; cat ENSFCAP00000002624; chicken XP_419621; rabbit ENSOCUP00000002189; bushbaby ENSOGAP00000004719; squirrel ENSSTOP00000008083; zebrafish ENSDARP00000048889. Accession numbers are either from the ENSEMBL genome server (http://www.ensembl.org; numbers starting with "ENS") or from the "Protein" database at http://www.ncbi.nlm.nih.gov/entrez. Sequences from elephant, cat, rabbit, bushbaby, and squirrel are in part incomplete

et al. 2008; Minuesa et al. 2009; Volk et al. 2009). Whereas the affinities of the low-affinity substrate binding sites are in the same range as the respective Michaelis-Menten constant values, the high-affinity binding sites may have a 10,000-fold higher affinity. The different substrate and inhibitor binding sites overlap and may exhibit competitive or allosteric interactions. Both the low- and high-affinity sites may be inhibitory (Minuesa et al. 2009). High-affinity binding sites may be also

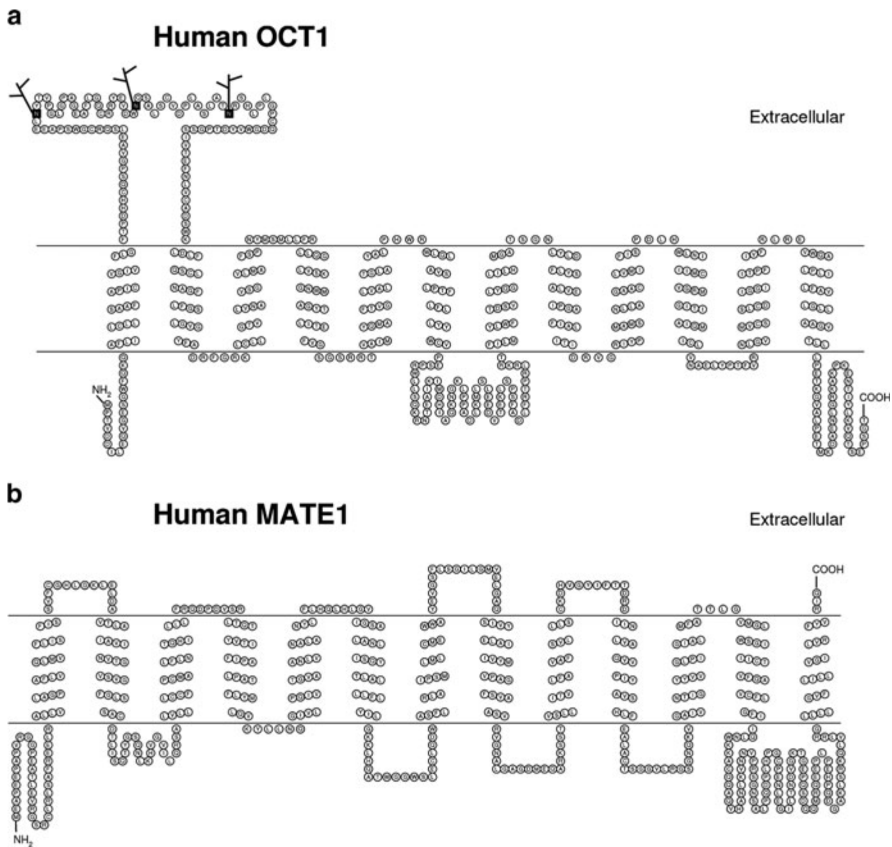

Fig. 3 Predicted membrane topology models of human OCT1 (**a**) and human MATE1 (**b**). Topology prediction was performed with the TMHMM algorithm (http://www.cbs.dtu.dk/services/TMHMM-2.0) and the model was drawn with TOPO2 (http://www.sacs.ucsf.edu/TOPO-run/wtopo.pl). (**a**) Tree-like structures indicate the location of putative *N*-glycosylation sites in OCT1. OCT2 and OCT3 have similar predicted secondary structures as OCT1. (**b**) There are no putative *N*-glycosylation sites in MATE1. Thirteen transmembrane segments are also predicted for human MATE2 and for most of the orthologs from other mammalian species (Terada and Inui 2008)

involved in transport since for inhibition of organic cation transport different IC_{50} values may be obtained when the uptake measurements were performed using different substrate concentrations far below the respective K_m values (see e.g. Table 2: inhibition of OCT2-mediated MPP uptake by flecainide or quinidine). The existence of various substrate and inhibitor binding sites and the complex interactions between different sites explains why largely different IC_{50} values were obtained for individual transporters when different substrates were used for transport measurements (see, e.g., Tables 1–2: inhibition of OCT1-mediated TEA uptake versus MPP uptake by dopamine or histamine, or inhibition of OCT1-mediated TEA uptake vs. ASP uptake by quinidine). Many naturally occurring

Table 1 Physiological substrates and inhibitors of OCTs and MATEs

Compound	Physiological function	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
Corticosterone	Hormone	$7^{5\ \mathrm{TEA;\ B}}$ $8.9^{1\ \mathrm{YM;\ E}}$ $22^{0.025\ \mathrm{MPP;\ C}}$	$5.4^{1\ \mathrm{YM;\ E}}$ $34^{0.025\ \mathrm{MPP;\ C}}$	$0.12^{\mathrm{MPP;\ A}}$ $0.29^{0.025\ \mathrm{MPP;\ C}}$	$>20^{50\ \mathrm{TEA;\ D}}$		A: Gründemann et al. (1998), B: Zhang et al. (1998), C: Hayer-Zillgen et al. (2002), D: Otsuka et al. (2005) and E: Minematsu et al. (2010)
β-Estradiol	Hormone	$5.7^{0.025\ \mathrm{MPP}}$	$>30^{0.025\ \mathrm{MPP}}$	$2.9^{0.025\ \mathrm{MPP}}$			Hayer-Zillgen et al. (2002)
Progesterone	Hormone	$3.1^{0.025\ \mathrm{MPP}}$	$27^{0.025\ \mathrm{MPP}}$	$4.3^{0.025\ \mathrm{MPP}}$			Hayer-Zillgen et al. (2002)
Prostaglandin E_2	Hormone, locally acting	$\boldsymbol{K_m}$**: 0.66** controversial	$\boldsymbol{K_m}$**: 0.03** controversial				Kimura et al. (2002) and Harlfinger et al. (2005)
Prostaglandin $F_{2\alpha}$	Hormone, locally acting	$\boldsymbol{K_m}$**: 0.48** controversial	$\boldsymbol{K_m}$**: 0.33** controversial				Kimura et al. (2002) and Harlfinger et al. (2005)
Testosterone	Hormone	$10^{0.1\ \mathrm{MPP}}$	$3^{0.1\ \mathrm{MPP}}$	$44^{0.1\ \mathrm{MPP}}$			Koepsell et al. unpubl.
Choline	Metabolite	$3{,}540^{0.05\ \mathrm{TEA;\ B}}$ $16{,}700^{0.1\ \mathrm{MPP;\ D}}$ potential substrate	$\boldsymbol{K_m}$**: $210^{\mathrm{Ooc;\ A}}$**	$23{,}800^{0.1\ \mathrm{MPP;\ D}}$	$>5{,}000^{50\ \mathrm{TEA;\ C}}$		A: Gorboulev et al. (1997) B: Bednarczyk et al. (2003) C: Otsuka et al. (2005) and D: Koepsell et al. (unpublished)
Creatinine	Metabolite	$>1{,}000^{0.1\ \mathrm{MPP;\ D}}$	$6{,}060^{5\ \mathrm{TEA;\ C}}$ $42{,}400^{10\ \mathrm{AG;\ C}}$	$15{,}700^{0.1\ \mathrm{MPP;\ D}}$	Substrate$^{\mathrm{A}}$	Substrate$^{\mathrm{A,\ B}}$	A: Tanihara et al. (2007), B: Masuda et al. (2006), C: Kimura et al. (2009) and D: Koepsell et al. (unpublished)
Estrone sulfate	Metabolite				$\boldsymbol{K_m}$**: 470**	$\boldsymbol{K_m}$**: 850**	Tanihara et al. (2007)
Guanidine	Metabolite	$5{,}030^{0.1\ \mathrm{MPP;\ F}}$	$2{,}200^{5\ \mathrm{Crea;\ B}}$ $2{,}300^{10\ \mathrm{TEA;\ C}}$ $2{,}300^{0.1\ \mathrm{MPP;\ F}}$ $3{,}030^{5\ \mathrm{TEA;\ E}}$ potential substrate	$6{,}201^{0.03\ \mathrm{MPP;\ A}}$	$\boldsymbol{K_m}$**: $2{,}100^{\mathrm{D}}$**	$\boldsymbol{K_m}$**: $4{,}200^{\mathrm{D}}$**	A: Wu et al. (2000), B: Urakami et al. (2004), C: Suhre et al. (2005), D: Tanihara et al. (2007), E: Kimura et al. 2009 and F: Koepsell et al. (unpublished)
L-Carnitine	Metabolite	$12{,}400^{0.1\ \mathrm{MPP}}$	$13{,}000^{0.1\ \mathrm{MPP}}$	$5{,}590^{0.1\ \mathrm{MPP}}$			Koepsell et al. (unpublished)

(*continued*)

Table 1 (continued)

Compound	Physiological function	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
N-1-Methyl-nicotinamide	Metabolite	$1,035^{0.05\ \text{TEA; C}}$ $7,700^{5\ \text{TEA; B}}$	$266^{60\ \text{TEA Ooc; A}}$ $303^{10\ \text{TEA; E}}$ $310^{5\ \text{Crea; D}}$ $\sim 1,000^{61\ \text{YM; G}}$	$3,000^{0.1\ \text{MPP; H}}$		Substrate[F]	A: Gorboulev et al. (1997), B: Zhang et al. (1998), C: Bednarczyk et al. (2003), D: Urakami et al. (2004), E: Suhre et al. (2005), F: Masuda et al. (2006), G: Minematsu et al. (2010) and H: Koepsell et al. (unpublished)
Thiamine	Metabolite	$434^{0.05\ \text{TEA; A}}$			Substrate[C]	Substrate[B,C]	A: Bednarczyk et al. (2003), B: Masuda et al. (2006) and C: Tanihara et al. (2007)
Agmatine	Metabolite, neuromodulator	$24,000^{0.1\ \text{MPP}}$	**K_m: 1,400**	**K_m: 2,500**			Gründemann et al. (2003)
Cyclo(His-Pro)	Metabolite, neuromodulator	**K_m: 655**	**K_m: 74**	**K_m: 126**			Taubert et al. (2007)
Salsolinol	Metabolite, neuromodulator	**K_m: 440**	**K_m: 130**	**K_m: 139**			Taubert et al. (2007)
Tyramine	Metabolite, neuromodulator	$107^{0.05\ \text{TEA; B}}$		Substrate[A]			A: Gründemann et al. (1998) and B: Bednarczyk et al. (2003)
Acetylcholine	Neurotransmitter	$580^{0.2\ \text{MPP Ooc; A}}$	**K_m: $117^{\text{Ooc; A}}$** $149^{0.2\ \text{MPP Ooc; A}}$	$10,490^{0.1\ \text{MPP; B}}$			A: Lips et al. (2005) and B: Koepsell et al. (unpublished)
Dopamine	Neurotransmitter	$487^{0.05\ \text{TEA; B}}$ $>20,000^{0.1\ \text{MPP; E}}$	**K_m: $390^{\text{Ooc; A}}$** **K_m: $1,400^{D}$** $1,400^{5\ \text{Crea; C}}$	$1,200^{0.1\ \text{MPP; E}}$			A: Busch et al. (1998), B: Bednarczyk et al. (2003), C: Urakami et al. (2004), D: Amphoux et al. (2006) and E: Koepsell et al. (unpublished)

Epinephrine	Neurotransmitter	>30,000$^{0.1\ MPP;\ C}$	**K_m: 420^{B}**	**K_m: 240^{A}**		A: Gründemann et al. (1998), B: Amphoux et al. (2006), and C: Koepsell et al. (unpublished)
Histamine	Neurotransmitter	3,007$^{0.05\ TEA;\ C}$ >20,000$^{0.1\ MPP;\ E}$	**K_m: 940^{D}** **K_m: 1,300$^{Ooc;\ A}$**	**K_m: 180^{B}** **K_m: 220^{D}**		A: Busch et al. (1998), B: Gründemann et al. (1998), C: Bednarczyk et al. (2003), D: Amphoux et al. (2006) and E: Koepsell et al. (unpublished)
Norepinephrine	Neurotransmitter	7,100$^{0.1\ MPP;\ D}$	**K_m: 1,500^{C}** **K_m: 1,900$^{Ooc;\ B}$**	**K_m: 510^{A}** **K_m: 2,630^{C}**		A: Gründemann et al. (1998), B: Busch et al. (1998), C: Amphoux et al. (2006) and D: Koepsell et al. (unpublished)
Serotonin	Neurotransmitter	>20,000$^{0.025\ MPP;\ C}$	**K_m: 80$^{Ooc;\ A}$** **K_m: 290^{C}**	1,000$^{0.025\ MPP;\ C}$	<100$^{50\ TEA;\ B}$	A: Busch et al. (1998), B: Otsuka et al. (2005) and C: Amphoux et al. (2006)

IC_{50} values and K_m values (explicitly stated) were measured in oocytes of *Xenopus laevis* or mammalian cell lines transfected with the respective transporter. Expression in oocytes is indicated (Ooc) when different results were obtained in the oocyte system. The substrates employed for inhibition measurements are indicated; abbreviations used are: *AG* aminoguanidine, *Crea* creatinine, *MPP* 1-methyl-4-phenylpyridinium, *TEA* tetraethylammonium, *YM* YM155. The employed substrate concentration is indicated when different results were obtained using different substrate concentrations far below the respective Michaelis-Menten constant. Bold face indicates cations, for which transport has been demonstrated. For example, corticosterone is an inhibitor of OCT1 with an IC_{50} value of 22 μM when measured with 0.025 μM MPP as the substrate

Table 2 Clinically used drugs as substrates and inhibitors of OCTs and MATEs

Therapeutic use	Compound	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
Anesthetic	Ketamine	$115^{0.025\ MPP}$	$23^{0.025\ MPP}$	$226^{0.025\ MPP}$			Amphoux et al. (2006)
Anesthetic, local	Cocaine	$85^{0.025\ MPP}$	$113^{0.025\ MPP}$	$>1{,}000^{0.025\ MPP}$			Amphoux et al. (2006)
Anesthetic, local	Lidocaine		$294^{10\ Metf;\ B}$	$\mathbf{K_m: 139^{A}}$ $656^{0.2\ His;\ A}$			A: Hasannejad et al. (2004) and B: Umehara et al. (2008)
Antiallergic	Cetirizine				$371^{5\ TEA}$	$818^{5\ TEA}$	Tsuda et al. (2009b)
Antiallergic	Chlorpheniramine				$88^{5\ TEA}$	$191^{5\ TEA}$	Tsuda et al. (2009b)
Antiallergic	Clemastine	$4.9^{1\ ASP}$					Ahlin et al. (2008)
Antiallergic	Desloratidine		$60^{10\ MPP}$				Zolk et al. (2008)
Antiallergic	Fexofenadine				Substrate		Matsushima et al. (2009)
Antiallergic	Promethazine	$35^{1\ ASP}$					Ahlin et al. (2008)
Antiarrhythmic	Amiodarone			$<100^{10\ MPP}$			Sata et al. (2005)
Antiarrhythmic	Disopyramide	$15–30^{5\ TEA;\ A}$ $82^{1\ ASP;\ C}$	$324^{10\ MPP;\ D}$	$457^{0.2\ His;\ B}$ substrate	$84^{5\ TEA;\ E}$	$292^{5\ TEA;\ E}$	A: Zhang et al. (1998), B: Hasannejad et al. (2004), C: Ahlin et al. (2008), D: Zolk et al. 2008 and E: Tsuda et al. (2009b)
Antiarrhythmic	Flecainide	$42^{0.001\ MPP;\ A}$	$<191^{10\ MPP;\ B}$ $>1{,}000^{0.001\ MPP;\ A}$	$60^{0.001\ MPP;\ A}$			A: Umehara et al. (2008) and B: Zolk et al. (2008)
Antiarrhythmic	Mexiletine		$55^{10\ MPP;\ B}$	$260^{0.2\ His;\ A}$			A: Hasannejad et al. (2004) and B: Zolk et al. (2008)
Antiarrhythmic	Phenytoin			$0.75^{0.2\ His}$			Hasannejad et al. (2004)
Antiarrhythmic	Pilsicainide			$66^{0.2\ His}$			Hasannejad et al. (2004)
Antiarrhythmic	Procainamide	$14.5^{0.05\ TEA;\ D}$ $51^{1\ YM;\ K}$ $74^{5\ TEA;\ B}$	$28^{5\ Crea;\ E}$ $50^{60\ TEA\ Ooc;\ A}$ $92^{1\ YM;\ K}$ $406^{10\ Metf;\ I}$	$355^{0.2\ His;\ F}$ $738^{0.03\ MPP;\ C}$ substrate^{F}	$217^{5\ TEA;\ J}$ $\mathbf{K_m: 1{,}230^{H}}$	$178^{5\ TEA;\ J}$ $\mathbf{K_m: 1{,}580^{H}}$ $\mathbf{K_m: 4{,}100^{G}}$	A: Gorboulev et al. (1997), B: Zhang et al. (1998), C: Wu et al. (2000), D: Bednarczyk et al. (2003), E: Urakami et al. (2004), F: Hasannejad et al. (2004), G: Masuda et al. (2006), H: Tanihara et al. (2007), I: Umehara et al. (2008), J: Tsuda et al. (2009b) and K: Minematsu et al. (2010)
Antiarrhythmic	Propafenone	$11^{1\ ASP;\ A}$	$25^{10\ MPP;\ B}$				A: Ahlin et al. (2008) and B: Zolk et al. (2008)

Antiarrhythmic	Quinidine	$5.4^{0.05 \text{ TEA; B}}$ $5.7^{1 \text{ MPP; L}}$ $6.7^{1 \text{ MPP Ooc; G}}$ $7.1^{1 \text{ YM; K}}$ $17^{0.001 \text{ MPP; J}}$ $18^{5 \text{ TEA; A}}$ $114^{1 \text{ ASP; I}}$	$7.1^{1 \text{ YM; K}}$ $8.7^{1 \text{ MPP Ooc; G}}$ $10^{5 \text{ Crea; D}}$ $13^{1 \text{ MPP; L}}$ $17^{10 \text{ Metf; E}}$ $87^{10 \text{ MPP; H}}$ $446^{0.001 \text{ MPP; J}}$	$14^{0.001 \text{ MPP; J}}$ $18^{1 \text{ MPP Ooc; G}}$ $22^{1 \text{ MPP; L}}$ $124^{0.2 \text{ His; C}}$ $\boldsymbol{K_m}$**: 216**^{C}	$29^{5 \text{ TEA; F}}$	$23^{5 \text{ TEA; F}}$	A: Zhang et al. (1998), B: Bednarczyk et al. (2003), C: Hasannejad et al. (2004), D: Urakami et al. (2004), E: Kimura et al. (2005a), F: Tsuda et al. 2009b, G: Bourdet et al. (2005), H: Zolk et al. (2008), I: Ahlin et al. (2008), J: Umehara et al. (2008), K: Minematsu et al. (2010) and L: Ming et al. (2009)
Antiarrhythmic	Verapamil	$1.2^{1 \text{ YM; F}}$ $2.9^{5 \text{ TEA; A}}$	$13.4^{1 \text{ YM; F}}$ $85^{10 \text{ MPP; D}}$		$28^{5 \text{ TEA; E}}$ $<100^{50 \text{ TEA; B}}$	$32^{5 \text{ TEA; E}}$ substrate^{C}	A: Zhang et al. (1998), B: Otsuka et al. (2005), C: Masuda et al. (2006), D: Zolk et al. (2008), E: Tsuda et al. (2009b) and F: Minematsu et al. (2010)
Antiarrhythmic, antihypertensive	Oxprenolol	$29^{1 \text{ ASP; A}}$ $87^{0.001 \text{ MPP; B}}$	$>1{,}000^{0.001 \text{ MPP; B}}$	$326^{0.001 \text{ MPP; B}}$			A: Ahlin et al. (2008) and B: Umehara et al. (2008)
Antiarrhythmic, antihypertensive	Propranolol	$63^{1 \text{ ASP; B}}$ $113^{0.001 \text{ MPP; C}}$	$8.3^{10 \text{ Metf; E}}$ $229^{10 \text{ MPP; D}}$ $>300^{0.001 \text{ MPP; C}}$ substrate^{A}	$133^{0.001 \text{ MPP; C}}$			A: Dudley et al. (2000), B: Ahlin et al. (2008), C: Umehara et al. (2008), D: Zolk et al. (2008) and E: Bachmakov et al. (2009)
Antiasthmatic	Beclomethasone		$4.4^{1 \text{ TEA}}$				Lips et al. (2005)
Antiasthmatic	Budesonide		$7.3^{1 \text{ TEA}}$				Lips et al. (2005)
Antibacterial	Cephalexin				6,500		Tanihara et al. (2007)
Antibacterial	Cephradine				4,040	10,400	Tanihara et al. (2007)
Antibacterial	Levofloxacin		$127^{5 \text{ Crea; A}}$		Substrate^{B}		A: Okuda et al. (2006) and B: Tanihara et al. 2007
Antibacterial	Tetracycline					Substrate	Tanihara et al. (2007)
Antibacterial	Trimethoprim	$20^{\text{MPP; D}}$ $57^{1 \text{ ASP; C}}$	$21^{5 \text{ Crea; A}}$ $51^{\text{MPP; D}}$ $1{,}318^{10 \text{ MPP; E}}$	$<100^{10 \text{ MPP; B}}$			A: Urakami et al. (2004), B: Sata et al. (2005), C: Ahlin et al. (2008), D: Jung et al. (2008) and E: Zolk et al. (2008)
Anticoagulant	Nafamostat		20^{TEA}				Li et al. (2004)
Antidepressant	Citalopram	$3.1^{0.1 \text{ MPP; B}}$ $19^{1 \text{ ASP; A}}$	$12^{0.1 \text{ MPP; B}}$	$145^{0.1 \text{ MPP; B}}$			A: Ahlin et al. (2008) and B: Koepsell et al. (unpublished)
Antidepressant, tricyclic	Amitriptyline	$17^{1 \text{ ASP; B}}$	$14^{10 \text{ MPP; C}}$	$>100^{10 \text{ MPP; A}}$			A: Sata et al. (2005)B: Ahlin et al. (2008) and C: Zolk et al. (2008)

(continued)

Table 2 (continued)

Therapeutic use	Compound	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
Antidepressant, tricyclic	Clomipramine	$19^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antidepressant, tricyclic	Desipramine	$5.4^{5\ \mathrm{TEA;\ B}}$ $57^{1\ \mathrm{ASP;\ D}}$	$16^{60\ \mathrm{TEA;\ A}}$	$14^{0.03\ \mathrm{MPP;\ C}}$	$56^{5\ \mathrm{TEA;\ E}}$	$283^{5\ \mathrm{TEA;\ E}}$	A: Gorboulev et al. (1997), B: Zhang et al. (1998), C: Wu et al. (2000), D: Ahlin et al. (2008) and E: Tsuda et al. (2009b)
Antidepressant, tricyclic	Doxepin		$13^{10\ \mathrm{MPP}}$				Zolk et al. (2008)
Antidepressant, tricyclic	Imipramine	$17^{1\ \mathrm{ASP;\ B}}$	$6^{10\ \mathrm{MPP;\ C}}$	$42^{0.03\ \mathrm{MPP;\ A}}$	$42^{5\ \mathrm{TEA;\ D}}$	$183^{5\ \mathrm{TEA;\ D}}$	A: Wu et al. (2000), B: Ahlin et al. (2008), C: Zolk et al. (2008) and D: Tsuda et al. (2009b)
Antidepressant, tricyclic	Trimipramine	$28^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antidiarrheal	Loperamide	$24^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antiemetic	Diphenylhydramine	$3.4^{0.02\ \mathrm{MPP;\ A}}$	$15^{0.02\ \mathrm{MPP;\ A}}$	$695^{0.02\ \mathrm{MPP;\ A}}$	$87^{5\ \mathrm{TEA;\ B}}$	$267^{5\ \mathrm{TEA;\ B}}$	A: Müller et al. (2005) and B: Tsuda et al. (2009b)
Antiemetic	Granisetron	$<100^{0.1\ \mathrm{MPP}}$	$<100^{0.1\ \mathrm{MPP}}$	$<100^{0.1\ \mathrm{MPP}}$			Koepsell et al. (unpublished)
Antiemetic	Metoclopramide	$95^{1\ \mathrm{ASP;\ B}}$		$<100^{10\ \mathrm{MPP;\ A}}$			A: Sata et al. (2005) and B: Ahlin et al. (2008)
Antiemetic	Ondansetron	$20^{1\ \mathrm{ASP;\ A}}$	$<100^{0.1\ \mathrm{MPP;\ B}}$				A: Ahlin et al. (2008) and B: Koepsell et al. (unpublished)
Antiemetic	Promethazine	$17^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antiemetic	Ramosetron			$<100^{10\ \mathrm{MPP}}$			Sata et al. (2005)
Antiemetic	Tropisetron	$<100^{0.1\ \mathrm{MPP}}$	$<100^{0.1\ \mathrm{MPP}}$	$<100^{0.1\ \mathrm{MPP}}$			Koepsell et al. (unpublished)
Antihypertensive	Bisoprolol		$2.4^{10\ \mathrm{Metf}}$				Bachmakov et al. (2009)
Antihypertensive	Bucindolol	$27^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antihypertensive	Captopril					Substrate	Masuda et al. (2006)
Antihypertensive	Carvedilol		$2.3^{10\ \mathrm{Metf;\ B}}$ $63^{10\ \mathrm{MPP;\ A}}$				A: Zolk et al. (2008) and B: Bachmakov et al. (2009)
Antihypertensive	Clonidine	$0.6^{1\ \mathrm{TEA;\ A}}$ $0.7^{0.05\ \mathrm{TEA;\ C}}$ $6.5^{0.02\ \mathrm{MPP;\ D}}$ $23^{1\ \mathrm{ASP;\ F}}$	$2.2^{10\ \mathrm{TEA;\ E}}$ $16^{10\ \mathrm{MPP;\ G}}$ $23^{0.02\ \mathrm{MPP;\ D}}$	$110^{0.02\ \mathrm{MPP;\ D}}$ $373^{0.03\ \mathrm{MPP;\ B}}$			A: Zhang et al. (1998), B: Wu et al. (2000), C: Bednarczyk et al. (2003), D: Müller et al. (2005), E: Suhre et al. (2005), F: Ahlin et al. (2008) and G: Zolk et al. (2008)
Antihypertensive	Debrisoquine		$\boldsymbol{K_m}$**: 7.3**$^{\mathrm{Ooc}}$				Koepsell et al. (unpublished)
Antihypertensive	Diltiazem	$12^{1\ \mathrm{ASP;\ B}}$ $16^{0.001\ \mathrm{MPP;\ A}}$	$>1{,}000^{0.001\ \mathrm{MPP;\ A}}$	$50^{0.001\ \mathrm{MPP;\ A}}$	$12.5^{5\ \mathrm{TEA;\ C}}$	$117^{5\ \mathrm{TEA;\ C}}$	A: Umehara et al. (2008), B: Ahlin et al. (2008) and C: Tsuda et al. (2009b)

Antihypertensive	Phenoxybenzamine	$2.7^{0.025\text{ MPP; A}}$ $15^{1\text{ ASP; B}}$	$4.9^{0.025\text{ MPP; A}}$	$6.1^{0.025\text{ MPP; A}}$			A: Hayer-Zillgen et al. (2002) and B: Ahlin et al. (2008)
Antihypertensive	Pindolol	$9.7^{0.05\text{ TEA; A}}$ $39^{0.001\text{ MPP; B}}$		$>1{,}000^{0.001\text{ MPP; B}}$			A: Bednarczyk et al. (2003) and B: Umehara et al. (2008)
Antihypertensive	Prazosin	$1.6^{1\text{ YM; C}}$ $1.8^{0.025\text{ MPP; A}}$ $9.9^{1\text{ ASP; B}}$	$80^{1\text{ YM; C}}$ $>100^{0.025\text{ MPP; A}}$	$13^{0.025\text{ MPP; A}}$			A: Hayer-Zillgen et al. (2002), B: Ahlin et al. (2008) and C: Minematsu et al. (2010)
Antihypertensive	Terazosine	$24^{1\text{ ASP}}$					Ahlin et al. (2008)
Antihypertensive, antiarrhythmic	Acebutolol	$96^{5\text{ TEA}}$					Zhang et al. (1998)
Antihypertensive, antiarrhythmic	Metoprolol	$268^{0.001\text{ MPP; A}}$	$50^{10\text{ Metf; B}}$ $>1{,}000^{0.001\text{ MPP; A}}$	$804^{0.001\text{ MPP; A}}$			A: Umehara et al. (2008) and B: Bachmakov et al. (2009)
Antihypotensive	Etilefrine	$447^{0.02\text{ MPP}}$	$4{,}009^{0.02\text{ MPP}}$	**K_m: 2,800**			Müller et al. (2005)
Anti-inflammatory	Diclofenac	$<2{,}000^{5\text{ TEA}}$	$<2{,}000^{5\text{ TEA}}$				Khamdang et al. (2002)
Anti-inflammatory	Ibuprofen	$<2{,}000^{5\text{ TEA}}$	$2{,}000–5{,}000^{5\text{ TEA}}$				Khamdang et al. (2002)
Anti-inflammatory	Indomethacin	$<2{,}000^{5\text{ TEA}}$	$<2{,}000^{5\text{ TEA}}$				Khamdang et al. (2002)
Anti-inflammatory	Ketoprofen	$<2{,}000^{5\text{ TEA}}$	$<2{,}000^{5\text{ TEA}}$				Khamdang et al. (2002)
Anti-inflammatory	Mefenamic acid	$<2{,}000^{5\text{ TEA}}$	$\sim 2{,}000^{5\text{ TEA}}$				Khamdang et al. (2002)
Anti-inflammatory	Piroxicam	$<2{,}000^{5\text{ TEA}}$	$<2{,}000^{5\text{ TEA}}$				Khamdang et al. (2002)
Anti-inflammatory	Salicylic acid				Substrate		Tanihara et al. (2007)
Anti-inflammatory	Sulindac	$<1{,}000^{5\text{ TEA}}$	$<1{,}000^{5\text{ TEA}}$				Khamdang et al. (2002)
Antimalarial	Chloroquine		$1{,}096^{0.01\text{ MPP}}$				Zolk et al. (2008)
Antimalarial	Mefloquine		$204^{0.01\text{ MPP}}$				Zolk et al. (2008)
Antimalarial	Pyrimethamine	$3.8^{30\text{ TEA}}$	$10^{30\text{ TEA}}$		$0.077^{30\text{ TEA}}$	$0.046^{30\text{ TEA}}$	Ito et al. (2010)
Antimalarial	Quinine	$13^{0.02\text{ MPP; E}}$ $23^{5\text{ TEA; B}}$ $45^{1\text{ ASP; D}}$ $52^{1\text{ ASP; H}}$	$3.4^{60\text{ TEA Ooc; A}}$ $6.7^{1\text{ ASP; C}}$ $23^{0.02\text{ MPP; E}}$	$37^{0.02\text{ MPP; E}}$	Substrate^{G}	Substrate^{F}	A: Gorboulev et al. (1997), B: Zhang et al. (1998), C: Cetinkaya et al. (2003), D: Ciarimboli et al. (2004), E: Müller et al. (2005), F: Masuda et al. (2006), G: Tanihara et al. (2007) and H: Ahlin et al. (2008)
Antineoplastic	Amsacrine	$5.0^{1\text{ ASP}}$					Ahlin et al. (2008)
Antineoplastic	Cisplatin	$1{,}000–5{,}000^{50\text{ TEA; B}}$	$1.5^{1\text{ ASP; A}}$ $5{,}000–10{,}000^{50}$	$1{,}000–5{,}000^{50\text{ TEA; B}}$	$1{,}000–5{,}000^{50\text{ TEA; B}}$ substrate^{B}	$>4{,}000^{50\text{ TEA; B}}$ substrate^{B}	A: Ciarimboli et al. (2005) and B: Yonezawa et al. (2006)

(*continued*)

Table 2 (continued)

Therapeutic use	Compound	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
			$^{\mathrm{TEA;\ B}}$ substrate$^{\mathrm{A}}$				
Antineoplastic	Imatinib	Potential substrate$^{\mathrm{A,\ B}}$ $<50^{100\ \mathrm{TEA;\ C}}$					A: Wang et al. (2008a), B: Hu et al. (2008) and C: Nies et al. (unpublished)
Antineoplastic	Irinotecan			$1.8^{1\ \mathrm{MPP}}$			Shnitsar et al. (2009)
Antineoplastic	Melphalan			$366^{1\ \mathrm{MPP}}$			Shnitsar et al. (2009)
Antineoplastic	Mitoxantron	$16^{0.1\ \mathrm{MPP}}$	$800^{0.1\ \mathrm{MPP}}$	$440^{0.1\ \mathrm{MPP}}$			Koepsell et al. (unpublished)
Antineoplastic	Oxaliplatin	Substrate	Substrate	Substrate	$2{,}000\text{–}5{,}000^{50}_{\mathrm{TEA}}$		Yonezawa et al. (2006)
Antineoplastic	Tamoxifen		$87^{10\ \mathrm{MPP}}$				Zolk et al. (2008)
Antineoplastic	Topotecan				$\boldsymbol{K_m}$**: 70**	$\boldsymbol{K_m}$**: 60**	Tanihara et al. (2007)
Antineoplastic	Vincristine			$17^{1\ \mathrm{MPP}}$			Shnitsar et al. (2009)
Antineoplastic; radiopharmaceutical	Metaiodobenzylguanidine	Substrate	Substrate	Substrate			Bayer et al. (2009)
Antiobesity	Fenfluramine		$10^{10\ \mathrm{MPP}}$				Zolk et al. (2008)
Antiobesity	Sibutramine		$29^{10\ \mathrm{MPP}}$				Zolk et al. (2008)
Antiparasitic	Furaminidine two positive charges	$7.4^{1\ \mathrm{MPP}}$ $\boldsymbol{K_m}$**: 6.1**	$182^{1\ \mathrm{MPP}}$	$20^{1\ \mathrm{MPP}}$			Ming et al. (2009)
Antiparasitic	Pentamidine two positive charges	$0.4^{0.1\ \mathrm{MPP;\ A}}$ $16^{1\ \mathrm{MPP;\ B}}$ $\boldsymbol{K_m}$**: 36**$^{\mathrm{B}}$	$3.8^{0.1\ \mathrm{MPP;\ A}}$ $11^{1\ \mathrm{MPP;\ B}}$	$15^{1\ \mathrm{MPP;\ B}}$			A: Jung et al. (2008) and B: Ming et al. (2009)
Anti-Parkinson	Memantine	$3.7^{0.025\ \mathrm{MPP;\ B}}$ $27^{1\ \mathrm{ASP;\ C}}$	$7.3^{0.025\ \mathrm{MPP;\ B}}$ $\boldsymbol{K_m}$**: 34**$^{\mathrm{Ooc;\ A}}$	$236^{0.025\ \mathrm{MPP;\ B}}$			A: Busch et al. (1998), B: Amphoux et al. (2006) and C: Ahlin et al. (2008)
Anti-Parkinson	Pramipexole				$141^{5\ \mathrm{TEA}}$	$24^{5\ \mathrm{TEA}}$	Tsuda et al. (2009b)
Anti-Parkinson	Talipexole				$66^{5\ \mathrm{TEA}}$	$120^{5\ \mathrm{TEA}}$	Tsuda et al. (2009b)
Anti-Parkinson; antiviral	Amantadine	$18^{0.05\ \mathrm{TEA;\ B}}$ $40^{1\ \mathrm{YM;\ E}}$ $236^{0.025\ \mathrm{MPP;\ D}}$	$20^{10\ \mathrm{TEA;\ C}}$ $23^{200\ \mathrm{Dop;\ A}}$ $\boldsymbol{K_m}$**: 27**$^{\mathrm{A}}$ $28^{0.025\ \mathrm{MPP;\ D}}$ $46^{1\ \mathrm{YM;\ E}}$	$>1{,}000^{0.025}_{\mathrm{MPP;\ D}}$	$112^{5\ \mathrm{TEA;\ F}}$	$1{,}167^{5\ \mathrm{TEA;\ F}}$	A: Busch et al. (1998), B: Bednarczyk et al. (2003), C: Suhre et al. (2005), D: Amphoux et al. (2006), E: Minematsu et al. (2010) and F: Tsuda et al. (2009b)
Antipsychotic	Chlorpromazine	$4.3^{0.05\ \mathrm{TEA;\ A}}$ $27^{1\ \mathrm{ASP;\ C}}$	$14^{10\ \mathrm{MPP;\ D}}$	$<100^{10\ \mathrm{MPP;\ B}}$			A: Bednarczyk et al. (2003), B: Sata et al. (2005), C: Ahlin et al. (2008) and D: Zolk et al. (2008)

Antipsychotic	Chlorprotixen	$78^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antipsychotic	Flupentixol	$90^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antipsychotic	Fluphenazine	$110^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antipsychotic	Haloperidol	$142^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antipsychotic	Prochlorperazine	$50^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antipsychotic	Sulpiride			$<100^{10\ \mathrm{MPP}}$			Sata et al. (2005)
Antipsychotic	Tiapride			$>100^{10\ \mathrm{MPP}}$			Sata et al. (2005)
Antispasmodic	Butylscopolamine	$16^{0.02\ \mathrm{MPP}}$	$764^{0.02\ \mathrm{MPP}}$				Müller et al. (2005)
Antispasmodic	Propantheline			$\sim100^{10\ \mathrm{MPP}}$			Sata et al. (2005)
Antiulcer	Cimetidine	$95^{1\ \mathrm{ASP;\ D}}$ $166^{5\ \mathrm{TEA;\ A}}$	$14^{1\ \mathrm{Amil;\ I}}$ $26^{1\ \mathrm{ASP;\ I}}$ $27^{5\ \mathrm{Crea;\ E}}$ $36^{1\ \mathrm{ASP;\ C}}$ $\boldsymbol{K_m}$**: 67**$^{\mathrm{B}}$ $70^{5\ \mathrm{TEA;\ G}}$ $\boldsymbol{K_m}$**: 73**$^{\mathrm{H}}$ $120^{10\ \mathrm{MPP;\ M}}$ $510^{10\ \mathrm{Metf;\ L}}$ $1{,}380^{1\ \mathrm{Et;\ N}}$	$240^{1\ \mathrm{Et;\ N}}$	$1.1^{5\ \mathrm{TEA;\ O}}$ $\sim10^{50\ \mathrm{TEA;\ F}}$ $\boldsymbol{K_m}$**: 170**$^{\mathrm{K}}$	$7.3^{5\ \mathrm{TEA;\ O}}$ $\boldsymbol{K_m}$**: 120**$^{\mathrm{K}}$ $\boldsymbol{K_m}$**: 370**$^{\mathrm{J}}$	A: Zhang et al. (1998), B: Motohashi et al. (2002), C: Cetinkaya et al. (2003), D: Ciarimboli et al. (2004), E: Urakami et al. (2004), F: Otsuka et al. (2005), G: Suhre et al. (2005), H: Tahara et al. (2005), I: Biermann et al. (2006), J: Masuda et al. (2006), K: Tanihara et al. (2007), L: Umehara et al. (2008), M: Zolk et al. (2008), N: Lee et al. (2009) and O: Tsuda et al. (2009b)
Antiulcer	Famotidine		$\boldsymbol{K_m}$**: 56**$^{\mathrm{D}}$ $70^{5\ \mathrm{Crea;\ A}}$ $111^{10\ \mathrm{TEA;\ C}}$	$\sim20^{10\ \mathrm{MPP;\ B}}$	$0.6^{5\ \mathrm{TEA;\ E}}$	$9.7^{5\ \mathrm{TEA;\ E}}$	A: Urakami et al. (2004), B: Sata et al. (2005), C: Suhre et al. (2005), D: Tahara et al. (2005) and E: Tsuda et al. (2009b)
Antiulcer	Mepenzolate	$65^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Antiulcer	Ranitidine	$22^{0.05\ \mathrm{TEA;\ A}}$ $28^{0.02\ \mathrm{MPP;\ C}}$	$31^{10\ \mathrm{Fam;\ E}}$ $38^{5\ \mathrm{Crea;\ B}}$ $40^{10\ \mathrm{TEA;\ D}}$ $79^{10\ \mathrm{Cim;\ E}}$ $\boldsymbol{K_m}$**: 265**$^{\mathrm{E}}$ $1{,}617^{0.02\ \mathrm{MPP;\ C}}$	$372^{0.02\ \mathrm{MPP;\ C}}$	$25^{5\ \mathrm{TEA;\ F}}$	$25^{5\ \mathrm{TEA;\ F}}$	A: Bednarczyk et al. (2003), B: Urakami et al. (2004), C: Müller et al. (2005), D: Suhre et al. (2005), E: Tahara et al. (2005) and F: Tsuda et al. (2009b)
Antiviral	Acyclovir	$\boldsymbol{K_m}$**: 151**$^{\mathrm{A}}$			$\boldsymbol{K_m}$**: 2,640**$^{\mathrm{B}}$	$\boldsymbol{K_m}$**: 4,320**$^{\mathrm{B}}$	A: Takeda et al. (2002) and B: Tanihara et al. (2007)
Antiviral	Ganciclovir	$\boldsymbol{K_m}$**: 516**$^{\mathrm{A}}$			$\boldsymbol{K_m}$**: 5,120**$^{\mathrm{B}}$	$\boldsymbol{K_m}$**: 4,280**$^{\mathrm{B}}$	A: Takeda et al. (2002) and B: Tanihara et al. (2007)
Antiviral HIV	Abacavir	$7.2 \times 10^{-5},_{0.0013\ \mathrm{MPP}}$	$4.1 \times 10^{-5},_{0.0013\ \mathrm{MPP}}$	$5 \times 10^{-5},_{0.0013\ \mathrm{MPP}}$			Minuesa et al. (2009)

(*continued*)

Table 2 (continued)

Therapeutic use	Compound	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
Antiviral HIV	Azidothymidine	1.6×10^{-4}, $_{0.0013\ \mathrm{MPP}}$	2.7×10^{-4}, $_{0.0013\ \mathrm{MPP}}$	4×10^{-4}, $_{0.0013\ \mathrm{MPP}}$			Minuesa et al. (2009)
Antiviral HIV	Emtricitabine	2×10^{-5}, $_{0.0013\ \mathrm{MPP}}$	2.4×10^{-3}, $_{0.0013\ \mathrm{MPP}}$	5.3×10^{-4}, $_{0.0013\ \mathrm{MPP}}$			Minuesa et al. (2009)
Antiviral HIV	Indinavir	$37^{0.1\ \mathrm{MPP;\ B}}$ $62^{10\ \mathrm{MPP;\ A}}$	$275^{0.1\ \mathrm{MPP;\ B}}$				A: Zhang et al. (2000) and B: Jung et al. (2008)
Antiviral HIV	Nelfinavir	$7^{0.1\ \mathrm{MPP;\ B}}$ $22^{10\ \mathrm{MPP;\ A}}$	$13^{0.1\ \mathrm{MPP;\ B}}$				A: Zhang et al. (2000) and B: Jung et al. (2008)
Antiviral HIV	Ritonavir	$5.2^{10\ \mathrm{MPP;\ A}}$ $14^{0.1\ \mathrm{MPP;\ B}}$	$25^{0.1\ \mathrm{MPP;\ B}}$				A: Zhang et al. (2000) and B: Jung et al. (2008)
Antiviral HIV	Saquinavir	$8.3^{10\ \mathrm{MPP;\ A}}$ $37^{0.1\ \mathrm{MPP;\ B}}$	$205^{0.1\ \mathrm{MPP;\ B}}$				A: Zhang et al. (2000) and B: Jung et al. (2008)
Antiviral HIV	Tenofovir	8.5×10^{-4}, $_{0.0013\ \mathrm{MPP;\ B}}$	5.7×10^{-4}, $_{0.0013\ \mathrm{MPP;\ B}}$	5×10^{-6}, $_{0.0013\ \mathrm{MPP;\ B}}$	Substrate^{A}		A: Tanihara et al. (2007) and B: Minuesa et al. (2009)
Antiviral HIV	Zalcitabine	$24^{0.1\ \mathrm{MPP}}$ **K_m: 242**	$131^{0.1\ \mathrm{MPP}}$ **K_m: 232**				Jung et al. (2008)
Antiviral HIV, HBV	Lamivudine	1.2×10^{-5}, $_{0.0013\ \mathrm{MPP;\ B}}$ $17^{0.1\ \mathrm{MPP;\ A}}$ **K_m: 249**^{A} **K_m: 1,250**^{B} $1{,}900^{0.0013\ \mathrm{MPP;\ B}}$	8.1×10^{-6}, $_{0.0013\ \mathrm{MPP;\ B}}$ $33^{0.1\ \mathrm{MPP;\ A}}$ K_m: 248^{A} **K_m: 1,900**^{B} $3{,}450^{0.0013\ \mathrm{MPP;\ B}}$	2×10^{-5}, $_{0.0013\ \mathrm{MPP;\ B}}$ $2{,}400^{0.0013\ \mathrm{MPP;\ B}}$ **K_m: 2,140**^{B}			A: Jung et al. (2008) and B: Minuesa et al. (2009)
Bronchodilator	Ipratropium		$15^{10\ \mathrm{MPP}}$				Zolk et al. (2008)
Cardiotonic	Denopamine	$47^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
CNS stimulant	3,4-Methylenedioxy-methamphetamine	$24^{0.025\ \mathrm{MPP}}$	$1.6^{0.025\ \mathrm{MPP}}$	$74^{0.025\ \mathrm{MPP}}$			Amphoux et al. (2006)
CNS stimulant	D-Amphetamine	$202^{0.025\ \mathrm{MPP}}$	$11^{0.025\ \mathrm{MPP}}$	$460^{0.025\ \mathrm{MPP}}$			Amphoux et al. (2006)
CNS stimulant	Phencyclidine	$4.4^{0.025\ \mathrm{MPP}}$	$25^{0.025\ \mathrm{MPP}}$	$333^{0.025\ \mathrm{MPP}}$			Amphoux et al. (2006)
Diuretic	Amiloride	$57^{1\ \mathrm{ASP;\ A}}$	$23^{1\ \mathrm{ASP;\ B}}$ **K_m: 95**^{B}				A: Ahlin et al. (2008) and B: Biermann et al. (2006)

Emetic	Apomorphine	$21^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Hypoglycemic	Metformin	**K_m: 1,470**^{C} $2{,}010^{1\ \mathrm{Cim\ Ooc;\ A}}$ **K_m: 2,160**^{H} $3{,}420^{0.1\ \mathrm{MPP;\ G}}$ $9{,}480^{10\ \mathrm{AG;\ H}}$	$339^{10\ \mathrm{TEA;\ D}}$ **K_m: 990**^{C} **K_m: 1,380**^{B} $1{,}700^{1\ \mathrm{Cim;\ A}}$ $2{,}370^{10\ \mathrm{AG;\ H}}$	**K_m: 2,260**^{G} $2{,}980^{0.1\ \mathrm{MPP;\ G}}$	$667^{5\ \mathrm{TEA;\ I}}$ **K_m: 780**^{F}	$667^{5\ \mathrm{TEA;\ I}}$ **K_m: 1,050**^{E} **K_m: 1,980**^{F}	A: Dresser et al. (2002), B: Kimura et al. (2005a), C: Kimura et al. (2005b), D: Suhre et al. (2005), E: Masuda et al. (2006), F: Tanihara et al. (2007), G: Kimura et al. (2009), H: Nies et al. (2009) and I: Tsuda et al. (2009b)
Hypoglycemic	Phenformin	$10^{1\ \mathrm{Cim\ Ooc;\ A}}$	$15^{10\ \mathrm{TEA;\ B}}$ $65^{1\ \mathrm{Cim\ Ooc;\ A}}$				A: Dresser et al. (2002) and B: Suhre et al. (2005)
Hypoglycemic	Repaglinide	$1.6^{10\ \mathrm{Metf;\ A}}$ $1.8^{30\ \mathrm{MPP;\ A}}$ $9.2^{1\ \mathrm{ASP;\ B}}$					A: Bachmakov et al. (2008) and B: Ahlin et al. (2008)
Hypoglycemic	Rosiglitazone	$6.9^{10\ \mathrm{Metf}}$ $30^{30\ \mathrm{MPP}}$					Bachmakov et al. (2008)
Muscle relaxant	Orphenadrine	$13^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Muscle relaxant	Vecuronium	$127^{1\ \mathrm{MPP;\ B}}$ $232^{5\ \mathrm{TEA;\ A}}$					A: Zhang et al. (1997) and B: Zhang et al. (1998)
Mydriatic	Atropine	$1.2^{0.02\ \mathrm{MPP;\ A}}$ $12^{1\ \mathrm{ASP;\ B}}$	$29^{0.02\ \mathrm{MPP;\ A}}$	$466^{0.02\ \mathrm{MPP;\ A}}$			A: Müller et al. (2005) and B: Ahlin et al. 2008
Narcotic	Morphine	$28^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Narcotic; analgesic	Tramadol	$53^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Sedative	Midazolam	$3.7^{5\ \mathrm{TEA}}$					Zhang et al. (1998)
Smoking cessation	Varenicline		**K_m: 370**				Feng et al. (2008)
Tranquilizer	Flurazepam		$60^{10\ \mathrm{MPP}}$				Zolk et al. (2008)

IC_{50} values and K_m values (explicitly stated) were measured in oocytes of *Xenopus laevis* or mammalian cell lines transfected with the respective transporter. Expression in oocytes is indicated (Ooc) when different results were obtained in the oocyte system. The substrates employed for inhibition measurements are indicated; abbreviations used are: *Amil* amiloride, *ASP* 4-(4-(Dimethylamino)styryl)-*N*-methylpyridinium, *AG* aminoguanidine, *Cim* cimetidine, *Crea* creatinine, *Dop* dopamine, *Et* ethidium bromide, *Fam* famotidine, *His* histamine, *Metf* metformin, *MPP* 1-methyl-4-phenylpyridinium, *TEA* tetraethylammonium, *YM* YM155. The employed substrate concentration is indicated when different results were obtained using different substrate concentrations far below the respective Michaelis-Menten constant. Bold face indicates cations, for which transport has been demonstrated. For example, ketamine is an inhibitor of OCT1 with an IC_{50} value of 115 μM when measured with 0.025 μM MPP as the substrate. Drug classification is according to the standard handbook Goodman & Gilman's: The Pharmacological Basis of Therapeutics (Hardman et al. 2001)

Table 3 Other selected xenobiotics as substrates and inhibitors of OCTs and MATEs

Compound	Classification	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
Aflatoxin B1	Mycotoxin	Substrate $64^{5\ \mathrm{TEA}}$	Substrate $121^{5\ \mathrm{TEA}}$				Tachampa et al. (2008)
Aminoguanidine	Model cation	Substrate $<10{,}000^{5\ \mathrm{TEA}}$	**K_m: 4,100** $800^{5\ \mathrm{TEA}}$				Kimura et al. (2009)
Azidoprocainamide	Model cation	**K_m: 101**					van Montfoort et al. (2001)
Berberine	Fluorescent cation	**K_m: 14.8**	**K_m: 4.4**				Nies et al. (2008)
N-Butylpyridinium chloride	Model cation		$2.3^{0.015\ \mathrm{TEA}}$ **K_m: 18**				Cheng et al. (2009)
Citreoveridine	Mycotoxin	$6.6^{5\ \mathrm{TEA}}$	$18^{5\ \mathrm{TEA}}$				Tachampa et al. (2008)
Decynium 22	Model cation	$1.0^{0.025\ \mathrm{MPP;\ D}}$ $2.7^{5\ \mathrm{TEA;\ C}}$ $4.7^{1\ \mathrm{MPP;\ A}}$	$0.1^{60\ \mathrm{TEA\ Ooc;\ B}}$ $1.1^{0.025\ \mathrm{MPP;\ D}}$	$0.09^{0.025\ \mathrm{MPP;\ D}}$			A: Zhang et al. (1997), B: Gorboulev et al. (1997), C: Zhang et al. (1998) and D: Hayer-Zillgen et al. (2002)
4-4-Dimethylaminostyryl-*N*-methylpyridinium (ASP)	Fluorescent model cation	**K_m: 2.3^{B}**	$7^{1\ \mathrm{Amil;\ A}}$ **K_m: 42^{A}**				A: Biermann et al. (2006) and B: Ahlin et al. (2008)
Disprocynium 24	Model cation			$0.015^{1\ \mathrm{MPP}}$			Gründemann et al. (1998)
Ethidium	Fluorescent xenobiotic	$0.6^{0.1\ \mathrm{MPP}}$ **K_m: 0.8**	$1.2^{0.1\ \mathrm{MPP}}$ **K_m: 1.7**	$1.4^{0.1\ \mathrm{MPP}}$ **K_m: 2.0**			Lee et al. (2009)
Gliotoxin	Mycotoxin	$584^{5\ \mathrm{TEA}}$	$117^{5\ \mathrm{TEA}}$				Tachampa et al. (2008)
1-Methyl-4-phenylpyridinium (MPP)	Model cation	**K_m: 15^{A}** $16^{0.05\ \mathrm{TEA;\ E}}$ $30^{1\ \mathrm{YM;\ L}}$ **K_m: 32^{D}**	$2.4^{10\ \mathrm{TEA;\ F}}$ $2.4^{60\ \mathrm{TEA;\ B}}$ **K_m: 3.1^{J}** $4.4^{1\ \mathrm{YM;\ L}}$ **K_m: 7.8^{D}** **K_m: 19$^{\mathrm{Ooc;\ B}}$** $20^{10\ \mathrm{MPP;\ K}}$ $43–54^{\mathrm{Et;\ J}}$	**K_m: 47^{C}** **K_m: 83^{G}**	**K_m: 100^{I}**	**K_m: 94^{H}** **K_m: 111^{I}**	A: Zhang et al. (1997), B: Gorboulev et al. (1997), C: Wu et al. (2000), D: Gründemann et al. (2003), E: Bednarczyk et al. (2003), F: Suhre et al. (2005), G: Sata et al. (2005), H: Masuda et al. (2006), I: Tanihara et al. (2007), J: Lee et al. (2009), K: Zolk et al. (2008) and L: Minematsu et al. (2010)
N-Methylquinidine	Model cation	**K_m: 12**					van Montfoort et al. (2001)
N-Methylquinine	Model cation	**K_m: 20**					van Montfoort et al. (2001)
Nandrolone	Anabolic steroid	$35^{1\ \mathrm{ASP}}$					Ahlin et al. (2008)
Nicotine	Tobacco toxin	$53^{0.05\ \mathrm{TEA;\ A}}$ $186^{1\ \mathrm{TEA;\ B}}$	$42^{1\ \mathrm{TEA;\ B}}$	$101^{1\ \mathrm{TEA;\ B}}$	$>500^{50\ \mathrm{TEA;\ C}}$		A: Bednarczyk et al. (2003), B: Lips et al. (2005) and C: Otsuka et al. (2005)
Paraquat	Herbicide (two positive charges)		**K_m: 114**		**K_m: 212**		Chen et al. 2007

Rhodamine 123	Fluorescent cation				$<10^{50\ \text{TEA}}$		Otsuka et al. (2005)
Tetrabutylammonium	Model cation	$6.5^{0.05\ \text{TEA; B}}$ $30^{1\ \text{MPP Ooc; A}}$	$20^{10\ \text{TEA; C}}$ $120^{1\ \text{MPP Ooc; A}}$		$18^{4.5\ \text{PQ; D}}$		A: Dresser et al. (2002), B: Bednarczyk et al. (2003), C: Suhre et al. (2005) and D: Chen et al. (2007)
Tetraethylammonium TEA	Model cation	$158^{1\ \text{MPP Ooc; E}}$ $\boldsymbol{K_m}$**: 168**^{F} $173^{1\ \text{MPP; A}}$ $216^{1\ \text{MPP Ooc; H}}$ $\boldsymbol{K_m}$**: 229**^{I} $470^{0.1\ \text{MPP; N}}$ $673^{1\ \text{ASP; G}}$ $1{,}390^{10\ \text{AG; O}}$	$\boldsymbol{K_m}$**: 27**^{Q} $30^{1\ \text{ASP; K}}$ $\boldsymbol{K_m}$**: 46**^{J} $\boldsymbol{K_m}$**: 76**$^{\text{Ooc; B}}$ $98^{1\ \text{Amil; K}}$ $156^{1\ \text{MPP Ooc; E}}$ $160^{10\ \text{AG; O}}$ $161^{5\ \text{TEA; N}}$ $189^{0.1\ \text{MPP; N}}$ $222^{1\ \text{MPP Ooc; H}}$	$1{,}237^{1\ \text{MPP Ooc; H}}$ $1{,}372^{0.03\ \text{MPP; D}}$ $1{,}477^{0.1\ \text{MPP; N}}$	$121^{4.5\ \text{PQ; P}}$ $\boldsymbol{K_m}$**: 220**^{I} $\boldsymbol{K_m}$**: 380**^{M}	$\boldsymbol{K_m}$**: 760**^{M} $\boldsymbol{K_m}$**: 830**^{L}	A: Zhang et al. (1997), B: Gorboulev et al. (1997), C: Zhang et al. (1998), D: Wu et al. (2000), E: Dresser et al. (2002), F: Bednarczyk et al. (2003), G: Ciarimboli et al. (2004), H: Bourdet et al. (2005), I: Otsuka et al. (2005), J: Suhre et al. (2005), K: Biermann et al. (2006), L: Masuda et al. (2006), M: Tanihara et al. (2007), N: Ming et al. (2009), O: Kimura et al. (2009), P: Chen et al. (2007) and Q: Cheng et al. (2009)
Tetramethylammonium	Model cation	$12{,}400^{1\ \text{MPP Ooc; B}}$	$24^{\text{Crea; C}}$ $150^{1\ \text{MPP Ooc; B}}$ $180^{60\ \text{TEA Ooc; A}}$ $525^{10\ \text{TEA; D}}$		$5{,}073^{4.5\ \text{PQ; E}}$		A: Gorboulev et al. (1997), B: Dresser et al. (2002), C: Urakami et al. (2004), D: Suhre et al. (2005) and E: Chen et al. (2007)
Tetrapentylammonium	Model cation	$1.8^{0.05\ \text{TEA; C}}$ $5.5^{1\ \text{ASP; E}}$ $7.5^{5\ \text{TEA; B}}$	$1.5^{60\ \text{TEA Ooc; A}}$ $2^{1\ \text{ASP; G}}$ $2.7^{1\ \text{ASP; D}}$ $7^{1\ \text{Amil; G}}$ $11^{10\ \text{TEA; F}}$	$4.5^{0.1\ \text{MPP; H}}$			A: Gorboulev et al. (1997), B: Zhang et al. (1998), C: Bednarczyk et al. (2003), D: Cetinkaya et al. (2003), E: Ciarimboli et al. (2004), F: Suhre et al. (2005), G: Biermann et al. (2006) and H: Koepsell et al. (unpublished)
Tetrapropylammonium	Model cation	$22^{0.05\ \text{TEA; B}}$ $102^{1\ \text{MPP Ooc; A}}$	$20^{10\ \text{TEA; C}}$ $128^{1\ \text{MPP Ooc; A}}$		$63^{4.5\ \text{PQ; D}}$		A: Dresser et al. (2002), B: Bednarczyk et al. (2003), C: Suhre et al. (2005) and D: Chen et al. (2007)
Tubocurarine		$62^{1\ \text{YM}}$	$>100^{1\ \text{YM}}$				Minematsu et al. 2010
YM155	Survivin suppressant, experimental antineoplastic	$\boldsymbol{K_m}$**: 22**^{B} $24^{0.02\ \text{MPP; B}}$ $\boldsymbol{K_m}$**: 39**^{A}	$\boldsymbol{K_m}$**: 2.7**^{B} $16^{0.02\ \text{MPP; B}}$	$108^{0.02\ \text{MPP; B}}$			A: Iwai et al. (2009) and B: Minematsu et al. (2010)

(*continued*)

Table 3 (continued)

Compound	Classification	OCT1	OCT2	OCT3	MATE1	MATE2-K	References
YM758	I_f channel inhibitor	$41^{0.001\ MPP}$	$16^{10\ Metf}$ $651^{0.001\ MPP}$	$16^{0.001\ MPP}$			Umehara et al. (2008)
α-Zearalenol	Mycotoxin	$1.7^{5\ TEA}$	$34^{5\ TEA}$				Tachampa et al. (2008)
Zearalenone	Mycotoxin	$0.6^{5\ TEA}$	$25^{5\ TEA}$				Tachampa et al. (2008)

IC_{50} values and K_m values (explicitly stated) were measured in oocytes of *Xenopus laevis* or mammalian cell lines transfected with the respective transporter. Expression in oocytes is indicated (Ooc) when different results were obtained in the oocyte system. The substrates employed for inhibition measurements are indicated; abbreviations used are: *Amil* amiloride, *ASP* 4-(4-(Dimethylamino)styryl)-*N*-methylpyridinium, *AG* aminoguanidine, *Crea* creatinine, *Et* ethidium bromide, *Metf* metformin, *MPP* 1-methyl-4-phenylpyridinium, *PQ* paraquat, *TEA* tetraethylammonium, *YM* YM155. The employed substrate concentration is indicated when different results were obtained using different substrate concentrations far below the respective Michaelis-Menten constant. Bold face indicates cations, for which transport has been demonstrated. For example, aflatoxin B1 is an inhibitor of OCT1 with an IC_{50} value of 64 μM when measured with 5 μM MPP as the substrate

genetic variants of human OCT1, OCT2, and OCT3 exist that encode transporters with changed functions (Sect. 6).

2.2 MATE Transporters

The human MATE1 gene (SLC47A1) and the MATE2 gene (SLC47A2) are located in tandem on chromosome 17p11.2 and encode proteins of 570 and 602 amino acids, respectively (Otsuka et al. 2005). The amino acid sequence identity of MATE1 and MATE2 is 47.5%. Two additional human MATE2 isoforms have been cloned: MATE2-K (NM_001099646) coding for a 566-amino acid protein and MATE2-B encoding a truncated protein of 220 amino acids (Masuda et al. 2006). Of note, MATE2-K is currently the only isoform in the MATE2 subfamily, for which function has been demonstrated; MATE2-B is nonfunctional and MATE2 function has not been tested (Masuda et al. 2006; Tanihara et al. 2007). MATE orthologs have also been cloned from other mammalian species, including mouse (Otsuka et al. 2005; Kobara et al. 2008), rat (Terada et al. 2006; Ohta et al. 2006), and rabbit (Zhang et al. 2007).

The hydropathy analysis performed by Otsuka et al. (2005) suggested that MATE1 consists of 12 transmembrane helices. However, most of the current topology analysis programs predict 13 transmembrane helices with an extracellular location of the carboxyl terminus (Zhang et al. 2007; Terada and Inui 2008) (Fig. 3b). Immunocytochemical analyses using accessibility of an antibody to a carboxyl-terminal tag in nonpermeabilized cells proved the extracellular location of the carboxyl terminus of rabbit Mate1 (Zhang et al. 2007). Whether this holds true for other MATE orthologs awaits investigation. Several histidine, cysteine, and glutamate residues in different transmembrane helices of human MATE1 and MATE2-K are apparently involved in substrate binding and/or transport (Asaka et al. 2007; Matsumoto et al. 2008). As for the OCTs, naturally occurring genetic variants have been identified in human MATEs that lead to synthesis of functionally impaired transporters (Sect. 6).

3 Tissue Distribution and Subcellular Localization

By screening the abundance of human transcript sequences (“UniGene” database at http://www.ncbi.nlm.nih.gov) one can assess the approximate gene expression pattern for each OCT and MATE transporter. Northern blot and real-time quantitative PCR analyses have revealed the different mRNA expression profiles in more detail (Koepsell et al. 2007; Okabe et al. 2008). In addition to the mRNA expression profiles, knowledge of the protein expression profiles and the subcellular localization of each transporter in distinct cell types of a given tissue are of equal importance, and they have been analyzed to some extent as well. Although each

cell is equipped with a number of different transporters, it is of particular interest to identify transporters in the absorptive and secretory cells of the small intestine, liver, and kidney, because these are the major organs of drug absorption, metabolism, and excretion. The combined action of electrogenic OCT uptake and MATE efflux transporters, which function as proton/cation antiporters, results in the transcellular movement of organic cations in the small intestine, liver, and kidney (Fig. 4).

Because OCTs and MATEs also transport cationic cytostatic drugs such as platinum drugs (see Sect. 4), transporter expression may affect intracellular levels of anticancer drugs and, thus, response to chemotherapy. Therefore, several studies have analyzed transporter expression profiles in cancer-derived cells as well as in normal tissue in comparison to cancerous tissue (Hayer-Zillgen et al. 2002; Zhang et al. 2006; Ballestero et al. 2006; Yokoo et al. 2008; Okabe et al. 2008). Only recently, OCT1 expression was identified as an important clinical determinant of the response to imatinib in chronic myeloid leukemia (Wang et al. 2008a) (see Sect. 6).

3.1 OCT1

Rat Oct1, the first cloned member of the SLC22A family, is strongly expressed in liver, kidney, and intestine (Gründemann et al. 1994). In humans, on the contrary, OCT1 mRNA is most prominently expressed in the liver (Gorboulev et al. 1997; Nishimura and Naito 2005; Jung et al. 2008; Nies et al. 2009). The OCT1 protein has been localized in the sinusoidal (basolateral) membrane of rat and human hepatocytes (Meyer-Wentrup et al. 1998; Nies et al. 2008), where it mediates the uptake of substrates from the blood and, thereby, mediates the first step in hepatic excretion of many cationic drugs (Fig. 4a). Other reported locations of human OCT1 include the lateral membrane of intestinal epithelial cells (Müller et al. 2005) and the luminal (apical) membrane of ciliated cells in the lung (Lips et al. 2005) and of tubule epithelial cells in the kidney (Tzvetkov et al. 2009).

3.2 OCT2

Human OCT2 mRNA is most strongly expressed in kidney (Gorboulev et al. 1997; Nishimura and Naito 2005; Jung et al. 2008), where the OCT2 protein has been localized in the basolateral membrane of proximal tubule epithelial cells (Motohashi et al. 2002; Nies et al. 2008). Analogous to OCT1 in hepatocytes, OCT2 plays an important role in the secretion of organic cations in the kidney by mediating the first step, that is, the uptake of organic cations across the basolateral membrane (Fig. 4b). OCT2 transcripts were also detected in several other human organs, including small intestine, lung, and different brain regions, and the inner ear

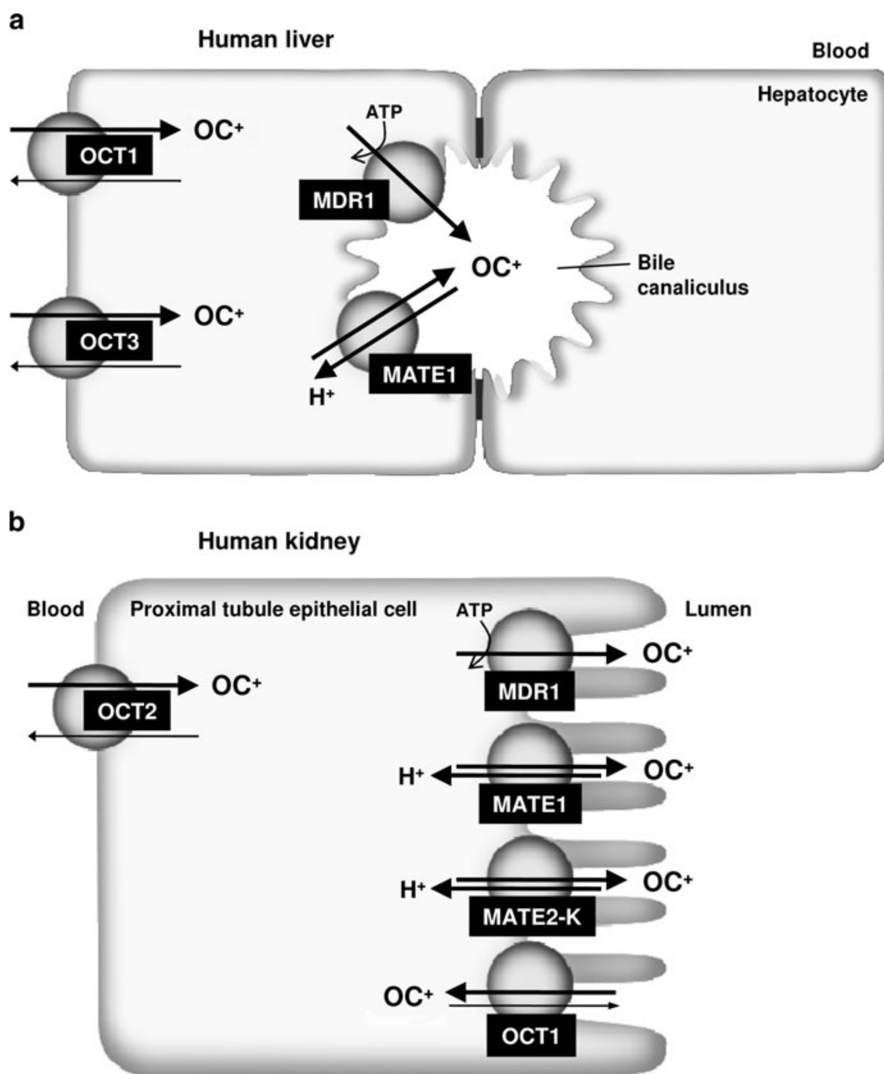

Fig. 4 Localization of OCT and MATE transporters in human hepatocytes (**a**) and proximal tubule epithelial cells in the kidney (**b**). The basolateral localization of OCT1 and OCT3 in hepatocytes and of OCT2 in proximal tubule epithelial cells together with the apical localization of MATE transporters results in the transcellular movement and, thereby, secretion of organic cations into bile and urine. MDR1 P-glycoprotein (ABCB1) is an ATP-dependent efflux pump for organic cations. In addition, OCTN1 (SLC22A4) and OCTN2 (SLC22A5) are present in the luminal membrane of proximal tubule cells, where they may exchange luminal carnitine plus sodium or luminal cations against intracellular cations. An apical OCT1 localization in proximal tubule cells was recently reported and was suggested to be involved in reabsorption of metformin from the urine

(Gorboulev et al. 1997; Busch et al. 1998; Lips et al. 2005; Taubert et al. 2007; Ciarimboli et al. 2010). The human OCT2 protein has been localized in the luminal membrane of ciliated epithelial cells in the lung (Lips et al. 2005) and in pyramidal cells of the hippocampus (Busch et al. 1998).

3.3 OCT3

Human OCT3 was initially cloned from a kidney-derived cell line and termed extraneuronal monoamine transporter (EMT) because substrate specificity is similar to monoamine uptake measured in extraneuronal tissues, neuronal expression of OCT3 was not established, and it was not known that monoamines are also transported by OCT2 (Gründemann et al. 1998); for discussion see Koepsell et al. (2003). Unlike OCT1 and OCT2, OCT3 has a broad tissue distribution (Verhaagh et al. 1999; Nies et al. 2009) and transcripts have been detected, among others, in placenta, adrenal gland, liver, kidney, heart, lung, brain, and intestine (Koepsell et al. 2007). The human OCT3 protein was identified in basolateral membrane vesicles from placenta (Sata et al. 2005), in the plasma membrane of normal human astrocytes (Inazu et al. 2003), in the luminal membrane of bronchial and intestinal epithelial cells (Müller et al. 2005; Lips et al. 2005), and in the sinusoidal membrane of hepatocytes (Nies et al. 2009) (Fig. 4).

3.4 MATE1 and MATE2-K

Human MATE1 is strongly expressed in liver and kidney as well as in skeletal muscle, adrenal gland, and testis (Otsuka et al. 2005; Masuda et al. 2006). Immunolocalization analyses identified the MATE1 protein in the canalicular membrane of hepatocytes (Otsuka et al. 2005) and in the luminal membrane of tubular epithelial cells in the kidney (Otsuka et al. 2005; Masuda et al. 2006). Human MATE2-K is almost exclusively expressed in the kidney and is localized in the luminal membrane of proximal tubular epithelial cells (Masuda et al. 2006) (Fig. 4).

4 Functional Characterization of OCT and MATE Transporters

4.1 Common Functional Properties of OCTs

The functional characteristics of OCTs have been studied in detail using cRNA-injected *Xenopus laevis* oocytes or OCT-transfected mammalian cell lines. Several transport characteristics are shared by all OCTs irrespective of their subtype or the

species. OCTs transport a broad range of organic cations with diverse molecular structures exhibiting K_m values in the micro- to millimolar range (Tables 1–3). Typically, the relative molecular masses of the substrates are below 500 (Suhre et al. 2005; Schmitt and Koepsell 2005; Ahlin et al. 2008; Zolk et al. 2008). OCTs are electrogenic facilitative diffusion systems that translocate organic cations in both directions across the plasma membrane (Busch et al. 1996; Nagel et al. 1997; Kekuda et al. 1998; Budiman et al. 2000; Lips et al. 2005). Transport of organic cations by OCTs is driven by the electrochemical potential but not accelerated by gradients of sodium or protons. For rat Oct2, a nonselective cotranslocation of inorganic cations together with transported organic cation substrates has been observed under depolarized conditions (Schmitt et al. 2009). OCTs are inhibited by a large number of cations and uncharged compounds that are not transported themselves. Partial or total inhibition of transport activity may be achieved (Volk et al. 2009). Transport inhibition may be competitive, partial competitive, or noncompetitive. Importantly, the affinities of the inhibitors are also dependent on the transported substrate (Tables 1–3). For human OCTs, IC_{50} values between 10 pM and 24 mM have been determined. Transported substrates and inhibitors of OCTs are of endogenous origin, xenobiotics, and clinically used drugs.

4.2 Substrate and Inhibitor Specificities of Human OCTs

Human OCT1, OCT2, and OCT3 have largely overlapping but distinctly different substrate and inhibitor specificities (Tables 1–3). The substrates of human OCTs (hOCT) are typically organic cations with one positive charge or two positive charges (furamidine and paraquat) or weak bases that are positively charged at physiological pH (Tables 1–3). Noncharged compounds such as cimetidine at alkaline pH (Barendt and Wright 2002) may also be transported. Whether OCTs may be also able to transport organic anions remains to be clarified. Transport of prostaglandins by hOCT1 and hOCT2 has been reported by Kimura et al. (2002) but was not confirmed by Harlfinger et al. (2005).

Transported endogenous substrates of human OCTs include monoamine neurotransmitters, neuromodulators, and other compounds such as choline, creatinine, and guanidine. Among the >120 clinically used drugs that were shown to interact with human OCTs, about 20 were identified as transport substrates (Table 2). These include antineoplastic platinum compounds, the histamine H_2 receptor antagonist cimetidine, the antiviral drugs acyclovir, ganciclovir, lamivudine, and zalcitabine, the antidiabetic drug metformin, and the antiarrhythmic drug quinidine. The neurotoxin 1-methyl-4-phenyl pyridinium (MPP), the antidiabetic drug metformin, and the antiviral drug lamivudine are transported with similar affinities by the three human OCT orthologs. The model cation TEA is transported with similar affinities by hOCT1 and hOCT2 but shows low-affinity interaction with hOCT3. At variance, epinephrine and norepinephrine are transported with similar affinity by hOCT2 and hOCT3, and only exhibit low-affinity interactions with hOCT1. Histamine is

transported with higher affinity by hOCT3 compared to hOCT2 and is apparently not transported by hOCT1 (Koepsell et al. unpublished data).

Inhibitors of OCTs may have larger molecular weights compared to substrates. They may bind to the central substrate binding pockets of the OCTs or to more peripheral regions in the clefts. Two or more inhibitor molecules may bind at the same time. Transport of a specific substrate may be inhibited partially after inhibitor binding to a high-affinity site and total inhibition may be observed when the inhibitor has bound to the low-affinity site (Minuesa et al. 2009).

It may be difficult to distinguish whether a compound that inhibits an OCT transporter is translocated or not. The reasons are (1) that transport rates may be low, (2) that the expression of endogenous cation transporters may be different in transfected and nontransfected cell lines, and (3) that OCT inhibitors that inhibit control substrates may have different affinities for other substrates. It has to be kept in mind that a correlation between transporter expression and the effect of a drug that interacts with the transporter does not prove that the drug is transported because the transporter inhibition may block cellular uptake of an endogenous compound that may critically influence drug effects on cell functions.

Thomas et al. (2004) observed that compounds that inhibit OCTs decreased uptake of imatinib, a first-generation tyrosine kinase inhibitor, into a human T-cell lymphoblast-like cell line. Similarly, imatinib uptake into blood cells from patients with chronic-phase chronic myeloid leukemia (CML) was blocked by OCT inhibitors (White et al. 2006). When the CML cell line KCL22 was transfected with hOCT1, imatinib uptake was about 1.6-fold higher compared to uptake into control transfectants (Wang et al. 2008a). At variance, expression of hOCT1 in *X. laevis* oocytes or in human embryonic kidney cells did not lead to a significant increase of imatinib uptake (Hu et al. 2008 and Koepsell, Nies, et al. unpublished data). Independent from the conflicting transport data, it was demonstrated that OCT1 mRNA levels and OCT1 genotype are important clinical determinants of treatment response in CML patients (Wang et al. 2008a; Kim et al. 2009) (Sect. 6.3).

4.3 Drug–Drug Interactions Involving OCTs

Various clinically used drugs were identified as inhibitors of OCT-mediated transport by investigating their potency to inhibit in vitro uptake of transported cations (Table 2). When these inhibitory drugs are coprescribed with drugs that are transported by OCTs, drug pharmacokinetics may be altered. Several studies, therefore, investigated the ability of drugs to inhibit transport of the OCT drug substrates metformin or cimetidine in vitro. For example, OCT2-mediated cimetidine transport is inhibited by ranitidine (Tahara et al. 2005) and OCT2-mediated metformin transport by sodium channel blockers (Umehara et al. 2008), β-adrenergic receptor antagonists (Bachmakov et al. 2009), and cimetidine (Zolk et al. 2009). The oral antidiabetics repaglinide and rosiglitazone inhibit OCT1-mediated metformin transport (Bachmakov et al. 2008).

Clinical studies suggest that drug–drug interactions involving OCTs also occur in vivo and may mainly affect cationic drugs that are predominantly eliminated by renal secretion (Ayrton and Morgan 2008; Kindla et al. 2009). For example, cimetidine decreases the renal tubular secretion of ranitidine (van Crugten et al. 1986), procainamide (Lai et al. 1988), dofetilide (Abel et al. 2000), and varenicline (Feng et al. 2008). The inhibition of tubular secretion of metformin by cimetidine was first described more than 20 years ago (Somogyi et al. 1987), but only recently this drug–drug interaction was attributed to OCT2 (Wang et al. 2008b). Other in vivo drug–drug interactions were reported between lamivudine and trimethoprim and between cisplatin and cimetidine or imatinib. It was shown that renal lamivudine clearance was decreased after coadministration of trimethoprim (Moore et al. 1996) and that the concomitant administration of imatinib has a protective effect against cisplatin-induced nephrotoxicity and ototoxicity (Tanihara et al. 2009; Ciarimboli et al. 2010).

4.4 Common Functional Properties of MATEs

MATE transporters are electroneutral transporters that operate independently of a sodium gradient, but use an oppositely directed proton gradient as driving force; translocation of organic cations across the plasma membrane may occur in both directions (Otsuka et al. 2005; Tanihara et al. 2007). MATEs are apparently the functionally long known but searched for proton-driven cation efflux transporters of the canalicular hepatocyte membrane and the luminal membrane of proximal tubule epithelial cells, which have been functionally described for many years (Koepsell 1998; Otsuka et al. 2005).

4.5 Substrate and Inhibitor Specificities of MATEs

MATE1 and MATE2-K have similar substrate and inhibitor specificities, which overlap with those of OCTs (Tables 1–3). The OCT substrates MPP and TEA are also transported by the two MATE orthologs. Endogenous substrates include the organic cations creatinine, guanidine, thiamine, and also the organic anion estrone sulfate. About 30 clinically used drugs have been shown to interact with MATE transporters, and several were identified as transport substrates such as metformin, cimetidine, oxaliplatin, acyclovir, and fexofenadine (Table 2).

4.6 Drug–Drug Interactions Involving MATEs

Information of drug–drug interactions involving MATEs is currently limited. In vitro, cimetidine inhibits MATE1-mediated transport of fexofenadine (Matsushima

et al. 2009) and metformin (Tsuda et al. 2009b). Thus, the clinical observation that metformin tubular secretion is inhibited by cimetidine (Somogyi et al. 1987) may not only be due to inhibition of OCT2-mediated metformin uptake (Wang et al. 2008b) but also to inhibition of MATE1-mediated luminal metformin efflux (Tsuda et al. 2009b).

5 Knockout Mouse Models

Knockout mouse models are valuable tools to identify the physiological and pharmacokinetic roles of transporters in vivo. For that purpose, *Oct1* (Jonker et al. 2001; Shu et al. 2007), *Oct2* (Jonker et al. 2003), *Oct3* (Zwart et al. 2001; Wultsch et al. 2009), and *Mate1* (Tsuda et al. 2009a) single-knockout mice and *Oct1*/*Oct2* double-knockout mice (Jonker et al. 2003) have been generated. All strains are viable and fertile and show no apparent phenotypical abnormalities, indicating that none of the transporters is essential for obvious physiological functions in mice. However, the tissue distribution and disposition of endogenous or exogenous organic cations may differ significantly between wild-type mice and the knockout mouse strains. These knockout mouse models may be used for the prediction of pharmacokinetics in humans, especially in those carrying genetic variants that encode transporters with reduced function (Sect. 6).

5.1 Oct1 Knockout Mice

Intravenous injection of the model cation TEA into *Oct1*(−/−) mice resulted in a fourfold to sixfold reduced hepatic accumulation and in a twofold reduced direct intestinal excretion of TEA in comparison to wild-type mice (Jonker et al. 2001). On the other hand, urinary TEA excretion was increased, probably because lack of hepatic Oct1 leads to increased availability of TEA to the kidney. Similar to TEA, the levels of the anticancer drug *meta*-iodobenzylguanidine, the neurotoxin MPP (Jonker et al. 2001), and the antidiabetic drug metformin (Wang et al. 2002; Shu et al. 2007) were also lower in livers from *Oct1*(−/−) mice than in those from wild-type mice. The decreased hepatic metformin uptake resulted in a reduced effect on AMP-activated protein kinase phosphorylation and gluconeogenesis, and, in consequence, the glucose-lowering effect of metformin was completely abolished (Shu et al. 2007). Thus, mouse Oct1 – as well as human OCT1 (see Sect. 6) – is a major determinant of the pharmacodynamic responses to metformin. It is of interest that *Oct1*(−/−) mice do not develop metformin-induced lactic acidosis, which is a severe and rare adverse drug reaction of metformin treatment in humans (Wang et al. 2003).

5.2 Oct2 Single-Knockout and Oct1/Oct2 Double-Knockout Mice

In contrast to the absence of Oct1, the targeted disruption of the murine *Oct2* gene had only little effect on the pharmacokinetics of intravenously injected TEA (Jonker et al. 2003). The hepatic and renal concentrations of TEA and the excretion of TEA in the urine and feces were similar in *Oct2*(−/−) and wild-type mice. Because Oct1 is expressed in mouse kidney in addition to Oct2 (Alnouti et al. 2006) and Oct1 and Oct2 have overlapping substrate specificities (Gründemann et al. 1999), renal Oct1 expression is apparently sufficient for secretion of most organic cations even in the absence of Oct2. In order to develop a mouse model for studying the renal secretion of organic cations, *Oct1*/*Oct2* double-knockout mice have been generated (Jonker et al. 2003). Renal tubular secretion of TEA was completely abolished and TEA was only eliminated by glomerular filtration in these double-knockout mice, which resulted in significantly elevated TEA plasma levels compared to wild-type mice. Similarly, urinary excretion of cisplatin was significantly impaired in *Oct1*/*Oct2*(−/−) mice so that the animals were protected from severe cisplatin-induced renal tubular damage and from cisplatin-induced loss of hearing (Filipski et al. 2009; Ciarimboli et al. 2010).

5.3 Oct3 Knockout Mice

After cloning human OCT3 it was hypothesized that the functional defined corticosterone-sensitive extraneuronal transport activity for monoamine neurotransmitters is mainly mediated by OCT3 (Gründemann et al. 1998; Koepsell et al. 2003). Interestingly, steady-state norepinephrine and dopamine levels did not differ between several tissues from wild-type and *Oct3*(−/−) mice whereas differences in MPP accumulation were observed (Zwart et al. 2001). Intravenous injection of MPP into *Oct3*(−/−) mice resulted in significantly reduced MPP levels in heart, but not in small intestine, liver, kidney, brain, and placenta in comparison to tissues from wild-type mice. Moreover, fetuses from pregnant *Oct3*(−/−) mice had three times lower MPP levels. Because MPP is a substrate of murine Oct1, Oct2, and Oct3, these data suggest a prominent role of Oct3 in the heart and fetoplacental interface, whereas in other tissues the lack of Oct3 is apparently well compensated by the function of other Octs. Although *Oct3*(−/−) mice did not show overt phenotypical abnormalities, Oct3 is probably critically involved in central nervous function. Vialou et al. (2004) showed that Oct3 is implicated in the appropriate neural and behavioral responses to environmentally induced changes in osmolarity. Whether Oct3 also plays a role in the regulation of fear and anxiety is being discussed (Vialou et al. 2008; Wultsch et al. 2009). Of note, there is compensatory upregulation of Oct3 in the brain of mice that lack the neuronal serotonin transporter Slc6a4/Sert (Schmitt et al. 2003; Baganz et al. 2008).

5.4 *Mate1 Knockout Mice*

Pharmacokinetic characterization of *Mate1*(−/−) mice (Tsuda et al. 2009a) was carried out with metformin, a typical drug substrate of human MATE1 (Table 2). After intravenous injection, renal and hepatic metformin concentrations were markedly increased in the *Mate1*(−/−) mice compared to wild-type mice. In addition, plasma metformin levels were increased in *Mate1*(−/−) mice, whereas urinary metformin excretion was significantly decreased. These data indicate a crucial role of Mate1 in the renal clearance of metformin and probably other drugs as well.

6 Pharmacogenomics of OCT and MATE Transporters

It is well accepted that drug response to the same medication differs among individuals (Kerb 2006). Besides factors such as age, organ function, concomitant therapy, drug–drug interactions, and the nature of the disease, genetic factors have been recognized as important determinants of interindividual variability of drug response. Because OCTs and MATEs function as drug uptake and efflux transporters, respectively (Sect. 4), genetic variants in these transporters may account for interindividual variability of pharmacokinetics of many drugs (Ho and Kim 2005; Giacomini and Sugiyama 2006; Kerb 2006). At present, major research efforts are being taken to identify OCT and MATE variants, to analyze their potential functional consequences, and to determine their contribution to a patient's response to pharmacotherapy.

6.1 *Identification of Genetic Variants, Their Predicted Consequences, and Their Effects In Vitro*

More than 1,100 and 450 single-nucleotide polymorphisms (SNPs) are currently listed for the OCT and MATE genes, respectively, in the NCBI-SNP database (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/SNP; build 130, January 2010). The Pharmacogenetics and Genomics Knowledge Base (PharmGKB, http://www.pharmgkb.org) is another public database comprising data and information related to all areas of pharmacogenetics including a large collection of DNA samples from ethnically diverse populations (Giacomini et al. 2007). Moreover, the International HapMap Consortium (http://www.hapmap.org) has generated a haplotype map of the human genome by identifying more than 3.1 million SNPs genotyped in 270 individuals from four geographically diverse populations (International HapMap Consortium et al. 2007). It is expected that the current next generation sequencing projects aiming at the complete sequencing

of 1,000 human genomes (Kaiser 2008; Siva 2008) will identify more variants, especially those with low frequencies between 0.1% and 1% (Ionita-Laza et al. 2009).

Whereas most sequence variants are present in the introns, others are located in the 5´- and 3´-flanking regions and may lead to an altered expression level of the respective OCT or MATE transporter (Ogasawara et al. 2008; Nies et al. 2009; Hesselson et al. 2009; Ha Choi et al. 2009). Sequence variants within the exons (coding SNPs, cSNPs) may result in amino acid substitutions. These nonsynonymous or missense variants are of considerable interest because they may affect the transport function of the OCT and MATE transporters. A comprehensive list of the currently known cSNPs in the genes encoding human OCTs and MATEs are given in Table 4.

PolyPhen (polymorphism phenotyping, http://genetics.bwh.harvard.edu/pph2; Ramensky et al. 2002) and SIFT (Sorting Intolerant from Tolerant, http://sift.jcvi.org/; Kumar et al. 2009) are two commonly used algorithms, with which the potential functional effects of single amino acid substitutions can be predicted in silico. Based on multiple sequence alignments and in part on information from known three-dimensional protein structures, the algorithms predict the probability that an amino acid substitution has an impact on protein structure and function. However, these in silico predictions cannot substitute for the experimental analysis of each amino acid variant to proof functional changes of the respective OCT or MATE transporter. For comparison, Table 4 lists the predicted functional consequences as well as in vitro transport data for many of the known nonsynonymous OCT and MATE variants. SIFT and PolyPhen predictions are similar for most variants though they differ for some (e.g., OCT1-Ser14Phe, OCT1-Leu23Val, OCT1-Pro341Leu, OCT2-Lys432Gln, MATE1-Leu125Phe). Moreover, amino acid substitutions that are predicted to be tolerated have no transport activity in vitro (e.g., OCT1-Gly220Val, MATE1-Val480Met). This shows limitations of the in silico predictions, which did not include recent structural analysis data (Popp et al. 2005; Volk et al. 2009). The differences may partly be due to the fact that several variants are not properly incorporated into the plasma membrane but are rather retained intracellularly (Shu et al. 2007; Kajiwara et al. 2009; Chen et al. 2009b). The observations that variants alter transport function in a substrate-dependent manner (e.g., OCT1-Ser189Leu, OCT1-Met420del) illustrate the difficulty to predict complex effects of mutagenesis on functions of polyspecific transporters.

6.2 *Interethnic Variability*

Geographic, ethnic, and racial differences in the frequency of genetic variants are well known and several examples in the field of ADME genes have been reported as a mechanistic basis for differences in drug response and/or drug toxicity (Schaeffeler et al. 2001; Klein et al. 2005).

Table 4 Characteristics of nonsynonymous *SLC22A1-A3/OCT1-3* and *SLC47/MATE* variants, their predicted functional consequences, and in vitro transport data

Gene	rs#	Amino acid change	SIFT prediction (score)	Polyphen prediction	Transport in vitro in comparison to refseq 60712.2 (MATE1)		References
					TEA	Metformin	
SLC22A1	rs34447885	Ser14Phe	Tolerated (1.00)	Possibly damaging	~190%[A]	~60%[B]	A: Shu et al. (2003) and B: Shu et al. (2007)
	rs72552761	Gln18His fs	NA	NA			
	rs34570655	Leu23Val	Deleterious (0.04)	Benign			
	rs35888596	Gly38Asp	Deleterious (0.01)	Possibly damaging			
	rs2297373	Phe41Leu	Deleterious (0.00)	Probably damaging			
	rs12208357	Arg61Cys	Deleterious (0.02)	Probably damaging	30%[A,B]	~5%[C]	A: Kerb et al. (2002), B: Shu et al. (2003) and C: Shu et al. (2007)
	rs35546288	Leu85Phe	Tolerated (0.30)	Benign	Similar		Shu et al. (2003)
	rs55918055	Cys88Arg	Deleterious (0.00)	Probably damaging	1.4%		Kerb et al. (2002)
	No refSNP ID	Pro117Leu	Deleterious (0.01)	Possibly damaging			Sakata et al. (2004)
	rs683369	Phe160Leu	Tolerated (0.66)	Benign	Similar[A, B]	Similar[C]	A: Kerb et al. (2002), B: Shu et al. (2003) and C: Shu et al. (2007)
	rs34104736	Ser189Leu	Tolerated (0.49)	Benign	Similar[A]	~20%[B]	A: Shu et al. (2003) and B: Shu et al. (2007)
	rs36103319	Gly220Val	Tolerated (0.10)	Benign	0%[A]	0%[B]	A: Shu et al. (2003) and B: Shu et al. (2007)
	rs4646277	Pro283Leu	Deleterious (0.00)	Probably damaging	0%		Itoda et al. (2004)
	rs4646278	Arg287Gly	Deleterious (0.00)	Probably damaging	0%		Itoda et al. (2004)
	rs2282143	Pro341Leu	Tolerated (0.07)	Probably damaging	~65%[A]	Similar[B]	A: Shu et al. (2003) and B: Shu et al. (2007)
	rs34205214	Arg342His	Tolerated (0.06)	Benign	Similar[A]	Similar[B]	A: Shu et al. (2003) and B: Shu et al. (2007)

	rs34130495	Gly401Ser	Tolerated (0.19)	Benign	0.9%,[A] 0%[B]	~10%[C]	A: Kerb et al. (2002), B: Shu et al. (2003) and C: Shu et al. (2007)
	rs628031	Met408Val	Tolerated (0.27)	Benign	Similar[A]	Similar[B]	A: Shu et al. (2003) and B: Shu et al. (2007)
	rs72552762	Gly414Ala	Deleterious (0.00)	Benign			
	No refSNP ID	Met420del	NA	NA	Similar[A,B]	~30%[C]	A: Kerb et al. (2002), B: Shu et al. (2003) and C: Shu et al. (2007)
	rs35956182	Met440Ile	Tolerated (0.12)	Probably damaging			
	rs34295611	Val461Ile	Tolerated (1.00)	Benign	Similar		Shu et al. (2003)
	rs34059508	Gly465Arg	Deleterious (0.00)	Probably damaging	0%[A]	0%[B]	A: Shu et al. (2003) and B: Shu et al. (2007)
	rs35270274	Arg488Met	Tolerated (0.14)	Benign	Similar[A]	Similar[B]	A: Shu et al. (2003) and B: Shu et al. (2007)
					Transport in vitro in comparison to refseq NP_003049.1 (OCT2)		
					MPP	Metformin	
SLC22A2	rs72552765	Phe45Leu fs	NA	NA			
	rs8177505	Phe45Ile fs	NA	NA			
	rs8177504	Pro54Ser	Deleterious (0.00)	Possibly damaging	Similar		Fujita et al. (2006)
	rs8177509	Phe161Leu	Tolerated (1.00)	Benign	Similar		Fujita et al. (2006)
	rs8177507	Met165Ile	Tolerated (0.44)	Benign	Decrease		Leabman et al. (2002)
	rs8177508	Met165Val	Tolerated (0.09)	Benign	Similar		Fujita et al. (2006)
	rs57371881	Arg176His	Deleterious (0.00)	Probably damaging			
	No refSNP ID	Thr199Ile	Deleterious (0.02)	Possibly damaging	~42%[A]	~31%[B]	A: Kang et al. (2007) and B: Song et al. (2008)
	No refSNP ID	Thr201Met	Tolerated (0.23)	Benign	~50%[A]	~40%[B]	A: Kang et al. (2007) and B: Song et al. (2008)
	rs316019	Ala270Ser	Tolerated (0.69)	Benign	Decrease,[A] ~62%[B]	~60%[C]	A: Leabman et al. (2002), B: Kang et al. (2007) and C: Song et al. (2008)

(*continued*)

Table 4 (continued)

Gene	rs#	Amino acid change	SIFT prediction (score)	Polyphen prediction	Transport in vitro in comparison to refseq 60712.2 (MATE1)		References
					TEA	Metformin	
	rs8177513	Ala297Gly	Tolerated (0.34)	Benign	Similar		Fujita et al. (2006)
	rs45599131	Leu351Trp	Deleterious (0.01)	Possibly damaging			
	rs8177516	Arg400Cys	Deleterious (0.00)	Benign	Decrease		Leabman et al. (2002)
	rs8177517	Lys432Gln	Tolerated (0.56)	Possibly damaging	Decrease		Leabman et al. (2002)
	rs3907239	Arg463Lys	Deleterious (0.00)	Probably damaging			
	rs17853948	Val502Gly	Deleterious (0.03)	Possibly damaging			
	rs17853948	Val502Glu	Deleterious (0.05)	Possibly damaging			
					Norepinephrine transport in vitro in comparison to refseq NP_068812.1 (OCT3)		
SLC22A3	rs8187715	Thr44Met	Tolerated (0.09)	Benign			
	rs8187717	Ala116Ser	Tolerated (1.00)	Benign			
	No refSNP ID	Met370Ile	Tolerated (0.21)	Probably damaging	~50%		Lazar et al. (2008)
	rs8187725	Thr400Ile	Tolerated (0.35)	Benign			
	rs12212246	Ala439Val	Tolerated (0.45)	Benign			
	rs9365165	Gly475Ser	Tolerated (1.00)	Benign			
					Transport in vitro in comparison to refseq NP_060712.2 (MATE1)		
					TEA	Metformin	
SLC47A1	No refSNP ID	Val10Leu	Tolerated (0.58)	Benign	Similar	Similar	Kajiwara et al. (2009)
	rs77630697	Gly64Asp	Deleterious (0.00)	Probably damaging	~1%,[A] ~14%[B]	~2%[A,B]	A: Kajiwara et al. (2009) and B: Chen et al. (2009b)
	rs77474263	Leu125Phe	Deleterious (0.00)	Benign	~50%	~50%	Chen et al. (2009b)
	rs11551331	Pro148Arg	Deleterious (0.00)	Benign			
	rs35646404	Thr159Met	Tolerated (0.33)	Benign			

	No refSNP ID	Ala310Val	Tolerated (0.31)	Benign	~20%	~41%	Kajiwara et al. (2009)
	No refSNP ID	Asp328Ala	Deleterious (0.02)	Probably damaging	~20%	~27%	Kajiwara et al. (2009)
	rs35790011	Val338Ile	Tolerated (0.51)	Benign	~40%	~40%	Chen et al. (2009b)
	No refSNP ID	Asn474Ser	Tolerated (0.35)	Benign	~63%	Similar	Kajiwara et al. (2009)
	rs76645859	Val480Met	Tolerated (0.16)	Benign	0%	0%	Chen et al. (2009b)
	rs35395280	Cys497Ser	Tolerated (0.64)	Benign	Similar	~65%	Chen et al. (2009b)
	rs35395280	Cys497Phe	Tolerated (0.12)	Probably damaging			
	rs78700676	Gln519His	Tolerated (0.12)	Benign	Similar	Similar	Chen et al. (2009b)
					Transport in vitro in comparison to refseq NP_001093116.2 (MATE2-K)		
					TEA	Metformin	
SLC47A2	No refSNP ID	Lys64Asn	Deleterious (0.00)	Benign	~48%	~66%	Kajiwara et al. (2009)
	No refSNP ID	Gly211Val	Deleterious (0.00)	Probably damaging	0%	0%	Kajiwara et al. (2009)
	rs34399035	Gly393Arg	Deleterious (0.00)	Probably damaging			

fs frameshift, *MPP* 1-methyl-4-phenylpyridinium, *NA* not applicable, *Polyphen* polymorphism phenotyping algorithm, http://genetics.bwh.harvard.edu/pph2. (Ramensky et al. 2002). Default settings were used for calculations, *SIFT* sorting intolerant from tolerant algorithm, http://sift.jcvi.org (Kumar et al. 2009). For prediction, the "SIFT sequence" single protein tool was used with default settings and the UniProt-Swiss Prot 56.6 database. Substitution values $\leq$0.05 are predicted to be intolerant and, thus, deleterious, *TEA* tetraethylammonium

Significant ethnic differences exist also in the frequency of allele and genotype distributions of SLC22A1, SLC22A2, and SLC47A1 variants as listed in Tables 5–8. For instance, whereas in European-Americans and Caucasians the allele frequency of the SLC22A1-Arg61Cys polymorphism is approximately 8%, in African-Americans as well as Asian-Americans, no variant subject including 260 individuals tested were identified. In contrast, for the SLC22A1-Pro341Leu variant a significant higher allele frequency was found in African-Americans and Asian-Americans (8% and 17%) than in Caucasians (up to 2%). Finally, the Met408Val polymorphism was detected with high-frequency distributions in all ethnic groups (Caucasians, Africans, Asians). Currently it is unclear whether these differences in allele frequencies between various ethnic groups are of any clinical importance and potentially may render individuals more susceptible to certain xenobiotics and/or environmental factors. For example, aflatoxin B1 is a substrate of OCT1 and it is well recognized that the incidence of hepatocellular carcinoma is significantly more frequent in Asians compared to Caucasians. One may assume that such a difference in disease frequency may be explained by functional relevant genetic variants of the uptake transporter OCT1 that are more common in Asian populations.

6.3 Phenotype–Genotype Correlations

Currently data on tissue expression of OCTs and MATEs correlated to genetic variants are limited. The only polymorphism identified so far that affects OCT1 expression in human liver on mRNA and protein levels is Arg61Cys (Nies et al. 2009; Table 9) after correction for nongenetic factors (such as cholestasis) and additional SLC22A1 variants. Of note, a total of 36 variants in the *SLC22A1* gene were tested including some SNPs, which showed reduced function in vitro (Table 4). It would be of major interest to analyze whether expression of OCT2, which is the predominant OCT uptake transporter in human kidney and involved in renal excretion of several drugs (e.g., metformin), is also influenced by genetic factors.

Several studies addressed the association of OCT genotypes with pharmacokinetic parameters of OCT substrates in humans (Tables 9–12). These investigations were based on initial observations that some variants alter OCT function in in vitro cell experiments (Table 4). A key publication in this field showed that metformin AUC and $C_{\max}$ are significantly higher in OCT1-variant healthy subjects as compared to individuals with *OCT1* reference gene sequence (Shu et al. 2008). In addition and in line with *Oct1* knockout mice experiments, OCT1 variant human subjects revealed poor response to metformin measured by the oral glucose tolerance test (Shu et al. 2007). These data suggested for the first time that OCT1 may be a promising candidate gene for better prediction of the response to the antidiabetic agent metformin. Although some studies including type 2 diabetes patients were subsequently performed, unfortunately the results are inconsistent (Tables 9–12). A second clinically highly relevant agent, which was related to OCT1 expression and

Table 5 Allele frequencies of *SLC22A1* (*OCT1*) genetic variants in different ethnic populations

SLC22A1 (OCT1)[a]	rs#	Allele frequency (%)				References
		European-American/Caucasian	African-American	Asian-American	Chinese, Japanese, Korean, Vietnamese	
5′ near gene	rs6935207	14 ($n = 55$)[A] 27 ($n = 150$)[B]			59 ($n = 150$ Koreans)[C]	A: Kerb et al. (2002), B: Nies et al. (2009) and C: Kang et al. (2007)
5′ near gene	rs9457840	2 ($n = 150$)				Nies et al. (2009)
5′ near gene	rs6899549	0 ($n = 150$)				Nies et al. (2009)
5′ UTR, C>A g.160462864	No refSNP ID				0.4 ($n = 116$ Japanese)	Itoda et al. (2004)
Ser14Phe	rs34447885	0 ($n = 200$)[A] 0 ($n = 150$)[B]	3.1 ($n = 200$)[A]	0 ($n = 60$)[A]		A: Shu et al. (2003) and B: Nies et al. (2009)
Leu23Val	rs34570655	0 ($n = 150$)				Nies et al. (2009)
Gly38Asp	rs35888596	0.3 ($n = 150$)				Nies et al. (2009)
Phe41Leu	rs2297373	0 ($n = 150$)[A]			0.4 ($n = 116$ Japanese)[B]	A: Nies et al. (2009) and B: Itoda et al. (2004)
Ser52Ser	rs1867351	23 ($n = 243$)[A]			44.4 ($n = 116$ Japanese)[B] 35 ($n = 150$ Koreans)[C]	A: Kerb et al. (2002), B: Itoda et al. (2004) and C: Kang et al. (2007)
Arg61Cys	rs12208357	9.1 ($n = 243$)[A] 7.2 ($n = 200$)[B] 9.7 ($n = 150$)[C]	0 ($n = 200$)[B]	0 ($n = 60$)[B]		A: Kerb et al. (2002), B: Shu et al. (2003) and C: Nies et al. (2009)
Leu85Phe	rs35546288	0 ($n = 200$)[A] 0 ($n = 150$)[B]	1 ($n = 200$)[A]	0 ($n = 60$)[A]		A: Shu et al. (2003) and B: Nies et al. (2009)
Cys88Arg	rs55918055	0.6 ($n = 243$)[A] 0.3 ($n = 150$)[B]				A: Kerb et al. (2002) and B: Nies et al. (2009)
Pro117Leu, C>T g.160463307	No refSNP ID				0.4 ($n = 116$ Japanese)	Itoda et al. (2004)
Intron	rs4646272	6.7 ($n = 150$)[A]			62.9 ($n = 116$ Japanese)[B]	A: Nies et al. (2009) and B: Itoda et al. (2004)
Intron, T>C g.160471091					8.2 ($n = 116$ Japanese)	Itoda et al. (2004)

(*continued*)

Table 5 (continued)

SLC22A1 (OCT1)[a]	rs#	Allele frequency (%)				References
		European-American/Caucasian	African-American	Asian-American	Chinese, Japanese, Korean, Vietnamese	
Phe160Leu	rs683369	22 ($n = 241$)[A] 6.5 ($n = 200$)[B] 23 ($n = 150$)[C]	0.5 ($n = 200$)[B]	1.7 ($n = 60$)[B]	8.6 ($n = 116$ Japanese)[D] 13 ($n = 150$ Koreans)[E]	A: Kerb et al. (2002), B: Shu et al. (2003), C: Nies et al. (2009), D: Itoda et al. (2004) and E: Kang et al. 2007
Intron	rs4646273				45.7 ($n = 116$ Japanese)	Itoda et al. (2004)
Intron	rs3737088				1.7 ($n = 116$ Japanese)	Itoda et al. (2004)
Ala187Ala, G>A g.160473299					0.9 ($n = 116$ Japanese)	Itoda et al. (2004)
Intron	rs4646276				47 ($n = 116$ Japanese)	Itoda et al. 2004
Intron	rs2282142				16.8 ($n = 116$ Japanese)	Itoda et al. (2004)
Ser189Leu	rs34104736	0.5 ($n = 200$)[A] 0 ($n = 150$)[B]	0 ($n = 200$)[A]	0 ($n = 60$)[A]		A: Shu et al. (2003) and B: Nies et al. (2009)
Gly220Val	rs36103319	0 ($n = 200$)[A] 0 ($n = 150$)[B]	0.5 ($n = 200$)[A]	0 ($n = 60$)[A]		A: Shu et al. (2003) and B: Nies et al. (2009)
Pro283Leu	rs4646277	0 ($n = 150$)[A]			1.3 ($n = 150$ Koreans)[B] 0 ($n = 100$ Vietnamese)[B] 0.5 ($n = 100$ Chinese)[B]	A: Nies et al. (2009) and B: Kang et al. (2007)
Arg287Gly	rs4646278	0 ($n = 150$)				Nies et al. (2009)
Pro341Leu	rs2282143	0 ($n = 200$)[A] 1.7 ($n = 150$)[B]	8.2 ($n = 200$)[A]	11.7 ($n = 60$)[A]	16.8 ($n = 116$ Japanese)[C] 16.7 ($n = 150$ Koreans)[D] 5.5 ($n = 100$ Vietnamese)[D] 11 ($n = 100$ Chinese)[D]	A: Shu et al. (2003), B: Nies et al. (2009), C: Itoda et al. (2004) and D: Kang et al. (2007)
Intron, C>G g.160477747					2.2 ($n = 116$ Japanese)	Itoda et al. (2004)
Arg342His	rs34205214	0 ($n = 200$)[A] 0 ($n = 150$)[B]	3.1 ($n = 200$)[A]	0 ($n = 60$)[A]		A: Shu et al. (2003) and B: Nies et al. (2009)

Gly401Ser	rs34130495	$3.2\ (n = 232)^{A}$ $1.1\ (n = 200)^{B}$ $1\ (n = 150)^{C}$	$0.7\ (n = 200)^{B}$	$0\ (n = 60)^{B}$		A: Kerb et al. 2002, B: Shu et al. (2003) and C: Nies et al. (2009)
Met408Val	rs628031	$59.7\ (n = 232)^{A}$ $59.8\ (n = 200)^{B}$ $42.9\ (n = 150)^{C}$	73.5 $(n = 200)^{B}$	$76.2\ (n = 60)^{B}$	$81\ (n = 116$ Japanese$)^{D}$ $74\ (n = 150$ Koreans$)^{E}$	A: Kerb et al. (2002), B: Shu et al. (2003), C: Nies et al. (2009), D: Itoda et al. (2004) and E: Kang et al. (2007)
Ala413Ala	rs34888879	$0\ (n = 150)$				Nies et al. (2009)
Gly414Ala	rs72552762	$0.2\ (n = 232)$				Kerb et al. (2002)
Intron	rs4646281				$81\ (n = 116$ Japanese)	Itoda et al. (2004)
Met420del g.160480871-160480873delATG	No refSNP ID	$15.7\ (n = 232)^{A}$ $18.5\ (n = 200)^{B}$ $17\ (n = 150)^{C}$	$2.9\ (n = 200)^{B}$	$0\ (n = 60)^{B}$		A: Kerb et al. (2002), B: Shu et al. (2003) and C: Nies et al. (2009)
Met440Ile	rs35956182	$0\ (n = 200)$	$0.5\ (n = 200)$	$0\ (n = 60)$		Shu et al. (2003)
Intron, A>G g.160484779					$0.4\ (n = 116$ Japanese)	Itoda et al. (2004)
Intron	rs2297374				$30.2\ (n = 116$ Japanese)	Itoda et al. (2004)
Intron	rs622591				$59.1\ (n = 116$ Japanese)	Itoda et al. (2004)
Val461Ile	rs34295611	$0\ (n = 200)^{A}$ $0\ (n = 150)^{B}$	$1\ (n = 200)^{A}$	$0\ (n = 60)^{A}$		A: Shu et al. (2003) and B: Nies et al. (2009)
Gly465Arg	rs34059508	$1.5\ (n = 236)^{A}$ $4\ (n = 200)^{B}$ $4.3\ (n = 150)^{C}$	$0\ (n = 200)^{B}$	$0\ (n = 60)^{B}$		A: Kerb et al. (2002), B: Shu et al. (2003) and C: Nies et al. (2009)
Arg488Met	rs35270274	$0\ (n = 200)^{A}$ $0\ (n = 150)^{B}$	$5\ (n = 200)^{A}$	$0\ (n = 60)^{A}$		A: Shu et al. (2003) and B: Nies et al. (2009)
Val501Val	rs41267797	$3.6\ (n = 56)$				Kerb et al. (2002)
3′ UTR, delATG, g.160499610-160499612					$0.4\ (n = 116$ Japanese)	Itoda et al. (2004)
3′ UTR	rs9457846	$0\ (n = 150)$				Nies et al. (2009)
3′ UTR	rs34108432	$0\ (n = 150)$				Nies et al. (2009)

UTR untranslated region

The work by Itoda et al. (2004) describes arrhythmic patients

[a]In case that no refSNP ID is available, the genomic localization on chromosome 6 is given (NC_000006.10)

Table 6 Allele frequencies of *SLC22A2* (*OCT2*) genetic variants in different ethnic populations

SLC22A2 (OCT2)[a]	rs#	Allele frequency (%)				References
		European-American/ Caucasian	African-American	Asian-American	Chinese, Japanese, Korean, Vietnamese	
5′ UTR	rs8177506	0 ($n = 200$)	0 ($n = 200$)	1.7 ($n = 60$)		Leabman et al. (2002)
Phe45 fs	rs8177505	0.5 ($n = 200$)[A]	0 ($n = 200$)[A]	0 ($n = 60$)[A]	0 ($n = 116$ Japanese)[B]	A: Leabman et al. (2002) and B: Fukushima-Uesaka et al. (2004)
Pro54Ser	8177504	0 ($n = 200$)[A]	0.5 ($n = 200$)[A]	0 ($n = 60$)[A]	0 ($n = 116$ Japanese)[B]	A: Leabman et al. (2002) and B: Fukushima-Uesaka et al. (2004)
Thr130Thr	rs624249	39.4 ($n = 200$)[A]	20.5 ($n = 200$)[A]	18.3 ($n = 60$)[A]	15 ($n = 150$ Koreans)[B] 18.4 ($n = 112$ Chinese)[C]	A: Leabman et al. (2002), B: Kang et al. (2007) and C: Wang et al. (2008b)
Phe161Leu	rs8177509	0.5 ($n = 200$)	0 ($n = 200$)	0 ($n = 60$)		Leabman et al. (2002)
Met165Val	rs8177508	0 ($n = 200$)	0.5 ($n = 200$)	0 ($n = 60$)		Leabman et al. (2002)
Met165Ile	rs8177507	0 ($n = 200$)[A]	1 ($n = 200$)[A]	0 ($n = 60$)[A]	0 ($n = 116$ Japanese)[B]	A: Leabman et al. (2002) and B: Fukushima-Uesaka et al. (2004)
Intron	rs2774230	29.6 ($n = 200$)[A]	41.9 ($n = 200$)[A]	3.3 ($n = 60$)[A]	15.8 ($n = 112$ Chinese)[B]	A: Leabman et al. (2002) and B: Wang et al. (2008b)
Intron	rs8177511	0 ($n = 200$)	2.5 ($n = 200$)	0 ($n = 60$)		Leabman et al. (2002)
Thr199Ile, C>T g.160591647	No refSNP ID				0.9 ($n = 116$ Japanese)[A] 0.7 ($n = 150$ Koreans)[B] 0 ($n = 100$ Vietnamese)[B] 0 ($n = 100$ Chinese)[B]	A: Fukushima-Uesaka et al. (2004) and B: Kang et al. (2007)
Thr201Met, C>T g.160591641	No refSNP ID				1.3 ($n = 116$ Japanese)[A] 0.7 ($n = 150$ Koreans)[B] 1.5 ($n = 100$ Vietnamese)[B] 0 ($n = 100$ Chinese)[B] 0.4 ($n = 112$ Chinese)[C]	A: Fukushima-Uesaka et al. (2004), B: Kang et al. (2007) and C: Wang et al. (2008b)
Ile223Ile	rs8177510	0 ($n = 200$)	0 ($n = 200$)	1.7 ($n = 60$)		Leabman et al. (2002)
Ala270Ser	rs316019	15.7 ($n = 200$)[A] 8.7 ($n = 150$)[B]	11 ($n = 200$)[A]	8.6 ($n = 60$)[A]	16.8 ($n = 116$ Japanese)[C] 11.0 ($n = 150$ Koreans)[D] 13.5 ($n = 100$	A: Leabman et al. (2002), B: Nies et al. (2009), C: Fukushima-

					Vietnamese)[D] 14.0 (n = 100 Chinese)[D] 13.3 (n = 112 Chinese)[E]	Uesaka et al. (2004), D: Kang et al. (2007) and E: Wang et al. (2008b)
Intron	rs8177514	0 (n = 200)	0.5 (n = 200)	0 (n = 60)		Leabman et al. (2002)
Intron	rs2279463				12.4 (n = 112 Chinese)	Wang et al. (2008b)
Ala297Gly	rs8177513	0.5 (n = 200)	0 (n = 200)	0 (n = 60)		Leabman et al. (2002)
Intron	rs45437591	13.1 (n = 200)	14.5 (n = 200)	1.7 (n = 60)		Leabman et al. (2002)
Intron	rs8177512	13.1 (n = 200)	14.5 (n = 200)	1.7 (n = 60)		Leabman et al. (2002)
Intron	rs3219195				13.4 (n = 112 Chinese)	Wang et al. (2008b)
Intron	rs316021	23 (n = 150)[A]			17.4 (n = 112 Chinese)[B]	A: Nies et al. 2009 and B:Wang et al. 2008b
Intron	rs11422119				75.3 (n = 112 Chinese)	Wang et al. (2008b)
Arg400Cys	rs8177516	0 (n = 200)[A]	1.5 (n = 200)[A]	0 (n = 60)[A]	0 (n = 116 Japanese)[B]	A: Leabman et al. (2002) and B: Fukushima-Uesaka et al. (2004)
Ile401Ile	rs8177515	0.5 (n = 200)	0 (n = 200)	0 (n = 60)		Leabman et al. (2002)
Intron	rs8177518	0 (n = 200)[A]	0.5 (n = 200)[A]	6.7 (n = 60)[A]	4.5 (n = 112 Chinese)[B]	A: Leabman et al. (2002) and B: Wang et al. (2008b)
Lys432Gln	rs8177517	0 (n = 200)[A]	1 (n = 200)[A]	0 (n = 60)[A]	0 (n = 116 Japanese)[B]	A: Leabman et al. (2002) and B: Fukushima-Uesaka et al. (2004)
Gly466Gly	rs8177520	0 (n = 200)	0.5 (n = 200)	0 (n = 60)		Leabman et al. (2002)
Intron	rs11342198				15.5 (n = 112 Chinese)	Wang et al. (2008b)
Intron	rs315991				15.5 (n = 112 Chinese)	Wang et al. (2008b)
Intron	rs10532482				15.5 (n = 112 Chinese)	Wang et al. (2008b)
Intron	rs8177519	0 (n = 200)	0.5 (n = 200)	6.7 (n = 60)		Leabman et al. (2002)
Intron	rs3219197				15.5 (n = 112 Chinese)	Wang et al. (2008b)
Val502Val	rs316003	29 (n = 200)[A] 22 (n = 150)[B]	50 (n = 200)[A]	11.7 (n = 60)[A]	15 (n = 150 Koreans)[C] 15.5 (n = 112 Chinese)[D]	A: Leabman et al. (2002), B: Nies et al. (2009), C: Kang et al. (2007) and D: Wang et al. (2008b)
Ala529Ala	rs8177521	0 (n = 200)	0 (n = 200)	1.7 (n = 60)		Leabman et al. (2002)
3′ UTR, C>A	rs8177524	2 (n = 200)	0.5 (n = 200)	0 (n = 60)		Leabman et al. (2002)
3′ UTR, G>A	rs8177523	0 (n = 200)	0.5 (n = 200)	0 (n = 60)		Leabman et al. (2002)
	No refSNP ID	0 (n = 200)	3 (n = 200)	0 (n = 60)		Leabman et al. (2002)

(continued)

Table 6 (continued)

SLC22A2 (OCT2)[a]	rs#	Allele frequency (%)				References
		European-American/Caucasian	African-American	Asian-American	Chinese, Japanese, Korean, Vietnamese	
3′ UTR, insT g.160558192						
3′ UTR	rs3219198	0.5 ($n = 200$)	0.5 ($n = 200$)	0 ($n = 60$)		Leabman et al. (2002)

fs frameshift, *UTR* untranslated region

[a] In case that no refSNP ID is available, the genomic localization on chromosome 6 is given (NC_000006.10)

Table 7 Allele frequencies of *SLC22A3*(*OCT*) genetic variants in different ethnic populations

SLC22A3 (OCT3)[a]	rs#	Allele frequency (%)		References
		Caucasian	Korean	
5′ near gene	rs10455781	3 ($n = 150$)		Nies et al. (2009)
5′ near gene	rs555754	47.5 ($n = 100$)		Lazar et al. (2003)
Thr44Met	rs8187715	0 ($n = 150$)		Nies et al. (2009)
Arg120Arg	rs668871	48.5 ($n = 100$)[A]	73 ($n = 150$)[B]	A: Lazar et al. (2003) and B: Kang et al. (2007)
Gly193Gly g.160748108	rs76026925		2 ($n = 150$)	Kang et al. (2007)
Phe201Phe	rs3918291	0.5 ($n = 100$)		Lazar et al. (2003)
Met370Ile g.160778055	No refSNP ID	0 ($n = 100$)[A] 0 ($n = 150$)[B]		A: Lazar et al. (2008) and B: Nies et al. (2009)
Thr400Ile	rs8187725	0.3 ($n = 150$)		Nies et al. (2009)
Ala411Ala	rs2292334	36.5 ($n = 100$)[A] 34.3 ($n = 150$)[B]	50 ($n = 150$)[C]	A: Lazar et al. (2003), B: Nies et al. (2009) and C: Kang et al. (2007)
Ala439Val	rs12212246	0 ($n = 150$)		Nies et al. (2009)
Gly475Ser	rs9365165	0 ($n = 150$)		Nies et al. (2009)
Leu498Leu	rs8187722	0.7 ($n = 150$)		Nies et al. (2009)
3′ UTR	rs3088442	33.7 ($n = 150$)		Nies et al. (2009)

UTR untranslated region

[a]In case that no refSNP ID is available, the genomic localization on chromosome 6 is given (NC_000006.10)

Table 8 Allele frequencies of *SLC47/MATE* genetic variants in different ethnic populations.

SLC47A1 (MATE1)[a]	rs#	Allele frequency (%)					References
		European-American/Caucasian	Mexican-American	African-American	Chinese-American	Japanese	
5′ UTR	rs2252281	32.1 ($n = 68$)	28.9 ($n = 68$)	44.5 ($n = 68$)	23.1 ($n = 68$)		Ha Choi et al. (2009)
5′ UTR	rs78572621	5.4 ($n = 68$)	7.8 ($n = 68$)	1.7 ($n = 68$)	3.1 ($n = 68$)		Ha Choi et al. (2009)
5′ UTR	rs76654011	0 ($n = 68$)	0 ($n = 68$)	2.5 ($n = 68$)	0 ($n = 68$)		Ha Choi et al. (2009)
5′ UTR	rs75517315	1.5 ($n = 68$)	0.8 ($n = 68$)	1.5 ($n = 68$)	2.9 ($n = 68$)		Ha Choi et al. (2009)
Val10Leu, G>T g.19377872	No refSNP ID					2.2 ($n = 89$)	Kajiwara et al. (2009)
Arg11Arg, C>T g.19377877	No refSNP ID					0.6 ($n = 89$)	Kajiwara et al. (2009)
Ser29Ser	rs61733934	2.2 ($n = 68$)	0.7 ($n = 68$)	0 ($n = 68$)	0 ($n = 68$)		Chen et al. (2009b)
Ala42Ala, T>C g.19377970	No refSNP ID					0.6 ($n = 89$)	Kajiwara et al. (2009)
Gly64Asp	rs77630697	0 ($n = 68$)[A]	0 ($n = 68$)[A]	0 ($n = 68$)[A]	0.7 ($n = 68$)[A]	0.6 ($n = 89$)[B]	A: Chen et al. (2009b) and B: Kajiwara et al. (2009)
Phe90Phe	rs34012597	0 ($n = 68$)	0 ($n = 68$)	5.1 ($n = 68$)	0.7 ($n = 68$)		Chen et al. (2009b)
Leu125Phe	rs77474263	0 ($n = 68$)	5.1 ($n = 68$)	0 ($n = 68$)	0.7 ($n = 68$)		Chen et al. (2009b)
Leu236Leu	rs16960203	0 ($n = 68$)[A]	7.6 ($n = 68$)[A]	0.7 ($n = 68$)[A]	8.3 ($n = 68$)[A]	9.6 ($n = 89$)[B]	A: Chen et al. (2009b) and B: Kajiwara et al. (2009)
Ile297Ile,	rs76420645	0 ($n = 68$)	0 ($n = 68$)	0.8 ($n = 68$)	0 ($n = 68$)		Chen et al. (2009b)
Ala310Val, C>T g.19404100	No refSNP ID					2.2 ($n = 89$)	Kajiwara et al. (2009)
Asp328Ala, A>C g.19404154	No refSNP ID					0.6 ($n = 89$)	Kajiwara et al. (2009)
Val338Ile	rs35790011	0 (n = 68)	0 (n = 68)	5.1 (n = 68)	0 (n = 68)		Chen et al. (2009b)
Asn474Ser, A>G g.19416701	No refSNP ID					0.6 ($n = 89$)	Kajiwara et al. (2009)

Val480Met	rs76645859	0 ($n = 68$)	0 ($n = 68$)	0 ($n = 68$)	0.8 ($n = 68$)	Chen et al. (2009b)
Cys497Ser	rs35395280	0 ($n = 68$)	0 ($n = 68$)	2.4 ($n = 68$)	0 ($n = 68$)	Chen et al. (2009b)
Gln519His	rs78700676	0 ($n = 68$)	0 ($n = 68$)	0.8 ($n = 68$)	0 ($n = 68$)	Chen et al. (2009b)

The work by Kajiwara et al. (2009) describes subjects with renal diseases

[a]In case that no refSNP ID is available, the genomic localization on chromosome 17 is given (NC_000017.9). *UTR*, untranslated region

Table 9 Phenotype-genotype correlations of *SLC22A1*(*OCT1*) in humans

	SLC22A1 (OCT1)	Population (n)	Results	References
Tissue expression				
Liver	rs4646272 (intron) Met408Val	Nondiabetic donors (Caucasian $n = 33$, Japanese $n = 25$)	OCT1 mRNA tended to be lower in 408Met carriers	Shikata et al. (2007)
Liver	36 variants	Caucasian surgical liver samples ($n = 150$)	By multivariate analysis adjusted for multiple testing, Arg61Cys significantly correlated with decreased OCT1 protein expression ($p < 0.0001$)	Nies et al. (2009)
Pharmacokinetics/pharmacodynamics				
Metformin (two doses, total 1,850 mg)	Arg61Cys Gly401Ser Met420del Gly465Arg	Healthy subjects ($n = 20$)	Plasma glucose AUC for OGTT ($p = 0.004$) and insulin levels 2 h after glucose administration ($p < 0.05$) were higher in OCT1-variant subjects (carrier of any of the SNPs tested) vs. subjects with only OCT1-reference alleles	Shu et al. (2007)
Metformin (two doses, total 1,850 mg)	Arg61Cys Gly401Ser Met420del Gly465Arg	Healthy subjects ($n = 20$)	Significant higher AUC, higher $C_{max,}$ and lower V/F in OCT1-variant subjects (carrier of any of the SNPs tested) vs. subjects with only OCT1-reference alleles	Shu et al. (2008)
Metformin (single dose, 500 mg)	Ser52Ser Arg61Cys Gly401Ser Met420del Gly465Arg and other tagging SNPs	Healthy male caucasians ($n = 103$)	CL_{ren} ($p = 0.032$) and net CL by tubular secretion ($p = 0.03$) increased with the number of inactive OCT1 alleles defined by the presence of one or more of the amino acid substitutions at positions 61, 401, 420, and 465	Tzvetkov et al. (2009)
Imatinib	Arg61Cys Gly465Arg	Patients with GIST ($n = 74$)	No difference in oral clearance at steady state in patients with at least one of both variants compared to patients with the reference allele on both positions	Hu et al. (2008)
Treatment outcome				
Metformin	Phe41Leu Ser52Ser Gly81Gly Pro117Leu rs4646272 (intron)	Patients with type 2 diabetes ($n = 33$)	The intron variant (rs4646272 T>G) was a negative and Met408Val a positive outcome predictor in a stepwise discriminant functional analysis	Shikata et al. (2007)

	Phe160Leu Pro341Leu Met408Val rs36056065 (intron) rs622591 (intron)			
Metformin	11 tagging SNPs (Illumina 550k SNP array) rs3798174 (intron) rs6937722 (intron) rs3798168 (intron) rs628031 (Met408Val) rs9457843 (intron) rs3798167 (intron) rs2197296 (intron) rs622342 (intron) rs1443844 (intron) rs2297374 (intron) rs1564348 (intron) rs622591 (intron)	Incident metformin users ($n = 102$, Rotterdam Study)	Only the rs622342 A>C variant was associated with metformin response. For each minor C allele, the reduction in HbA1c levels was 0.28% less ($p = 0.005$). After Bonferroni correction, the *p*-value was 0.045	Becker et al. (2009b)
Metformin	Arg61Cys Met420del	Patients with type 2 diabetes and definable metformin response ($n = 1531$, GoDARTS study)	No clinically significant reduction to lower HbA1c levels, to influence the chance of achieving a treatment target or the hazard of therapy failure in patients carrying both SNPs compared to reference genotype	Zhou et al. (2009)
Metformin	OCT1: rs622342 (intron) MATE1: rs2289669 (intron)	Incident metformin users ($n = 98$, Rotterdam Study)	The effect of MATE 1 rs2289669 polymorphism on glucose-lowering effect was larger in patients with the OCT1 rs622342 CC genotype ($p = 0.005$) than in patients with the AA genotype	Becker et al. (2010)
Imatinib	Arg61Cys	Patients with CML ($n = 32$)	No association with the Arg61Cys variant and imatinib response (cytogenetic/major molecular response)	Zach et al. (2008)
Imatinib oral				

(*continued*)

Table 9 (continued)

	SLC22A1 (OCT1)	Population (n)	Results	References
	Arg61Cys Ser52Ser Phe160Leu Pro341Leu Met408Val	Patients with CML ($n = 229$), median duration of therapy 40.8 months, median follow-up 47.3 months	Patients with the GG genotype for Phe160Leu showed a higher risk of LOR (HR, 4.86; $p = 0.0008$) or treatment failure (HR, 3.24; $p = 0.02$) compared to patients carrying at least one C allele. No correlation with SLC22A1 haplotypes	Kim et al. (2009)

CML chronic myeloid leukemia, *GIST* gastrointestinal stromal tumor, *OGTT* oral glucose tolerance test, *LOR* loss of response, *HR* hazard ratio

Table 10 Phenotype-genotype correlations of *SLC22A2* (*OCT2*) in humans

	SLC22A2 (OCT2)	Population (n)	Results	References
Pharmacokinetics/pharmacodynamics				
Metformin (single dose, 500 mg)	Thr199Ile Thr201Met Ala270Ser	Healthy Korean subjects ($n = 26$)	Subjects with variant genotypes for the three SNPs showed higher values for C_{max} ($p = 0.0005$) and for AUC ($p = 0.0003$), but lower values for CL/F ($p = 0.0335$), Vd/F ($p = 0.0316$), CL_{ren} ($p = 0.0018$), and net CL by tubular secretion ($p = 0.001$) as compared to the reference genotype group	Song et al. (2008)
Metformin (single dose, 500 mg)	Ala270Ser	Healthy Chinese subjects ($n = 14$)	Mean CL_{ren} and net CL by tubular secretion were 26.1% ($p = 0.022$) and 28% ($p = 0.036$), resp., lower in TT vs. GG carriers After cimetidine coadministration CL_{ren} and net CL were significantly decreased in GG and GT carriers, respectively. Metformin $AUC_{0-\infty}$ increased in GG carriers ($p = 0.043$).	Wang et al. (2008b)
Metformin (single dose, 850 mg)	Ala270Ser	Healthy subjects ($n = 23$)	Mean CL_{ren} ($p = 0.005$) net CL by tubular secretion ($p = 0.002$) were lower in GG vs GT carriers	Chen et al. (2009a)
Metformin (single dose, 500 mg)	14 variants including Ala270Ser	Healthy male Caucasians ($n = 103$)	No significant association between CL_{ren} and OCT2 variants	Tzvetkov et al. (2009)
Treatment outcome				
Metformin	Thr201Met Ala270Ser	Patients with type 2 diabetes ($n = 33$)	No association with metformin response was found	Shikata et al. (2007)
Cisplatin	Ala270Ser	Patients with solid tumors and cisplatin-based therapy ($n = 78$)	Ala270Ser variant was associated with reduced cisplatin-induced nephrotoxicity and only patients with the reference sequence showed significant increase in serum creatinine level ($n = 68$, $p = 0.0009$)	Filipski et al. (2009)
Susceptibility				
Essential hypertension	Ala270Ser	Caucasian patients with cardiovascular diseases ($n = 607$)	Essential hypertension was less prevalent among patients carrying at least one Ser270 allele compared to patients with the reference sequence ($p = 0.028$). The effect was more prominent in patients without type 2 diabetes ($p = 0.013$)	Lazar et al. (2006)

Table 11 Phenotype-genotype correlations of *SLC22A3* (*OCT3*) in humans

	SLC22A3 (OCT3)	Population (n)	Results	References
Tissue expression				
Liver	34 variants including rs3088442 (3′ UTR)	Caucasian surgical liver samples ($n = 150$)	By multivariate analysis adjusted for multiple testing, four variants (rs2292334, rs2048327, rs1810126, rs3088442) were associated with reduced mRNA levels ($p = 0.03$)	Nies et al. (2009)
Pharmacokinetics/pharmacodynamics				
Metformin (single dose, 500 mg)	6 variants	Healthy male Caucasians ($n = 103$)	No significant association between CL_{ren} and OCT3 variants	Tzvetkov et al. (2009)
Susceptibility				
Methamphetamine dependence	rs655185 (intron) rs509707 (intron) rs4709426 (intron) rs7745775 (intron) rs3106164 (intron) rs2292334 (Ala411Ala) rs3918286 (intron) rs3088442 (3′ UTR)	Japanese subjects with methamphetamine (MAP) dependence ($n = 213$) and healthy controls ($n = 443$)	Genotype ($p = 0.024$) and allele ($p = 0.011$) frequency of rs509707, allele frequency of rs4709426 ($p = 0.037$), and haplotypic frequencies for both SNPs ($p = 0.0438$) differed significantly between polysubstance and single-MAP users	Aoyama et al. (2006)
Obsessive-compulsive disorder (OCD)	rs60515630 (5′ near gene) rs555754 (5′ near gene) rs668871 (Arg120Arg) rs3918291 (Phe201Phe) Met370Ile rs2292334 (Ala411Ala) rs3918287 (intron) rs2457574 (intron) rs1810126 (3′ UTR)	Children/adolescents ($n = 84$) with childhood-onset OCD vs healthy Caucasian subjects ($n = 100$)	Known SNPs and frequent haplotypes were not associated with OCD. Two novel variants (rs60515630, Met370Ile) were exclusively found in OCD patients	Lazar et al. (2008)
Prostate cancer	GWA study	Prostate cancer patients vs population-screened controls stage 1: 1854 cases vs 1894 controls Stage 2: 3268 cases vs 3366 controls	Significant association of variant rs9364554 (intron) with prostate cancer susceptibility (stage 1: $p = 9.3 \times 10^{-7}$, stage 1 + 2: $p = 5.5 \times 10^{-10}$)	Eeles et al. (2008)

Coronary artery disease (CAD)	GWHA study	Stage 1: WTCC CAD study (1,926 CAD cases vs 2,938 controls) Stage 2: GerMIFS I study (875 CAD cases vs 1,644 controls) Stage 3: four additional studies (total numbers: 6,198 CAD cases vs 5,681 controls)	Significant association of the haplotypes derived from 4 SNPs of SLC22A3 (rs2048327)-LPAL2-LPA gene cluster with CAD in independent studies	Tregouet et al. (2009)

GWA genomewide association, *GWHA* genomewide haplotype association

Table 12 Phenotype-genotype correlations of *SLC47/MATE* in humans

	SLC47A1 (MATE1)	Population (n)	Results	References
Tissue expression				
Kidney Liver	rs2252281 (5′ UTR)	Surgical kidney samples ($n = 38$), post-mortem liver samples ($n = 34$)	mRNA in TC ($n = 21$) or CC ($n = 5$) kidneys were significantly lower ($p = 0.015$) compared to TT genotype ($n = 12$). No effect was found in human liver	Ha Choi et al. (2009)
Pharmacokinetics/pharmacodynamics				
Metformin (single dose, 500 mg)	rs2289669 (intron)	Healthy male Caucasians ($n = 103$)	No significant association between CL_{ren} and the rs2289669 G>A variant	Tzvetkov et al. (2009)
Treatment outcome				
Metformin	12 tagging SNPs (Illumina 550k SNP array) rs894680 (intron) rs2018675 (intron) rs2440154 (intron) rs2440155 (intron) rs16960201 (intron) rs2453568 (intron) rs2244280 (intron) rs2289669 (intron) rs1961669 (intron) rs2453594 (intergenic region) rs2453589 (intergenic region) rs2165894 (intergenic region)	Incident metformin users ($n = 116$, Rotterdam Study)	Only the rs2289669 G>A variant was associated with metformin response. For each minor A allele, the reduction in HbA1c levels was 0.30% larger ($p = 0.005$). After Bonferroni correction, the p-value was 0.045	Becker et al. (2009a)

activity (White et al. 2007; Wang et al. 2008a), is the tyrosine kinase inhibitor imatinib, a mainstay in treatment of patients with chronic myeloid leukemia (CML). Although one study suggests a significant contribution of the OCT1-Phe160Leu variant related to loss of response to imatinib or treatment failure (Kim et al. 2009), further confirmatory studies are still missing, which are mandatory to support such an association.

Regarding OCT2 variants, the Ala270Ser polymorphism was investigated in several pharmacokinetic metformin studies with discrepant results (Table 10). The study with the most representative number of subjects included ($n = 103$) did not show any association (Tzvetkov et al. 2009). Interestingly, the OCT2-Ala270Ser variant was also related to a significantly reduced cisplatin-induced nephrotoxicity in patients with solid tumors, which fits to the fact that cisplatin is indeed an OCT2 substrate and OCT2 is highly expressed in human kidney (Filipski et al. 2009).

Although the physiological role of OCTs and MATEs is not fully resolved, it is conceivable that membrane transporters determine intracellular concentration of potentially efficient and/or toxic agents and metabolites. In this context it is plausible to hypothesize that genotype-dependent OCT/MATE expression may also contribute to a certain disease susceptibility. Of interest, susceptibility for diseases was repeatedly related to OCT3 (Table 11), whereas convincing data for both, OCT1 and OCT2, are lacking. The *SLC22A3* gene was identified as a potential risk factor for prostate cancer as well as coronary artery disease by genomewide association studies (GWA), including thousands of index cases and confirmed by independent control groups (Eeles et al. 2008; Tregouet et al. 2009).

Taken together, compared with other transport proteins the research on the impact of OCT and MATE variants is only at the beginning. Comprehensive genotype–phenotype correlation studies including different human tissues as well as clinical response data are required in the future.

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Role of the Intestinal Bile Acid Transporters in Bile Acid and Drug Disposition

Paul A. Dawson

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Abstract Membrane transporters expressed by the hepatocyte and enterocyte play critical roles in maintaining the enterohepatic circulation of bile acids, an effective recycling and conservation mechanism that largely restricts these potentially cytotoxic detergents to the intestinal and hepatobiliary compartments. In doing so, the hepatic and enterocyte transport systems ensure a continuous supply of bile acids to be used repeatedly during the digestion of multiple meals throughout the day. Absorption of bile acids from the intestinal lumen and export into the portal circulation is mediated by a series of transporters expressed on the enterocyte apical and basolateral membranes. The ileal apical sodium-dependent bile acid

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cotransporter (abbreviated ASBT; gene symbol, *SLC10A2*) is responsible for the initial uptake of bile acids across the enterocyte brush border membrane. The bile acids are then efficiently shuttled across the cell and exported across the basolateral membrane by the heteromeric Organic Solute Transporter, OSTα–OSTβ. This chapter briefly reviews the tissue expression, physiology, genetics, pathophysiology, and transport properties of the ASBT and OSTα–OSTβ. In addition, the chapter discusses the relationship between the intestinal bile acid transporters and drug metabolism, including development of ASBT inhibitors as novel hypocholesterolemic or hepatoprotective agents, prodrug targeting of the ASBT to increase oral bioavailability, and involvement of the intestinal bile acid transporters in drug absorption and drug–drug interactions.

Keywords Bile acids · Intestine · Transporter · Drug absorption · Prodrug targeting

Abbreviations

ABC	ATP-binding cassette
ASBT	Apical-dependent bile acid transporter
BA	Bile acid
BARI	Bile acid reabsorption inhibitor
BCRP	Breast cancer resistance protein
BDDCS	Biopharmaceutics Drug Disposition Classification System
BLM	Basolateral membrane
BSEP	Bile salt export pump
CHO	Chinese hamster ovary
CM	Canalicular membrane
FDA	United States Food and Drug Administration
FGF	Fibroblast growth factor
FHTG	Familial Hypertriglyceridemia
FXR	Farnesoid X-receptor
GWAS	Genome-Wide Association Study
IBAM	Idiopathic bile acid malabsorption
ILBP	Ileal lipid binding protein
LDL	Low density lipoprotein
MDCK	Madin-Darby canine kidney
MDR	Multidrug resistance protein
MRP	Multidrug resistance-associated protein
norUDCA	Nor-ursodeoxycholic acid
NTCP	Na^+-taurocholate cotransporting polypeptide
OATP	Organic anion transporting polypeptide
OMIM	Online Mendelian Inheritance in Man
OST	Organic solute transporter
PBAM	Primary bile acid malabsorption

POSCH	Program on the Surgical Control of Hyperlipidemias
PSC	Primary Sclerosing Cholangitis
QSAR	Quantitative structure–activity relationship
SLC	Solute carrier
SNP	Single nucleotide polymorphism
UDCA	Ursodeoxycholic acid

1 Overview of the Enterohepatic Circulation of Bile Acids

Bile acids are synthesized from cholesterol in the liver, conjugated (N-acyl amidated) to taurine or glycine, secreted into bile, and stored in the gallbladder. During a meal, the gallbladder contracts and bile acids enter the small intestine, where they facilitate absorption of fat-soluble vitamins and cholesterol (Hofmann and Hagey 2008). The majority (>90%) of bile acids are reabsorbed from the intestine and returned to the liver via the portal venous circulation. The bile acids are then transported across the sinusoidal membrane of the hepatocyte and resecreted across the canalicular membrane into bile (Dawson et al. 2009). Because these processes, that is, intestinal absorption, return to the liver in the portal circulation, and hepatic extraction of bile acids are so efficient, the majority of the bile acids secreted across the canalicular membrane into bile are derived from the recirculating pool, with less than 10% from new *de novo* hepatic synthesis. In the small intestine, bile acids are absorbed by passive and active mechanisms, with active transport accounting for the majority of conjugated bile acid uptake (Dietschy 1968; Lewis and Root 1990; Marcus et al. 1991; Aldini et al. 1996). The passive absorption occurs down the length of the intestine, whereas active absorption of bile acids is largely restricted to the distal small intestine (ileum) (Schiff et al. 1972; Krag and Phillips 1974). In man and all other vertebrates examined to date, the ileal epithelium has developed an efficient transport system for active reclamation of bile acids (Hofmann and Hagey 2008; Hofmann et al. 2010). This scheme ensures that the intraluminal concentration of conjugated bile acids will remain sufficiently high in proximal intestine to promote lipid absorption as well as reduce the small intestinal bacterial load. Overall, the enterohepatic circulation maintains a bile acid pool size of approximately 4 mg in mice and 2–4 g humans. This pool cycles multiple times per meal (Hofmann et al. 1983; Hulzebos et al. 2001) and intestinal bile acid absorption may be as great as 20 mg/day in mice and 30 g/day in humans. The bile acids that escape intestinal absorption (<0.5 g/day in humans) are excreted into the feces. The bile acid pool size is carefully maintained by hepatic conversion of cholesterol to bile acid, and this process represents a major route for elimination of cholesterol from the body (Dietschy et al. 1993; Dietschy and Turley 2002). Over the past two decades, investigators have identified all the major hepatic and intestinal transporters that function to maintain the enterohepatic circulation of bile acids (Dawson

et al. 2009). The cellular location and properties of these transporters are summarized in Fig. 1 and Table 1, respectively.

2 Overview of Intestinal Bile Acid Transport

Bile acids are reclaimed through a combination of passive absorption in the proximal small intestine, active transport in the distal ileum, and passive absorption in the colon. Several observations support the concept that the terminal ileum is the major site of bile acid reabsorption in man and experimental animal models. These observations include the finding that there is little decrease in intraluminal bile acid concentration prior to the ileum (Dietschy 1968) and the appearance of bile acid malabsorption after ileal resection (Hofmann and Poley 1972). Subsequent studies using in situ perfused intestinal segments to measure bile acid absorption (Marcus et al. 1991; Aldini et al. 1994, 1996) demonstrated that ileal bile acid transport is a high capacity system sufficient to account for the hepatobiliary output of bile acids. The general consensus from these studies was that ileal active transport is the major route for conjugated bile acid uptake, whereas the intestinal passive or facilitative absorption may be significant for unconjugated and some glycine-conjugated bile acids. The ileal apical sodium-dependent bile acid cotransporter (abbreviated ASBT; gene symbol, *SLC10A2*) mediates the initial uptake of bile acids across the ileal enterocyte apical brush border membrane (Dawson et al. 2009). After entering the cytosolic compartment, the bile acids bind to the ileal lipid binding protein (abbreviated ILBP; also called the ileal bile acid binding protein or IBABP; gene symbol, *FABP6*), an abundant 14 kDa soluble protein (Oelkers and Dawson 1995). ILBP is believed to be involved in the transcellular transport of bile acids (Lin et al. 1990; Kramer et al. 1993, 1997) or possibly protection of the enterocyte from the cytotoxic properties of bile acids. However, the Farnesoid X-receptor (FXR) null mouse lacks appreciable intestinal ILBP expression but still exhibits normal levels of intestinal bile acid absorption. These results suggest that ILBP is not essential for intestinal bile acid transport (Kok et al. 2003). Nevertheless, resolving the questions regarding ILBP's function will require further study, including the generation and analysis of *Fabp6*-null mice. Regardless of their intracellular route, the bile acids are ultimately shuttled across the ileal enterocyte (Lewis and Root 1990) and exported across the basolateral membrane into the portal circulation by the heteromeric Organic Solute Transporter, OSTα–OSTβ (Ballatori et al. 2005; Dawson et al. 2005).

3 The Apical Sodium-Dependent Bile Acid Transporter: ASBT

3.1 ASBT General Properties and Tissue Expression

ASBT (also called IBAT, ISBT, ABAT; gene symbol *SLC10A2*) was the second member identified of the SLC10 family of solute carrier proteins. The SLC10

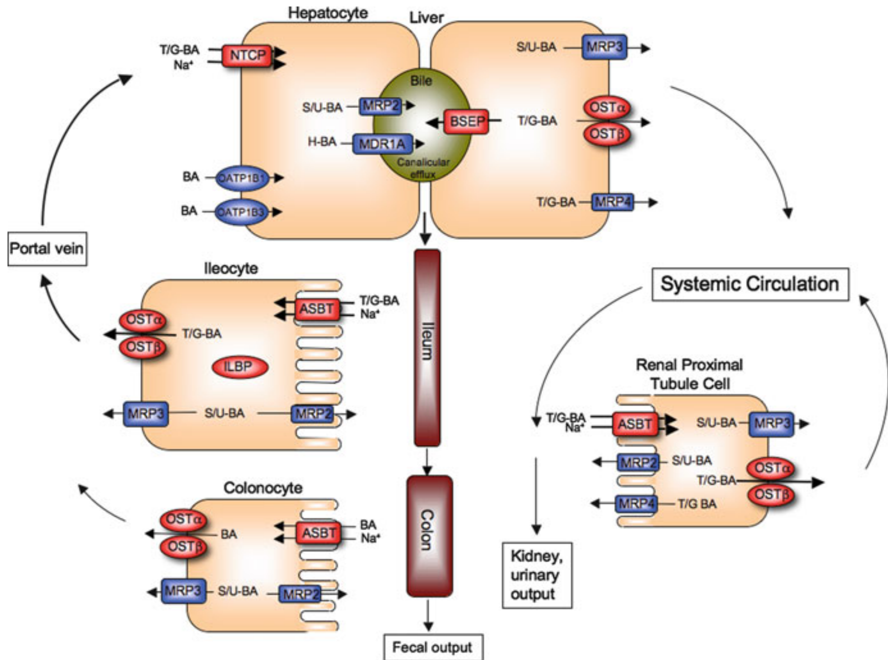

Fig. 1 Enterohepatic circulation of bile acids showing the individual transport proteins in hepatocytes, ileocytes (ileal enterocytes), and renal proximal tubule cells. After their synthesis or reconjugation, taurine and glycine (T/G) conjugated bile acids (BA) are secreted into bile by the canalicular bile salt export pump (BSEP; gene symbol *ABCB11*). The small amount of bile acids that have been modified by the addition of sulfate or glucuronide (S/U) are secreted by the multidrug resistance-associated protein-2 (MRP2; gene symbol *ABCC2*), whereas those modified by additional hydroxylation (H) are secreted by MRP2 and possibly P-glycoprotein (MDR1; gene symbol *ABCB1A*). These divalent (S/G) or tetrahydroxylated (H) bile acids are present in very small quantities under normal physiological conditions, but may accumulate in disease states such as cholestasis. The bile acids are stored in the gallbladder and empty into the intestinal lumen in response to a meal. Bile acids are poorly absorbed in the proximal small intestine, but efficiently taken up by the apical sodium-dependent bile acid transporter (ASBT; gene symbol *SLC10A2*) in the ileum. The bile acids bind to the ileal lipid binding protein (ILBP; gene symbol *FABP6*) in the cytosol, and are efficiently exported across the basolateral membrane into the portal circulation by the heteromeric transporter OSTα–OSTβ. The multidrug resistance-associated protein-3 (MRP3; gene symbol *ABCC3*) is a minor contributor to basolateral export of native bile acids from the enterocyte, but may have a more significant role in export of any modified (glucuronidated or sulfated) bile acids that may be formed. MRP2 may also serve to export modified bile acids across the apical brush border membrane. Although most bile acids are absorbed in the small intestine, colonocytes express very low levels of ASBT and appreciable levels of MRP3 and OSTα–OSTβ; these carriers may serve to absorb a fraction of the unconjugated bile acids from the lumen of the colon. After their absorption from the intestine, bile acids travel back to the liver where that are cleared by the Na$^+$-taurocholate cotransporting polypeptide (NTCP; gene symbol *SLC10A1*). Members of the Organic Anion Transport Protein family, OATP1B1 (gene symbol *SLCO1B1*) and OATP1B3 (gene symbol *SLCO1B3*) also participate, and are particularly important for unconjugated bile acids. Under cholestatic conditions, unconjugated, conjugated, or modified bile acids can be effluxed across the basolateral (sinusoidal) membrane

Table 1 Function of transport proteins in the enterohepatic circulation of bile acids

Transporter (*Gene*)	Location	Function
Hepatocyte		
Process: hepatocyte sinusoidal to canalicular bile acid transport		
NTCP (*SLC10A1*)	BLM	Na^+-dependent uptake of BA
OATP1B1 (*SLCO1B1*)	BLM	Na^+-independent uptake of BA
OATP1B3 (*SLCO1B3*)	BLM	Na^+-independent uptake of BA
BSEP (*ABCB11*)	CM	ATP-dependent export of BA
MRP2 (ABCC2)	CM	ATP-dependent export of BA sulfates/glucuronides
MDR1 (*ABCB1*)	CM	ATP-dependent export of tetrahydroxylated BA
Process: hepatocyte sinusoidal bile acid export		
MRP3 (*ABCC3*)	BLM	ATP-dependent export of BA, BA sulfates/glucuronides
MRP4 (*ABCC4*)	BLM	ATP-dependent export of BA
OSTα–OSTβ	BLM	BA export
Cholangiocyte		
Process: ductular secretion and bile remodeling		
ASBT (*SLC10A2*)	APM	Bile acid uptake (cholehepatic shunt)
OSTα–OSTβ	BLM	Bile acid export
MRP3 (*ABCC3*)	BLM	Bile acid export of BA, BA sulfates/glucuronides
Renal proximal tubule cell		
Process: reclamation of bile acids from the renal tubules		
ASBT (*SLC10A2*)	APM	Bile acid uptake
OSTα–OSTβ	BLM	Bile acid export
MRP3 (*ABCC3*)	BLM	ATP-dependent export of BA, BA sulfates/glucuronides
MRP4	BLM	ATP-dependent BA export
Ileal enterocyte		
Process: reclamation of bile acids from the intestinal lumen		
ASBT (*SLC10A2*)	APM	Bile acid uptake
OSTα–OSTβ	BLM	Bile acid export
MRP3 (*ABCC3*)	BLM	Bile acid export

ABC ATP-binding cassette, *ASBT* apical sodium-dependent bile acid transporter, *BA* bile acid, *BLM* basolateral membrane, *BSEP* bile salt export pump, *CM* canalicular membrane, *MDR* multidrug resistance protein, *MRP* multidrug resistance-associated protein, *NTCP* Na^+-taurocholate cotransporting polypeptide, *OATP* organic anion transporting polypeptide, *OST* organic solute transporter, *SLC* solute carrier

Fig. 1 (continued) of the hepatocyte by OSTα–OSTβ, MRP3, or multidrug resistance-associated protein-4 (MRP4; gene symbol *ABBC4*) into the systemic circulation. Under normal physiological conditions, a fraction of the bile acid escapes first pass hepatic clearance and enters the systemic circulation. The free bile acids are filtered by the renal glomerulus, efficiently reclaimed by the ASBT in the proximal tubules, and exported back into the systemic circulation, thereby minimizing their excretion in the urine. This efficient renal reabsorption occurs even under cholestatic conditions for unconjugated and conjugated bile acids, when serum bile acid concentrations are dramatically elevated. Overall, this integrated transport system minimizes fecal and urinary bile acid loss and functions to largely restrict these potentially cytotoxic detergents to the intestinal and hepatobiliary compartments (Reprinted with permission from PA Dawson et al. Getting the most from OST: Role of the organic solute transporter, OSTα–OSTβ, in bile acid and steroid metabolism. Biochim Biophys Acta 2010; 1801: 994–1004.)

family is comprised of six members, *SLC10A1* (NTCP), *SLC10A2* (ASBT), *SLC10A3* (P3), *SLC10A4* (P4), *SLC10A5* (P5), and *SLC10A6* (SOAT), that share between 19% and 42% amino acid sequence identity (Geyer et al. 2006). *SLC10A1* (NTCP) and *SLC10A2* (ASBT) are the best characterized family members and have important physiological functions as bile acid transporters (Hagenbuch and Dawson 2004). The related *SLC10A6* (SOAT) transports steroid sulfates but not bile acids (Geyer et al. 2007), and little is known about the physiological function, substrates, or transport properties of *SLC10A3*, *SLC10A4*, and *SLC10A5* (Geyer et al. 2006; Splinter et al. 2006).

ASBT is expressed at tissue sites that enable the enterohepatic circulation of bile acids, including the apical membrane of ileal enterocytes, proximal renal convoluted tubule cells, large cholangiocytes, and gallbladder epithelial cells (Wong et al. 1994; Christie et al. 1996; Alpini et al. 1997; Lazaridis et al. 1997; Chignard et al. 2001). In the intestine, sodium-dependent bile acid transport activity and ASBT expression is found predominantly in villus but not crypt enterocytes (Kapadia and Essandoh 1988; Shneider et al. 1995). ASBT expression in small intestine is restricted to the terminal ileum (distal ~30% of the small intestine) in the mouse, rat, hamster, and monkey, with negligible expression in proximal small intestine (Wong et al. 1994; Shneider et al. 1995; Dawson et al. 2005). For humans, several lines of evidence suggest that the gradient of expression along the longitudinal axis of the intestine is qualitatively similar with highest levels of ASBT expression in terminal ileum. This evidence includes intestinal perfusion studies demonstrating active bile acid absorption in human ileum but not in proximal small intestine (Krag and Phillips 1974), and studies showing that ASBT mRNA or protein expression is higher in ileum than proximal intestine or colon (Hruz et al. 2006; Meier et al. 2007; Balesaria et al. 2008). Unlike rodents or monkeys, humans express low but readily detectable levels of ASBT in duodenum (Hruz et al. 2006; Balesaria et al. 2008). Nevertheless, the low level of ASBT expression in proximal intestine is insufficient to maintain the enterohepatic circulation of bile acids as evidenced by the clinically significant bile acid malabsorption following ileal resection (Hofmann and Poley 1972). The factors that control ASBT expression along the longitudinal (cephalocaudal) axis of the small intestine are not well understood. But the recent discovery that the transcription factor GATA4 is essential for silencing ASBT expression in proximal small intestine has provided an important new insight to underlying mechanism (Bosse et al. 2006; Battle et al. 2008).

In addition to small intestine, ASBT is expressed in renal proximal tubule cells (Christie et al. 1996; Craddock et al. 1998) and biliary epithelium (Alpini et al. 1997; Lazaridis et al. 1997). In the kidney, the ASBT acts as a salvage mechanism to prevent urinary excretion of bile acids that have undergone glomerular filtration (Wilson et al. 1981). In the ileum and kidney, ASBT functions to absorb the majority of the bile acids available in the adjacent lumen (almost quantitative reclamation). Only a very small fraction escapes absorption and is excreted in the feces or urine. In contrast, almost all the bile acids that are secreted by the liver into bile will ultimately move down the biliary tract and enter the gallbladder or small intestine, indicating that ASBT's function in this compartment is not

quantitative reclamation of bile acids. As such, the physiological significance of active bile acid absorption from the biliary tract is unclear. One possibility is that the ASBT functions in the cholehepatic shunt pathway. The term *cholehepatic shunt* was originally coined by Dr. Alan Hofmann to describe the cycle whereby unconjugated dihydroxy bile acids secreted into bile are passively absorbed by the epithelial cells (cholangiocytes) lining the bile ducts, returned to the hepatocyte via the periductular capillary plexus, and resecreted into bile (Gurantz et al. 1991). Absorption of the protonated unconjugated bile acid molecule generates a bicarbonate anion, resulting in a bicarbonate-rich choleresis. Premature absorption and resecretion of the bile acid also promotes bile formation by increasing bile acid-dependent bile flow. This cycle explains the hypercholeresis (increased bile secretion) observed for unconjugated C-24 dihydroxy bile acids such as ursodeoxycholic acid (UDCA), for unconjugated C-23 bile acid analogs such as norursodeoxycholate (norUDCA) (Halilbasic et al. 2009), and for certain drugs such as the nonsteroidal anti-inflammatory drug sulindac (Hofmann et al. 2005). Because the original description of the cholehepatic shunt pathway included only a passive component, its physiological significance for hepatic secretion was unclear. Although exogenously administered UDCA or norUDCA may only be partially conjugated to glycine or taurine, the endogenous bile acids are efficiently conjugated by the liver prior to secretion by the bile salt export pump (BSEP) into bile. As such, the majority of the bile acids in the biliary tract are ionized and unable to diffuse passively across the biliary epithelium. The findings that ASBT and the basolateral bile acid transporter OSTα–OSTβ are expressed by the biliary epithelium provide an important physiological mechanism for cholehepatic shunting of ionized conjugated bile acids (Alpini et al. 1997; Lazaridis et al. 1997; Ballatori et al. 2005). In vivo data in support of this pathway for conjugated bile acids has been obtained using a rat model (Alpini et al. 2005), but the quantitative significance in humans or under different physiological or pathophysiological conditions still remains to be determined (Xia et al. 2006). Nevertheless, this is an area of increased interest following the publication of recent exciting preclinical results obtained using norUDCA as a therapy for Primary Sclerosing Cholangitis (PSC) (Fickert et al. 2006), and renewed investigation should yield important insights to the physiological role and therapeutic potential of the cholehepatic shunt pathway (Glaser and Alpini 2009).

3.2 ASBT Structure

ASBT is a 348 amino acid membrane glycoprotein with a glycosylated extracellular amino terminus and cytosolic carboxyl terminus, indicating an odd number of transmembrane domains. Early membrane topology models favored seven transmembrane segments for the ASBT and NTCP (Hagenbuch and Meier 1994; Dawson and Oelkers 1995), however subsequent in vitro translation/membrane insertion scanning and alanine-scanning mutagenesis studies carried out by Hallén

and Sachs yielded conflicting results unable to distinguish between models with seven or nine membrane-spanning regions (Hallen et al. 1999, 2002; Mareninova et al. 2005). Swaan and colleagues ultimately resolved this question by providing irrefutable evidence supporting a seven transmembrane segment model for the ASBT (Zhang et al. 2004; Banerjee and Swaan 2006). Those studies included the introduction of N-linked glycosylation sites at residues 113–118 (loop 1) and 266–272 (loop 3) (Zhang et al. 2004), and dual epitope insertion scanning mutagenesis (Banerjee and Swaan 2006).

In contrast to its primary structure, the subunit stoichiometry and assembly of the functional ileal bile acid transporter complex is poorly understood. Extensive photoaffinity labeling and SDS-PAGE studies using monomeric and dimeric bile acid analogues labeled a variety of proteins in ileal brush border membrane preparations, but predominantly a 93 kDa protein, a dimer of the ASBT, and a 14 kDa protein identified as the cytosolic ileal lipid binding protein (ILBP; IBABP; *FABP6*) (Kramer et al. 1993, 1997, 1998). Furthermore, radiation inactivation studies found large target sizes for the bile acid transporters in ileal brush border and hepatic sinusoidal membranes, suggesting that the functional units are multimeric complexes. The apparent sizes of the transporters determined by cloning, photoaffinity labeling, and radiation inactivation are summarized in Table 2. The molecular mass differences between the cloned transporters and the large functional complexes are difficult to interpret in these crude systems. However, the results are generally consistent with the ASBT functioning as a homomultimer or perhaps even a heteromultimer that includes the ILBP. Alternatively, the large target sizes may reflect interaction of the transporters with specialized regions of plasma membrane such as lipid rafts (Annaba et al. 2008). The requirement for other protein subunits is also unclear and needs to be weighed in light of the widespread observation that overexpression of the ASBT alone in heterologous systems such as *Xenopus* oocytes, COS, CHO, and MDCK cells is sufficient to recapitulate

Table 2 Determination of bile acid transporter size in ileum and liver

Method of analysis	Ileum (ASBT)	References	Liver (NTCP)	References
Cloning: native protein	38 kDa	Wong et al. (1994, 1995) and Shneider et al. (1995)	38 kDa	Hagenbuch et al. (1991) and Hagenbuch and Meier (1994)
Glycosylated protein	40–50 kDa		40–50 kDa	
Photoaffinity labeling	93 kDa	Kramer et al. (1993)	46 kDa	Kramer et al. (1982)
Radiation inactivation/ photoaffinity labeling	230 kDa	Kramer et al. (1995)	ND	
Radiation inactivation/bile acid transport	450 kDa	Kramer et al. (1995)	170 kDa	Elsner and Ziegler (1989)

ND not determined

membrane targeting and robust sodium-dependent bile acid transport (Craddock et al. 1998; Balakrishnan et al. 2006b). So in contrast to the basolateral bile acid transporter OSTα–OSTβ (discussed in the following), ASBT can function as a monomer or homomultimer.

3.3 ASBT Structure–Function Relationships

A variety of approaches have been taken to identify protein regions and sequences important for transport function within the ASBT and related SLC10 family members, and these structure–activity relationships have been reviewed recently (Balakrishnan and Polli 2006; Geyer et al. 2006; Sievanen 2007). Kramer and coworkers used a combination of affinity labeling with photolabile bile acid analogs, enzymatic fragmentation, and epitope-specific antibodies to identify the terminal 67 amino acids of the rabbit Asbt as a region that strongly interacts with the bile acid 7-hydroxy position (Kramer et al. 2001). Several groups have carried out general mutagenesis studies that targeted cysteine residues, negatively charged amino acids, and threonine residues in the ASBT and NTCP as a first step toward identifying functionally important regions (Hallen et al. 2000; Zahner et al. 2003; Sun et al. 2006). Swaan and coworkers have carried out the most systematic examination of ASBT structure–function relationships. In the absence of a crystal structure for the ASBT, this group used the structure of bacteriorhodopsin (PDB 1AT9) as a scaffold for ASBT modeling and mutagenesis studies (Zhang et al. 2002, 2004). The experimental strategies included using a combination of exposure to exoplasmic-specific modifying agents (methanethiosulfonates) and site-directed mutagenesis, substitute cysteine accessibility mutagenesis, different bile acid substrates, and high affinity specific inhibitors to finely map the solute binding site and translocation sites in the ASBT (Hallen et al. 2000, 2002; Banerjee et al. 2005; Hussainzada et al. 2006, 2008; Ray et al. 2006; Khantwal and Swaan 2008).

The interaction of sodium with NTCP and ASBT was studied using similar mutagenesis strategies that focused on the negatively charged amino acids (Zahner et al. 2003; Sun et al. 2006). These studies implicated similar negatively charged residues in extracellular loop 1 (Asp115 in rat Ntcp, Asp122 in rat Asbt) and extracellular loop 3 (Glu257 in rat Ntcp, Glu261 in rat Asbt) as potential extracellular sodium sensors. Again, those observations were extended and refined by Swaan and coworkers for the human ASBT (Banerjee et al. 2008; Hussainzada et al. 2008, 2009). In aggregate, the results are consistent with a model where the larger extracellular loops 1 and 3 and the exofacial half of transmembrane segment 7 participate directly in bile acid binding and substrate entry/translocation. Complementing those results are very recent findings suggesting that the cytosolic half of transmembrane segment 3 forms part of the substrate exit route (Hussainzada et al. 2009).

3.4 ASBT Substrate Specificity and Native Bile Acid Pharmacophore Models

ASBT functions as an electrogenic sodium-solute cotransporter, moving 2 or more sodium ions per molecule of solute (Weinman et al. 1998). In contrast to the strict requirement for sodium (i.e., other cations such potassium, lithium, rubidium, cesium cannot substitute for sodium to support transport), there is no apparent anion specificity, arguing against a role for a cotransported anion (Craddock et al. 1998). The driving force for solute transport is provided by the inwardly directed sodium gradient maintained by the basolateral Na^{+}, K^{+}-ATPase as well as the negative intracellular potential. Studies using patch clamped transfected cells demonstrated that ASBT can mediate bidirectional bile acid transport and the directionality was determined by the sodium-gradient and membrane potential; uptake was voltage-dependent and stimulated by a negative intracellular potential (Weinman et al. 1998). Although these studies provided important insights regarding the mechanism of transport, it should be noted that the transmembrane sodium and electrical gradients present under physiological conditions force the ASBT to operate solely as an uptake mechanism for bile acids.

ASBT's major physiological substrates include the major unconjugated bile acids, cholic acid, deoxycholic acid, chenodeoxycholic acid, and ursodeoxycholic acid, as well as their glycine and taurine conjugates (Craddock et al. 1998; Kramer et al. 1999). There is limited published information regarding transport of sulfated or glucuronidated bile acid conjugates by the ASBT. However, results from studies using in situ perfused guinea pig ileum (De Witt and Lack 1980) or human ASBT-transfected cells (Craddock et al. 1998) indicated that sulfated di- and trihydroxy bile acids were poor substrates. Based on the known structure–activity relationships for ASBT substrates, it is also predicted that glucuronidated bile acids are not ASBT substrates (Kramer et al. 1999). To date, no nonbile acid transport substrate has been identified for the ASBT. This contrasts with the related liver bile acid transporter NTCP, which also transports estrone-3-sulfate (Craddock et al. 1998; Ho et al. 2004) and the HMG CoA reductase inhibitor, rosuvastatin (Ho et al. 2006).

Lack and colleagues first examined the solute structural features important for the ileal sodium-dependent bile acid transporter using intestinal perfusions and everted gut sac models (Lack and Weiner 1966; Lack et al. 1970; Bundy et al. 1977; Lack 1979). Based on these findings, a hypothetical model for the ASBT substrates was proposed that included: (1) a negatively charged side chain for coulombic interaction with a positively charged moiety in the ASBT, (2) at least one axial hydroxyl group on the steroid nucleus at positions 3, 7, or 12, such that trihydroxy bile acids are better transported than dihydoxy bile acids, and (3) a *cis* configuration of the cyclohexyl rings A and B of the steroid nucleus (Lack 1979). With cloning of the ASBT by Dawson and coworkers (Wong et al. 1994, 1995), cell-based transport assays became possible using cell lines transfected with human ASBT or Asbt from other species (Craddock et al. 1998; Kramer et al. 1999; Balakrishnan et al. 2006b). This permitted an in-depth analysis of ASBT substrate specificity and resulted in

significant changes to the original Lack model. The new results confirmed the requirement for the α-hydroxyl groups at the 7 and 12 positions. However, no requirement was found for a *cis* configuration of the A/B rings of the steroid nucleus or a 3α-hydroxyl group. The new findings included: (1) 6-hydroxylation (a common modification found with rodent bile acids such as α, β, or ω-muricholic acid and hyodeoxycholic acid) dramatically reduced transport, and (2) glycine or taurine conjugation enhanced affinity, as did the presence of fewer hydroxyl groups on the steroid nucleus such that the rank ordering of affinity for the ASBT was monohydroxy > dihydroxy > trihydroxy (Balakrishnan and Polli 2006; Balakrishnan et al. 2006b). Kramer and coworkers went on to develop a detailed 3D pharmacophore (QSAR) conformational model for rabbit Asbt substrates using training sets of various bile acid-based inhibitors and the CATALYST software (Baringhaus et al. 1999). These results increased the predictive properties of the pharmacophore model that had been generated earlier using comparative molecular field analysis and a more limited set of bile acid analogs (Swaan et al. 1997b). Modification of the bile acid side chain on transport was also explored in these early studies (Lack et al. 1970; Kramer et al. 1999) and more recently in studies by Polli and colleagues (Tolle-Sander et al. 2004; Balakrishnan et al. 2006a), who generated a conformationally sampled pharmacophore structure–activity relationship model to predict these interactions (Gonzalez et al. 2009). This work confirmed that shortening of the side chain by one methylene group (a nor-bile acid) decreased transport. Although monoanionic conjugates were favored for ASBT-mediated transport, the presence of a single negative charge at the C24 position was not essential for interaction with the ASBT (Balakrishnan et al. 2006a). Notable with regard to prodrug development, these transport and modeling studies showed that the 3α-hydroxy group of natural bile acids is not essential for interaction with the ASBT or NTCP and that the steroid A-ring, preferably the 3 position, could serve as an attachment site for compounds to target their delivery via the sodium-dependent bile acid transporters (Sievanen 2007). These relationships are discussed further in the later section on targeting the ASBT for prodrug delivery.

A limitation of these earlier modeling studies was that the training sets of compounds included only bile acids, bile acid derivatives, and benzothiazaepine-based ASBT inhibitors rather than more diverse sets of chemical structures or FDA-approved drugs. This limitation has been addressed by a recent elegant transport and modeling study by Polli and coworkers that examined the interaction of a large set of FDA-approved drugs with the ASBT (Zheng et al. 2009). These findings are discussed in more detail in the later section on the role of ASBT and OSTα–OSTβ in drug absorption and drug interactions.

3.5 ASBT Genomics and Pathophysiology

The *SLC10A2* gene is organized in six exons spanning approximately 24 kb of DNA sequence on human chromosome 13q33. The first exon encodes the 5′ untranslated

region and amino acids 1–126. Exons 2–6 encompass the remaining coding sequence (amino acids 126–348) and exon 6 includes a long 3′ untranslated region (Oelkers et al. 1997). Analysis of the *SLC10A2* gene has revealed a variety of single nucleotide polymorphisms (SNPs) and dysfunctional mutations. The coding SNPs previously identified in the ASBT gene are indicated in Fig. 2. These sequence variations are discussed below in the context of the pathophysiology associated with ASBT defects.

The enterohepatic circulation efficiently conserves and compartmentalizes bile acids, thereby maintaining bile flow and adequate intraluminal bile acid concentrations for micellular solubilization and absorption of lipids. Considering its central role in the enterohepatic circulation, inherited defects or dysfunctional regulation of the ASBT may play a role in the pathogenesis or clinical presentation of a variety of gastrointestinal disorders. Indeed, in the course of cloning and characterizing the *SLC10A2* gene, the first dysfunctional mutation (P290S) associated with diminished transporter activity was identified in a single patient diagnosed with Crohn's disease (Wong et al. 1995). ASBT mutations were later identified as a cause of Primary Bile Acid Malabsorption (PBAM; Online Mendelian Inheritance in Man: OMIM#6013291; http://www.ncbi.nlm.nih.gov/omim/613291), a rare idiopathic disorder associated with interruption of the enterohepatic circulation of bile acids, chronic diarrhea beginning in early infancy, steatorrhea, fat-soluble vitamin malabsorption, intracranial hemorrhage (as a result of the vitamin K deficiency), and

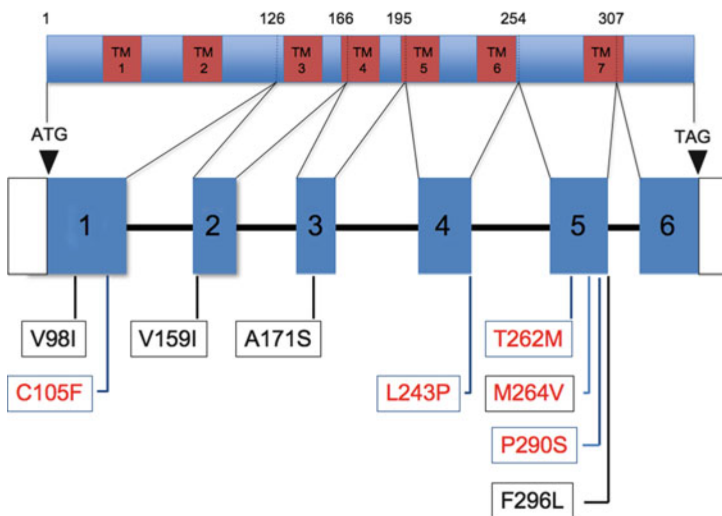

Fig. 2 Location of coding region single nucleotide polymorphisms and mutations in the human ileal bile acid transporter gene (SLC10A2). A schematic diagram depicting the human ASBT protein and gene is shown. The amino acid positions disrupted by the intron/exon junctions are indicated above the schematic of the ASBT protein. The seven predicted transmembrane domains (TM1–TM7) are shown as brown bars. The location of coding region polymorphisms (V98I, V159I, A171S, F296L) and dysfunctional bile acid transporter mutations (C105F, L243P, T262M, M264V, P290S) identified to date are indicated below each exon

lifelong reduced plasma cholesterol levels (Heubi et al. 1982; Oelkers et al. 1997). The affected PBAM patient was heterozygous for a splice junction mutation on one allele as well as two dysfunctional missense mutations, L243P and T262M, on the other allele (Oelkers et al. 1997). In contrast to PBAM, ASBT mutations have not been found in patients with adult-onset bile acid malabsorption, chronic diarrhea, and a morphologically functional ileum, a condition known as Idiopathic Bile Acid Malabsorption (IBAM) (Montagnani et al. 2001, 2006; Montagnani et al. 2006). For IBAM patients, it is still unclear as to whether altered regulation of the ASBT contributes to the phenotype in a subset of those patients (Balesaria et al. 2008). Indeed, a recent genetic study identified an ASBT haplotype that was associated with significantly reduced ileal expression of ASBT mRNA and protein (Renner et al. 2009).

Other disorders associated with intestinal bile acid malabsorption that potentially involve the ASBT include Familial Hypertriglyceridemia (Angelin et al. 1987; Duane et al. 2000; Love et al. 2001), Idiopathic Chronic Diarrhea (Schiller et al. 1987), Chronic Ileitis (Meihoff and Kern 1968), Cholesterol and Black Pigment Gallstone disease (Vitek and Carey 2003; Holzer et al. 2008), Postcholecystectomy Diarrhea (80), Crohn's disease (Krag and Krag 1976; Farivar et al. 1980; Tougaard et al. 1986; Nyhlin et al. 1994; Fujisawa et al. 1998), Irritable Bowel Syndrome (Camilleri et al. 2009), and susceptibility to Colon Cancer (Wang et al. 2001b). In the example of Familial Hypertriglyceridemia (FHTG), a screen of 20 FHTG patients with abnormal bile acid metabolism identified only a single patient with a frame-shift mutation at codon 216 (646insG) (Love et al. 2001). Thus, as with IBAM, dysfunctional mutations of the *SLC10A2* gene were largely excluded as a common genetic cause of decreased ASBT expression in hypertriglyceridemia. In the case of gallstone disease, ASBT expression is decreased in nonobese gallstone patients and associated with decreased expression of ILBP and OSTα–OSTβ (Bergheim et al. 2006; Renner et al. 2008). Considering that the ileal transport system is a major determinant of bile acid return in the enterohepatic circulation, reduced ASBT expression could decrease hepatic bile acid secretion, increase the cholesterol saturation of bile, and promote cholesterol gallstone formation (Portincasa et al. 2006). Bile acid malabsorption has also been associated with black pigment (bilirubin) gallstones (Brink et al. 1996, 1999; Vitek and Carey 2003). The postulated mechanism is that increased bile acid concentrations in the colon results in solubilization of precipitated bilirubin glucuronide conjugates, deconjugation by the colonic flora, and passive absorption. Analysis of the ASBT coding region in 39 gallstone patients (mixture of cholesterol and pigment gallstone patients) in a pilot study identified only a single pigment gallstone patient with a dysfunctional missense mutation (C105F) (Montagnani, Carey, and Dawson, unpublished results), suggesting that ASBT coding region SNPs are not a common genetic contributor to black pigment gallstones.

Major limitations of these earlier studies included insufficient patients and the fact that only small portions of the *SLC10A2* gene were examined. With the availability of improved SNP maps, high-density SNP arrays, and technical breakthroughs in DNA sequencing technology, genetic analyses of large numbers of

subjects are now tractable. These same tools are used in Genome-Wide Association Studies (GWAS), an unbiased powerful approach to identify genetic factors such as transporter genes that influence disease phenotypes and respond to therapy, or quantitative traits (http://www.genome.gov/20019523). In fact, a recent genome-wide association study designed to identify genetic contributors to variability in serum bilirubin levels showed a weak association with the *SLC10A2* locus in addition to the highest scoring loci, UGT1A1 and SLCO1B1 (Johnson et al. 2009). So although the relationship between common polymorphisms in the ASBT and drug metabolism remains to be carefully explored, the tools for such analysis are now readily available.

4 The Basolateral Bile Acid and Organic Solute Transporter: OSTα–OSTβ

4.1 OSTα–OSTβ General Properties and Tissue Expression

The proteins responsible for bile acid export across the basolateral membrane of the ileal enterocyte, cholangiocytes, and renal proximal tubule cell have only recently been identified. The breakthrough in this area came with the elegant expression cloning of an unusual transporter, OSTα–OSTβ, from the little skate (*Raja erinacea*) by Ballatori and coworkers (Wang et al. 2001a). Subsequently, the human and mouse orthologs of skate OSTα–OSTβ were cloned and expressed in *Xenopus* oocytes where they transport bile acids as well as a variety of steroids (Seward et al. 2003). As with the skate, solute transport by the human ortholog required coexpression of two different subunits: OSTα and OSTβ. The human OSTα gene encodes a 340 amino acid polytopic membrane protein with an extracellular amino terminus, 7 predicted transmembrane domains, and a cytoplasmic carboxyl terminus. The human OSTβ encodes a 128 amino acid predicted type I membrane protein with an extracellular amino terminus and cytoplasmic carboxyl terminus. Although the functional role of the individual subunits has not yet been determined, coexpression and assembly of both subunits into a complex is required for their trafficking to the plasma membrane and solute transport (Dawson et al. 2005; Li et al. 2007). In contrast to the ASBT, no systematic studies of OSTα–OSTβ structure–function relationships have been published yet.

OSTα–OSTβ was identified as a candidate ileal basolateral bile acid transporter using a transcriptional profiling approach (Dawson et al. 2005). Support for a role of OSTα–OSTβ in basolateral bile acid transport includes: (1) intestinal expression of OSTα and OSTβ mRNA that generally follows that of the ASBT, with highest levels in ileum (Ballatori et al. 2005; Dawson et al. 2005; Balesaria et al. 2008), (2) appropriate cellular localization on the lateral and basal plasma membranes of ileal enterocyte (Dawson et al. 2005), (3) expression of OSTα–OSTβ on the basolateral plasma membrane of hepatocytes, cholangiocytes, and renal proximal tubule cells, other cells important for bile acid transport (Ballatori et al. 2005), (4) efficient

transport of the major bile acid species (Ballatori et al. 2005; Dawson et al. 2005), and (5) positive regulation of OSTα–OSTβ expression by bile acids via activation of farnesoid X-receptor (FXR) (Frankenberg et al. 2006; Landrier et al. 2006), and (6) targeted inactivation of the *Ostα* gene in mice results in impaired intestinal absorption of bile acids and impaired bile acid homeostasis (Ballatori et al. 2008; Rao et al. 2008).

In contrast to the ASBT, which operates as an electrogenic sodium-cotransporter, the mechanism for OSTα–OSTβ mediated transport has not been fully elucidated (Ballatori 2005; Ballatori et al. 2005). When expressed in *Xenopus laevis* oocytes, OSTα–OSTβ mediated transport was unaffected by depletion of intracellular ATP, by alterations in transmembrane electrolyte concentration gradients, or by changes in the pH gradient (Ballatori et al. 2005). OSTα–OSTβ exhibits both solute uptake and efflux properties and transport is *trans*-stimulated by known substrates (Ballatori et al. 2005; Dawson et al. 2005). The general consensus from these studies is that OSTα–OSTβ operates by facilitated diffusion and mediates solute uptake or efflux, depending on the solute's electrochemical gradient.

Another important difference between the ASBT and OSTα–OSTβ lies in their substrate specificities. Although no nonbile acid substrates have been identified for the ASBT, several compounds in addition to the major taurine and glycine-conjugated bile acids have been identified as substrates for OSTα–OSTβ (Ballatori et al. 2005). A systematic screen to identify OSTα–OSTβ transport substrates has not yet been published. Nevertheless, the existing list of nonbile acid substrates for OSTα–OSTβ includes estrone-3-sulfate, digoxin, prostaglandin E2, and dehydroepiandrosterone-3-sulfate. The list of inhibitors of OSTα–OSTβ-mediated transport of taurocholate or estrone-3-sulfate is also broad and includes a variety of compounds such as spironolactone, bromosulfophthalein, probenecid, and indomethacin (Seward et al. 2003). Although preliminary, these results suggest that the substrate specificity for OSTα-OSTβ is relatively broad and is consistent with a direct role of OSTα-OSTβ in drug transport.

In humans, the expression of OSTα and OSTβ generally parallel one another with highest levels in small intestine, liver, kidney, and testis (Seward et al. 2003; Ballatori et al. 2005). Lower levels of OSTα and OSTβ mRNA are also detected by real-time PCR in other human tissues including colon, adrenal gland, ovary, with lowest levels in heart, lung, brain, pituitary gland, and prostate. Analysis of samples from mouse small intestine, cecum and colon, and human ileum showed that expression of OSTα and OSTβ mRNA are highly correlated (Frankenberg et al. 2006; Renner et al. 2008); in human ileal biopsy samples, a strong positive correlation was also observed between mRNA expression and protein levels for OSTα and OSTβ (Renner et al. 2008). An important tissue expression difference between rodents and humans is the significantly higher level of OSTα–OSTβ expression in human liver. Under basal conditions, Ostα and Ostβ mRNA expression is almost undetectable in mouse, rat, or hamster liver, but is readily measured in primate and human liver. In humans and rodents, OSTα–OSTβ expression is induced dramatically in response to cholestasis (Boyer et al. 2006; Zollner et al. 2006).

4.2 OSTα–OSTβ Genomics and Pathophysiology

The OSTα and OSTβ genes are encoded on human chromosomes 3q29 and 15q22, respectively. Both are relatively small genes, with OSTα consisting of 9 exons spanning approximately 17 kb and OSTβ consisting of 4 exons spanning approximately 8 kb of DNA. Examination of the most recent Ensembl database for OSTα shows three nonsynonymous SNPs resulting in the following conservative amino acid changes, V197G, V202I, and R241H; the frequency and functional significance of these SNPs has not been determined. No nonsynonymous SNPs have yet been reported for OSTβ.

No inherited defects have been reported for the OSTα or OSTβ genes in humans; however, targeted inactivation of the *Ostα* gene in mice resulted in impaired intestinal bile acid absorption and altered bile acid metabolism (Ballatori et al. 2008; Rao et al. 2008). As predicted for a major intestinal basolateral bile acid transporter, studies using everted gut sacs (Rao et al. 2008) or intraileal administration of [^{3}H]taurocholate (Ballatori et al. 2008) demonstrated a significant reduction in transileal transport in *Ostα* null mice. However fecal bile acid excretion was not increased in *Ostα* null mice, as had been observed in Asbt (*Slc10a2*) null mice (Dawson et al. 2003). These results were particularly perplexing because the whole body bile acid pool size was significantly decreased (Rao et al. 2008), a hallmark of intestinal bile acid malabsorption (Oelkers et al. 1997; Jung et al. 2007). Examination of the Fibroblast growth factor (FGF)15/19 signaling pathway (Inagaki et al. 2005) provided a solution to this conundrum. In the *Ostα* null mice, bile acids are taken up by the ileal enterocyte but their efflux across the basolateral membrane is impaired. As a result, bile acids accumulate within the ileal enterocyte, constitutively activating FXR and inducing greater expression and secretion of FGF15/19 into the portal circulation. FGF15/19 then signals at the hepatocyte to downregulate hepatic bile acid synthesis. The net result is that hepatic bile acid synthesis is paradoxically repressed rather than induced, which is the normal physiological response to a block in intestinal bile acid absorption (Davis and Attie 2008; Rao et al. 2008).

5 Development of ASBT Inhibitors

As mentioned in the previous section, disruption of the bile acid enterohepatic circulation normally stimulates de novo synthesis of bile acids in the liver. The resulting demand for cholesterol by the liver is met by increasing the number of hepatic low density lipoprotein (LDL) receptors to clear additional plasma LDL, and by increasing hepatic cholesterol synthesis (Brown and Goldstein 1986). If the malabsorption is significant, hepatic bile acid production may be unable to compensate for bile acid loss, leading to decreased bile acid concentrations in the intestinal lumen and a reduced ability to solubilize and absorb biliary and dietary

cholesterol. The net result is a decrease in plasma total and LDL cholesterol levels. This is the basis for the reduced morbidity and mortality from cardiovascular disease associated with ileal resection in the POSCH study (Program on the Surgical Control of Hyperlipidemias) (Buchwald et al. 1990) or from the use of polymeric bile acid sequestrants, such as cholestyramine or colestipol (1984; Bays and Goldberg 2007). The bile acid sequestrants are large nonabsorbable polymeric resins, and as such have few systemic side effects. Prior to the development of HMG CoA reductase inhibitors (Statins), bile acid sequestrants were one of the mainstays of therapy for hypercholesterolemia (Bays and Goldberg 2007). However, gastrointestinal side effects such as constipation, indigestion, dyspepsia, and flatulence are very common. These side effects together with the high doses required (15–30 g/day) adversely affect patient compliance and the efficacy of these agents. The recent development of a higher affinity bile acid binder Colesevelam decreased the required dose (Steinmetz and Schonder 2005), but the efficacy, patience compliance, and side effect profile still favors the use of statins as a first-line therapy. Based on its remarkable substrate specificity and expression on the intestinal brush border membrane, blocking the ASBT using high affinity, nonabsorbable inhibitors represented an attractive strategy and alternative to the sequestrants for treatment of hypercholesterolemia (Kramer and Glombik 2006).

The general properties of the ASBT inhibitors are summarized in Table 3. These compounds fall into two general classes, bile acid derivatives including bile acid dimers, and nonbile acid compounds including benzothiazepine and benzothiepine analogs. The first compounds termed Bile Acid Reabsorption Inhibitors (BARI) were developed by Kramer and colleagues at Hoechst AG (now part of Sanofi-Aventis) in Frankfurt (Wess et al. 1994). This group developed a wide variety of dimeric and trimeric bile acids and extensively characterized their interaction with the ASBT using in situ ileal perfusions, isolated ileal brush border membranes, ASBT-transfected cell lines, and bile acid photoaffinity labeling (Baringhaus et al.

Table 3 Specific ASBT inhibitors

Compound	IC_{50}	Manufacturer	Description (references)
S9060	~30 μM	Hoechst AG (Aventis)	Bile acid dimer; (Wess et al. 1994; Baringhaus et al. 1999)
R-146224	~0.02 μM	Sankyo	Amphiphilic 4-oxo-1-phenyl-1,4-dihydroquinoline derivative; (Kurata et al. 2004; Kitayama et al. 2006)
S-8921	~66 μM	Shionogi	Substituted napthol derivative; (Hara et al. 1997); glucuronidation significantly increases potency (Sakamoto et al. 2007)
2164U90	~7 μM	Burroughs Wellcome (GlaxoSmithKline)	Benzothiazepine derivative; (Root et al. 1995; Root et al. 2002)
264W94	~0.4 μM		
SC-435	~2 nM	Monsanto-Searle (Pfizer)	Benzothiepin derivative; (Bhat et al. 2003; Huang et al. 2005; Tremont et al. 2005)
PR835	~0.15 μM	AstraZeneca	Benzothiazepine derivative; (Galman et al. 2003)

1999; Kramer and Glombik 2006). This mechanistic approach utilized elegant chemistry, but little was ever published regarding the ability of these compounds to reduce plasma LDL cholesterol levels or prevent the development of atherosclerosis in animal models. Nevertheless, newer bile acid derivatives have been developed as potential ASBT inhibitors by Marin (Vicens et al. 2007) as well as by Polli (Gonzalez et al. 2009), and this general strategy remains an area of interest.

Researchers working at several Japanese pharmaceutical companies developed a variety of ASBT inhibitors. For example, researchers at Tanabe Seiyaku (Osaka, Japan) described their investigations of a series of arylnapthelene lignans for their hypocholesterolemic properties. The compound TA-7552 [1-(3,4-dimethoxyphenyl)-2,3-bis(methoxycarbonyl)-4-hydroxy-6,7,8-trimethoxynaphthalene] was effective in increasing fecal bile acid excretion, inducing hepatic bile acid synthesis, and decreasing plasma cholesterol levels in a rat model. The agent appeared to inhibit intestinal absorption of both cholesterol and bile acids, but the interaction of TA-7552 with the ASBT was not characterized in those published studies (Takashima et al. 1994). More specific nonabsorbable ASBT inhibitors, amphiphilic 4-oxo-1-phenyl-1,4-dihydroquinoline derivatives, were developed at Sankyo Co (Tokyo, Japan) (Kurata et al. 2004). The lead compound, R-146224 [1-{7-[(1-(3,5-Diethoxyphenyl)-3-{[(3,5-difluorophenyl)(ethyl) amino]carbonyl}-4-oxo-1,4-dihydroquinolin-7-yl)oxy]heptyl}-1-methylpiperidinium bromide] potently inhibited the human ASBT (IC_{50} ~23 nM) and significantly reduced plasma non-HDL cholesterol levels in hamster and monkey models (Kitayama et al. 2006). Mizui, Hara, and coworkers at Shinogi and Company (Osaka, Japan) developed the ASBT inhibitor S-8921 [methyl 1-(3,4-dimethoxyphenyl)-3-(3-ethylvaleryl)-4-hydroxy-6,7,8-trimethoxy-2-naphthoate]. The interaction of S-8921 with the ASBT was characterized (competitive/noncompetitive inhibitor; IC_{50} ~66 μM), and S-8921 was shown to reduce serum cholesterol levels in several animal models as well as prevent the development of atherosclerosis in rabbits (Hara et al. 1997; Higaki et al. 1998). Interestingly, S-8921 is glucuronidated in vivo and this modification converts the parent S-8921 compound to a 6,000-fold more potent inhibitor of the human ASBT (K_i = 18 nM vs. 109 μM) (Sakamoto et al. 2007). S-8921 entered phase I trials but does not appear to have progressed beyond that stage (Booker 2001).

Lewis and coworkers at Burroughs Wellcome (now part of GlaxoSmithKline) identified the first series of benzothiazepine-based ASBT inhibitors and published their characterization of 2164U90 [(−)-(3R,5R)-*trans*-3-butyl-3-ethyl-2,3,4,5-tetrahydro-5-phenyl-1,4-benzothiazepine1,1-dioxide] and 264W94 [(-)-(3R,5R)-*trans*-3-butyl-3-ethyl-2,3,4,5-tetrahydro-7,8-dimethoxy-5-phenyl-1,4-benzothiazepine1,1-dioxide]. The compounds were potent competitive inhibitors of the ASBT; in cholesterol-fed rat and mouse models, the compounds inhibited intestinal bile acid absorption, induced hepatic bile acid synthesis, and effectively lowered plasma VLDL and LDL cholesterol levels (Lewis et al. 1995; Root et al. 1995, 2002). Additional benzothiepin-based inhibitors such as [1-[4-[4-[(4*R*,5*R*)-3,3-dibutyl-7-(dimethylamino)-2,3,4,5-tetrahydro-4-hydroxy-1,1-dioxido-1-benzothiepin-5-yl] phenoxy]butyl]-4-aza-1-azoniabicyclo[2.2.2] octane methanesulfonate (salt)] SC-435 (IC_{50} ~1.5 nM) and benzothiazepine-based inhibitors such as PR835

(IC_{50} ~0.15 μM) were developed by Monsanto-Searle (now part of Pfizer) and Astra-Zeneca, respectively (Galman et al. 2003; Tremont et al. 2005). The Monsanto-Searle group initially developed a new series of 2,3-disubstituted-4-phenylquinolines (similar to the Shinogi ester substituted napthol compounds) and benzothiazepine-based compounds (Tollefson et al. 2003) that inhibited the ASBT at micromolar and nanomolar concentrations, respectively. This group later developed a series of potent benzothiepine-based inhibitors, demonstrating their activity as potent competitive inhibitors of the ASBT (Bhat et al. 2003; Huang et al. 2005; Tremont et al. 2005) and characterizing the mechanisms responsible for the LDL lowering in various animal models (Huff et al. 2002; Bhat et al. 2003; Telford et al. 2003; West et al. 2003). The Monsanto-Searle compound entered the clinical trial phase but does not appear to have progressed further, potentially due to questions over efficacy and dose-related diarrhea (Hofmann and Hagey 2008).

Development of the ASBT inhibitors focused on their potential for treating hypercholesterolemia. However, because the ASBT is also is expressed by the renal proximal tubule cells, similar inhibitors could be used to block renal reclamation of bile acids and increase urinary bile acid output. This would create a shunt for elimination of hepatotoxic bile acids that cannot be excreted efficiently via the normal biliary route. The predicted decrease in serum and hepatic bile acid concentrations may relieve cholestasis-associated pruritus and slow the progression of the hepatocellular degeneration. Long before the mechanism for renal reabsorption of bile acids was elucidated, Billings and colleagues had suggested a variation on this therapeutic approach (Summerfield et al. 1977; Corbett et al. 1981). Interestingly, although the use of ASBT inhibitors for eliminating excess bile acids in patients with cholestasis is as yet untested, a similar approach in concept is currently being exploited using renal sodium-glucose cotransporter inhibitors to create a shunt for elimination of excess plasma glucose in patients with diabetes (Bakris et al. 2009). Of course, the potential hepatoprotective effects of such an intervention must be balanced against risk of increased bile acid-induced kidney cell injury (Morgan et al. 2008).

6 Targeting the ASBT for Prodrug Delivery

The specificity and high capacity of the ileal and hepatic bile acid transport systems has stimulated numerous attempts to utilize bile acids or bile acid derivatives as platforms (or "Trojan Horses") for prodrug design. This section will provide an overview of the studies targeting the ASBT to enhance oral bioavailability, and the reader is referred to several recent review articles specific to this topic for additional details (Balakrishnan and Polli 2006; Sievanen 2007). Because of their stability and unique structure, bile acids are useful building blocks for synthetic chemistry (Kuhajda et al. 2006; Davis 2007); drugs and other large molecules have been attached via different chemical bonds or linkers to the side chain and the C-3, C-7, or C-12 positions on the bile acid steroid nucleus. Although this general

approach dates to 1948 when Berczeller published the synthesis of p-aminobenzene sulphonamide conjugated to cholic acid (United States Patent 2,441,129), systematic development of bile acid prodrugs did not begin until much later through the efforts of the group led by Kramer and Wess at Hoechst in Frankfurt (Kramer and Wess 1996). Over approximately a decade dating from the early 1990s, this group published an extensive number of studies detailing its attempts to couple drugs, peptides, and oligonucleotides to bile acids for targeting to the liver and ileal transporters (Kramer et al. 1992; Kramer et al. 1994a, b; Kullak-Ublick et al. 1997; Starke et al. 2001). Based on their analysis of the structure–activity relationships for NTCP or ASBT substrates, the strategy employed avoided modifying the side chain and focused on targeting the C-3 position on the steroid nucleus (Baringhaus et al. 1999). The drugs or large molecules that were coupled to bile acids and analyzed included HMG CoA reductase inhibitors, an oxaprolylpeptide to inhibit hepatic collagen synthesis, the antineoplastic alkylating nitrogen mustard chlorambucil, small linear peptides up to 10 amino acid residues, and even oligonucleotides. For example, the bile acid–chlorambucil conjugates were absorbed from ileum and secreted by the liver, redirecting chlorambucil away from its normal route of renal clearance (Kramer et al. 1992). The bile acid-HMG CoA reductase inhibitor conjugates exhibited increased liver selectivity in a rat model; however, the conjugates were weak reductase inhibitors and it was not clear whether the conjugates were being transported by NTCP and ASBT or by other carriers in vivo (Kramer et al. 1994a). Small oligopeptide–bile acid conjugates were absorbed from the ileum and secreted intact by the liver, albeit with a low efficiency (Kramer et al. 1994b). Overall, the C-3-coupled prodrugs met with mixed success (Kramer and Wess 1996), and none appear to have proceeded beyond early stages of preclinical or clinical development. More recently, Gallop and colleagues at XenoPort revisited this general approach, synthesizing and evaluating a novel series of C2–C3 annulated bile acid pyrazoles coupled to large molecules such as naproxen. However, the conjugates showed weak affinity for the ASBT (as compared to the NTCP) and only weak to moderate transport activity in ASBT-expressing *Xenopus* oocytes (Bhat et al. 2005).

Other groups focused on using the native or a modified side chain of the bile acid as a platform for coupling agents. Many of these efforts are focused on targeting the NTCPs or OATPs to increase their liver selectivity (Sievanen 2007). For example, Marin and colleagues developed a large series of cisplatin analogs, termed *Bamets* (bile acid–metal hybrids) (Macias et al. 1998; Briz et al. 2002), and bile acids conjugated to metals such as gadolinium have been developed as potential contrast agents for magnetic resonance imaging (Anelli et al. 2004). With regard specifically to the ASBT, Swaan and colleagues had some success in targeting the ASBT by coupling small peptides or peptide-based HIV protease inhibitors to the side chain of cholic acid (Kagedahl et al. 1997; Swaan et al. 1997a). But the clearest example of increased oral bioavailability using a prodrug targeted to the ASBT is the study by Polli where acyclovir was conjugated to chenodeoxycholate via a valine side chain linker (acyclovir valyl-chenodeoxycholate), resulting in a twofold increase in oral bioavailability in rats (Tolle-Sander et al. 2004).

7 Role of the Intestinal Bile Acid Transporters in Drug Absorption and Drug Interactions

7.1 Role of ASBT in Drug Absorption and Drug Interactions

As discussed in the previous sections, a great deal has been learned regarding the substrate specificity of the ASBT in the course of developing potent high affinity inhibitors and prodrug platforms. In general, ASBT's substrate specificity is restricted to monovalent unconjugated, glycine-conjugated, and taurine-conjugated bile acids, with little evidence for the active transport of other endobiotics such as steroids or steroid sulfates (Lack 1979; Craddock et al. 1998; Kramer et al. 1999). This narrow substrate specificity agrees with ASBT's physiological role in the enterohepatic circulation. In the lumen of the ileum or renal proximal tubules, the ASBT is a high capacity system for almost quantitative recovery of specific solutes (bile acids), leaving other metabolites or xenobiotics for elimination in the feces or urine. Nevertheless, the conclusion that few nonbile acid substrates exist for the ASBT does not the exclude the possibility that other compounds, particularly drugs or drug metabolites, may interact as inhibitors. But several events conspired to promote the idea that few compounds unrelated to bile acids interacted with the ASBT. First, early studies comparing the inhibitor profile of NTCP versus ASBT found that the latter had a more restricted ability to interact with cholephilic compounds and drugs. In a study of 28 cholephilic organic compounds and drugs, the majority inhibited NTCP but very few inhibited ASBT-mediated transport (Kramer et al. 1999). The list of compounds that did not affect bile acid transport in rabbit ileal brush border membranes or in ASBT-transfected cells included antibiotics such as benzylpenicillin, cephalexin, cefixime, ofloxacin, rifampicin, tetracyclin, streptomycin, or novobiocin, and compounds such as β-estradiol, digitoxigenine, 6α-methylprednisolone, dexamthasone, cortisol, or reserpine (Kramer et al. 1999). Second, in developing ASBT inhibitors, the pharmaceutical industry focused on nonabsorbable compounds that fell within a few chemical classes, giving the impression that that number of interacting chemical structures may be small. Third, the early substrate molecular modeling studies (reviewed previously) focused on bile acids and their derivatives (Swaan et al. 1997b; Baringhaus et al. 1999); as such, the substrate models were difficult to extrapolate to other chemical structures or drugs. Fortunately, this question has recently been revisited in an elegant study carried out by Polli and colleagues (Zheng et al. 2009). The authors initially screened a training set of 30 FDA-approved drugs for their ability to inhibit the ASBT. The most potent inhibitors were used to develop a qualitative pharmacophore, which was then used to search a drug database to identify additional inhibitors. These compounds were used to develop 3D-QSAR and Bayesian models that were further validated by assessing their inhibitory potential in cell-based assays. Ultimately, the authors identified a diverse group of FDA-approved drugs that act as ASBT inhibitors, including dihydropyridine calcium channel blockers

and HMG CoA reductase inhibitors. A list of some of the most potent ASBT inhibitors identified by Polli and colleagues is shown in Table 4. These results raise the possibility that inhibition of the ASBT may account for some of the side effects associated with these drugs. As discussed previously, ASBT inhibition can result in increased spillage of bile acids into the colon, and thus is a possible mechanism for potential drug side effects such as diarrhea, gallstone disease, hypertriglyceridemia, or even susceptibility to colon cancer (Zheng et al. 2009).

7.2 Role of OSTα–OSTβ in Drug Absorption and Drug Interactions

Many of the intestinal apical brush border membrane uptake transporters have been identified such as OATP1A2, OATP2B1, PEPT1/2, MCT1, CNT1/2, OCTN1/2, and the role of apical efflux transporters such as Pgp (ABCB1), BCRP (ABCG2), and MRP4 (ABCC4) in limiting drug absorption has been an area of intense investigation, However, less is known about the basolateral membrane transporters important for completing the circuit and exporting various substrates from the enterocyte into the portal circulation (Shugarts and Benet 2009). Its significant expression down the length of the small intestine and colon and potentially broad substrate specificity raises the possibility that OSTα–OSTβ participates in the absorption of a variety of drugs or other xenobiotics in addition to bile acids. OSTα–OSTβ may play a particularly important role for drugs that fall into the Class 3 (high solubility, low permeability, poor metabolism) or Class 4 (Low solubility, low permeability, poor metabolism) categories in the Biopharmaceutics Drug Disposition Classification System (BDDCS) proposed by Benet and colleagues (Wu and Benet 2005). A systematic screen to identify OSTα–OSTβ transport substrates has not yet been published, but these drugs, particularly the Class 3 drugs (high solubility, low permeability, poor metabolism), would be excellent candidates to characterize initially. Such studies would be an important first step toward understanding the role of OSTα–OSTβ in drug absorption and potential drug–drug interactions.

In addition to a role in drug absorption from the small intestine, the observation that OSTα–OSTβ operates by facilitated diffusion and can mediate solute uptake or efflux raises the possibility that OSTα–OSTβ may particulate in intestinal drug secretion. In this paradigm, drug or drug metabolites would be taken up from blood across the basolateral membrane by transporters such as OSTα–OSTβ and then efficiently pumped out into the intestinal lumen by efflux transporters such as Pgp, BCRP, or MRP4. There is growing evidence for an important role of transintestinal secretion in the elimination of endobiotics such as cholesterol (van der Velde et al. 2007; Brown et al. 2008; van der Veen et al. 2009) or oxalate (Hatch and Freel 2008). In general, direct intestinal secretion is not thought to be a major route of drug elimination (Lennernas 2007; Fagerholm 2008), in part due to the lower blood

Table 4 Drug inhibitors of the ASBT

Compound	K_i	Description	Compound	K_i	Description
Nifedipine	4	Dihydropyridine CCB[a]; antihypertensive	Pioglitazone	55	Thiazolidinedione (TZD); hypoglycemic
Nisoldipine	5	Dihydropyridine CCB; antihypertensive	Propafenone	62	Sodium channel blocker; Class 1c antiarrhythmic
Nimodipine	6	Dihydropyridine CCB; antihypertensive	Indomethacin	62	Nonsteroidal anti-inflammatory
Simvastatin	10	Statin; hypolipidemic	Mevastatin	65	Statin; hypolipidemic
Latanoprost	11	Prostaglandin analog; glaucoma	Manidipine	73	Dihydropyridine CCB; antihypertensive
Fluvastatin	12	Statin; hypolipidemic	Pentamidine	76	Antimicrobial
Niguldipine	16	Dihydropyridine CCB; antihypertensive	Azelnidipine	86	Dihydropyridine CCB; antihypertensive
Mesoridazine	18	Piperidine neuroleptic; schizophrenia	Bendroflumethiazide	93	Thiazide diuretic; antihypertensive
Isradipine	19	Dihydropyridine CCB; antihypertensive	Doxorubicin	101	Anthracycline antibiotic; antineoplastic
Lovastatin	22	Statin; hypolipidemic	Spironolactone	110	Aldosterone antagonist; diuretic
Nemadipine A	23	Dihydropyridine CCB	Atropine	170	Tropane alkaloid; muscarine acetylcholine receptor antagonist
Cyclosporin A	24	Cyclic peptide; immunosuppressant	Ketoprofen	178	Nonsteroidal anti-inflammatory; propionic acid class
Nicardipine	33	Dihydropyridine CCB; antihypertensive	Diltiazem	211	Benzothiazepine CCB; antihypertensive; class IV antiarrhythmic
Ticonazole	33	Imidazole antifungal	Quinine	223	Alkaloid; antimalarial; antipyretic
Nitrendipine	34	Dihydropyridine CCB; antihypertensive	Bumetanide	225	Loop diuretic; antiedema; antihypertensive
Dibucaine	35	Amide local anesthetic	Verapamil	266	Phenylalkylamine CCB; antihypertensive
Thioridazine	37	Piperidine neuroleptic; schizophrenia	Torasemide	292	Pyridine-sulfonylurea; loop diuretic; antiedema; antihypertensive
Amlodipine	42	Dihydropyridine CCB; antihypertensive	Darifenacin	296	Muscarinic acetylcholine receptor blocker; urinary incontinence
Cilnidipine	45	Dihydropyridine CCB; antihypertensive	Trichlormethiazide	377	Thiazide diuretic; antiedema; antihypertensive
Felodipine	50	Dihydropyridine CCB; antihypertensive	Probenecid	385	Uricosuric; gout and hyperuricemia

[a]*CCB* Calcium Channel Blocker; Results from Zheng et al. (2009)

flow to the intestinal mucosa as compared to the liver and kidneys (~1/6th to 1/5th) and the postulated limited number of enterocytes involved. However, the available data is limited regarding the quantitative contribution of direct intestinal secretion. In light of the potential permeation pathway provided by OSTα–OSTβ working in conjunction with the well-characterized apical brush border efflux transporters (such as Pgp, BCRP), this question deserves greater exploration.

The broad substrate specificity of OSTα–OSTβ raises the concerns regarding drug interactions. Drugs, dietary constituents, or dietary supplements could act as OSTα–OSTβ inhibitors to slow bile acid export from the enterocyte and activate FXR to induce intestinal FGF15/19 expression. This would result in decreased hepatic bile acid synthesis and is predicted to increase the risk of developing hypercholesterolemia or gallstone disease. In fact, precedence already exists for part of this pathway. Cafestol, the active component in unfiltered coffee that has been described as one of the most potent cholesterol-elevating compounds known in the human diet, is believed to operate by directly activating intestinal FXR to induce FGF19 expression and reduce hepatic CYP7A1 expression (Ricketts et al. 2007). The phenotype of decreased efficiency for bile acid absorption coupled with an inability of the liver to synthesize additional bile acids to maintain a normal pool was described by the late Z. Reno Vlahcevic in a study of gallstone patients published in 1970 (Vlahcevic et al. 1970). Whether the inhibition of OSTα–OSTβ plays any role in the pathophysiology of gallstone disease is an important question that is beginning to be explored (Renner et al. 2008).

Finally, there may be pathophysiological conditions where inhibiting intestinal OSTα–OSTβ is clinically useful. This combination of reduced return of bile acids in the enterohepatic circulation and reduced hepatic bile acid synthesis that would result from inhibiting intestinal OSTα–OSTβ may have therapeutic benefit in various forms of cholestatic liver disease.

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The Role of the Sodium-Taurocholate Cotransporting Polypeptide (NTCP) and of the Bile Salt Export Pump (BSEP) in Physiology and Pathophysiology of Bile Formation

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Abstract Bile formation is an important function of the liver. Bile salts are a major constituent of bile and are secreted by hepatocytes into bile and delivered into the small intestine, where they assist in fat digestion. In the small intestine, bile salts are almost quantitatively reclaimed and transported back via the portal circulation to

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the liver. In the liver, hepatocytes take up bile salts and secrete them again into bile for ongoing enterohepatic circulation. Uptake of bile salts into hepatocytes occurs largely in a sodium-dependent manner by the sodium taurocholate cotransporting polypeptide NTCP. The transport properties of NTCP have been extensively characterized. It is an electrogenic member of the solute carrier family of transporters (SLC10A1) and transports predominantly bile salts and sulfated compounds, but is also able to mediate transport of additional substrates, such as thyroid hormones, drugs and toxins. It is highly regulated under physiologic and pathophysiologic conditions. Regulation of NTCP copes with changes of bile salt load to hepatocytes and prevents entry of cytotoxic bile salts during liver disease. Canalicular export of bile salts is mediated by the ATP-binding cassette transporter bile salt export pump BSEP (ABCB11). BSEP constitutes the rate limiting step of hepatocellular bile salt transport and drives enterohepatic circulation of bile salts. It is extensively regulated to keep intracellular bile salt levels low under normal and pathophysiologic situations. Mutations in the *BSEP* gene lead to severe progressive familial intrahepatic cholestasis. The substrates of BSEP are practically restricted to bile salts and their metabolites. It is, however, subject to inhibition by endogenous metabolites or by drugs. A sustained inhibition will lead to acquired cholestasis, which can end in liver injury.

Keywords Bile salt transporter · Bile formation · Cholestasis

1 Physiology of Bile Formation

Bile production is one of the key functions of the liver. Bile is delivered from the liver to the small intestine, where it is indispensable for the absorption of lipids and of fat-soluble vitamins. Lipids are an essential part of our diet and constitute an important energy source for the body. Fat-soluble vitamins are vital for many processes, for example, vitamin K deficiency leads to a disturbed balance of the blood clotting system. Hence, ongoing bile formation by the liver and undisturbed bile flow are vital processes to satisfy the energy needs of our body as well as to provide essential food constituents to our body. Major constituents of the bile fluid are bile salts, phospholipids, and organic anions (Esteller 2008). Bile salts are amphipathic molecules, display detergent properties, and form together with the phospholipids mixed micelles (Hofmann 2009; Hofmann and Hagey 2008). The mixed micelles reduce the detergent action and consequently the toxicity of bile salts in bile (Trauner et al. 1999) and act as carriers for the biliary elimination of poorly water soluble substances, for example, cholesterol. If bile salts accumulate within hepatocytes, they impair mitochondrial function and hence are cytotoxic (Krahenbuhl et al. 1994; Sokol et al. 2006).

Bile salts are synthesized from cholesterol in hepatocytes by a complex biosynthetic process comprising 17 reactions catalyzed by 16 different enzymes (Russell 2009). Bile salt biosynthesis constitutes the major catabolic pathway of cholesterol.

Bile salts are secreted from hepatocytes into the canaliculi, from where they flow via the biliary tree into the gall bladder (except in species, which have none) (Hofmann 2009; Hofmann and Hagey 2008). From the gall bladder, bile salts are released with bile into the duodenum where they promote further digestion of the chyme, in particular the absorption of fat. Along the small intestine, bile salts are absorbed by more than 90% both passively and actively and are transported back to the liver via the portal circulation. In the liver, bile salts are taken up into hepatocytes, from where they are secreted again into bile (Alrefai and Gill 2007; Dawson et al. 2009; Hofmann and Hagey 2008; Kosters and Karpen 2008; Kullak-Ublick et al. 2004; Meier and Stieger 2002; Pauli-Magnus et al. 2005; Pellicoro and Faber 2007). This circling of bile salts between the liver and the small intestine is called enterohepatic circulation. Overall, the total bile salt pool of humans (3–4 g) circulates 6–10 times per day between the liver and the small intestine, whereby only about 0.5 g of bile salts are not reclaimed, but lost instead via fecal excretion (Hofmann 1999). Hence, enterohepatic circulation is a very efficient process. The size of the bile salt pool is monitored in the enterocytes of the ileum, where the expression of the nuclear transcription factor FXR positively correlates with bile salt load. FXR in turn regulates the transcription of FGF15 (in rodents, FGF19 in man). FGF15 is secreted into the portal circulation and in the liver represses via a receptor-mediated process the biosynthesis of bile salts (Davis and Attie 2008; Rao et al. 2008). In humans, reduced serum levels of FGF19 lead to bile salt induced diarrhea, which highlights the importance of a tight control of the bile salt pool (Hofmann et al. 2009; Walters et al. 2009). In addition to its tight regulation, bile acid biosynthesis undergoes diurnal variation, as exemplified by a diurnal rhythm of biliary bile salt and lipid secretion (Nakano et al. 1990). In mice, disruption of the clock genes *Per1* and *Per2* leads to an impaired bile salt homeostasis with elevated serum bile salt levels (Ma et al. 2009).

Hepatocellular bile salt secretion requires the coordinate action of distinct transporters located in the sinusoidal and in the canalicular membrane of hepatocytes (Kullak-Ublick et al. 2004; Meier and Stieger 2002). In the basolateral hepatocyte membrane, uptake of bile salts occurs predominantly in a sodium-dependent manner via the sodium taurocholate cotransporting polypeptide (Ntcp in rodents, NTCP in humans, gene symbol Slc10a1 in rodents, SLC10A1 in humans) and to a minor extent in a sodium-independent manner mediated by organic anion-transporting polypeptides (Oatps in rodents, OATPs in humans, gene symbol Slco in rodents, SLCO in humans) (Meier and Stieger 2002). Transport across hepatocytes is a fast process: In rats, after injection of an intravenous bolus, taurocholate appears in bile in much less than 1 min and the bolus is almost completely recovered in bile within 10 min after injection (Crawford et al. 1988). The pathway across hepatocytes is not yet fully understood, and different mechanisms may step in depending on the bile salt load imposed to hepatocytes. At a physiologic exposition, the monomeric form of bile salts in the cytoplasm is likely <1 μM (Weinman and Maglova 1994). Part of the bile salts are bound to cytoplasmic bile salt binding proteins, for example, 3α-hydroxysteroid dehydrogenase, glutathione-S-transferase, rat liver fatty acid-binding protein, and human bile salt-binding protein (Agellon and Torchia 2000;

Stolz et al. 1989). These proteins may mediate bile salt diffusion from the basolateral to the apical membrane along an intracellular concentration gradient. Under a high bile salt load, bile salts partition due to their hydrophobicity into intracellular membranes and vesicles (Erlinger 1996; Lamri et al. 1988; Simion et al. 1984; Suchy et al. 1983). In this condition a transcellular vesicular transport route becomes relevant (Crawford et al. 1988), which under normal physiologic conditions seems not to play an important role (Agellon and Torchia 2000; Crawford 1996). The issue of vesicular bile salt transport is complicated by the fact that bile salts regulate microtubule function, which provides tracks for vesicle movement and is important to regulate carrier density in the canalicular membrane (El-Seaidy et al. 1997; Marks et al. 1995). Furthermore, several Rab-proteins can bind lithocholate (Ikegawa et al. 2008) and modulate vesicular trafficking within hepatocytes. Bile salt secretion across the canalicular membrane is known to be the rate-limiting step of hepatocellular bile salt secretion (Reichen and Paumgartner 1976), is an ATP-dependent process (Adachi et al. 1991; Muller et al. 1991; Nishida et al. 1991; Stieger et al. 1992), and is mediated by an ATP-binding cassette (ABC) transporter named bile salt export pump (Bsep in rodents, BSEP in humans, gene symbol Abcb11 in rodents, ABCB11 in humans) (Gerloff et al. 1998; Kullak-Ublick et al. 2004; Meier and Stieger 2002; Stieger 2009; Stieger et al. 2007). Hence, due to its strategic location in the enterohepatic bile salt circulation, BSEP drives and maintains enterohepatic circulation of bile salts.

Disturbed bile formation leads to a reduction of bile flow and is called cholestasis. Cholestasis will lead to retention of bile salts within hepatocytes. The accumulated bile salts will, as a consequence of their detergent properties, induce damage to mitochondria and consequently become cytotoxic to hepatocytes (Krahenbuhl et al. 1994; Sokol et al. 2006). If cholestasis persists, the liver will be damaged and serum bile salts will raise followed by an increase of plasma liver enzymes as a consequence of liver injury. In order to lower or prevent the toxicity of the retained bile salts, they are detoxified by sulfation (Alnouti 2009). An additional protective mechanism is downregulation of bile salt uptake systems as well as upregulation of salvage transport systems (Geier et al. 2007; Wagner et al. 2009).

2 The Sodium Taurocholate Cotransporting Polypeptide Ntcp/NTCP

2.1 Molecular Properties

The first Ntcp orthologue to be molecularly identified was cloned from rat liver using the *Xenopus laevis* expression cloning methodology (Hagenbuch et al. 1991). Ntcp/NTCP was subsequently cloned from many species including human (Hagenbuch and Meier 1994), mouse (Cattori et al. 1999), and rabbit (Kramer et al. 1999), and additional orthologues are predicted in the genomes of a variety of species (Geyer

et al. 2006). Rat Ntcp is the founding member of the solute carrier family (Hediger et al. 2004) number 10 (SLC10) of transporters (Hagenbuch and Dawson 2004). The SLC10 is comprised of seven members, three of which have been functionally characterized and four orphan transporters. The characterized transporters include Ntcp, the apical sodium bile acid transporter (ASBT; SLC10A2), and the steroid sulfate transporter (SOAT; SLC10A6) (Dawson et al. 2009; Geyer et al. 2006). The human and rat *NTCP* genes span 21.4 and 13.6 kb, map to chromosomes 6q24 and 14q24, and encode 349 and 362 amino acids, respectively (Cohn et al. 1995; Hagenbuch and Meier 1994). Mouse *Ntcp* covers 12.5 kb, is found on chromosome 12qD1, and is transcribed into two variants, which differ at their C-terminus (Cattori et al. 1999; Green et al. 1998). The predicted molecular weight of rat Ntcp is about 39 kDa, and in silico hydropathy analysis predicted seven transmembrane spanning domains and five putative N-linked glycosylation sites (Hagenbuch et al. 1991). Expression of Ntcp in *X. laevis* oocytes demonstrated a glycosylated protein with an apparent MW of ~35 kDa after enzymatic deglycosylation. Ntcp glycosylation was confirmed by in vitro translation in a reticulocyte lysate system yielding an apparent MW for the unglycosylated form of ~33 kDa. In vitro translation studies using a reticulocyte lysate system showed no evidence of a cleaved signal sequence in agreement with in silico analysis (Hagenbuch et al. 1991). N-linked glycosylation was experimentally confirmed in basolateral plasma membranes from rat hepatocytes (Ananthanarayanan et al. 1994; Stieger et al. 1994). The C-terminus of Ntcp was immunologically localized to the intracellular side of the plasma membrane and the native molecular weight in rat liver was determined as 51 kDa (Ananthanarayanan et al. 1994; Stieger et al. 1994). Site-directed mutagenesis experiments with the rat orthologue demonstrated the first two putative N-linked glycosylation sites to be glycosylated, confirming that the N-terminus is extracellular. (Hagenbuch 1997). Additional posttranslational modifications of Ntcp include phosphorylation (Mukhopadhyay et al. 1998a, b) and ubiquitination (Kuhlkamp et al. 2005).

The structure of Ntcp has not yet been resolved, but experimental evidence derived from translation/insertion scanning, alanine insertion scanning, and mutation of putative N-linked glycosylation site provides evidence for nine transmembrane spanning domains (Hallen et al. 2002), which were later refined to seven transmembrane spanning domains and helices 7 and 8 forming an extracellular loop at the plasma membrane. This loop is essential for function (Mareninova et al. 2005). Using site-directed mutagenesis in conjunction with expression in *X. laevis* oocytes (Zahner et al. 2003) or COS-7 cells (Saeki et al. 2002), Asp115, Glu257, Cys266 and Cys96, Cys250, Cys266 were found to be functionally essential for rat and mouse Ntcp, respectively.

2.2 Subcellular Expression and Tissue Distribution

Immunofluorescence experiments localized Ntcp to the basolateral plasma membrane of rat (Ananthanarayanan et al. 1994; Stieger et al. 1994) and human hepatocytes (Kullak-Ublick et al. 1997). It displays an even expression throughout

the liver acinus. Next to the liver, Northern blot analysis also indicated expression of Ntcp in rat kidney (Hagenbuch et al. 1991), which so far has not been confirmed at the protein level. Later, expression of Ntcp was observed in the luminal membrane of pancreatic acinar cells (Kim et al. 2002). An additional organ involved in bile salt transport is the placenta, where a fetal-to-maternal bile salt gradient exists (Macias et al. 2009). Whereas in the human placenta NTCP was reported to be absent (Marin et al. 2008; Patel et al. 2003), low levels of Ntcp mRNA have been reported for rat placenta (Leazer and Klaassen 2003; St-Pierre et al. 2004).

2.3 Phylogenetics and Ontogenesis

The phylogenetic analysis of Ntcp revealed that Ntcp is only found in mammalian species, but not in other vertebrates such as chicken, turtle, frog, or skate (Boyer et al. 1993). The ontogeny of Ntcp parallels the development of the bile salt pool (Little et al. 1979; Suchy et al. 1987) and first mRNA expression is seen around day 18 of gestation in rats (Boyer et al. 1993; St-Pierre et al. 2004) with protein clearly detectable in the basolateral plasma membrane of embryonic hepatocytes at day 20 of gestation (Gao et al. 2004). Complete glycosylation of the protein is only observed about 4 weeks after birth (Hardikar et al. 1995). Functionally, sodium-dependent bile salt transport is observed at day 20 of gestation (Suchy et al. 1986), coinciding with the first basolateral protein expression of Ntcp in fetal liver. This relatively late appearance of Ntcp during ontogenesis is reproduced in primary cultures of small hepatocytes, which expand and differentiate into mature hepatocytes (Oshima et al. 2008; Sidler Pfandler et al. 2004). Small hepatocytes are hepatocyte progenitor cells found in adult liver (Chen et al. 2007). In primary cultured rat hepatocytes, Ntcp is rapidly downregulated at the mRNA and protein level (Liang et al. 1993; Rippin et al. 2001). Therefore, Ntcp might serve as a sensitive marker for monitoring the degree of differentiation state of primary cultured hepatocytes.

2.4 Transport Properties

Functional characterization of Ntcp requires its expression in heterologous expression systems. The membrane environment, for example, via cholesterol content, may influence transporter activity, as recently demonstrated for Ntcp expressed in HEK-293 cells (Molina et al. 2008). A careful comparison of the characteristics of Ntcp delineated in heterologous expression systems with results obtained from studies in its "natural" environment, for example, from perfused liver, isolated hepatocytes, or subcellular fractions, is warranted. Whereas normal physiologic expression levels of Ntcp may vary between species and certainly vary between different expression systems, the affinity of Ntcp for its substrates as reflected by the K_m value should remain constant. A comparison of K_m values for hepatic and Ntcp/

NTCP-mediated sodium-dependent taurocholate transport is provided in Table 1. Comparison of the properties of the cloned protein with physiologic parameters determined in organ or cellular systems is feasible for rat Ntcp, as a vast literature exists on hepatic handling of bile salts by rats. It is evident from Table 1 that the K_m value of the cloned rat Ntcp matches very well the K_m values determined in the perfused rat liver, in isolated rat hepatocytes, and in isolated liver plasma membrane vesicles. These results strongly support the prevailing concept that Ntcp is the physiologically relevant sodium-dependent taurocholate (and bile salt) uptake system in rat liver. This conclusion is also supported by results from a study combining an antisense oligonucleotide approach with expression of total mRNA from rat liver in *X. laevis* oocytes. In this study, coexpression of antisense oligonucleotides against rat Ntcp with total rat liver mRNA led to a complete knockdown of sodium-dependent bile salt uptake into the oocytes (Hagenbuch and Meier 1996). This is evidence that Ntcp is the only sodium-dependent bile salt uptake system in rat liver. Although Ntcp appears to account for most, if not all hepatic Na^+-dependent bile salt transport, the presence of additional sinusoidal Na^+-dependent bile salt transporters has been suggested. The microsomal epoxide hydrolase has been postulated as an additional basolateral, sodium-dependent bile salt uptake system (von Dippe et al. 1993), albeit with conflicting and equivocal evidence (Honscha et al. 1995; Meier et al. 1997). Mice with a disrupted gene for microsomal epoxide hydrolase were reported to be phenotypically normal (Miyata et al. 1999), which may be taken as evidence that these mice had no obvious defect in bile salt homeostasis. A patient with high serum bile salt levels was described as having no apparent irregularities in the *NTCP* gene and its expression, but a mutation in the promoter of microsomal epoxide hydrolase. This mutation results in a marked reduction of the activity of this promoter in vitro and a pronounced reduction of microsomal epoxide hydrolase protein as assessed by Western blotting in liver tissue from this patient (Zhu et al. 2003). To date no Ntcp null mice have been described, and the role of microsomal expoxide hydrolase cannot be settled on grounds of genetic evidence. The data on rat Ntcp given in Table 1 are also strong evidence that the different expression systems seem to have only a minimal influence on the affinity of this transporter for taurocholate. The published K_m values for sodium-dependent taurocholate transport into human hepatocytes vary considerably (Table 1). A likely explanation for this finding are differences in procurement of human liver tissue samples and differences of the physiologic state of the donor tissue. Nevertheless, cloned human NTCP expressed in heterologous systems (Table 1) suggest a high affinity for the human transporter toward taurocholate.

Ntcp transports two sodium ions together with one bile salt molecule (Hagenbuch and Meier 1996), which makes it an electrogenic transporter (Weinman 1997). It therefore functions as a secondary active transporter and utilizes as a driving force both the out to in sodium gradient maintained by the (Na + K)-ATPase localized in the basolateral plasma membrane of hepatocytes and the inside negative membrane potential of about −35 mV to −40 mV, which is governed mainly by ion channels (Boyer et al. 1992; Fitz and Scharschmidt 1987). Hence, the energetics allows Ntcp to take up bile salts from the portal blood against a concentration gradient into

Table 1 K_m values of hepatocellular sodium-dependent taurocholate transport determined in different experimental systems

Experimental system	K_m (in µM)	References
Rat Ntcp		
Perfused liver	25	Reichen and Paumgartner (1976)
	61	Dietmaier et al. (1976)
Hepatocyte monolayer	33	Van Dyke et al. (1982)
	30	Liang et al. (1993)
	17	Kouzuki et al. (1998)
Hepatocyte suspension	19	Schwarz et al. (1975)
	26	Anwer et al. (1976)
	21	Blitzer et al. (1982)
	12	Kuhn and Gewirtz (1988)
	30	Follmann et al. (1990)
	24	Sandker et al. (1992)
	44	Sabordo and Sallustio (1997)
Isolated membrane vesicles	56	Inoue et al. (1982)
	52	Duffy et al. (1983)
	46	Zimmerli et al. (1989)
X. laevis oocytes	25	Hagenbuch et al. (1991)
COS-7 (transient)	29	Boyer et al. (1994)
HPTC (stable)	25	Platte et al. (1996)
McArdle RH-7777 (stable)	13	Torchia et al. (1996)
COS-7 (transient)	18	Kouzuki et al. (1998)
CHO 9-6 (stable)	34	Schroeder et al. (1998)
HepG2 (EGFP-tagged)	25	Kullak-Ublick et al. (2000a)
COS-7 (transient)	30	Sun et al. (2001a)
HeLa (stable)	8	Hata et al. (2003)
HepG (stable)		Gundala et al. (2004)
MDCK	14	Mita et al. (2005)
Human NTCP		
Hepatocyte suspension	46	Azer and Stacey (1993)
	62	Sandker et al. (1994)
	5	Shitara et al. (2003)
	84	Mita et al. (2005)
Isolated membrane vesicles	34	Novak et al. (1989)
X. laevis oocytes	6	Hagenbuch and Meier (1994)
WIF-B (hybrid between rat hepatoma and human fibroblasts)	6	Konieczko et al. (1998)
COS-1 (transient)	10	Craddock et al. (1998)
Hela (transient)	8	Kim et al. (1999)
CHO (stable)	16	de Waart et al. (2010)
Not specified	25	Mita et al. (2005)
Mouse Ntcp		
X. laevis oocytes	86 (Ntcp1)	Cattori et al. (1999)
	14 (Ntcp2)	Cattori et al. (1999)
COS-7 (transient)	18 (Ntcp1)	Saeki et al. (2002)

hepatocytes. In transport experiments using isolated basolateral plasma membrane vesicles, albumin stimulates sodium-dependent taurocholate uptake by lowering the K_m value (Blitzer and Lyons 1985; Zimmerli et al. 1989). Expression of total mRNA

from rat liver in *X. laevis* oocytes followed by determination of sodium-dependent taurocholate transport in the presence of albumin also results in a stimulation of the transport activity by low concentrations of albumin (Hagenbuch et al. 1990). This indicates that transport activity of rat Ntcp may be directly stimulated by albumin. This assumption was confirmed for human NTCP expressed in CHO cells (Kim et al. 1999).

Addition of EGFP to the C-terminus of Ntcp did not affect the targeting to the plasma membrane inHepG2 cells nor did it affect the K_m value of Ntcp (Kullak-Ublick et al. 2000a), demonstrating that a free C-terminus is not essential for function. Similar results were obtained for human NTCP (Sun et al. 2001b). Deleting the 56 amino acid C-terminus of rat Ntcp did not abolish its transport function (K_m for the truncated form: of 44 μM; for wild type: 30 μM), but severely impaired its plasma membrane targeting in COS-7 and MDCK cells (Sun et al. 2001a). This demonstrates that the C-terminus of Ntcp is important for efficient plasma membrane targeting.

The substrate specificity of cloned Ntcps has been studied to a great extent and has been covered and compared with data from in vivo systems in many reviews (Alrefai and Gill 2007; Dawson et al. 2009; Geyer et al. 2006; Kullak-Ublick et al. 2000b, 2004; Meier et al. 1997; Meier and Stieger 2002; Suzuki and Sugiyama 2000; Trauner and Boyer 2003). Table 2 gives an overview on these findings by listing Ntcp substrates different from taurocholate. Ntcp has a preference for glycine- and taurine-conjugated bile salts over their unconjugated counterparts. Additionally, the affinity is often higher for dihydroxy bile salts (conjugates of chenodeoxycholate and deoxycholate) than for trihydroxy bile salts (conjugates of cholate). Besides different bile salts, estrone-3-sulfate and bromosulfophthalein are good substrates. Ntcp is also able to transport drugs conjugated to bile salts and hence is a potential drug target (Marin et al. 2009; Vallejo et al. 2007). Thyroid hormones and their metabolites are additional substrates of Ntcp (Friesema et al. 1999; Visser et al. 2009). Of note, human NTCP is able to mediate transport of rosuvastatin and accounts for about 35% of total rosuvastatin uptake into isolated human hepatocytes (Ho et al. 2006). For high-throughput experiments with a transporter, fluorescent molecules are desirable. For potential application of such screenings to Ntcp, the following fluorescent bile salts have been shown to be substrates with heterologously expressed Ntcp: cholylglycylfluorescein and chenodeoxycholyllysyl-NBD (Boyer et al. 1994), NBD-cholyltaurine (Platte et al. 1996) and cholylglycylamidofluorescein and chenodeoxycholylglycylamidofluorescein (Mita et al. 2006a), but not cholyl-L-lysyl-fluorescein (de Waart et al. 2010). This latter finding highlights the importance of the side chain as a determinant for Ntcp substrates (Baringhaus et al. 1999).

2.5 NTCP/Ntcp Inhibitors

The liver is a major site for drug metabolism and elimination, particularly for poorly water-soluble drugs. As bile salts are also amphipathic molecules, Ntcp is

Table 2 Substrates of Ntcp identified in different expression systems

Experimental system	K_m (in μM)	References
Rat Ntcp		
Bile salts		
Cholate (*X. laevis*)		Schroeder et al. (1998)
Cholate (COS-7 transient)		Kouzuki et al. (1998)
Cholate (MDCK)		Mita et al. (2005)
Chenodeoxycholate (MDCK)		Mita et al. (2005)
Ursodeoxycholate (MDCK)		Mita et al. (2005)
Glycocholate (*X. laevis*)		Schroeder et al. (1998)
Glycocholate (CHO)	27	Schroeder et al. (1998)
Glycocholate (COS-7 transient)		Kouzuki et al. (1998)
Glycocholate (CHO)		Briz et al. (2002)
Glycocholate (HeLa)		Hata et al. (2003)
Glycocholate (MDCK)		Mita et al. (2005)
Glycochenodeoxycholate (MDCK)		Mita et al. (2005)
Glycoursodeoxycholate (MDCK)		Mita et al. (2005)
Tauromurocholate (HeLa)		Hata et al. (2003)
Taurohyodeoxycholate (HeLa)	7	Hata et al. (2003)
Taurolagodeoxycholate (HeLa)		Hata et al. (2003)
Taurodeoxycholate (HeLa)	7	Hata et al. (2003)
Tauro-*allo*-cholate (CHO)		Mendoza et al. (2003)
Taurochenodeoxycholate (*X. laevis*)		Schroeder et al. (1998)
Taurochenodeoxycholate (CHO)	5	Schroeder et al. (1998)
Taurochenodeoxycholate (MDCK)		Mita et al. (2005)
Tauroursodeoxycholate (*X. laevis*)		Schroeder et al. (1998)
Tauroursodeoxycholate (CHO)	14	Schroeder et al. (1998)
Tauroursodeoxycholate (MDCK)		Mita et al. (2005)
Bile salt and steroid metabolites		
Estrone-3-sulfate (*X. laevis*)		Schroeder et al. (1998)
Estrone-3-sulfate (CHO)	27	Schroeder et al. (1998)
Dehydroepiandosterone sulfate (HepG2, EGFP-tagged)		Kullak-Ublick et al. (2000a)
Others		
Sulfobromophthalein (*X. laevis*)		Meier et al. (1997)
Thyroxine (T4) (*X. laevis*)		Friesema et al. (1999)
3,3′,5-triiodothyronine (T3) (*X. laevis*)		Friesema et al. (1999)
3,3′,5′-triiodothyronine (rT3) (*X. laevis*)		Friesema et al. (1999)
3,3′-diiodothyronine (T2) (*X. laevis*)		Friesema et al. (1999)
Thyroxine sulfate (*X. laevis*)		Friesema et al. (1999)
3,3′,5-triiodothyronine sulfate (*X. laevis*)		Friesema et al. (1999)
3,3′,5′-triiodothyronine sulfate (*X. laevis*)		Friesema et al. (1999)
3,3′-diiodothyronine sulfate (*X. laevis*)		Friesema et al. (1999)
Thyroxine sulfamate (*X. laevis*)		Friesema et al. (1999)
3,3′,5-triiodothyronine sulfamate (*X. laevis*)		Friesema et al. (1999)
ONO-1301 (not specified)		Suzuki and Sugiyama (2000)
Bamet-R2 (CHO)	[a]	Briz et al. (2002)
Bamet-UD2 (CHO)	[a]	Briz et al. (2002)
Sulfobromophthalein (HeLa)	4	Hata et al. (2003)
α-Amanitin (Hep-G2)		Gundala et al. (2004)
Human NTCP		
Bile salts		
Cholate (LLC-PK_1)		Mita et al. (2006b)

(*continued*)

Table 2 (continued)

Experimental system	K_m (in μM)	References
Chenodeoxycholate (LLC-PK$_1$)		Mita et al. (2006b)
Chenodeoxycholate-3-sulfate (COS-1 transient)		Craddock et al. (1998)
Ursodeoxycholate (LLC-PK$_1$)		Mita et al. (2006b)
Glycocholate (LLC-PK$_1$)		Mita et al. (2006b)
Glycochenodeoxycholate (LLC-PK$_1$)		Mita et al. (2006b)
Glycoursodeoxycholate (HEK293)	0.4 and 25	Maeda et al. (2006)
Glycoursodeoxycholate (LLC-PK$_1$)	15	Mita et al. (2006b)
Tauroursodeoxycholate (HEK293)	10	Maeda et al. (2006)
Taurochenodeoxycholate (LLC-PK$_1$)		Mita et al. (2006b)
Bile salt and steroid metabolites		
Estrone-3-sulfate (COS-1 transient)	60	Craddock et al. (1998)
Others		
Chlorambucil-taurocholate (*X. laevis*)	11	Kullak-Ublick et al. (1997)
Bromosulfophthalein (*X. laevis*)		Meier et al. (1997)
Bamet-R2 (*X. laevis*)	27	Briz et al. (2002)
Bamet-UD2 Bamet-UD2 (*X. laevis*)	33	Briz et al. (2002)
Rosuvastatin NTCP*1 (HeLa transient)	65	Ho et al. (2006)
Rosuvastatin NTCP*2 (HeLa transient)	3	Ho et al. (2006)
3,3′,5-triiodothyronine sulfate (COS1)		Visser et al. (2009)
Thyroxine sulfate (COS1)		Visser et al. (2009)

The heterologus expression system is given in parenthesis
[a]Transport mediated by Ntcp was partially sodium-independent

subject to inhibition by various drugs. For example, the immunosuppressant cyclosporine leads to cholestatic liver injury (Arias 1993). Cyclosporine inhibits sodium-dependent taurocholate uptake into isolated rat (Stacey and Kotecka 1988) and human hepatocytes (Azer and Stacey 1993) as well as into isolated rat basolateral plasma membrane vesicles (Moseley et al. 1990; Zimmerli et al. 1989). Cyclosporine clearly inhibits rat Ntcp (Schroeder et al. 1998) and human NTCP (Mita et al. 2006a) in heterologous expression systems, with an IC_{50} value of 1 μM (Kim et al. 1999). However, it is not transported by rat Ntcp expressed in *X. laevis* oocytes (Schroeder et al. 1998). Similar results were obtained for bumetanide, which is also an inhibitor but not a transport substrate for rat Ntcp (Platte et al. 1996; Schroeder et al. 1998). Consequently and importantly, inhibition experiments should not be used with transporters as stand-alone assays to identify transported substrates. Rat Ntcp is also inhibited by probenicide (Platte et al. 1996). Inhibition of Ntcp should lead to an increase of serum bile salt levels, but the effects may be variable. The liver also expresses sodium-independent bile salt transporters, namely the organic anion-transporting polypeptides (OATPs), which may partially compensate for the inhibition of NTCP. However, it should be noted that these compounds can often inhibit OATPs in addition to NTCP, as demonstrated for rifampicin and rifamycin with rat Ntcp, Oatp1a1, and Oatp1a4 (Fattinger et al. 2000) and for cyclosporine with NTCP, OATP1B1, and OATP1B3 (Treiber et al. 2007). This inhibition of sodium-dependent and sodium-independent bile salt uptake into hepatocytes is predicted to increase serum bile salt levels, depending in part on the relative

affinities of the inhibitors to the different transporters (Fattinger et al. 2000). Such an inhibition could diminish intracellular bile salt concentrations in hepatocytes and consequently lower the cytotoxic action of bile salts. This concept has so far not been tested experimentally. Examples of other drugs reported to inhibit NTCP are: (R)-propanolol (IC_{50} 6 μM), (S)-propanolol (IC_{50} 6 μM), furosemide (IC_{50} 15 μM), ketokonazole (264 μM), renin inhibitors and a somatostatin analogue (Kim et al. 1999), rifamycin SV and glibenclamide (Mita et al. 2006a), the antiviral drugs ritonavir (IC_{50} 2 μM), saquinavir (IC_{50} 7 μM), and efavirenz (IC_{50} 43 μM) (McRae et al. 2006), or bosentan (IC_{50} 24 μM) (Leslie et al. 2007).

2.6 Pathophysiology

Currently, no human diseases caused by mutations in the *NTCP* gene are known, even though patients with primary hypercholanemia have been described (Carlton et al. 2003; Shneider et al. 1997). Information regarding changes for NTCP expression in liver disease is limited and frequently based on cholestatic liver diseases. In liver samples obtained from patients with progressive familial intrahepatic cholestasis, NTCP was downregulated at the protein, but not at the mRNA level, indicating a posttranslational regulation of NTCP in this disease (Keitel et al. 2005). As a consequence of progressing obstructive cholestasis, such as for example in biliary atresia, NTCP is downregulated at the mRNA level (Chen et al. 2008a; Shneider et al. 1997). The mRNA level of NTCP correlates inversely with serum bilirubin and serum bile salt levels, respectively (Shneider et al. 1997; Zollner et al. 2001). Restoration of bile flow in such patients increased mRNA levels for NTCP (Shneider et al. 1997). In patients with chronic hepatitis C or primary biliary cirrhosis stage I and II, no change in NTCP expression was observed (Kojima et al. 2003; Zollner et al. 2001, 2003), whereas in stage III it was downregulated (Kojima et al. 2003). If inflammation was the cause of cholestasis, NTCP was markedly downregulated (Zollner et al. 2001), which is reproduced at the mRNA and protein level in primary cultured human liver slices treated with LPS (Elferink et al. 2004) or in isolated and cultured human hepatocytes treated with IL-1β, tumor necrosis factor α, or IL-6 (Le et al. 2008, 2009). In hepatocellular carcinoma, NTCP is downregulated in comparison to the surrounding healthy tissue (Kullak-Ublick et al. 1997; Zollner et al. 2005). Complementing this finding, NCTP expression is absent in the hepatoma cell line HepG2 (Kullak-Ublick et al. 1996; Le et al. 2006). In summary, in human liver disease NTCP expression is often downregulated. This can be considered a protective adaptation as downregulation of NTCP should lower the load of potentially cytotoxic bile salts for hepatocytes. Liver transplantation involves a period of ischemic stress for the transplanted organ. It was found that after liver transplantation NTCP mRNA is increased significantly (Geuken et al. 2004).

Animal models of cholestasis have reproduced the human pattern of NTCP expression during liver disease and exhibit downregulation of Ntcp after bile duct ligation, which is more pronounced in the periportal areas (Donner et al. 2007),

treatment with ethinylestradiol, or administration of LPS (Dumont et al. 1997; Gartung et al. 1996; Green et al. 1996; Moseley et al. 1996; Simon et al. 1996; Slitt et al. 2007). In rats treated with trinitrobenzene sulfonic acid, an animal model of primary sclerosing cholangitis, Ntcp was transiently downregulated at the mRNA and protein level (Geier et al. 2002a). If animals were treated with a heat shock (42°C body temperature for 10 min) 2 h prior to the application of LPS, downregulation of Ntcp was prevented by a posttranslational mechanism (Bolder et al. 2002). The expression of Ntcp in animal models of liver disease is affected by hepatocellular retention of bile salts, but not by variations of transcellular bile salt flux (Gartung et al. 1997; Koopen et al. 1997; Wolters et al. 2002). CCl_4 treatment of rats as a model for toxic liver injury leads to a marked downregulation of Ntcp mRNA and protein (Geier et al. 2002b, 2003), whereas no change in obese Zucker rats is seen (Palmeira and Rolo 2004). In a model of fatty liver, the ob/ob mouse, Ntcp protein is downregulated in both males and females, but Ntcp mRNA is downregulated only in female mice (Cheng et al. 2008). Hypoxic liver injury after ischemia in rats and mice leads to downregulation of Ntcp at the mRNA and protein level (Fouassier et al. 2007; Hoekstra et al. 2008). This is also observed for cold ischemic injury in conjunction with liver transplantation in $Mdr2^{+/-}$ mice used as a model for bile duct injury (Hoekstra et al. 2006). Ntcp has also been shown to be downregulated in pregnancy (Arrese et al. 2003), though this has not been a universal finding (Cao et al. 2001). After delivery, the expression of Ntcp is increased (Cao et al. 2001; Ganguly et al. 1994). Finally, in the regenerating rat liver, Ntcp is transiently downregulated both at the mRNA, protein and functional level (Dransfeld et al. 2005; Gerloff et al. 1997, 1999; Green et al. 1997; Vos et al. 1999).

The expression of Ntcp is not only modulated in various pathophysiologic situations as outlined previously, but its expression in the basolateral hepatocyte membrane is also subject to regulation by posttranslational modification through phosphorylation/dephosphorylation (Anwer 2004). cAMP, but not cGMP stimulates basolateral taurocholate transport and increases Ntcp by translocating it into basolateral membrane (Mukhopadhayay et al. 1997). This process is independent of microtubules. Ntcp is phosphorylated at serine and threonine residues and the dephosphorylation of these residues is stimulated in a cAMP-dependent way (Mukhopadhyay et al. 1998a), which consequently leads to incorporation of dephosphorylated Ntcp into the basolateral membrane. The intracellular pool of the phosphorylated form resides in an endosomal compartment for recruitment to the basolateral membrane (Mukhopadhyay et al. 1998b). The translocation of Ntcp to the basolateral plasma membrane is facilitated in HuH cells expressing Ntcp by activation of Rab4 (Schonhoff et al. 2008).

2.7 Pharmacogenomics

In 48 Japanese individuals, 14 single-nucleotide polymorphisms were identified in the *NTCP* gene (Saito et al. 2002b) and four insertion/deletion polymorphisms.

Two of the polymorphisms were found in exons, namely p.T75T (c.307G>A) in exon 1 and p.S267F in exon 4. No variants were observed in the promoter region of the *NTCP* gene and the functional consequences of the alterations were not investigated. Screening of a cohort of 50 European Americans, 50 African Americans and 50 Chinese Americans identified several polymorphisms, 4 of which were nonsynonymous [c.668T>C (p.S267F), c.800C>T (p.S267F), c.836T>C (p.I279T), and c.940A>G (p.K314E)] (Ho et al. 2004). All these variants were expressed in HeLa cells and functionally compared to NTCP*1 for their transport properties with taurocholate, cholate, and estrone-3-sulfate as substrates. With the exception of the p.I223T variant, the others displayed significant cell surface expression. Of all variants, p.S267F displayed almost no transport of taurocholate or cholate, whereas the other variants displayed transport of all three substrates with comparable affinities. All the variants were later shown to mediate rosuvastatin transport (Ho et al. 2006). The p.S267F variant showed a higher transport capacity for rosuvastatin than NCTP*1 and a much higher affinity (3 μM vs. 65 μM) than NTCP*1, despite exhibiting almost no detectable bile salt transport. To date, no genotype phenotype correlations (e.g., serum bile salt levels) of NTCP variants have been published. Serum bile salt levels, if at all, would certainly not only be determined by NTCP variant function, but also by NTCP expression levels, which have been reported to be subject to considerable interindividual variability at the mRNA level (Ho et al. 2006).

In summary, Ntcp/NTCP is the major hepatocyte system for uptake of conjugated bile salts from the portal blood. Ntcp transports bile salts into hepatocytes in a sodium-dependent manner and works electrogenically against a concentration gradient. Ntcp prefers conjugated to nonconjugated bile salts and exhibits a substrate specificity largely restricted to bile salts, sulfated steroids, and a limited number of drugs or drug metabolites. Ntcp's expression is extensively regulated at the transcriptional and posttranscriptional level in many pathophysiologic situations. Ntcp expression is typically downregulated in situations where bile secretion is impaired to protect hepatocytes from accumulating cytotoxic bile salts. The transcriptional regulation of Ntcp will be covered elsewhere in this book.

3 The Bile Salt Export Pump Bsep/BSEP

3.1 Molecular Properties

Bsep was cloned based on the hypothesis that it belongs to the ABC transporter family (Brown et al. 1995; Lomri et al. 1996). PCR analysis of total rat liver mRNA with degenerate oligonucleotide primers spanning the Walker A and Walker B motif of ABC transporters identified a c DNA fragment that revealed a high identity with a published cDNA fragment isolated from pig liver (Gerloff et al. 1998). This partial cDNA had been named sister of p-glycoprotein (Childs et al. 1995; Gerloff et al. 1998; Stieger et al. 2007). Expression of the full-length cDNA of the rat liver

isoform of the sister of p-glycoprotein in *X. laevis* oocytes demonstrated a stimulation of taurocholate efflux and membrane vesicles isolated from Sf9 cells expressing this cDNA exhibit ATP-dependent bile salt transport (Gerloff et al. 1998). Sister of p-glycoprotein was therefore renamed bile salt export pump (Bsep). Analysis of the genome of patients with progressive familial intrahepatic cholestasis type 2 by positional cloning identified the genetic defect to be located in the human *ABCB11* or *BSEP* gene (Strautnieks et al. 1998). The *BSEP* gene is located on chromosome 2q24–31, spans 28 exons, and encodes 1,321 amino acids. Mouse and rat Bsep are more than 80% identical to human BSEP, whereby the difference between the species are mostly found in the predicted transmembrane helices, whereas the cytoplasmic loops display very little variability (Green et al. 2000; Lecureur et al. 2000; Noe et al. 2001). Mouse Bsep is located to a region on chromosome 2, which is syntenic to human 2q24–31 (Lecureur et al. 2000). Bsep has recently been cloned and characterized from dog and shown to be highly homologous to human and rat Bsep (Yabuuchi et al. 2008). BSEP is a glycoprotein with four putative N-linked glycosylation sites and with a molecular weight of 140–170 kDa (Byrne et al. 2002; Gerloff et al. 1998; Green et al. 2000; Meier et al. 2006; Noe et al. 2002). Rat Bsep is glycosylated at all four putative glycosylation sites (Mochizuki et al. 2007). In addition to glycosylation, phosphorylation of Bsep (Hayashi and Sugiyama 2009; Noe et al. 2001) and ubiquitination occur as additional posttranslational modifications.

The structure of BSEP has not yet been determined. It is a member of the ABCB family of the human ABC transporters, which also contains MDR1 or P-glycoprotein (Gerloff et al. 1998; Thompson and Strautnieks 2000), and it is predicted to have two transmembrane domains of six membrane spanning helices each. The transmembrane domains are separated by a large cytoplasmic domain and the second transmembrane domain is again followed by a large cytoplasmic domain (Gerloff et al. 1998). This structural organization constitutes the basic building principle of all ABC transporters (Borst and Elferink 2002; Higgins 1992; Klein et al. 1999). The cytoplasmic domains harbor the Walker A and Walker B motifs, which are part of the ATP-binding site as well as the family signature motif of ABC transporters. Analysis of inherited defects in the human BSEP gene has provided a wealth of information on the functional role of individual amino acids within the BSEP protein. These findings will be discussed in the following section. Recently, a first report of findings on quantitative structure–activity relationship analysis using BSEP inhibitors has been published (Hirano et al. 2006). Pursuing this approach will certainly provide most valuable information on the structural requirements of substrates and inhibitors for their interaction with Bsep.

3.2 Subcellular Expression and Tissue Distribution

Bsep is expressed homogenously throughout the liver acinus and located in the canalicular membrane of hepatocytes as demonstrated by immunohistochemical

methods at the light and electron microscopic level (Gerloff et al. 1998). Using imunogold labeling, Bsep was found to be expressed on canalicular microvilli, but absent from intermicrovillar parts of the cananicular membrane (Gerloff et al. 1998). Hence, Bsep could partition into subdomains of the canalicular plasma membrane. This assumption is supported by the observation that Bsep is concentrated in canalicular microdomains, which are resistant to extraction by Lubrol WX (Ismair et al. 2009). Bsep is also expressed in subapical vesicles in close proximity to the canalicular plasma membrane (Dombrowski et al. 2006; Gerloff et al. 1998).

In pig, rat, and mouse, Northern blot analysis revealed a predominant if not exclusive expression of Bsep in liver (Childs et al. 1995; Gerloff et al. 1998; Lecureur et al. 2000). Using a PCR approach, Bsep mRNA was found in liver, brain, small and large intestine but not kidney of rats (Torok et al. 1999). Another group identified Bsep both by PCR and by Western blotting in rat kidney (Heemskerk et al. 2007). In dog, very low levels of Bsep mRNA were demonstrated with PCR in testis, spleen, and stomach, whereas expression in heart, lung, kidney, skeletal muscle, large and small intestine was not detectable (Yabuuchi et al. 2008). In humans, BSEP mRNA was found at high levels in testis in addition to liver by quantitative PCR. Low levels were reported for trachea, colon, prostate, lung, and thymus (Langmann et al. 2003). In another study, moderate expression was found in kidney and colon, but jejunum was reported negative (Hilgendorf et al. 2007). Low BSEP expression has also been reported for the first trimester human placenta (Patel et al. 2003) and very low levels in rat placenta (St-Pierre et al. 2004). Taken together, although there is no doubt that Bsep is expressed at a high level in the liver, data on extrahepatic expression revealed conflicting results so far. In particular, with the exception of one study, no information is available on the expression BSEP protein in extrahepatic tissues.

3.3 Phylogenetics and Ontogenesis

Bsep is also found in lower vertebrates. Bsep of the small skate *Raja erinacea*, an ancient vertebrate, is 69% identical to human BSEP (Cai et al. 2001). Hence BSEP remained remarkably conserved during vertebrate evolution. A partial Bsep sequence was also cloned from flounder (*Pseudopleuronectus americanus*) (Childs et al. 1995) and a highly identical sequence could be amplified from rainbow trout (*Oncorhynchus mykiss*) hepatocytes (Zaja et al. 2008). Using Northern blot analysis, Bsep expression was also identified in liver from the two vertebrates chicken and turtle (Gerloff et al. 1998).

The ontogeny of Bsep has been extensively studied in rat liver. Bsep mRNA could be detected at day 15 (Gao et al. 2004; Tomer et al. 2003; St-Pierre et al. 2004; Zinchuk et al. 2002) and stayed at very low level until day 20 of gestation. In rats, low levels of Bsep staining can be detected at the canaliculi at embryonic day 20. From day 20 on, Bsep expression rapidly increases both at the mRNA and at the protein level, reaching almost adult levels within the first week after birth (Gao

et al. 2004; Tomer et al. 2003; Zinchuk et al. 2002). One group has reported a transient increase of Bsep mRNA above adult levels in rats in the first days after birth (de Zwart et al. 2008). In mouse liver, Bsep mRNA is reported at day 14 in developing liver (Lecureur et al. 2000). In humans, BSEP is expressed at mid-gestation, albeit at much lower levels than in adult liver (Chen et al. 2005). The fact that Bsep has not yet reached adult levels at birth explains the observation that healthy human neonates have a transient elevation of physiologic bile salts in their serum (Suchy et al. 1981). In summary, in rats ontogeny of the major bile salt uptake (Ntcp) and export (Bsep) in parallel one another and closely follow the development of the bile salt pool and the enterohepatic circulation of bile salts (Little et al. 1979; Suchy et al. 1987).

3.4 Transport Properties

Hepatocellular, ATP-dependent bile salt transport has been first described in isolated rat canalicular plasma membrane vesicles (Adachi et al. 1991; Muller et al. 1991; Nishida et al. 1991; Stieger et al. 1992). Molecular characterization of a bile salt export system is best done in a vesicular system. Isolated membrane vesicles are oriented in part inside out and do therefore allow direct experimental access to the cytoplasmic side of the transporter. Furthermore, transport in a vesicular system can be measured as uptake. Hence, isolated canalicular vesicles can be considered to resemble the physiologic characteristics of the bile salt export system. Characterization of this system demonstrated that bile salt transport in the canalicular membrane cannot be stimulated by nucleotides other than ATP and requires the hydrolysis of the γ-phosphate ester (Adachi et al. 1991; Muller et al. 1991; Nishida et al. 1991; Stieger et al. 1992). The transport is sensitive to orthovanadate and is electrogenic. It can be stimulated by the presence of the more permeable anion nitrate in the transport buffer as compared to gluconate (Stieger et al. 1992). Other indirect coupling mechanisms for transmembrane taurocholate transport such as an ATP-dependent pH gradient or an inside positive membrane potential were excluded as driving forces in the same study. Cloned rat Bsep expressed in Sf9 cells has comparable transport properties to ATP-dependent bile salt transport in isolated rat canalicular plasma membrane vesicles and is also strictly dependent on hydrolysable ATP and not stimulated by other nucleotides (Gerloff et al. 1998; Stieger and Meier 2001).

A comparison of the functional characteristics of the cloned Bsep isoforms from different species with the respective transporter data in canalicular vesicles (Table 3) demonstrates that Bsep is predominantly, if not exclusively, a bile salt transporter. No overt effect of different expression systems on the substrate pattern and on the affinity of the substrates is observed. With respect to glycocholate transport activity of rat Bsep, the results vary between research groups and expression systems, but this bile salt seems to be a poor substrate for rat Bsep. While taurolithocholate-3 sulfate is not transported by rat Bsep expressed in Sf9 cells

Table 3 Substrates of Bsep identified in different expression systems

Experimental system	K_m (in μM)	References
Rat canalicular plasma membrane vesicles		
Substrate		
Taurocholate	26	Nishida et al. (1991)
Taurocholate	8	Muller et al. (1991)
Taurocholate	47	Adachi et al. (1991)
Taurocholate	2	Stieger et al. (1992)
Taurocholate	6	Liu et al. (1997)
Taurocholate	13	Niinuma et al. (1999)
Taurocholate	2	Funk et al. (2001)
Glycocholate	Low	Gerloff et al. (1998)
Cholate	Low	Gerloff et al. 1998
Rat Bsep		
Bile salts		
Taurocholate (Sf9)	5	Gerloff et al. (1998)
Taurocholate (Sf9)	8	Akita et al. (2001)
Taurocholate (HEK293)	10	Hayashi et al. (2005b)
Taurocholate (Sf9)	22	Yabuuchi et al. (2008)
Taurocholate (Sf9)	12	Kis et al. (2009a)
Glycocholate (Sf9)	nt	Gerloff et al. (1998)
Glycocholate (HEK293)	10	Hayashi et al. (2005b)
Glycocholate (Sf9)	22	Kis et al. (2009a)
Tauroursodeoxycholate	4	Stieger et al. (2000)
Taurochenodeoxycholate (SF9)	2	Stieger et al. (2000)
Taurochenodeoxycholate (HEK293)	10	Hayashi et al. (2005b)
Taurochenodeoxycholate (Sf9)	12	Kis et al. (2009a)
Glycochenodeoxycholate (HEK293)	6	Hayashi et al. (2005b)
Glycochenodeoxycholate (Sf9)	16	Kis et al. (2009a)
Cholate (Sf9)	nt	Gerloff et al. (1998)
Bile salt and steroid metabolites		
Taurolithocholate-3-sulfate (Sf9)	nt	Stieger et al. (2000)
Taurolithocholate-3-sulfate (Sf9)	nt	Akita et al. (2001)
Taurolithocholate-3-sulfate (HEK293)	Low	Hayashi et al. (2005b)
Others		
Pravastatin (HEK293)	Yes	Hirano et al. (2005)
Human canalicular plasma membrane vesicles		
Taurocholate	5	Wolters et al. (1992)
Taurocholate	21	Niinuma et al. (1999)
Human BSEP		
Bile salts		
Taurocholate (Sf9)	8	Noe et al. (2002)
Taurocholate (high five)	4	Byrne et al. (2002)
Taurocholate (HEK293)	6	Hayashi et al. (2005b)
Taurocholate (Sf9)	20	Yabuuchi et al. (2008)
Taurocholate (Sf9)	15	Kis et al. (2009a)
Taurocholate (Sf9)	20	Yamaguchi et al. (2009)
Glycocholate (HEK293)	22	Hayashi et al. (2005b)
Glycocholate (Sf9)	11	Noe et al. (2002)
Glycocholate (Sf9)	36	Kis et al. (2009a)
Tauroursodeoxycholate (Sf9)	12	Noe et al. (2002)
Taurochenodeoxycholate (Sf9)	5	Noe et al. (2002)
Taurochenodeoxycholate (HEK293)	7	Hayashi et al. (2005b)

(*continued*)

Table 3 (continued)

Experimental system	K_m (in μM)	References
Taurochenodeoxycholate (Sf9)	4	Kis et al. (2009a)
Taurochenodeoxycholate	13	Yamaguchi et al. (2009)
Glycochenodeoxycholate (HEK293)	8	Hayashi et al. (2005b)
Glycochenodeoxycholate (Sf9)	2	Kis et al. (2009a)
Taurodeoxycholate	34	Yamaguchi et al. (2009)
Taurolithocholate	4	Yamaguchi et al. (2009)
Bile salt and steroid metabolites		
Taurolithocholate-3-sulfate (HEK293)	10	Hayashi et al. (2005b)
Others		
Pravastatin (HEK293)	124	Hirano et al. (2005)
Mouse total plasma membrane vesicles		
Taurocholate	10	Paulusma et al. (2009)[a]
Mouse Bsep		
Taurocholate (Balb-3T3)	11	Green et al. (2000)
Taurocholate, His-tagged (Sf9)	30	Noe et al. (2001)
Taurocholate His-tagged (Sf9)	7	Kis et al. (2009a)
Glycocholate, His-tagged (Sf9)	20	Noe et al. (2001)
Glycocholate His-tagged (Sf9)	9	Kis et al. (2009a)
Taurochenodeoxycholate, His-tagged (Sf9)	6	Noe et al. (2001)
Taurochenodeoxycholate, His-tagged (Sf9)	9	Kis et al. (2009b)
Taurochenodeoxycholate, His-tagged (Sf9)	17	Kis et al. (2009a)
Glycochenodeoxycholate, His-tagged (Sf9)	2	Kis et al. (2009a)
Dog Bsep		
Taurocholate (Sf9)	34	Yabuuchi et al. (2008)
Skate Bsep		
Taurocholate (Sf9)	15	Cai et al. (2001)

The heterologous expression system is given in parenthesis
nt not transported
[a]C.C. Paulusma, personal communication

(Akita et al. 2001; Gerloff et al. 1998; Stieger et al. 2000), it is minimally transported after Bsep expression in HEK 293 cells (Hayashi et al. 2005b). In contrast, taurolithocholate-3 sulfate is a substrate of human BSEP expressed in HEK293 cells (Hayashi et al. 2005b). Unconjugated bile salts are, if at all, poor substrates for rat Bsep, as demonstrated for isolated canalicular plasma membrane vesicles or for Bsep expressed in Sf9 cells (Gerloff et al. 1998). This observation was confirmed in MDCK cells simultaneously expressing rat Ntcp and Bsep in a polar manner, where transcellular transport of cholate was marginal, while considerable transport of chenodeoxycholate and ursodeoxycholate was observed and excellent transport for all conjugated bile salts tested (Mita et al. 2005). A similar study with human NTCP and BSEP expressed in LLC-PK1 cells identified again cholate, chenodeoxycholate, and ursodeoxycholate as BSEP substrates together with all conjugated bile salts tested (Mita et al. 2006a). In contrast, BSEP expressed in SF9 cell vesicles does not transport cholate (Mita et al. 2006a; Noe et al. 2002), which parallels the virtual absence of unconjugated bile acids in the bile from patients with a defect in bile acid conjugation (Carlton et al. 2003). Neither the human nor the rat orthologue of Bsep transport lithocholate (Mita et al. 2005,

2006a). The fluorescent bile salt derivatives cholylglycylamidofluorescein and chenodeoxycholylglycylamidofluorescein (Mita et al. 2006a), but not cholyl-L-lysyl-fluorescein (de Waart et al. 2010) are BSEP substrates. These results indicate that similar to Ntcp and also for Bsep the bile salt side chain is an important substrate determinant. Comparison of the side chains of cholylglycylamidofluorescein and cholyl-L-lysyl-fluorescein reveals that the fluorescent Bsep substrate has one negative charge, whereas the nontransported bile salt derivative carries two negative charges and is transported by mouse and rat Mrp2 (de Waart et al. 2010). Hence, it is highly suggestive that bile salt derivatives with two negative charges are not substrates for Bsep but rather for Mrp2 (Stieger et al. 2000) with the previously mentioned exception of taurolithocholate-3 sulfate. Although it becomes clear from Table 3 that different expression systems have only a minimal effect on Bsep transport activity of conjugated bile salts, it is interesting to note that the transport activity positively correlates with the cholesterol content of the system used for transport assay (Kis et al. 2009a, b; Paulusma et al. 2009). Different cholesterol content of the membrane system has a marked effect on the maximum transport velocity, but only a minor effect on the affinity of Bsep to bile salts. In rat canalicular membranes, Bsep partitions into Lubrol WX induced microdomains, which are enriched in cholesterol (Ismair et al. 2009). Hence, also in situ in rat liver, cholesterol content of the microenvironment of Bsep might modulate its transport activity. Whether Bsep activity is absolutely dependent on the presence of cholesterol is unknown at the moment, as insect cell membrane vesicles are not absolutely free of cholesterol, but contain a very low amount of this lipid (unpublished observation). A comparison of the intrinsic clearances of mBsep, rBsep, and BSEP showed the same rank order (Noe et al. 2002), indicating that the transport properties of Bsep are well preserved between different species. In general, there is a good correlation of the transported substrates between different species with one notable exception: taurolithocholate-3-sulfate is not transported by rat Bsep but by human BSEP (Akita et al. 2001; Hayashi et al. 2005b; Stieger et al. 2000). So far, only pravastatin has been identified as a nonbile salt substrate for BSEP whereby the human orthologue displays a higher transport capacity than its rat counterpart (Hirano et al. 2005). The different isoforms of Bsep do display bile salt-stimulated ATPase activity, again supporting a direct coupling of ATP hydrolysis to substrate transport (Byrne et al. 2002; Kis et al. 2009a, b; Noe et al. 2001).

3.5 BSEp/bSEP Inhibitors

The sequence of BSEP and MDR1 are closely related (Byrne et al. 2002; Dawson et al. 2009; Gerloff et al. 1998; Noe et al. 2001; Stieger et al. 2007). MDR1 is a key ABC transporter needed to protect individual cells and organs as well as the entire body from xenobiotics (Fung and Gottesman 2009). MDR1 can be considered a cellular vacuum cleaner (Higgins and Gottesman 1992). Therefore, substrates of

MDR1 can also interfere with BSEP function. For example, cyclosporine is associated with cholestasis in patients (Arias 1993). In rats, cyclosporine has been demonstrated to inhibit bile flow and ATP-dependent bile salt transport into canalicular vesicles (Bohme et al. 1994). After cloning of rat Bsep, it was possible to directly demonstrate that cyclosporine is an inhibitor of Bsep (Stieger et al. 2000).

Ongoing inhibition of BSEP will lead to reduced canalicular bile salt secretion and consequently to liver injury due to persistent cholestasis (Pauli-Magnus et al. 2010; Stieger 2010). Drug-induced liver injury including cholestasis is a relevant clinical issue leading to many hospital admissions and in severe cases can only be treated with liver transplantation (Bleibel et al. 2007). In addition, drug-induced liver injury is an important factor leading to attrition of drugs during development or to withdrawal of drugs from the market (Schuster et al. 2005; Smith and Schmid 2006). Among drug-induced liver injury in medical inpatients, about 30% are cholestatic or mixed (Meier et al. 2005). Unfortunately, it is not possible estimating from the literature to know what extent cholestatic liver injury is caused by direct BSEP inhibition (Andrade et al. 2005; Chalasani et al. 2008; Lucena et al. 2009). As inferred from patients with inherited nonfunctional BSEP, serum γ-glutamyltransferase should not be elevated as a consequence of direct BSEP inhibition by drugs (Davit-Spraul et al. 2009). It should, however, be noted that biliary excretion of toxic drug metabolites may lead to cholangiocyte injury and elevation serum γ-glutamyltransferase (Kaplowitz 2005), which complicates the picture. Therefore, the demonstration of direct BSEP inhibition by a given drug in a patient with drug-induced liver injury may be very difficult. Using Sf9 cell vesicles expressing Bsep, cyclosporine, rifamycin SV, rifampicin, and glibenclamide were shown to be competitive inhibitors of rat Bsep. The K_i value of Bsep inhibition was comparable to K_i values in rat canalicular plasma membrane vesicles (Table 4) (Stieger et al. 2000). Extending these studies to human BSEP expressed in insect cells demonstrated that cyclosporin A, rifampicin, and glibenclamide also inhibit human BSEP in a competitive manner (Table 4). The clinical relevance of Bsep inhibition by a drug was worked out in detail with bosentan. Bosentan and one of its main metabolites are dual-endothelin receptor antagonists. Their main route of elimination is via bile and they are taken up by OATP1B1 and OATP1B3 into hepatocytes (Treiber et al. 2007). During clinical trials, bosentan caused asymptomatic, reversible transaminase elevations in some patients (Fattinger et al. 2001). This incidence of liver injury was dose dependent, and plasma bile salt levels increased with increasing dose of bosentan. Individuals, which in addition to bosentan were taking glyburide, showed a higher incidence of liver injury than patients on bosentan alone. Studies with rat Bsep and human BSEP expressed in insect cell vesicles demonstrated that bosentan and one of its metabolites are competitive Bsep inhibitors (Fattinger et al. 2001; Noe et al. 2002). Bosentan treatment of rats led to an elevation of plasma bile salt levels, which was more pronounced if glibenclamide was coadministered (Fattinger et al. 2001). Therefore, the fact that the serum bile salt levels positively correlated with bosentan dose and the rapid spontaneous normalization of serum liver parameters after discontinuing the drug strongly suggests that also in vivo bosentan acts as a competitive BSEP inhibitor. This effect

Table 4 Inhibitors of Bsep identified in different expression systems

Inhibitor	Assay system	Potency (μM)	References
Bosentan	Rat Bsep (Sf9)	K_i 12	Fattinger et al. (2001)
	Rat Bsep (Sf9)	IC_{50} 101	Mano et al. (2007)
	BSEP (Sf9)	K_i 37	Noe et al. (2002)
	BSEP (Sf9)	IC_{50} 77	Mano et al. (2007)
Bosentan M2	Rat Bsep (Sf9)	K_i 9	Fattinger et al. (2001)
Bosentan	Primary human hepatocytes in sandwich culture	Concentration dependent	Kostrubsky et al. (2003)
	Primary rat hepatocytes in sandwich culture	Concentration dependent	Kemp et al. (2005)
Bromosulfophthalein	Rat canalicular vesicles	na	Nishida et al. (1991) and Stieger et al. (1992)
Clofazimine	BSEP (Sf9)	IC_{50} 2	Kis et al. (2009a)
Chlorpromazine	Rat canalicular vesicles	K_i 506	Horikawa et al. (2003)
Cloxacillin	Rat canalicular vesicles	K_i 75	Horikawa et al. (2003)
	Human canalicular vesicles	na	Horikawa et al. (2003)
Colchicine	Rat canalicular vesicles	K_i 539	Horikawa et al. (2003)
CI-1034	Primary human hepatocytes in sandwich culture	Concentration dependent	Kostrubsky et al. (2003)
CP-724,714	BSEP (Sf9)	IC_{50} 16	Feng et al. (2009)
Cyclosporine	Rat canalicular vesicles	K_i 0.2	Bohme et al. (1994)
		K_i 1.1	Horikawa et al. (2003)
		IC_{50} 0.8	Funk et al. (2001)
	Human canalicular vesicles	Na	Horikawa et al. (2003)
Cyclosporine	Rat Bsep (Sf9)	K_i 0.3	Stieger et al. (2000)
	Rat Bsep (Sf9)	IC_{50} 0.9	Kis et al. (2009a)
	BSEP (high five)	K_i 10	Byrne et al. (2002)
	BSEP (Sf9)	IC_{50} 19	Kis et al. (2009a)
	Mouse Bsep (Sf9)	IC_{50} 2	Kis et al. (2009a)
	Dog Bsep	na	Yabuuchi et al. (2008)
Cyclosporine	Primary human hepatocytes in sandwich culture	Concentration dependent	Kostrubsky et al. (2003)
Cyclosporine	BSEP (LLC-PK1)	Concentration dependent	Mita et al. (2006a)
Ciglitazone	Rat canalicular vesicles	Concentration dependent	Snow and Moseley (2007)
Efavirenz	BSEP (Sf9)	na	McRae et al. (2006)
Efavirenz	Primary human hepatocytes in sandwich culture	Concentration independent	McRae et al. (2006)
	Primary rat hepatocytes in sandwich culture	Concentration independent	McRae et al. (2006)
Ethinly-estradiol	BSEP (Sf9)	IC_{50} 16	Kis et al. (2009a)
DIDS	Rat canalicular vesicles	na	Nishida et al. (1991)
Doxycycline	Rat canalicular vesicles	K_i 530	Horikawa et al. (2003)
Erythromycin estolate	Primary human hepatocytes in sandwich culture	Concentration dependent	Kostrubsky et al. (2003)
Fendiline	BSEP (Sf9)	na	Hirano et al. (2006)
Fluvastatin	BSEP (Sf9)	IC_{50} 50	Lang et al. (2007)
Fusidate	Rat canalicular vesicles	K_i 2	Bode et al. (2002)
GSBSP	Rat canalicular vesicles	na	Nishida et al. (1991)

(*continued*)

Table 4 (continued)

Inhibitor	Assay system	Potency (μM)	References
Glibenclamide	Rat canalicular vesicles	K_i 6	Stieger et al. (2000)
		K_i 5	Horikawa et al. (2003)
		IC_{50} 9	Funk et al. (2001)
	Human canalicular vesicles	na	Horikawa et al. (2003)
Glibenclamide	Rat Bsep (Sf9)	K_i 6	Stieger et al. (2000)
	Rat Bsep (Sf9)	IC_{50} 26	Kis et al. (2009a)
	BSEP (high five)	K_i 28	Byrne et al. (2002)
	BSEP (SSf9)	IC_{50} 15	Kis et al. (2009a)
	Mouse Bsep (Sf9)	IC_{50} 150	Kis et al. (2009a)
	Dog Bsep (Sf9)	na	Yabuuchi et al. (2008)
Glibenclamide	Primary human hepatocytes in sandwich culture	Concentration dependent	Kostrubsky et al. (2003)
Glibenclamide	BSEP (LLC-PK1)	na	Mita et al. (2006a)
Lovastatin	Not specified	na	Funk (2008)
Mideamycin	Rat canalicular vesicles	K_i 154	Horikawa et al. (2003)
	Human canalicular vesicles	na	Horikawa et al. (2003)
Nefazodone	Primary human hepatocytes in sandwich culture	IC_{50} 14	Kostrubsky et al. (2006)
Nefadozone	BSEP (Sf9)	IC_{50} 9	Kostrubsky et al. (2006)
Nicardipine	BSEP (Sf9)	na	Hirano et al. (2006)
Nifedipine	BSEP (Sf9)	na	Hirano et al. (2006)
Oligomycin	Rat canalicular vesicles	na	Nishida et al. (1991) and Stieger and Meier (2001)
PKI166	Rat canalicular vesicles	na	Takada et al. (2004)
Pravastatin	BSEP (HEK293)	K_i 10	Hirano et al. (2005)
	BSEP (Sf9)	IC_{50} 240	Yabuuchi et al. (2008)
	Dog Bsep (Sf9)	IC_{50} 441	Yabuuchi et al. (2008)
Prenylamine	BSEP (Sf9)	na	Hirano et al. (2006)
Probenicide	Rat canalicular vesicles	na	Nishida et al. (1991)
Quinidine	Rat canalicular vesicles	K_i 542	Horikawa et al. (2003)
Reserpine	BSEP (Sf9)	IC_{50} 3	Kis et al. (2009a)
Rifampicin	Rat canalicular vesicles	K_i 6	Stieger et al. (2000)
Rifampicin	Rat Bsep (Sf9)	K_i 12	Stieger et al. (2000)
	BSEP (high five)	K_i 31	Byrne et al. (2002)
	BSEP (Sf9)	IC_{50} 11	Kis et al. (2009a)
	Dog Bsep	na	Yabuuchi et al. (2008)
Rifampicin	BSEP (LLC-PK1)	na	Mita et al. (2006a)
Rifamycin SV	Rat canalicular vesicles	K_i 0.9	Stieger et al. (2000)
Rifamycin SV	Rat Bsep in Sf9 cells	K_i 4	Stieger et al. (2000)
Rifamycin SV	BSEP (LLC-PK1)	na	Mita et al. (2006a)
Ritonavir	BSEP (Sf9)	na	McRae et al. (2006)
Ritonavir	Primary human hepatocytes in sandwich culture	Concentration dependent	McRae et al. (2006)
	Primary rat hepatocytes in sandwich culture	Concentration dependent	McRae et al. (2006)
Rosiglitazone	Rat canalicular vesicles	Concentration dependent	Snow and Moseley (2007)
Saquinavir	BSEP (Sf9)	na	McRae et al. (2006)
Saquinavir	Primary human hepatocytes in sandwich culture	Concentration independent	McRae et al. (2006)

(*continued*)

Table 4 (continued)

Inhibitor	Assay system	Potency (μM)	References
	Primary rat hepatocytes in sandwich culture	Concentration dependent	McRae et al. (2006)
Sulfinpyrazone	Rat canalicular vesicls	na	Nishida et al. (1991)
Troglitazone	Rat canalicular vesicles	K_i 1	Funk et al. (2001)
	Rat canalicualr vesicles	Concentration dependent	Snow and Moseley (2007)
Troglitazone	Rat Bsep (Sf9)	IC_{50} 60	Yabuuchi et al. (2008)
	Rat Bsep (Sf9)	IC_{50} 28	Kis et al. (2009a)
	BSEP (Sf9)	IC_{50} 20	Yabuuchi et al. (2008)
	BSEP(Sf9)	IC_{50} 10	Kis et al. (2009a)
	Mouse Bsep (Sf9)	IC_{50} 40	Kis et al. (2009a)
	Dog Bsep (Sf9)	IC_{50} 32	Yabuuchi et al. (2008)
Troglitazone	Primary rat hepatocytes in sandwich culture	Concentration dependent	Kemp et al. (2005)
	Primary rat hepatocytes in sandwich culture	Concentration dependent	Marion et al. (2007)
	Primary human hepatocytes in sandwich culture	Concentration dependent	Marion et al. (2007)
Troglitazone sulfate	Rat canalicuar vesicles	K_i 0.2	Funk et al. (2001)
Troleandomycin	Primary human hepatocytes in sandwich culture	Concentration dependent	Kostrubsky et al. (2003)
Valinomycin	Rat canalicular vesicles	na	Stieger et al. (1992)
Valinomycin	BSEP(Sf9)	IC_{50} 1	Kis et al. (2009a)
Verapamil	Rat canalicular vesicles	K_i 93	Horikawa et al. (2003)
Vinblastine	Rat Bsep (Sf9)	na	Yabuuchi et al. (2008)
	BSEP (Sf9)	na	Yabuuchi et al. (2008)
	BSEP (Sf9)	IC_{50} 57	Kis et al. (2009a)
	Dog Bsep (Sf9)	na	Yabuuchi et al. (2008)
Vincristine	Rat Bsep (Sf9)	na	Yabuuchi et al. (2008)
	BSEP (Sf9)	na	Yabuuchi et al. (2008)
	Dog Bsep (Sf9)	na	Yabuuchi et al. (2008)

The heterologous expression system is given in parenthesis
na not available

may be rather specific for BSEP, as no elevation of serum bilirubin was observed in these patients (Fattinger et al. 2001). The list of Bsep/BSEP inhibitors is continuously growing (Table 4). In general, the data are very coherent between studies using isolated canalicular plasma membrane vesicles and heterologously expressed Bsep. Also, species differences of the inhibition results are minor. Therefore, in vitro screening of the interaction of drugs with BSEP may be a valuable tool during development to assess their potential for inducing cholestasis and liver injury.

Estrogens, such as the oral contraceptive ethinylestradiol or estrogen metabolites are known to be cholestatic in humans and animals (Lindberg 1992; Vore 1987). In animal experiments, the estrogen metabolite estradiol-17β-glucuronide has been demonstrated to be strongly cholestatic. The cholestatic action of estradiol-17β-glucuronide was found to be dependent on the expression of Mrp2 in the canaliculus of rats (Huang et al. 2000). Interestingly, when tested as an inhibitor of Bsep in Sf9

cell vesicles, estradiol-17β-glucuronide did not inhibit ATP-dependent taurocholate transport (Stieger et al. 2000). However, in canalicular plasma membrane vesicles from normal rats and in Sf9 cell vesicles coexpressing Bsep and Mrp2, estradiol-17β-glucuronide inhibited Bsep-mediated taurocholate transport in a time- and dose-dependent manner (Stieger et al. 2000). This is in line with the observation from rat experiments that Mrp2 is strictly required for the cholestatic potential of estradiol-17β-glucuronide. Hence, Bsep can also be indirectly inhibited and it was postulated that estradiol-17β-glucuronide inhibits Bsep by *trans*-inhibition, that is, it needs to be secreted into the canalicular lumen to exert its inhibitory and consequently cholestatic property (Stieger et al. 2000). Indirect Bsep inhibition was later confirmed and extended to sulfated progesterone metabolites (Akita et al. 2001; Vallejo et al. 2006). The HER1/HER2 inhibitor PKI166 was also described to inhibit Bsep indirectly (Takada et al. 2004), extending the mechanism of indirect Bsep inhibition to drugs. Most interestingly, a follow-up study on the mechanism of bosentan-induced cholestasis in rats demonstrated that bosentan leads to a stimulation of bile flow and not – as expected – to a reduction of bile flow (Fouassier et al. 2002). Although bile flow was increased after administration of bosentan, bile salt output remained unchanged leading to a lower bile salt concentration in bile concomitant with a reduced biliary lipid secretion. The stimulation of the bile flow was again dependent on the presence of functional Mrp2 in the canaliculus. Hence, bosentan presents an additional albeit mechanistically different example of the involvement of Mrp2 in the cholestatic effect of substances. In Sf9 cell vesicles expressing rat and human Mrp2/MRP2 or Bsep/BSEP the inhibition of taurocholate transport by bosentan was confirmed for both isoforms of Bsep/BSEP (Mano et al. 2007). Moreover, this study demonstrated that the transport activity of Mrp2/MRP2 is directly stimulated by bosentan and hence provides a mechanistic explanation of the observed increase of bile salt-independent bile flow in rats. Taken together, drug-induced liver injury through inhibition of BSEP is now well established. Bsep can either be inhibited directly (competitively) by drugs acting from the cytoplasm or indirectly, most probably from the canalicular side. This latter process needs the presence of Mrp2, as the drugs need to be secreted into the canaliculus prior to their interaction with BSEP.

3.6 Pathophysiology

Liver disease as a consequence of impaired BSEP function can either be acquired or inherited (Pauli-Magnus et al. 2005). Acquired forms of liver disease affecting BSEP function include its inhibition by drugs and xenobiotics, obstructive or intrahepatic cholestasis, inflammatory liver diseases such as sepsis or acute and chronic hepatitis. For the purpose of this review, primary biliary cirrhosis and primary sclerosing cholangitis are also considered to be acquired forms of BSEP impairment, as in neither of these diseases BSEP alterations seem to be the primary cause.

Several studies have investigated the expression of BSEP in specimens from patients with liver disease. After percutaneous transhepatic biliary drainage, mRNA for BSEP was significantly downregulated in patients with poor drainage in comparison to patients that were well drained or to controls. Poorly drained patients had about sixfold higher serum bile salt levels in comparison to the well-drained patients (Shoda et al. 2001). Immunostaining of BSEP displayed the canalicular membrane, but was presenting with a fuzzy staining extending into the subcanalicular area. This fuzziness was very pronounced in poorly drained patients. Pediatric patients with biliary atresia show downregulation of BSEP mRNA at early stages and no mRNA change at late stages. The signal intensity and canalicular immunofluorescence staining of BSEP is comparable in all stages to control livers (Chen et al. 2008a). BSEP expression is also downregulated in patients with inflammatory cholestasis, but not with primary biliary cirrhosis stages I and II or primary sclerosing cholangitis (Kojima et al. 2003; Zollner et al. 2001, 2003), while another group reported upregulation of BSEP in primary biliary cirrhosis (Ros et al. 2003). Immunostaining of BSEP was conserved as well. Downregulation of BSEP in inflammatory liver disease is also observed at mRNA and protein level after incubation of human liver slices with LPS (Elferink et al. 2004) and in human hepatocytes after treatment with IL-1β (Le et al. 2008). In a small cohort of patients with cholesterol calculus, BSEP expression was reduced at the mRNA and protein level in comparison to control livers (Kong et al. 2006). Ursodeoxycholate is used to treat cholestatic liver disease (Beuers 2006). In a study with healthy gallstone patients, treatment with ursodeoxycholate, but not with rifampicin, leads to an upregulation of BSEP at the protein level, whereas mRNA level remained unchanged (Marschall et al. 2005). In patients who underwent liver transplantation, bile salt secretion is increased early after transplantation, which is paralleled by increased BSEP mRNA levels in these livers (Geuken et al. 2004). Alterations of BSEP expression in hepatocellular carcinomas seems to be highly variable and does not display a consistent pattern, but may tend to be reduced (Van der Borght et al. 2007; Zollner et al. 2005).

In rat models of obstructive cholestasis and of ethinylestradiol-induced cholestasis, Bsep is modestly downregulated (Lee et al. 2000) or practically unchanged (Donner et al. 2007). However, functional analysis of canalicular vesicles isolated from the liver of rats treated with ethinyl–estradiol showed a marked reduction of ATP-dependent taurocholate transport activity, suggesting a role of posttranslational processes in this model of cholestasis (Bossard et al. 1993). Supporting this assumption, relocation of Bsep into subapical vesicles has been observed in rats with estradiol-17β-glucuronide-induced cholestasis (Crocenzi et al. 2003a). Internalization of Bsep was also observed in rats treated with lithocholate and taurolithocholate, which can be prevented by preadministration of cAMP (Crocenzi et al. 2003b, 2005) or of tauroursodeoxycholate (Dombrowski et al. 2006). A modest downregulation of Bsep is also observed in primary cultured rat hepatocytes, which display a cholestatic expression pattern of organic anion transporters (Rippin et al. 2001). Treatment of rats with LPS has also been reported to alter the canalicular localization of Bsep in rats (Vos et al. 1998; Zinchuk et al. 2005). In mice, Bsep

tends to be upregulated after bile duct ligation (Slitt et al. 2007). The sepsis model of treating rats with LPS leads to a mild downregulation of Bsep at the mRNA and at the protein level (Donner et al. 2007; Lee et al. 2000; Vos et al. 1998) and a more fuzzy expression of Bsep in the canalicular membrane, whereas the downregulation is more pronounced in mice (Hartmann et al. 2002). Treating rats with CCl_4 is a model for toxic liver injury and has no effect on the expression of Bsep (Geier et al. 2002b). Cold ischemic injury prior to transplantation of mouse livers modeling bile duct injury leads to downregulation of Bsep. Hypoxia induces a downregulation of Bsep in rat and mouse liver (Fouassier et al. 2007; Hoekstra et al. 2008). Fatty liver disease in obsese Zucker rats is paralleled by a slight downregulation of Bsep (Pizarro et al. 2004), whereas in fatty livers of chronically ethanol-fed rats, Bsep is upregulated at the mRNA and protein level (Zinchuk et al. 2007). Taken together, in animal models of liver injury, Bsep is only mildly affected at the expression level but may alter its subcellular localization (Geier et al. 2007; Roma et al. 2008). This maintenance of Bsep, together with the upregulation of basolateral salvage systems, may be a cytoprotective effect as it will help keep intracellular cytotoxic bile salt concentrations at a low level.

During pregnancy, Bsep is not upregulated in the liver of the mother, but it increases postpartum (Cao et al. 2001). Functionally, prolactin treatment of ovarectomized rats leads to an increase of ATP-dependent bile salt transport into canalicular vesicles without a change of the K_m value (Liu et al. 1997). After partial hepatectomy as a model for the regenerating liver, Bsep expression remains practically unchanged at the mRNA and at the protein level (Vos et al. 1999) or is mildly upregulated (Dransfeld et al. 2005; Gerloff et al. 1999). This is paralleled by an unchanged ATP-dependent taurocholate transport into isolated canalicular plasma membrane vesicles from regenerating rat liver (Green et al. 1997).

Bile flow and in particular canalicular bile salt secretion is a highly regulated process. As Bsep is the rate-limiting step of hepatocellular bile salt transport (Reichen and Paumgartner 1976), it is also regulated by various posttranslational mechanisms. In isolated perfused rat livers, stimulation of biliary bile salt secretion by dibutyrylcAMP is paralleled by a stimulation of transcytotic vesicular transport processes (Hayakawa et al. 1990). This together with the observation that cholestasis can lead to a retrieval of Bsep into subapical vesicles is strongly suggestive for a regulation of Bsep via endo- and exocytosis. This stimulation of bile salt secretion is independent of Bsep biosynthesis but sensitive to the microtubule disrupting agent colchicine (Anwer 2004; Gatmaitan et al. 1997). Treatment of rats with taurocholate or dibutyl cAMP leads to a rapid insertion of Bsep into the canalicular membrane (Gatmaitan et al. 1997; Wakabayashi et al. 2006). As this process is sensitive to colchicine, Bsep is inserted from a pre-existing intracellular pool. cAMP-mediated stimulation of Bsep insertion can be inhibited by inhibition of PI3 kinase (Anwer 2004; Misra et al. 1999). Different from other canalicular ABC transporters such as, for example, p-glycoprotein, Bsep needs about 2 h from the endoplasmic reticulum to the canalicular plasma membrane (as opposed to about 30 min for others) (Kipp and Arias 2002; Kipp et al. 2001; Roma et al. 2008). Based on the kinetic analysis of the biosynthesis and on the short-term incorporation of

Bsep from an intracellular reservoir, two different intracellular vesicular pools for Bsep were postulated (Kipp and Arias 2002; Wakabayashi et al. 2006). The trafficking from the Golgi to the apical membrane in HepG2 cells requires the activity of MAP kinase p38 (Kubitz et al. 2004b). In the model hepatocyte cell line WifB, Bsep constitutively recycles between the canalicular membrane and a rab11 positive endosomal compartment (Wakabayashi et al. 2004). As an additional regulatory element, Ca^{2+}-dependent protein kinase C isoforms also modulate canalicular insertion/retrieval of Bsep (Anwer 2004; Beuers 2006; Dombrowski et al. 2006; Kubitz et al. 2004a; Roma et al. 2008). This complex intracellular trafficking of Bsep not only requires correct glycosylation (Mochizuki et al. 2007), but also proteins directly binding Bsep. Experiments in a polarized cell line revealed that Bsep needs myosin II regulatory light chain and HAX-1 for proper targeting to the apical membrane as well as for the regulation of Bsep density in the apical membrane of MDCK cells (Chan et al. 2005; Ortiz et al. 2004). In the Sf9 cell system, protein kinase C_{α} mediates phosphorlyation of mouse Bsep (Noe et al. 2001). It is hence conceivable that the activity of Bsep is also under the control of posttranslational modifications occurring within the canalicular plasma membrane (Anwer 2004). Although posttranslational regulation is usually a short-term process, adaptation to pathophysiologic situations needs much more time. The half-life of Bsep in rats is 4–6 days (Kipp et al. 2001). To what extent ubiquitination regulates the half-life of Bsep is not known in detail at this moment (Hayashi and Sugiyama 2009). In summary, the strategic position of Bsep in the enterohepatic circulation of bile salts is governed by complex adaptive and regulatory mechanisms of Bsep function in health and disease (Anwer 2004; Kipp and Arias 2002; Roma et al. 2008). Many studies have elucidated the factors and mechanisms governing transcriptional regulation of *BSEP/Bsep*. This topic is the focus of another chapter in this issue.

3.7 Mutations in the BSEP Gene

The key role of BSEP in hepatocellular bile salt secretion is highlighted in patients with mutations in the *BSEP* (Davit-Spraul et al. 2009; Oude Elferink et al. 2006). Such patients have less than 1% than normal of primary bile salts in their bile concomitant with absent Bsep expression (Jansen et al. 1999). They develop severe liver disease with progressive familial intrahepatic cholestasis type 2 (PFIC2) (Davit-Spraul et al. 2009; Oude Elferink et al. 2006; Strautnieks et al. 1998). This demonstrates that there exists no backup system for BSEP in the canalicular membrane. Many such patients are now described in the literature (Alissa et al. 2008; Davit-Spraul et al. 2009; Strautnieks et al. 2008). A milder variant of this disease often comes with recurring episodes of cholestasis and is called benign recurrent intrahepatic cholestasis. A group of such patients also presents with mutations in the *BSEP* gene (van Mil et al. 2004). Taken together, mutations in the *BSEP* gene represent a continuum from mild to severe forms of intrahepatic

cholestasis, which are often progressive (Lam et al. 2006; Pauli-Magnus et al. 2005; Takahashi et al. 2007). They lead to BSEP deficiency syndrome (Lam et al. 2006; Pauli-Magnus et al. 2005). The frequently occurring mutations in the *BSEP* gene can be grouped into missense mutations, nonsense mutations, deletions, insertions, and splice site mutations. A recent exhaustive study analyzed 109 families of patients with severe BSEP deficiency syndrome and identified 82 different mutations (Strautnieks et al. 2008). Mutations identified in these patients cluster in the two nucleotide binding domains of BSEP. This is in line with the high conservation of this domain between different species (Noe et al. 2001) and highlights the importance of a correct structure of the two nucleotide binding domains for proper functioning of ABC transporters (Locher 2009; Rees et al. 2009; Seeger and van Veen 2009). The former study also investigated BSEP expression by immunohistochemistry in the available biopsies and found that the vast majority of patients had abnormal or absent BSEP staining, whereby lack of Bsep staining was clearly dominating (Strautnieks et al. 2008). The number of known mutations continues to rise (Chen et al. 2008b; Treepongkaruna et al. 2009). Hence, mutations in the *BSEP* gene lead often to absent BSEP expression, which may be due to quality control of protein synthesis in the endoplasmic reticulum. Experimental support for this hypothesis is currently missing, as no large studies on mRNA levels in patients with BSEP deficiency syndrome are published so far. Another interesting finding of this study (Strautnieks et al. 2008) is the observation that the two common E297G and D482G mutants of BSEP varied most in their expression level among the respective carriers. This is notable, as E297G and D482G variants have been demonstrated to display residual (Noe et al. 2005) or normal (Hayashi et al. 2005a) transport activity, respectively. In an important continuation of the genetic and histological characterization of patients with bile salt deficiency syndrome, the consequence of mutations and single-nucleotide polymorphisms in the *BSEP* gene on the fidelity of pre-mRNA splicing and of the subsequent processing of BSEP protein was investigated (Byrne et al. 2009). In this study, 20 mutations/SNPs were identified, which resulted in reduced splicing activity compared to the wild-type BSEP gene and consequently reduced levels of normal mRNA in vitro. Furthermore, aberrant splicing and exon skipping was reported for *BSEP* mutations. The majority of the mutants, after expression in CHO-K1 cells, led to protein retention in the endoplasmic reticulum and subsequent degradation. This explains the lack of staining of BSEP expression in liver biopsies of such patients. Importantly and interestingly, the common European mutant D482G shows an enhanced aberrant splicing. This finding could provide a possible explanation for the wide variation of BSEP expression observed in patients with D482G mutations consequently leading to clinical phenotypes with variable severity. Recently, patients who developed antibodies against BSEP were reported (Jara et al. 2009; Keitel et al. 2009). These patients also developed persistent or transient severe progressive cholestasis. Although one patient died, the others could be rescued by a more aggressive immunosuppressive regimen. This constitutes a novel form of acquired (or primary) BSEP deficiency syndrome, as it was not anymore possible to determine the onset of the (auto)immune reaction.

Missing or nonfunctional BSEP leads to accumulation of bile salts within hepatocytes of the affected patients. Patients with severe forms of BSEP deficiency syndrome, who are often very young, are at significant risk to develop hepatocellular carcinoma very early in life (Knisely et al. 2006; Strautnieks et al. 2008). The exact mechanism of how chronically elevated intracellular bile salts are transforming hepatocytes into cancer cells is not yet known in detail. It is considered likely that bile salt-induced carcinogenesis involves mitochondrial damage, because bile salts are toxic to mitochondria (Palmeira and Rolo 2004; Sokol et al. 2006). Alternate or additional mechanisms of cell transformation may include interference of bile salts with signaling cascades controlling the cell cycle (Atherfold and Jankowski 2006) or activation of homeobox genes (Souza et al. 2008), or interference with DNA repair mechanisms. And finally, bile salts have been demonstrated to reversibly induce differentiation and cell polarization of rat hepatoma cells, again highlighting pleiotropic action of intracellular bile salts (Ng et al. 2000).

3.8 Pharmacogenomics of BSEP

Inborn errors of bile salt biosynthesis lead to the formation of aberrant bile salts (Sundaram et al. 2008). These metabolites inhibit ATP-dependent bile salt transport and hence lead to cholestasis (Stieger et al. 1997; Sundaram et al. 2008). Some patients with cholestasis of pregnancy were found to have a novel mutation in the BSEP gene, leading to a p.N591S variant (Dixon et al. 2009; Pauli-Magnus et al. 2004b). These two groups of patients have an acquired form of BSEP deficiency syndrome. Such inherited forms of acquired cholestasis are rare. In contrast, inhibition of BSEP by drugs (Table 4) is more frequent and leads to acquired BSEP deficiency syndrome and intrahepatic cholestasis. Drug-induced cholestasis is a clinically important and often severe problem (Pauli-Magnus and Meier 2006). However, the risk for such events is very low [e.g., 8.5 per 100,000 in the first 45 days for first-time users of flucloxacillin (Russmann et al. 2005)]. It is therefore evident that patients suffering from adverse drug reactions leading to acquired intrahepatic cholestasis could carry genetic susceptibility factors. To date, c.1331T>C (p.V444A) in exon 13 and c.2029A>G (p.M677V) in exon 17 of the *BSEP* gene are two nonsynonymous SNPs, which consistently have been found with frequencies higher than 0.5% (Lang et al. 2006; Pauli-Magnus et al. 2004a; Saito et al. 2002a; Stieger 2009; Stieger et al. 2007) in different, unrelated cohorts. Interestingly, the p.444A variant is found with a considerably higher frequency in the Japanese population than in Caucasians (Kim et al. 2009; Lang et al. 2006). A comparative study of protein expression levels of four canalicular ABC transporters demonstrated a considerable interindividual variability of protein expression for all the investigated transporters (BSEP, MDR1, MDR3, MRP2) (Meier et al. 2006). Strikingly, individuals carrying the p.444A variant are associated with a lower than mean BSEP expression level and all of the 17% of the 110 individuals having low or very low BSEP expression levels were carrying at least one c.1331T>C allele.

Kinetic characterization of these two BSEP variants expressed in Sf9 cells revealed no difference in their transport properties (Lang et al. 2007). From this finding, it can be postulated that individuals carrying the p.444A variant might be at a higher risk for developing acquired BSEP deficiency syndromes. This hypothesis is supported by findings in independent patient cohorts with drug-induced cholestasis (Lang et al. 2007) and in patients with intrahepatic cholestasis of pregnancy (Dixon et al. 2009; Meier et al. 2008), whereby in all studies the c.1331T>C variant of *BSEP* was significantly more frequent than in controls. These three studies therefore identified the c.1331T>C variant of *BSEP* as a susceptibility factor for acquired BSEP deficiency syndrome. Using CHO-K1 cells as a heterologous expression system, the p.444A variant was observed to lead to a lower BSEP protein expression than the p.444V variant (Byrne et al. 2009), thus corroborating the finding in human liver (Meier et al. 2006). It should, however, be mentioned that a study of a Swedish cohort with intrahepatic cholestasis of pregnancy did not find a difference in the distribution of common BSEP haplotypes compared to controls (Wasmuth et al. 2007). This discrepancy could in part relate to the fact that this study investigated haplotypes, whereas the previously mentioned studies investigated frequencies of SNPs. In further support of this hypothesis, five case reports (three patients with cholestasis of pregnancy and one patient with benign recurrent intrahepatic cholestasis type 2 and one patient with a heterozygous mutation in the ATP8B1 gene) observed homozygocity for p.444A three times and heterozygocity for p.444A twice (Favre et al. 2009; Hsu et al. 2009; Keitel et al. 2006; Kubitz et al. 2006; Muehlenberg et al. 2008). Clearly, although the association of a common BSEP variant with increased risk for acquired BSEP deficiency syndromes seems now well established, independent studies with large cohorts are needed to firmly establish the p.V444A polymorphism as a risk factor. In addition, given the frequency of this variant in the population, additional risk factors, such as, for example, DNA methylation as an epigenetic factor, are most likely involved as well (Imai et al. 2009). So far, one haplotype of the *BSEP* promoter was reported to lead to a lower transactivation of the *BSEP* gene in vitro (Lang et al. 2006). The clinical relevance of this variant has not been reported so far.

Two mutant forms of BSEP, which were identified in a patient heterozygous for the two mutants and who presented with benign recurrent BSEP deficiency syndrome, exhibited residual transport activity when expressed in Sf9 cells (Noe et al. 2005). This raises the important question as to what minimal level of functional BSEP is required to maintain canalicular bile salt secretion and prevent liver injury. It also indicates that in some patients additional endogenous or exogenous factors contribute to cholestatic episodes. Because BSEP constitutes the rate-limiting step of hepatocellular bile salt secretion, it is indirectly also controlling systemic bile salt concentrations, as reduced BSEP function will lead to a spillover of bile salts into the systemic circulation. In a large cohort analyzed for single nucleotide polymorphisms segregating with the physiologic control of fasting glucose levels, an SNP located in the vicinity of the *BSEP* gene was found to positively correlate with fasting glucose levels (Chen et al. 2008c). In the same cohort, two additional SNPs were identified in intron 19 of the *BSEP* gene. They also strongly associate

with fasting glucose levels. Additionally, serum bile salts control directly or indirectly via bile salt sensors such as TGR5 or transcription factors having bile salts as ligands body energy homeostasis (Houten et al. 2006; Keitel et al. 2008; Thomas et al. 2008, 2009; Wang et al. 2008b). The modulation of the activity of BSEP may therefore have far-reaching systemic effects on energy and glucose homeostasis.

In summary, the evidence from patients with inherited severe liver disease is now overwhelming that BSEP constitutes the most important if not the only transporter capable of mediating canalicular bile salt secretion.

3.9 In Vitro Characterization of BSEP Variants and Animal Models for Altered Bsep Expression

There is no doubt that a lot has been learned from patients on BSEP function and on important structural/functional elements of BSEP. However, direct mechanistic information on the influence of changes in the BSEP protein on its biosynthesis, targeting, and stability in human liver cannot be gained from this source. Nevertheless, information on these aspects can be obtained from heterologous expression systems, which can reproduce "in vivo" phenotypes as in "in vitro phenotypes." Originally, seven BSEP mutations leading to severe BSEP deficiency syndrome were characterized by this approach. All investigated mutations were situated at highly conserved amino acids in BSEP. These human mutations were introduced into the corresponding conserved positions of rat Bsep and the variant Bsep forms subsequently expressed in MDCK cells (Wang et al. 2002). Five of the studied mutations resulted in problems targeting the Bsep protein correctly to the apical membrane and/or showed reduced transport activity. Interestingly, the D482G mutation reduced transport activity for human BSEP but had no apparent effect on transport when introduced into mouse Bsep (Plass et al. 2004). This discrepancy could be due to species differences in the BSEP/Bsep sequence or to differences between expression systems. An alternate explanation is that the divergence of results could be explained by differences in RNA stability of this BSEP mutant (Byrne et al. 2009). These variable findings argue that multiple expression systems (cells and/or different species of BSEP/Bsep as templates for introducing of mutation) should be used before firm conclusions can be drawn regarding the mechanism underlying pathophysiology in the human disease. Different in vitro phenotyping studies (Table 5) from several groups using different BSEP mutants demonstrated alterations in BSEP targeting (Hayashi et al. 2005a; Plass et al. 2004), a shortening of the half-life of BSEP in the plasma membrane (Hayashi et al. 2005a), and differences in ubiquitin-dependent BSEP turnover (Hayashi and Sugiyama 2009). It was recently observed that the severity of BSEP deficiency syndrome phenotype in humans correlates with membrane expression and protein stability of the BSEP mutants in heterologous expression systems (Kagawa et al. 2008; Lam et al. 2007).

Table 5 Characterization of *BSEP* mutations in heterologous expression systems

Mutation	Expression cDNA	Expression system	Protein targeting	Protein stability	Transport function	References
G238V	rBsep	MDCK for targeting	No surface expression	Rapid degradation	na	Wang et al. (2002)
E297G			No surface expression	na	Absent	
C336S		Sf9 cells for transport	Normal	na	Normal	
D482G			Normal	na	Abolished	
G982R			No surface expression	na	Abolished	
R1153C			No surface expression	na	Abolished	
R1268Q			No surface expression	na	Abolished	
D482G	mBsep	HepG2 for targeting SF21 cells for transport	Reduced canalicular targeting and altered processing	Temperature-sensitive mRNA	Normal (slight reduction of ATPase activity)	Plass et al. (2004)
E297G	BSEP	MDCK for targeting	Partial surface expression	Shortened half-life of membrane form	Normal	Hayashi et al. (2005a)
D482G		HEK 293 for targeting and transport	No surface expression		Normal	
E297G	BSEP	Sf9 cells	na	na	Reduced	Noe et al. (2005)
R432T			na	na	Reduced	
E297G	rBsep	MDCK for targeting	Reduced surface expression	na	Absent	Lam et al. (2007)
D482G			No surface expression	na	Normal	
A570T		HEK 293 for targeting	Reduced surface expression	na	Normal	
N591S			Reduced surface expression	na	Normal	
R1050C		Sf9 cells for transport	Reduced surface expression	na	Normal	
D676Y	BSEP	Sf9 cells	na	na	Normal	Lang et al. (2007)
G855R					Reduced	
E297G	rBsep	MDCK	Normal	na	Reduced	Kagawa et al. (2008)
K461E			No surface expression	na	Absent	

(*continued*)

Table 5 (continued)

Mutation	Expression cDNA	Expression system	Protein targeting	Protein stability	Transport function	References
A570T			Normal	na	Reduced	
D482G			Reduced surface expression	Shortened half-life	Reduced	
G982R			No surface expression	na	Absent	
R1050C			Normal	na	Reduced	
R1153C			No surface expression	na	Absent	
R1057X			Normal	na	Absent	
R1286Q			No surface expression	na	Absent	
3467-8insC			No surface expression	na	Absent	
G238V	rBsep	HEK 293 cells	Reduced surface expression	Rescued by low temperature and glycerol	na	Wang et al. (2008a)
D482G			Reduced surface expression		na	
G982R			No surface expression	na	na	
R1153C			No surface expression	na	na	
R1286Q			No surface expression	na	na	
E297G D482G	BSEP	MDCK	Faster turnover of ubiquitinated BSEP from apical membrane	Increased ubiquitination		Hayashi and Sugiyama (2009)

Additional information on expression of BSEP mutants is found in Byrne et al. (2009). For an extensive characterization of BSEP expression in liver of patients with PFIC2 refer to Strautnieks et al. (2008)
na not assessed

Of note, it was recently demonstrated that levels of the E297G and the D482G mutants of BSEP could be increased at the apical membrane of MDCK cells, if the cells were treated with 4-phenylbutyrate (Hayashi and Sugiyama 2007) or with short- and medium-chain fatty acids (Kato et al. 2010). This suggests a possibility for a pharmacologic treatment of some forms of inherited BSEP deficiency syndrome.

An alternate approach to better understand the molecular pathophysiology of missing Bsep is the use of genetically modified mice. Mice with a disrupted *Bsep* gene do not develop severe cholestasis on a normal diet (Wang et al. 2001), but only after being fed with a high cholate diet (Wang et al. 2003). In mice lacking Bsep, novel tetrahydroxylated and hence more hydrophilic bile salts have been detected in their bile (Perwaiz et al. 2003; Wang et al. 2001). An extended screen of gene expression in these knockout animals found an upregulation of p-glycocprotein (Wang et al. 2003). As a consequence of this finding, p-glycoprotein was further investigated and found to be capable of mediating bile salt transport (Lam et al. 2005). Generating mice lacking both Bsep and p-glycoprotein indeed confirms this hypothesis (Lam et al. 2005; Wang et al. 2009). Such mice have a much more severe phenotype with jaundice, a more pronounced reduction of bile flow, and increased mortality. Very interestingly, these animals do nevertheless not have increased serum bile salt levels, but their bile salt levels tend to be lower than in wild-type animals. As alkaline phosphatase is highly elevated in the serum, these mice seem to have a high bile salt concentration in their hepatocytes. Instead of knocking out functional Bsep, it can be overexpressed in the liver. Such mice display an enhanced biliary lipid (phospholipid and cholesterol) secretion in parallel to their increased bile salt output (Figge et al. 2004). This supports the concept that canalicular bile salt and lipid secretion are tightly linked (Small 2003). Furthermore, this overexpression of Bsep markedly reduces steatosis in animals fed with a lithogenic diet (Figge et al. 2004). Feeding such mice with a lithogenic diet leads to formation of cholesterol crystals and gallstones (Figge et al. 2004; Henkel et al. 2005) confirming a role of Bsep in mice in cholesterol gallstone formation. Bsep has previously been identified in the *Lith1* locus (Bouchard et al. 1999).

4 Conclusion

Ntcp and Bsep are the two most important bile salt transporters in liver and both are strategically located in the enterohepatic circulation of bile salts. This is also reflected in results from the analysis of the hepatic proteome, which shows that hepatocellular bile salt transporters are highly abundant in hepatocytes at the protein level (Chinese Human Liver Proteome Profiling Consortium 2010). Consequently, both transporters are extensively regulated in health and disease. The regulation of these transporters is important for coping with changes in hepatic bile salt load, for example, after a meal or in pathophysiologic situations. The general principle of the regulatory mechanisms is to keep bile salt concentrations

within hepatocytes low to minimize their cytotoxic potential. Therefore, uptake, efflux, and salvage transporters for bile salts are interconnected at the regulatory and functional level. Bsep constitutes the rate-limiting step in hepatocellular bile salt transport and could also be called the guardian of hepatocellular bile salt concentrations. Thus, inherited or acquired forms of liver diseases, which impair the proper functioning of Bsep, lead to an accumulation of bile salts within hepatocytes and consequently to hepatocellular damage or even cell death. Although our understanding of substrate specificity as well as inhibitory mechanisms of bile salt transporters is advanced, structural information on these transporters is still missing. Unfortunately, high-resolution structural information, in particular for ABC transporters, is difficult to obtain and has remained elusive up to now (Locher 2009; Rees et al. 2009; Seeger and van Veen 2009; Zhou 2008). Furthermore, much more information is needed about factors (genetic and acquired), which make individuals susceptible to adverse drug reactions in the liver in the context of bile salt transport. This will require large genome-wide association studies, such as those already performed for common diseases (Wellcome Trust Case Control Consortium 2007) or for specific adverse effects of a statin (Link et al. 2008). In addition, more information is needed on regulatory genetic mechanisms such as epigenetics as well as on biochemical and cell biological processes underlying drug-induced liver injury.

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P-glycoprotein: Tissue Distribution, Substrates, and Functional Consequences of Genetic Variations

Ingolf Cascorbi

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Abstract P-glycoprotein (ABCB1, MDR1) belongs to the ABC transporter family transporting a wide range of drugs and xenobiotics from intra- to extracellular at many biological interfaces such as the intestine, liver, blood–brain barrier, and kidney. The ABCB1 gene is highly polymorphic. Starting with the observation of lower duodenal protein expression and elevated digoxin bioavailability in relation to the 3435C>T single nucleotide polymorphism, hundreds of pharmacokinetic and outcome studies have been performed, mostly genotyping 1236C>T, 2677G>T/A, and 3435C>T. Though some studies pointed out that intracellular concentrations of anticancer drugs, for example, within lymphocytes, might be affected by ABCB1 variants resulting in differential outcome, current knowledge of the functional significance genetic variants of ABC membrane transporters does not allow selection of a particular SNP to predict an individual's pharmacokinetics.

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1 Introduction

The ATP binding cassette (ABC) membrane transporter P-glycoprotein (P-gp) is one of the best characterized human efflux transporters. There is increasing understanding of its function, regulation, and impact of genetic variants. The gene encoding P-gp was formerly termed multidrug resistance gene (MDR1) due to the observation that P-gp were overexpressed in tumor cells conferring to the commonly known phenomenon of multidrug resistance against certain antineoplastic agents (Juranka et al. 1989). Today the common ABC transporter nomenclature is applied terming the gene and protein of P-glycoprotein ABCB1 (Figs. 1 and 2).

Mice have two closely related homologues of ABCB1 (*Abcb1a*, *Abcb1b*). Absence of the gene, as being the case in double-knockout mice, is conformable

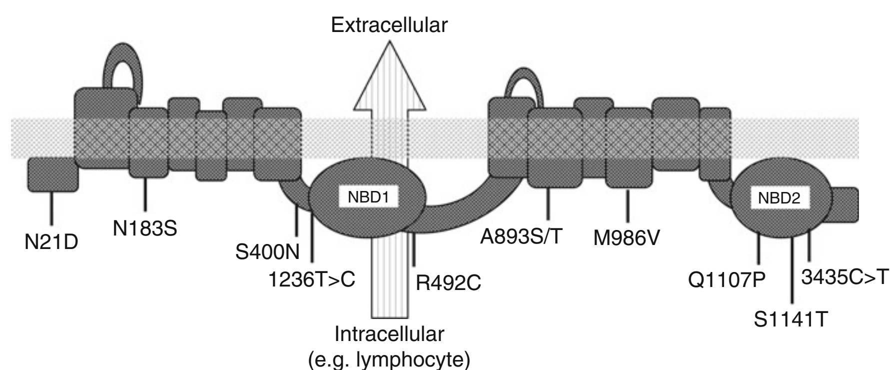

Fig. 1 Two-dimensional structure of ABCB1 with locations of amino acid replacements and two frequent synonymous SNPs, NBD = nucleotide binding domain [adapted from Cascorbi and Haenisch (2010)]

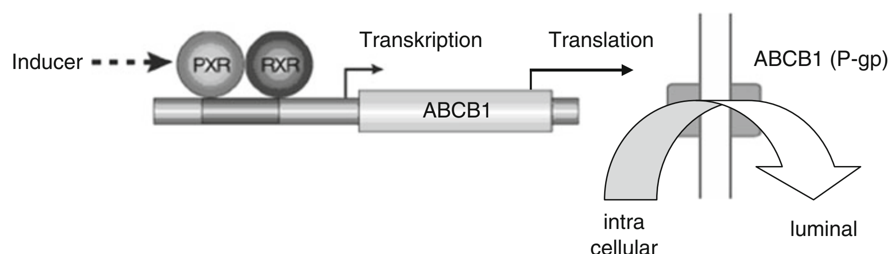

Fig. 2 Induction of ABCB1 via the nuclear PXR/RXR receptor leading to accelerated extrusion of P-glycoprotein substrates

with life. Double-knockout mice are viable and fertile but are highly sensitive to certain neurotoxins such as ivermectin (Schinkel et al. 1997), indicating the important role of ABCB1 in transport across the blood–brain barrier. Interestingly, such knockout mice develop an inflammatory bowel disease, similar to Crohn's disease (Panwala et al. 1998).

2 Tissue Distribution

2.1 Intestine

ABCB1 is expressed at the apical side of many barriers mediating an inside-out extrusion mainly of xenobiotic compounds. Thus ABCB1 can be considered as a defense mechanism against the penetration of xenobiotics into the body or deeper compartments. Following the way of an orally administered drug though the body, it first has to pass the brush-border membranes of intestinal enterocytes. However, ABCB1 serves not only as a functional barrier against drug entry but contributes also to the active excretion (Schinkel et al. 1996; Terao et al. 1996; Zhang and Benet 2001). P-gp is expressed in the apical membrane of the entire intestine from duodenum to rectum (Canaparo et al. 2007; Thorn et al. 2005) thereby contributing to the lowering of bioavailability of a large number of drugs. Interestingly, it is coexpressed with proteins of the cytochrome P450 3A family, a further defense mechanism against environmental compounds, changing the lipophilicity to more hydrophilic and less permeable derivatives. In the duodenum and jejunum there is a higher expression of P-gp than in the liver (von Richter et al. 2004) and a coregulation with CYP3A4. In rectum and sigma we found a significant correlation of CYP3A5 and ABCB1 expression among individuals being CYP3A5 expressors; 79% of the variability in ABCB1 mRNA expression was explained by the corresponding CYP3A5 expression levels (Ufer et al. 2008). Such a strong correlation between the expression of a drug-metabolizing enzyme and a drug transporter is most likely attributable to a coregulation mediated by the pregnane X receptor (PXR) (Geick et al. 2001; Tirona et al. 2003). Furthermore, ABCB1 is a subject of regulation by certain cytokines. In patients suffering from ulcerative colitis, there was a significant reduction of ABCB1 mRNA and protein expression, dependent on the extent of clinical activity index, whereas the cytokines IL1β and IL8 were upregulated (Ufer et al. 2009a). The downregulation of ABCB1 could be partly explained by the interaction of IL8 and ABCB1 in vitro.

2.2 Liver

P-gp is also expressed at the canalicular site of hepatocytes; however, sevenfold lower than in small-intestinal enterocyte-homogenates (von Richter et al. 2004). Overall, there is a large interindividual variability of hepatic P-gp expression,

which differs reportedly up to 50-fold (Schuetz et al. 1995), contributing to broad differences in the bioavailability of a wide range of drugs.

2.3 Kidney

Finally, despite the liver as major excretion organ, transporters play a substantial role in tubular cells in the kidney. ABCB1 is again expressed at the luminal side of cells thereby contributing to the active secretion of lipophilic drugs and other xenobiotics (del Moral et al. 1998). In cancerous kidney tissue, ABCB1 expression was found to be lower as compared to normal kidney tissue, possibly due to less extent of functional tubular cells (Haenisch et al. 2007).

2.4 Blood–Brain Barrier

The role of P-glycoprotein at the blood–brain barrier as defense mechanism against damages of the brain by toxins appears to be even more important, protecting against drug penetration into the CNS (Fromm 2000).

Inhibition of P-glycoprotein by verapamil or downregulation after inflammatory stimuli led to a dramatic increment of typical substrates such as digoxin in the liquor (Goralski et al. 2003). Accordingly, the antinociceptive effects of morphin-6-glucuronide increased after inhibition of P-gp by PSC833 in a rat model (Lotsch et al. 2000) or central nervous effects of loperamide occurred after coadministration of quinidine (Skarke et al. 2003). Overexpression of P-gp resulting in lowering local concentrations of anticonvulsants is hypothesized as one cause of drug resistance in epilepsy (Loscher et al. 2009).

2.5 Placenta

The maternal–fetal interface in the placenta is again an important blood-tissue barrier expressing multiple drug transporters, including P-glycoprotein (Young et al. 2003). These transporters are assumed to contribute to the protection of the fetus against xenobiotics or drugs ingested by the mother during pregnancy (Behravan and Piquette-Miller 2007). In a knockout mice model, fetuses exhibited substantially higher levels of typical P-glycoprotein substrates such as digoxin, saquinavir or paclitaxel than wild-type fetuses, after intravenous administration of those drugs to the mothers. As a consequence knockout mice are more susceptible to birth defects caused by exposure of the mothers to toxic agents (Lankas et al. 1998).

2.6 Lymphocytes

ABCB1 is also expressed in lymphocytes, but interestingly to a high extent in hematopoietic stem cells, where it may serve to protect these cells from toxins (Chaudhary et al. 1992; Chaudhary and Roninson 1991; Schinkel et al. 1997). Moreover, ABCB1 may be involved in the migration of dendritic cells (Randolph 2001). However, the efflux transporter contributes to the phenomenon of drug resistance in treatment of HIV (Chandler et al. 2007) or leukemia (Svirnovski et al. 2009). Though to some extent controversially discussed, protease inhibitors like saquinavir used in HIV treatment are subject of elevated efflux, leading to subtherapeutic intracellular levels (Jones et al. 2001). Therefore, the introduction of the concept of inhibition of P-glycoprotein by ritonavir to decrease the efflux of lopinavir or saquinar was a further milestone in HIV therapy (Janneh et al. 2007; Lucia et al. 2001). A fixed combination of lopinavir and ritonavir was approved by the FDA in 2000.

3 Substrates

The ABCB1 protein has a size of 170 Dalton and consists of two transmembranal domains and two intracellular nucleotide binding regions. The substrate binding site is located at the intracellular part of the proteins. After phosphorylation of ABCB1, the substrate molecule is extruded by a “flip-flop” mechanism to the luminal side. Dephosphorylation leads to transformation of the protein back into the initial state.

Like cytochrome P450 3A4, P-glycoprotein is nonspecific for a large variety of drugs from many different indication areas (Table 1). Moreover, there is an overlap of the substrate specificity particularly of cytostatics with other ABC transporters like ABCC1, ABCC2, and ABCG2 (Cascorbi 2006) and a wide overlap with the drug-metabolizing enzyme CYP3A4, making P-glycoprotein and CYP3A4 a synergistic defense mechanism against the intrusion of xenobiotics. Because both genes are coregulated by the nuclear receptor PXR (von Richter et al. 2004), they are both induced by PXR/CAR ligands like rifampicin (Geick et al. 2001) or St. John’s wort (Johne et al. 1999). High-affinity substrates also act as inhibitors, with the most prominent calcium-channel blocker verapamil used also in a number of experimental settings. The inhibition of P-glycoprotein at various barriers in particular at the blood–brain barrier by verapamil may lead to central nervous effects of loperamide, an opioid used against diarrhea with usually no systemic effects (Elsinga et al. 2004). Also quinidine is a potent P-gp inhibitor; in healthy volunteers loperamide plasma concentrations were about twice as high after coadministration of quinidine, and pupil size decreased, indicating increased concentration in the central spinal fluid (Skarke et al. 2003) (Table 1).

Table 1 Substrates of P-glycoprotein (ABCB1)

Class	Drug
Substrate	
Anticancer drugs	Docetacel, doxorubicin, etoposide, imatinib, paclitaxel, teniposide, vinblastine, vincristine
Steroids	Dexamethasone, methylprednisolone
Immunosuppressants	Cyclosporine, sirolimus, tacrolimus
HIV protease inhibitors	Amprenavir, indinavir, nelfinavir, saquinavir, ritonavir
Antibiotics	Erythromycin, levofloxacin, ofloxacin
β-blockers	Bunitrolol, carvedilol, celiprolol, tanilolol
Ca^{2+}-channel blockers	Diltiazem, verapamil
Cardiac drugs	Digoxin, digitoxin, quinidine
HMG-CoA inhibitors	Artovastatin, lovastatin
H_1-antihistamins	Fexofenadine, terfenadine
Antiemetics	Ondansetron
Diverse	Amitryptiline, colchicine, itraconazole, lansoprazole, loperamide, losartan, morphine, phenytoin, rifampicin
Fluorescent dyes	Rhodamine 123
Inducers	
Anticonvulsants	Carbamazepine, phenytoin, phenobarbital, primidon
Tuberculostatics	Rifampicin
Herbals	Hyperforin (constituent of St. John's wort)
Inhibitors	
Calcium channel antagonisten	Verapamil
Makrolide antibiotics	Erythromycin, clarythromycin, *not* azithromycin
HIV protease inhibitors	Ritonavir
Immunosuppressents	Cyclosporin
Antiarrhythmics	Chinidin, propafenon

Sources: Fromm (2004) and Cascorbi and Haenisch (2010)

4 ABCB1 Genetic Polymorphisms

4.1 Functional Consequences of Genetic Variations

ABCB1 spans 28 exons, and the cDNA consists of 3,843 bp. The first evidence for genetic variability was found in 1994 (Stein et al. 1994). Investigating genetic reasons for multidrug resistance, two single nucleotide polymorphisms in the ABCB1 promoter region of human osteosarcoma cells were detected. In 1998, Mickley et al. discovered two sense SNPs, a 2677G>T transversion in exon 21, and a 2995G>A transition in exon 24 (Mickley et al. 1998). These SNPs caused an Ala893Ser and Ala999Thr exchange, respectively. Notably, the different alleles showed similar expression in normal tissue, unselected cell lines, and untreated malignant lymphomas, but there was loss of heterozygosity for 2677G>T in a number of selected cell lines and relapsed malignant lymphomas.

Today about 100 SNPs have been identified in the coding region. The total number of genetic variants including intronic and the 5′ and 3′-region is estimated in the several hundreds, though deep sequencing has not been performed so far. Moreover,

the frequency is known only of selected variants, having putatively functional impact (Table 2). The first systematic investigation on ABCB1 SNPs revealed a significant correlation of a silent polymorphism in exon 26 (3435C>T; rs1045642) with intestinal P-gp expression levels and oral bioavailability of digoxin, showing significantly decreased intestinal P-gp expression and increased digoxin plasma levels after oral administration among homozygote 3435T carriers (Hoffmeyer et al. 2000). 2677G>T turned out to have three allelic variants (2677G>T/A; rs2032582) being in linkage disequilibrium with 3435C>T (Horinouchi et al. 2002; Kim et al. 2001; Tanabe et al. 2001). 2677T SNP in exon 21 occurred with 41.6%, whereas 2677A (893Thr) at the same position had a frequency of only 1.7% in a German cohort of 461 healthy volunteers (Cascorbi et al. 2001).

The frequency of the putatively most interesting 3435C>T SNP differs significantly between ethnicities. The variant 3435T allele has a prevalence of 0.17–0.27 in African blacks, 0.41–0.47 in Asian populations, and 0.52–0.57 among Caucasians (Ameyaw et al. 2001; Cascorbi et al. 2001; Kim et al. 2001; Lee et al. 2005; Schaeffeler et al. 2001). It was discussed whether such genotypic differences may contribute to interethnic differences of drug responses in certain populations, as far as the variants have functional relevance.

The functional significance of the ABCB1 variants is still under discussion. Though a number of efforts have been made, there is currently only weak evidence that 3435C>T has any significant impact on mRNA or protein expression in vivo (Cascorbi 2006). The most recent hypothesis on the functional impact suggests that the timing of cotranslational folding could be affected in presence of the nucleotide codon, requiring a rare tRNA for the synonymous amino acid. As a consequence, this deceleration of the translation may alter the protein structure and substrate affinity (Kimchi-Sarfaty et al. 2007).

The missense variant 2677G>T/A coding for the three different amino acids A893S/T exhibits altered transport properties in membrane vesicles from Sf9 insect cells, overexpressing human ABCB1. 893T had a higher $v_{\max}$ for the anticancer drug vincristine than 893S, and $v_{\max}$ of 893S was higher than the wild-type 893A, whereas K_M was higher for 893S compared to 893T or A (Schaefer et al. 2005). The functional difference could also be observed investigating the ATPase activity in a similar expression system (Ishikawa et al. 2005).

The rare missense SNP 1199G>T (Ser400Asn) was associated with lower transport capacity in vitro leading to putatively higher sensitivity against cytostatics. In contrast, HEK cells transfected with ABCB1 carrying 1199G>A exhibited elevated chemoresistance indicating elevated transport of the modified P-glycoprotein (Crouthamel et al. 2006).

4.2 Association to Drug Bioavailability

There is a large number of studies investigating the impact of ABCB1 genetic variants on the bioavailability of various drugs known to underlie P-gp-mediated transport (Eichelbaum et al. 2004; Sakaeda 2005).

Table 2 Frequency of ABCB1 genetic variants in Caucasians, position on DNA, putative effect, and frequencies [according to Cascorbi (2006) and Cascorbi and Haenisch (2010)]

Position	Amino acid or effect	Frequency of the variant allele	Association to expression, kinetics or drug response
5′-flanking −2903 T>C		0.02[a]	
5′-flanking −2410 T>C		0.10[a]	Decreased mRNA[a]
5′-flanking −2352 G>A		0.28[a]	
5′-flanking −1910 T>C		0.10[a]	
5′-flanking −1717 T>C		0.02[a]	
5′-flanking −1325 A>G		0.02[a]	
5′-flanking −934 A>G		0.10[a]	
5′-flanking −692 T>C		0.10[a]	Decreased mRNA[a]
5′-flanking −41 A>G		0.09[b]	
IVS 1a −145 C>G		0.02[b]	
IVS 1b −129 T>C		0.06[b]	
IVS 1b 12 T>C		0.06[c]	
IVS 2 −1 G>A		0.09[d]	
c. 61 A>G	N21D	0.11[d]	
IVS 5 −35 G>C	Intronic	0.006[c]	
IVS 5 −25 G>T	Intronic	0.16[c]	
IVS 6 +139 C>T	Intronic	0.37[d]	
c. 548 A>G	N183S	0.01[e]	
c. 571 G>A	G191R	0.07[f]	Reduced chemotherapy resistance[f]
c. 1199 G>A	S400N	0.05[d]	
c. 1199 C>T	S400I	0.02[g]	Elevated activity[g]
c. 1236 C>T	Synonymous	0.41[d]	Increased imatinib disposition and therapy response[h]
IVS 12 +44 C>T	Intronic	0.05[d]	
c. 1474 C>T	R492C	0.01[e]	
IVS 17 −76 T>A	Intronic	0.46[d]	
IVS 17 +137 A>G	Intronic	0.006[c]	
c. 2650 C>T	Synonymous	0.03[e]	
c. 2677 G>T/A	A893S/T	0.42[d]/0.02[d]	In vitro increased v_{max},[i] increased imatinib response in CML[h]
c. 2956 A>G	M986V	0.005[b]	
c. 3320 A>C	Q1107P	0.002[d]	
c. 3396 C>T	Synonymous	0.03[c]	
c. 3421 T>A	S1141T	0.00[c]	
c. 3435 C>T	Synonymous	0.54[d]	Decreased mRNA and protein expression,[e, k] decreased in vitro transport,[l] no effect on expression and bioavailability of talinolol,[m] no effect on in vitro transport,[n, o] decreased digoxin

(*continued*)

Table 2 (continued)

Position	Amino acid or effect	Frequency of the variant allele	Association to expression, kinetics or drug response
			bioavailability,[p] increased etoposid disposition,[q] no effect on AML or ALL outcome,[o] better prognosis of multiple myeloma,[r] better chemotherapy response in breast cancer,[s] no effect in colon cancer[t]
c. 4030	Synonymous	0.005[b]	
c. 4036	Synonymous	0.30[b]	

[a]Taniguchi et al. (2003)
[b]Tanabe et al. (2001)
[c]Kim et al. (2001)
[d]Cascorbi et al. (2001)
[e]Hoffmeyer et al. (2000)
[f]Yang et al. (2008)
[g]Crouthamel et al. (2006)
[h]Dulucq et al. (2008)
[i]Schaefer et al. (2005)
[k]Seedhouse et al. (2007)
[l]Hitzl et al. (2001)
[m]Siegmund et al. (2002b)
[n]Kimchi-Sarfaty et al. (2002)
[o]Hur et al. (2008), Semsei et al. (2008) and van der Holt et al. (2006)
[p]Kurata et al. (2002) and Sakaeda et al. (2001)
[q]Kishi et al. (2004)
[r]Buda et al. (2007)
[s]Kafka et al. (2003)
[t]Ho et al. (2006) and Petrova et al. (2008)

4.2.1 Digoxin

The heart glycoside digoxin is widely accepted as typical P-glycoprotein substrate. The study in 2000 revealed a strong impact of 3435C>T on digoxin plasma distribution with elevated levels among 3435TT carriers after intake of a single dose of 1 mg (Hoffmeyer et al. 2000). In subjects being in steady state with 0.25 mg digoxin/day, 3435T carriers had a 20% elevated area under the curve (AUC) within the first 4 h. Interestingly, the effect was more pronounced in 2677GG/3435TT carriers (Johne et al. 2002). Though a first meta-analysis of different studies performed in Caucasians, including some African blacks, supported these observations with similar differences between 3435TT and CC (Verstuyft et al. 2003), the results are inconsistent in a number of further studies (Gerloff et al. 2002). Interestingly, in Asians the opposite tendency was also observed in three independent studies, with lower digoxin AUC in the CC group than in subjects homozygous for TT (Horinouchi et al. 2002; Kurata et al. 2002; Morita et al. 2003; Sakaeda et al. 2001) suggesting different haplotype constellations among these ethnicities. However, in 2008 a study in 195 Dutch patients with chronic digoxin treatment revealed

again a significant influence not of single SNPs, but of the ABCB1 haplotype 1236T/2677T/3435T on digoxin trough levels (Aarnoudse et al. 2008).

The reasons for the discrepancies observed for ABCB1 variants and digoxin kinetics are currently unclear. The majority of studies revealed no differences in digoxin kinetics, and it was concluded that it is unlikely that digoxin bioavailability is modulated by ABCB1 polymorphisms (Chowbay et al. 2005) due to various hormonal and immunological influences on ABCB1 activity (Castagne et al. 2004; Sakaeda et al. 2002; Siegmund et al. 2002a) and possibly a circadian rhythm as could be demonstrated in a mouse model (Ando et al. 2005). Moreover, saturation of the intestinal ABCB1 transport capacity may surpass any genetic effects (Sakaeda 2005). Finally other uptake and efflux transporters may contribute to the disposition of digoxin in humans.

4.2.2 Talinolol

Further thorough investigations on the beta-blocker talinolol in German volunteers showed no impact of 3435C>T, but slightly elevated talinolol levels compared in variant homozygote carriers of 2677G>T/A compared to all other genotypes ($P < 0.05$). However, multiple comparisons with combinations of putative functional SNPs did not confirm a significant influence of the ABCB1 genotype on talinolol disposition (Siegmund et al. 2002b).

4.2.3 Antihistaminics

Conflicting results are reported for the antihistaminic drug fexofenadine (Kim et al. 2001) and again no effects were observed among Germans (Drescher et al. 2002)

4.2.4 Protease Inhibitors

As outlined previously, protease inhibitors used in the treatment of HIV infections are clearly P-glycoprotein substrates of ABCB1. However, there is only weak evidence that ABCB1 genetic variants may have an impact on plasma concentrations of lopinavir (Winzer et al. 2003). For indinavir only small studies have been undertaken showing results that are difficult to interpret, for example, ABCB1 CC carriers led to moderately lower absorption but CYP3A5 had an additional impact (Curras et al. 2009; Solas et al. 2007). For nelfinavir no influence of ABC transporter variants was observed (Colombo et al. 2005), but there was significant impact of CYP2C19 (Hirt et al. 2008; Saitoh et al. 2010). The more recent protease inhibitor atazanavir is also a substrate of a P450 enzyme, namely CYP3A5. Thus this confounder has to be taken into account when determining the impact of ABCB1 genotypes. Interestingly, in a study in U.S. Caucasians and blacks,

ABCB1 1236C/2677G/3435C haplotype carriers had a decreased clearance, but the study was too small to prove the ABCB1 effects in subgroups of CYP3A5 expressor and nonexpressors alone (Anderson et al. 2009). Strikingly, another study pointed in the same direction of an opposite effect of 3435C>T toward lower plasma concentrations (Rodriguez-Novoa et al. 2007).

4.2.5 Anticonvulsants

As mentioned previously, the overexpression of ABCB1 at the blood–brain barriers is one of the major hypotheses of therapy resistance in epilepsy treatment (Hughes 2008). Indeed a study in 115 British treatment-responsive and 200 nonresponsive patients revealed an association of ABCB1 3435C>T with treatment responsiveness in epilepsy (Siddiqui et al. 2003). The frequency of carriers being homozygous for the putatively low-active 3435T-allele was significantly higher among responsive patients than in controls and responsive patients. Though in this study no functional analyses on ABCB1 expression were performed and no data on the medication were provided. Assuming that further candidate SNPs could be causal for the ABCB1 expression, an additional attempt was made within a cohort partially overlapping with the study of Siddiqui (Siddiqui et al. 2003). Again ABCB1 34335C>T turned out to be associated with treatment responsiveness, but an SNP at IVS 26 + 80 T>C was additionally identified that was 1.5-fold less causal than 3435C>T (Soranzo et al. 2004). A further study from Austria found a result going in the same direction, although the definition of therapy response differed (Zimprich et al. 2004). A small study from Egypt was also in line with the initial study (Ebid et al. 2007). All further studies performed within Caucasian populations (nine were identified comprising 2,772 cases) failed to confirm any association between genetic variants of ABCB1 and therapy responsiveness of epilepsy. Only one study from Croatia showed an impact of 3435C>T on phenobarbital cerebrospinal fluid-plasma ratios. In this study 3435CC carriers had a significantly lower CSF/PB concentration ratio than CT or TT carriers. Moreover, low CSF/S PB concentration ratio correlated with increased seizure frequency (Basic et al. 2008).

There are two studies from Asian populations showing a positive association with ABCB1 3435C>T, one with a similar result as the Siddiqui study (Hung et al. 2005), whereas a Japanese study pointed in the opposite direction with an overrepresentation of variant T allele carriers among drug-resistant patients (Seo et al. 2006). All other studies revealed lack of evidence of any association (four were identified comprising 1,074 cases). Also a study in a mixed cohort from Scottish, Australian, and Hong Kong origin failed to find an association (Szoeke et al. 2009).

A meta-analysis summarizing eleven studies involving a total of 3,371 patients (1,725 responsive and 1,646 drug-resistant patients) calculated an odds ratio for drug responsiveness of T-allele carriers of 1.15 (0.78–1.70) $p = 0.48$, indicating lack of association. Stratification to ethnic groups revealed no further evidence (Bournissen et al. 2009) (Table 3).

Table 3 Association of ABCB1 3435C>T (rs1045642) to therapy responsiveness in the treatment of epilepsy [adapted from (Cascorbi (2010))]

Author	Year	Origin	Size	Responders/ nonresponders	Association of 3435C>T to nonresponse	Association of other ABCB1 variants to nonresponse	References
Positive association in Caucasians							
Siddiqui	2003	UK	315	115/200	C more frequent	None	Siddiqui et al. (2003)
Soranzo	2004	UK overlapping with Siddiqui et al. (2003)	421	135/286	C more frequent	IVS 26 + 80	Soranzo et al. (2004)
Zimprich	2004	Austria	193	44/83/66[a]	C more frequent	Haplotype CGC	Zimprich et al. (2004)
Ebid	2007	Egypt	100	37/63	C more frequent	n.a.	Ebid et al. (2007)
Negative association in Caucasians							
Tan	2004	Australia	609	208/401	None	n.a.	Tan et al. (2004)
Sills	2005	UK	400	230/170	None	n.a.	Sills et al. (2005)
Shahwan	2006	Ireland	366	242/124	None	None	Shahwan et al. (2007)
Leschziner	2007	UK	149	76/73	None	None	Leschziner et al. (2007)
Dericioglu	2008	Turkey	189	100[b]/89	None	n.a.	Dericioglu et al. (2008)
Ozgon	2008	Turkey	271	174[b]/97[c]	None	n.a.	Ozgon et al. (2008)
Ufer	2009	Germany	221	103/118	None	None	Ufer et al. (2009b)
Lakhan	2009	North-India	325	231/94	None	None	Lakhan et al. (2009)
Vahab	2009	South-India	242	129/113	One	n.a.	Vahab et al. (2009)
Positive association in Asians							
Hung	2005	Taiwan	331	223/108	C more frequent	Haplotype CGC. TGC, TTT	Hung et al. (2005)
Seo	2006	Japan	210	84/126	T more frequent	Haplotype TTT	Seo et al. (2006)
Negative association in Asians							
Kim	2009	Korea	199	100/99	None	None	Kim et al. (2009)
Kim	2006	Korea	160	101/59	None	n.a.	Kim et al. (2006b)
Kwan	2007	China	501	286/215	None	None	Kwan et al. (2007)
Chen	2007	China	214	164/50	None	n.a.	Chen et al. (2007)
Negative association in a mixed cohort							
Szoeke	2009	Australia, China, UK	542	208/334	None	n.a.	Szoeke et al. (2009)
Meta-analysis							
Bournissen	2009	Various	3,370	1,725/1,645	None	n.a.	Bournissen et al. (2009)

n.a. not applied
[a]Stratification according to seizure frequency
[b]Healthy controls
[c]Carbamazepine-resistant

From the current data it can be concluded that ABCB1 variants lack evidence of an association of drug responsiveness to anticonvulsants. This discrepancy may be explained by the weak impact of ABCB1 variants on the pharmacokinetics on anticonvulsants or on ABCB1 expression in brain tissue (Mosyagin et al. 2008).

Moreover most anticonvulsants are only weak substrates of human P-gp in vitro, though some in vivo studies reported an inverse correlation between some anticonvulsant and P-gp expression in the brain.

4.2.6 Immunosuppressants

Immunosuppressive drugs like the calcineurin inhibitors cyclosporine and tacrolimus or the TOR inhibitor sirolimus are mandatory in the long-term prevention of rejection of solid organ transplants. All three are known P-glycoprotein substrates. Due to their narrow therapeutics range, a large number of studies have been performed to investigate the impact of ABCB1 variants on plasma concentration but also on the clinical outcome. In a meta-analysis from 14 papers published between 1997 and 2007, a total of 1,036 individuals were included (Jiang et al. 2008). Overall there was lack of evidence of a major influence of 3435C>T on the pharmacokinetics of cyclosporine, though the authors noticed a somewhat lower AUC within the first 12 h in subjects homozygous for 3435C and minor ethnic differences. All changes of the plasma concentrations appeared to be clinically not relevant. But it should be taken into account that intracellular concentrations of lymphocytes are crucial for immunosuppression rather than plasma concentrations. In a recent study determining intracellular lymphocyte concentrations from 64 stable renal, liver, or lung transplant recipients, there was only a slight correlation between plasma levels and intracellular concentration and a 1.7-fold elevation among 3435TT carriers as compared to CC carriers (Crettol et al. 2008). Such changes are by far much stronger than observed in any study on plasma concentrations indicating that ABCB1 expression in lymphocytes is of major importance for intracellular levels, whereas plasma concentrations are also dependent on the CYP3A4 activity in the liver and intestine.

In our own study on tacrolimus there was also lack of any impact of ABCB1 variants on any pharmacokinetic parameter, but a significant dependency on the expressor status of the drug-metabolizing enzyme CYP3A5 (Renders et al. 2007). A recent study investigating tacrolimus-related neurotoxicity revealed a major impact of CYP3A5 genotype, but not of ABCB1 (Yanagimachi et al. 2009). There are a number of further studies partly showing effects of ABCB1 SNPs; however, they were not always controlled for CYP3A5. Based on our observation in sigmoidal tissue that ABCB1 mRNA expression is significantly correlated with CYP3A5 mRNA expression (Ufer et al. 2008), a combined analysis appears to be crucial.

Data available for sirolimus are smaller. We investigated only 20 patients (Renders et al. 2007) without finding any association; other studies did not observe any relation to ABCB1 genotypes or haplotypes (Anglicheau et al. 2005; Miao et al.

2008; Mourad et al. 2005) and to CYP3A5 only in a Chinese study (Miao et al. 2008).

Concluding, the impact of ABCB1 genotypes on immunosuppressants seems to be negligible, and there are no algorithms available applying the CYP3A5 status for tacrolimus dosing. Consequently Burckhardt and Liu stated in 2006 that the presently available pharmacogenetic information does not permit any pharmacokinetic predictions for cyclosporine, sirolimus, and corticosteroids (Burckhardt and Liu 2006).

4.2.7 Cytostatics

In accordance with the role of ABCB1 for immunosuppressants in prevention of transplant rejection, the expression of ABCB1 in lymphocyte membrane might be of major importance in the treatment outcome of lymphatic leukemia. Indeed, there is an increasing number of studies suggesting an association between ABCB1 genotypes and clinical outcome (Illmer et al. 2002; Jamroziak et al. 2004; Kim et al. 2006a; Stanulla et al. 2005). In fact, ABCB1 expression was significantly higher among the upper percentile lymphocytes from British acute myeloid leukemia (AML) patients being 3435C carriers than among T carriers (Seedhouse et al. 2007). In a study in 101 Korean AML patients 3435CC was significantly correlated with lower functional ABCB1 function in a daunorubicin intracellular accumulation assay (Kim et al. 2006a). Surprisingly against all expectations, the 3435CC and 2677GG genotype was strongly associated with a higher probability of complete remission and 3-year event-free survival. However, no differences were noted in overall survival according to the ABCB1 SNPs. This lack of association was confirmed in a clinical study among elderly AML patients. ABCB1 variants failed to show any association to the treatment outcome or ABCB1 expression and function, as evidenced by rhodamine efflux experiments controlled with the P-gp inhibitor PSC833 (van der Holt et al. 2006).

In French chronic myeloid leukemia (CML) patients, however, the molecular response to the BRC-ABL-inhibitor imatinib was dependent on ABCB1 genotypes (Dulucq et al. 2008). Although 3435C>T failed to show a significant influence, patients with 1236T had higher imatinib plasma concentrations and had a better therapy response, whereas presence of the wild-type 2677G variant worsened the clinical response. In another study in a small sample of CML patients and gastrointestinal stromal tumors (GIST), 1236C>T, 2677G>T/A, and 3435C>T led to decreased imatinib clearance among variant carriers.

In a Korean study on acute lymphoblastic leukemia (ALL), however, there was lack of association of clinical endpoints such as complete remission rates, or relapse-free and event-free survival rates to ABCB1 variants (Hur et al. 2008). Only rare ABCB1 haplotypes of 2677G>T/A and 3435C>T differed in a large Hungarian ALL study, but overall the genotype distribution was not statistically different (Semsei et al. 2008). In multiple myelomas treated with dexamethasone,

doxorubicin and vincristine, ABCB1 3435CT or TT carriers had a better prognosis than 3435CC carriers ($p = 0.02$) (Buda et al. 2007), and the pharmacokinetics of vincristine were only marginally influenced by the ABCB1 SNPs 2677G>T/A and 3435C>T in another study in childhood ALL (Plasschaert et al. 2004).

The attempt to find any further ABCB1 variant influencing the activity or being associated to the clinical response revealed a novel ABCB1 571G>A missense variant detected in 6.4% of leukemia patients, causing a Gly191Arg amino acid change (Yang et al. 2008). The functional impact was investigated in a stable recombinant expression cell model. Among 571A carriers, there was a selectively reduced degree of ABCB1-mediated resistance against the anticancer drugs doxorubicin, daunorubicin, vinblastine, vincristine, paclitaxel, and etoposide. In particular, the resistance on vinblastine, vincristine, paclitaxel, and etoposide was fivefold reduced, indicating lower transport capacity of the protein carrying the 191Arg-variant. It was suggested that individuals with the ABCB1 571A genotype may be more sensitive to the specific anticancer drugs that are P-gp substrates but may also exhibit a higher risk of side effects.

5 Conclusion

The current knowledge of functional significance genetic variants of ABC membrane transporters does not allow selection of a particular SNP to predict an individual's pharmacokinetics. However, the large number of studies achieving associations of ABCB1 variants to clinical outcomes strongly supports the necessity of further investigation of the role of ABC transporters for intracellular drug bioavailability as well as clinical outcomes of lymphatic and chronic leukemia in large, prospective trials.

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Importance of P-glycoprotein for Drug–Drug Interactions

Hartmut Glaeser

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Abstract P-glycoprotein (ABCB1) is one of the most extensively studied transporters regarding drug resistance and drug–drug interactions. P-glycoprotein is expressed in multiple key organs in drug disposition such as small intestine, blood–brain barrier, kidney, and liver. Therefore, P-glycoprotein mediated drug–drug interactions can occur at various organs and tissues. This chapter will mainly focus on drug–drug interactions that are mediated by the intestinal P-glycoprotein.

During the last decade, many in vitro and in vivo studies reported that the induction or inhibition of P-glycoprotein can lead to drug–drug interactions. For instance, induction of the intestinal P-glycoprotein activity can cause reduced bioavailability of orally administered drugs and decreased therapeutic efficacy. On the other hand, the inhibition of the intestinal P-glycoprotein activity can lead to increased bioavailability, thus leading to an increased risk of adverse side effects.

Keywords Digoxin · Drug-drug interactions · Fexofenadine · Induction · Inhibition · P-glycoprotein · Rifampin · Small intestine · St. John's wort · Talinolol

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1 Induction of P-glycoprotein

The important role of the human intestinal P-glycoprotein in drug–drug interactions was first demonstrated in a study with the P-glycoprotein substrates digoxin and rifampin (Greiner et al. 1999). This study reported that intestinal P-glycoprotein is a major determinant of the bioavailability of orally administered digoxin and that the induction of P-glycoprotein is a mechanism for drug interactions between rifampin and digoxin. Upon the oral intake of rifampin (600 mg/day for 10 days), the AUC_{0-114h} and bioavailability of orally administered digoxin (1 mg) were significantly reduced by 30% and 19%, respectively (Fig. 1). The AUC_{0-114h} of intravenously administered digoxin (1 mg) was also reduced after rifampin intake, but to much less extent, suggesting the importance of the intestinal P-glycoprotein for interactions between rifampin and digoxin. The clinical relevance of the digoxin– rifampin interaction was already previously observed. It was reported

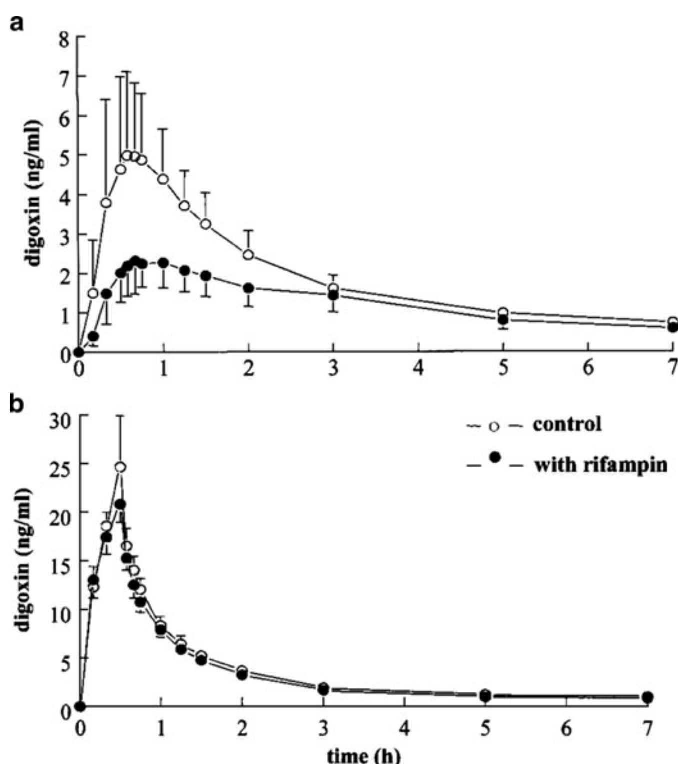

Fig. 1 Influence of rifampin on the pharmacokinetic of digoxin. (**a**) Mean ($n = 8$) plasma concentration (mean ± SD) time curves of orally administered digoxin (1 mg) before (*open circles*) and during (*filled circles*) coadministration of rifampin (600 mg). (**b**) Mean ($n = 8$) plasmaconcentration (mean ± SD) time curves of intravenously administered digoxin (1 mg) before (*open circles*) and during (*filled circles*) coadministration of rifampin (600 mg). This figure is being republished from Greiner et al. (1999)

that in two patients undergoing dialysis higher digoxin doses were required during treatment with rifampin in order to maintain therapeutic serum concentrations of digoxin (Gault et al. 1984).

A further drug–drug interaction study with digoxin, rifampin, and quinidine using a multilumen perfusion catheter (Drescher et al. 2003) highlighted the significance of P-glycoprotein for the intestinal secretion of drugs in humans. Rifampin caused an increased secretion of digoxin into a 20 cm long intestinal segment from 0.45% ± 0.24% to 0.83% ± 0.60% of the administered dose of digoxin (i.v.). In addition, the $AUC_{0–96h}$ of 1 mg digoxin (i.v.) significantly decreased from 62.2 ± 14.8 to 45.2 ± 16.2 ng h ml^{-1} after the administration of rifampin for 14 day (600 mg/day). The observed data confirm the relevance of the induction of intestinal P-glycoprotein by rifampin for drug–drug interactions.

In addition to rifampin, St. John's wort used for treatment of mild depression is also a clinically relevant inducer of P-glycoprotein and cytochrome P450 3A4. It was shown that St. John's wort influences the pharmacokinetics of the P-glycoprotein substrate digoxin (Johne et al. 1999). The coadministration of hypericum extract (300 mg/t.i.d.) with digoxin (0.25 mg/day at steady state) for ten days resulted in a significant reduction of $AUC_{0–24h}$ (−25%), C_{max} (−26%), and trough plasma concentrations (−33%) of digoxin. The authors hypothesized that the induction of the intestinal P-glycoprotein may be involved in the observed interaction. A subsequent study supported this hypothesis in that an increased expression of intestinal P-glycoprotein, CYP3A4, and hepatic CYP3A4 was observed after the intake of St. John's (300 mg/t.i.d.) for 14 days (Dürr et al. 2000).

Around this time a case report about the drug–drug interaction between cyclosporine A and St. John's wort underlined the clinical significance of drug interactions caused by St. John's wort (Ruschitzka et al. 2000). Two patients received heart transplantation and showed no signs of organ rejection during the follow-up under an immunosuppressant therapy with cyclosporine A, azathioprine, and corticosteroids. Both patients started to take St. John's wort (300 mg/t.i.d.) for 3 weeks. Thereupon both patients showed acute heart transplant rejection diagnosed by endomyocardial biopsy, which was associated with decreased plasma concentrations of cyclosporine A. Cyclosporine A is metabolized by CYP3A4 (Wu et al. 1995) and is a substrate of P-glycoprotein (Schinkel et al. 1995). Today it is well accepted that the induction of intestinal P-glycoprotein and CYP3A4 by St. John's wort pivotally contributes to the observed decrease in plasma concentrations of cyclosporine A (Ruschitzka et al. 2000).

In addition to the studies with digoxin, several human studies reported the influence of rifampin or St. John's wort on the pharmacokinetics of other clinically used P-glycoprotein substrates. For example, talinolol is a β_1-adrenoceptor blocker and a P-glycoprotein substrate that undergoes only a negligible metabolism (Trausch et al. 1995; Westphal et al. 2000b). Therefore, talinolol served as a probe drug in investigating intestinal P-glycoprotein as a major contributing factor for drug–drug interactions between rifampin and talinolol. A study by Westphal et al. (2000b) reported a significant reduction in the AUC of orally and intravenously administered talinolol during the coadministration with rifampin. Additionally the

systemic clearance of talinolol (i.v.) correlated with the intestinal P-glycoprotein expression indicating that P-glycoprotein significantly contributes to the secretion of talinolol into the gut.

In a study with talinolol and St. John's wort it became clear that the inductive effect of St. John's wort was associated with *ABCB1* genotype. The long-term intake of St. John's wort for a 12-day period (300 mg t.i.d.) resulted in a significant increase of nonrenal clearance of i.v. talinolol and a significant decrease in the $AUC_{0-\infty}$ of orally administered talinolol. Subjects with the variant MDR1 genotype (1236C>T, 2677G>T/A, and 3435C>T) were found to have an attenuated response to St. John's wort intake with respect to changes in $AUC_{0-\infty}$ of orally administered talinolol (Schwarz et al. 2007). This study highlights that the effect of Saint John's wort can be influenced by the *ABCB1* genotype. These results suggest that additional factors need to be considered in order to predict St. John's wort-mediated drug–drug interactions in humans. Furthermore, the increase in nonrenal clearance of intravenously administered talinolol confirmed the notion that intestinal P-glycoprotein is a major determinant of talinolol disposition in man (Schwarz et al. 2007).

Another example of P-glycoprotein-mediated drug–drug interactions includes the antihistaminic drug fexofenadine. Fexofenadine is another P-glycoprotein substrate that undergoes negligible metabolism. Several studies have examined the role of P-glycoprotein for drug–drug interactions with fexofenadine. A drug interaction study using fexofenadine and rifampin revealed an increased clearance of orally administered fexofenadine (60 mg) after rifampin intake (600 mg/day for 6 days) (Hamman et al. 2001). Interestingly, the increase of oral clearance of fexofenadine was independent of age and sex (Hamman et al. 2001).

It should be noted that the effect of an inducer such as St. John's wort on the pharmacokinetics depends on the particular drug and the relative contributions of CYP3A and P-glycoprotein in its disposition. This was demonstrated in a study that investigated the influence of St. John's wort on the pharmacokinetics of midazolam (CYP3A4 substrate), fexofenadine (P-glycoprotein substrate), and cyclosporine (CYP3A4/5 and P-glycoprotein substrate) following 12 days of pretreatment with St. John's wort (300 mg/t.i.d.) (Dresser et al. 2003). It was expected that the change in oral clearance of cyclosporine would be at least comparable to that of midazolam. In fact, a larger increase in oral clearance of cyclosporine seemed to be likely due to the additional induction of intestinal P-glycoprotein. However, the increase in oral clearance of cyclosporine was comparable to that of fexofenadine indicating that the change in pharmacokinetics of cyclosporine is more closely associated with the induction of intestinal P-glycoprotein rather than CYP3A4 (Dresser et al. 2003).

In addition, the schedule of St. John's wort intake relative to coadministered drug seems to impact the type of drug interaction. Oral single dose administrations of St. John's wort extract (900 mg) 1 h before fexofenadine intake led to a significant increase in C_{max} and decrease in oral clearance of fexofenadine without affecting the $t_{1/2}$ and renal clearance (Wang et al. 2002). These observations are consistent with an inhibition of intestinal P-glycoprotein and a corresponding increase in fexofenadine bioavailability. In this study the long-term effect of St. John's wort on the pharmacokinetics of orally administered fexofenadine was

also investigated, whereas after an intake of St. John's wort extract for 14 days (300 mg/t.i.d.) a 300 mg dose of St. John's wort extract was given one hour before fexofenadine intake. It was shown that compared with single-dose administration of St. John's wort, long-term administration resulted in reduced plasma concentrations of fexofenadine, without changing $t_{1/2}$. These observations are consistent with an induction of P-glycoprotein and reduced bioavailability of fexofenadine (Wang et al. 2002). In comparison to other studies with St. John's wort it can be concluded that St. John's wort inhibits P-glycoprotein during a period after its administration and as this effect fades away induction of P-glycoprotein becomes more apparent. These studies highlight that, in addition to *ABCB1* genotype (see previous), the schedule of St. John's administration plays a role in the effect of St. John's wort.

The prediction of St. John's wort-mediated drug–drug interactions is still difficult because different St. John's wort extracts are available on the market, which may cause drug interactions with various extents.

Simultaneous to the clinical studies investigating the induction of P-glycoprotein a lot of effort was put into the clarification of mechanism of induction. Using the human colon adenocarcinoma cell line LS180 it was shown that rifampin led to an induction of CYP3A and P-glycoprotein (Schuetz et al. 1996). This coinduction of intestinal P-glycoprotein and CYP3A4 in vitro and in vivo (Greiner et al. 1999; Schuetz et al. 1996) and the already reported involvement of the nuclear receptor PXR in induction of CYP3A genes (Goodwin et al. 1999) led to the investigation whether PXR is also involved in the induction of *ABCB1*. The DR4 (direct repeat) element AG(G/T)TCA 8 kb upstream of the *ABCB1* gene was identified as a PXR response element and it was shown that this element is necessary for induction of *ABCB1* by rifampin (Geick et al. 2001). A further study investigated the interaction between PXR and St. John's wort. The crystallization of the PXR-hyperforin complex revealed that the St. John's wort constituent hyperforin is responsible for the PXR-mediated induction of intestinal P-glycoprotein and CYP3A4 (Watkins et al. 2003). In addition, it was also demonstrated that the ligand-binding pocket of PXR can be induced to adopt different shapes depending upon which ligand is bound (Watkins et al. 2003).

2 Inhibition of P-glycoprotein

The inhibition of P-glycoprotein displays the second major mechanism of P-glycoprotein-mediated drug–drug interactions. Several in vitro and in vivo studies were performed to elucidate the inhibition of P-glycoprotein. The human epithelial colorectal adenocarcinoma cell line Caco-2 expresses P-glycoprotein on the apical membrane when grown in a polarized manner (Hunter et al. 1993b). Therefore this cell line represents a good model for the investigation of the inhibition of P-glycoprotein as well as for the identification of P-glycoprotein substrates. In a study using Caco-2 cells, the inhibition of the transcellular transport of the

P-glycoprotein substrate digoxin (Schinkel et al. 1995) by verapamil, a known P-glycoprotein inhibitor (Hunter et al. 1993a; Hunter et al. 1993b) was observed (Cavet et al. 1996). Therefore, it was concluded that P-glycoprotein is involved in the transcellular transport of digoxin in Caco-2 cells.

In a later study, a combination of mouse models and P-glycoprotein-expressing cell lines such as Caco-2 cells and L-MDR1 cells [LLC-PK1 cells overexpressing P-glycoprotein, (van Helvoort et al. 1996)] shed more light into the drug interaction between digoxin and quinidine (Fromm et al. 1999). The combination of the cardiac glycoside digoxin and the antiarrhythmic agent leads to one of the most common and serious drug interactions in clinical practice (Leahey et al. 1978), which causes a two to threefold increase in serum digoxin concentrations and frequently results in symptoms of digoxin toxicity. Using L-MDR1 cells and Caco-2 cells it was possible to show that quinidine is a substrate of P-glycoprotein and inhibits the P-glycoprotein-mediated transport of digoxin (Fromm et al. 1999). Furthermore, the effect of quinidine on digoxin disposition was studied in wild-type and in mdr1a(−/−) knockout mice. It was shown that quinidine (i.p.) increased plasma digoxin (i.v.) concentrations by 73.0% in wild-type animals, compared with 19.5% in mdr1a(−/−) knockout mice in which the quinidine dose was reduced because it is a P-glycoprotein substrate. Taken together, this study demonstrated that the inhibition of the P-glycoprotein-mediated digoxin transport by quinidine is a major mechanism for the clinically observed drug interaction between digoxin and quinidine.

In addition to the studies using P-glycoprotein-expressing cell lines and animal models many studies in humans were also performed in order to investigate the impact of P-glycoprotein inhibition by clinically used drugs on pharmacokinetics of other coadministered drugs. The already mentioned study by Drescher et al. also investigated the inhibitory effect of orally administered quinidine on the intestinal P-glycoprotein-mediated secretion of digoxin. Oral quinidine significantly reduced the intestinal secretion of digoxin (i.v., 1 mg) into a 20 cm long intestinal segment from 0.45% ± 0.24% to 0.23% ± 0.08% of the administered dose (Drescher et al. 2003). In this study the modulation of intestinal P-glycoprotein also demonstrated the relevance of intestinal P-glycoprotein for the direct secretion of drugs into the gut.

Due to the already discussed investigations it seems comprehensible that a lot of P-glycoprotein-mediated drug–drug interactions caused by inhibition can be attributed to intestinal P-glycoprotein. This was also demonstrated in a drug–drug interaction study with the two P-glycoprotein model substrates digoxin and talinolol (Westphal et al. 2000a). The AUC of orally administered digoxin (0.5 mg) given alone or concomitantly with intravenous (30 mg) or oral (100 mg) talinolol increased only following the oral administration of talinolol (Fig. 2). This demonstrates that P-glycoprotein-mediated drug–drug interactions are dependent from the route of application and dose.

The frequent oral administration of drugs in clinical use increases the likelihood of drug–drug interactions in the intestine. A study using the P-glycoprotein substrate talinolol showed that simultaneous oral administration of talinolol with

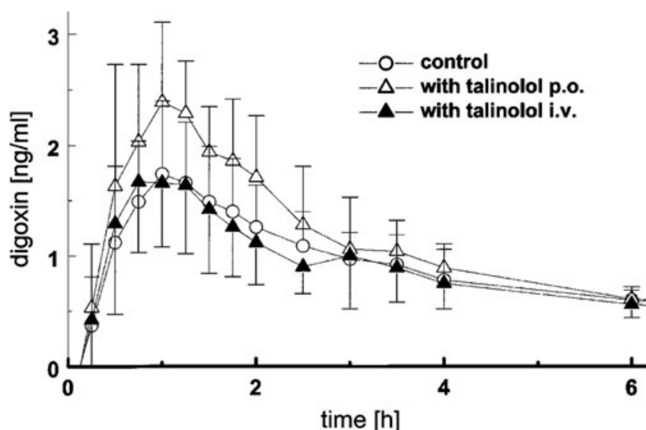

Fig. 2 Drug–drug interaction between oral digoxin and talinolol (oral and i.v.). Mean ± SD ($n = 10$) serum concentration–time curves of orally administered digoxin (0.5 mg) without (*open circles*) and with coadministration of 30 mg intravenous talinolol (*solid triangles*) or with 100 mg oral talinolol (*open triangles*). This figure is being republished from Westphal et al. (2000a)

erythromycin resulted in an increased AUC of talinolol in humans, whereas the renal clearance of talinolol was unchanged and a small decrease in elimination half-life was observed (Schwarz et al. 2000). These data suggest that increased bioavailability of talinolol is caused by increased net absorption due to the inhibition of intestinal P-glycoprotein by erythromycin.

However, the effect of drug–drug interactions between known inhibitors and substrates of P-glycoprotein was not always an increase in the bioavailability of the P-glycoprotein substrate. Such unexpected results were observed with the P-glycoprotein inhibitor R-verapamil (120 mg), which was divided into two doses and administered 15 min before and after talinolol (50 mg) administration (Schwarz et al. 1999). The AUC_{0-24h} of talinolol was significantly decreased with R-verapamil versus placebo. Also, the maximum serum concentration of talinolol was reached significantly earlier with R-verapamil intake compared to placebo. Because the renal clearance of talinolol was not affected by verapamil it can be concluded that verapamil inhibited uptake mechanisms of talinolol in the small intestine because an inhibition of P-glycoprotein would have led to increased bioavailability. Even though the intestinal uptake transporters of talinolol in humans are still unknown, it was speculated that uptake transporters of the human organic anion transporting peptide family such as OATP1A2 and OATP2B1, which are expressed on the apical membrane of enterocytes (Glaeser et al. 2007; Kobayashi et al. 2003), may be involved in absorption of talinolol (Schwarz et al. 2005, 2007). Verapamil also efficiently inhibits OATP1A2 (Bailey et al. 2007), which suggests that OATP1A2 might be involved in the absorption of drugs such as talinolol. Furthermore, talinolol was identified as a substrate of rOatp1a5, which is known to be expressed at the apical membrane of enterocytes (Walters et al. 2000) indicating that Oatp1a5 contributes to absorption of talinolol in rats (Shirasaka et al.

2009). This example shows that prediction of drug–drug interactions between P-glycoprotein inhibitors and substrates becomes more complicated if additional uptake transporters and/or drug metabolizing enzymes are involved in disposition of these drugs.

Fexofenadine is a substrate of P-glycoprotein and OATPs/Oatps (Cvetkovic et al. 1999; Dresser et al. 2002; Glaeser et al. 2007). This makes the prediction of the influence of P-glycoprotein on drug–drug interactions difficult. Analogical to talinolol a drug interaction study between fexofenadine and verapamil was performed (Yasui-Furukori et al. 2005). Verapamil was orally taken (80 mg, t.i.d) for 6 days. On day six 120 mg fexofenandine 1 h after the last dose of verapamil was administered. The verapamil treatment significantly increased the $C_{\max}$ of fexofenadine by 2.9-fold, the AUC_{0-48h} of fexofenadine by 2.5-fold, and the $AUC_{0-\infty}$ of fexofenadine by 2.5-fold, whereas the renal clearance of fexofenadine did not change during verapamil administration (Yasui-Furukori et al. 2005). For the interaction between fexofenadine and verapamil it can be concluded that the inhibition of intestinal P-glycoprotein plays the most important role, whereas the involvement of OATPs is negligible. However, the food–drug interaction between grapefruit juice and fexofenadine demonstrated that the inhibition of intestinal OATPs has to be considered. Using in vitro studies the inhibition of P-glycoprotein by grapefruit juice and its constituents such as 6′,7′-epoxybergamottin, 6′,7′-dihydroxybergamottin, naringin, and naringenin was observed (de Castro et al. 2007; Takanaga et al. 1998). Therefore, it was expected that grapefruit juice increased the bioavailability of fexofenadine. Surprisingly, the AUC of fexofenadine decreased when given simultaneously with grapefruit juice (Dresser et al. 2002; Glaeser et al. 2007). This effect was still prominent when grapefruit juice was given 2 h before the fexofenadine intake (Glaeser et al. 2007). Further detailed investigations revealed that OATP1A2 is expressed in the apical membrane of enterocytes and that its inhibition by naringin contributes to the decrease in absorption of fexofenadine when given together with grapefruit juice (Bailey et al. 2007; Glaeser et al. 2007). This example also demonstrates that the inhibition of intestinal P-glycoprotein is difficult to predict when other transporters such as OATPs are involved in the interaction between drugs and/or food. Therefore, detailed clinical studies are necessary to improve the predictability of drug–drug or drug–food interactions.

It seems obvious that the inhibition of intestinal P-glycoprotein is a major determinant of drug–drug interactions when drugs are given orally. It also has to be considered that P-glycoprotein is also expressed in several other tissues and organs such as the liver, kidney, blood–brain barrier, and placenta. However, the interactions with P-glycoprotein at these organs and tissues seem to be less frequently observed. This could be attributed to the different study designs that were used to investigate drug interactions. For example, during steady-state administration of digoxin (0.375 mg/day) with coadministration of the following regimens (doses three times a day) in a randomized sequence for 2 weeks each: verapamil (80 mg) and nifedipine (10 mg), verapamil (120 mg) and gallopamil (50 mg), or propafenone (150 mg) and quinidine (250 mg) increases in digoxin plasma

concentrations were observed (Belz et al. 1983). Interestingly, these increases of digoxin plasma concentrations correlated closely to decreases in renal digoxin clearances (Belz et al. 1983). Such data suggest that at steady state the inhibition of renal P-glycoprotein becomes identifiable. A lot of studies using a single oral dose administration of P-glycoprotein substrates and/or inhibitors just observed a change in nonrenal clearance and no change in renal clearance indicating that intestinal P-glycoprotein was inhibited.

Besides the intestine, P-glycoprotein plays an important role in the blood–brain barrier, limiting the entry of drugs into the brain. P-glycoprotein-mediated exclusion of drug molecules from the brain may provide a protection from central side effects or result in a failure of pharmacological and therapeutic objectives. For example, the μ-opioid receptor agonist loperamide is a substrate of P-glycoprotein (Schinkel et al. 1996). Loperamide is extensively used as an antidiarrhea drug without central side effects because the entry of loperamide into the central compartment is limited by P-glycoprotein. In a study with healthy volunteers, the simultaneous administration of loperamide with the P-glycoprotein inhibitor quinidine led to respiratory depression due to a central opioid effect of loperamide (Sadeque et al. 2000). In children whose blood–brain barrier function is not fully developed, loperamide can cause serious central side effects like constipation and respiratory depression (Turck et al. 1999). Additional studies tried to clarify the influence of polymorphisms in the *ABCB1* gene on the central side effects of loperamide (Pauli-Magnus et al. 2003; Skarke et al. 2003). However, the investigated polymorphisms in the *ABCB1* gene had only limited impact on the pharmacokinetics and central pharmacodynamics of loperamide in humans. Using PET imaging these results were also confirmed with the P-glycoprotein substrate verapamil (Brunner et al. 2005; Takano et al. 2006). The inhibition of P-glycoprotein-mediated transport of loperamide in the blood–brain barrier may also have the potential for misuse because increased access of loperamide to the brain by inhibition of P-glycoprotein could lead to euphoric effects.

The impact of P-glycoprotein on HIV therapy was well demonstrated by a study investigating the brain entry of HIV protease inhibitors using *mdr1* knockout mice (Choo et al. 2000; Kim et al. 1998). These data indicate that P-glycoprotein expressed in the blood–brain barrier limits access of HIV protease inhibitors to the brain, thereby possibly contributing to virus persistence in this sanctuary site. However, the inhibition of P-glycoprotein and CYP3A4 is clinically used in highly active antiretroviral therapy (HAART). For example, indinavir, lopinavir, or atazanavir are generally used with low-dose ritonavir in the HAART regimen. Ritonavir is a protease inhibitor and is a potent inhibitor of both P-glycoprotein and CYP3A. With this potent inhibition property, low-dose ritonavir has been demonstrated clinically to boost the bioavailability of other concomitant administered protease inhibitors (Johnson et al. 2005; Ribera et al. 2006; Young et al. 2002). The increase in bioavailability led to a clinically significant reduction of plasma HIV RNA and increase in $CD4^+$ cell count. Moreover, it was also reported that ritonavir is a potent inducer of P-glycoprotein and CYP3A in vitro (Perloff et al. 2001). This fact further complicates P-glycoprotein-mediated drug–drug interactions and

amplifies the clinical difficulties of HIV patients whose HIV infection must be controlled by ritonavir-boosted HAART.

P-glycoprotein is also expressed in the syncytiotrophoblast of the human placenta transporting toxins and drugs from the fetal into maternal circulation. The expression of P-glycoprotein in the human placenta was extensively investigated (Atkinson et al. 2003; Cordon-Cardo et al. 1990; Gil et al. 2005; Hitzl et al. 2004). The fact that P-glycoprotein is expressed in the trophoblast facing the maternal side supports the role of P-glycoprotein in protecting the fetus from toxic substances. In fact, a study investigating the P-glycoprotein expression in human placenta during pregnancy revealed a twofold lower P-glycoprotein expression at late terms compared to earlier time points during pregnancy, suggesting the ability of fetal protection was reduced during pregnancy (Gil et al. 2005). Consideration of the placental P-glycoprotein expression in the context of fetal accessibility to HIV protease inhibitors adds additional clinical relevance of placental P-glycoprotein in affecting the drug disposition and effectiveness of HIV drugs (Choo et al. 2000; Kim et al. 1998; Lee et al. 1998) and also in drug–drug interactions caused by HIV drugs. This notion was confirmed by investigating the transplacental passage of protease inhibitors at delivery (Marzolini et al. 2002).

3 Summary

Several studies demonstrated that the induction and inhibition by P-glycoprotein can lead to clinically relevant drug–drug interactions. It became clear that intestinal P-glycoprotein plays a pivotal role for these drug interactions. Most of the studies were performed with typical P-glycoprotein substrates such as digoxin, talinolol, and fexofenadine, which are characterized by negligible metabolism. However, even with some of these drugs it was recognized that drug uptake transporters need to be taken into account when drug–drug interaction of P-glycoprotein substrates are investigated. Taken together, P-glycoprotein-mediated drug–drug interactions display a high significance for the clinical use of drugs. The fact that most of the drugs are also substrates of CYP3A4 and/or OATPs may complicate the predictability of such drug–drug interactions. Furthermore, the possibility that a given drug is able to induce and inhibit P-glycoprotein has to be considered when such drugs are used in clinic.

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Multidrug Resistance Proteins (MRPs, ABCCs): Importance for Pathophysiology and Drug Therapy

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Abstract The nine multidrug resistance proteins (MRPs) represent the major part of the 12 members of the MRP/CFTR subfamily belonging to the 48 human ATP-binding cassette (ABC) transporters. Cloning, functional characterization, and cellular localization of most MRP subfamily members have identified them as ATP-dependent efflux pumps with a broad substrate specificity for the transport of endogenous and xenobiotic anionic substances localized in cellular plasma membranes. Prototypic substrates include glutathione conjugates such as

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leukotriene C_4 for MRP1, MRP2, and MRP4, bilirubin glucuronosides for MRP2 and MRP3, and cyclic AMP and cyclic GMP for MRP4, MRP5, and MRP8. Reduced glutathione (GSH), present in living cells at millimolar concentrations, modifies the substrate specificities of several MRPs, as exemplified by the cotransport of vincristine with GSH by MRP1, or by the cotransport of GSH with bile acids or of GSH with leukotriene B_4 by MRP4.

The role of MRP subfamily members in pathophysiology may be illustrated by the MRP-mediated release of proinflammatory and immunomodulatory mediators such as leukotrienes and prostanoids. Pathophysiological consequences of many genetic variants leading to a lack of functional MRP protein in the plasma membrane are observed in the hereditary MRP2 deficiency associated with conjugated hyperbilirubinemia in Dubin-Johnson syndrome, in pseudoxanthoma elasticum due to mutations in the *MRP6 (ABCC6)* gene, or in the type of human earwax and osmidrosis determined by single nucleotide polymorphisms in the *MRP8 (ABCC8)* gene. The hepatobiliary and renal elimination of many drugs and their metabolites is mediated by MRP2 in the hepatocyte canalicular membrane and by MRP4 as well as MRP2 in the luminal membrane of kidney proximal tubules. Therefore, inhibition of these efflux pumps affects pharmacokinetics, unless compensated by other ATP-dependent efflux pumps with overlapping substrate specificities.

Keywords ABCC subfamily · ATP-binding cassette (ABC) transporters · ATP-dependent efflux · Bilirubin glucuronosides · Cyclic AMP · Cyclic GMP · Dubin-Johnson syndrome · Earwax (human) · Hyperbilirubinemia · Leukotriene B_4 · Leukotriene C_4 · MRP1 · MRP2 · MRP3 · MRP4 · MRP5 · MRP8 · Multidrug resistance proteins (MRPs) · Osmidrosis · Prostanoids · Pseudoxanthoma elasticum · Radixin · Tissue distribution

1 Introduction

The identification and characterization of the drug and conjugate efflux pumps of the multidrug resistance protein (MRP, ABCC) subfamily was initiated by the cloning of MRP1 (ABCC1) in 1992 (Cole et al. 1992), and by its functional characterization as an ATP-dependent unidirectional efflux pump for anionic conjugates in 1994 (Jedlitschky et al. 1994; Leier et al. 1994a). The term "multidrug resistance protein" or "multidrug resistance-associated protein" relates to the demonstration that overexpression of the *MRP1 (ABCC1)* gene increases the resistance of cells to natural product drugs (Grant et al. 1994; Cole et al. 1994). Important for the understanding of MRP1-mediated resistance to drugs like Vinca alkaloids and anthracyclins was the discovery in Susan Cole's laboratory that reduced glutathione is required for coefflux of these drugs (Loe et al. 1996; Cole and Deeley 2006; Loe et al. 1998). Clearly, MRP1 as well as the other members of the MRP (ABCC)

subfamily are primarily ATP-dependent efflux pumps for physiological substrates, including glutathione conjugates such as leukotriene C_4 (LTC_4), many glucuronosides, and cyclic nucleotides (for review see Nies et al. 2007). In addition, several MRPs can function as efflux pumps for natural and synthetic toxins and drugs and, thus, can confer resistance of cells to these toxic substances. The efflux of conjugates, often generated in phase II reactions of drug metabolism, represents the final step in the pathway of detoxification of many xenobiotics and some endogenous substances. Thus, the drug and conjugate efflux pumps of the MRP subfamily represent the most important and widespread group of proteins determining the removal of conjugates from cells as indicated by their elimination from hepatocytes into bile, or from kidney proximal tubule epithelia into urine, or from intestinal epithelia into the intestinal lumen. Moreover, the biosynthetic release of many mediators, such as the glutathione conjugate LTC_4, to their extracellular site of action is mediated by members of the MRP subfamily.

The human CFTR/MRP (ABCC) subfamily comprises 12 members and includes nine MRPs, in addition to the cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7) and two sulfonylurea receptors SUR1 (ABCC8) and SUR2A/B (ABCC9). The nomenclature and some properties of the human *MRP* (*ABCC*) genes and MRP (ABCC) proteins are presented in Table 1. Amino acid sequence identities in comparison with MRP1 range from 33% for MRP8 (ABCC11) to 58% for MRP3 (ABCC3). The predicted topology for ABCC proteins indicates two

Table 1 Nomenclature and some properties of the human *MRP (ABCC)* genes and MRP (ABCC) proteins

Symbol	Alternate name	Gene accession number[a]	Chromosomal localization[a]	Protein accession number[b]	Amino acids[b]	Amino acid identity[b](%)
ABCC1	MRP1	NM_004996	16p13.1	NP_004987	1531	100
ABCC2	MRP2	NM_000392	10q24	NP_000383	1545	50
ABCC3	MRP3	NM_003786	17q22	NP_003777	1527	58
ABCC4	MRP4	NM_005845	13q32	NP_005836	1325	41
ABCC5	MRP5	NM_005688	3q27	NP_005679	1437	38
ABCC6	MRP6	NM_001171	16p13.1	NP_001162	1503	46
ABCC10	MRP7	NM_033450	6p12.1	NP_258261	1492	35
ABCC11	MRP8	NM_033151	16q12.1	NP_149163	1382	33
ABCC12	MRP9	NM_033226	16q12.1	NP_150229	1356	36
CFTR	ABCC7	NM_000492	7q31.2	NP_000483	1480	30
ABCC8	SUR1	NM_000352	11p15.1	NP_000343	1581	36
ABCC9	SUR2A	NM_005691	12p12.1	NP_005682	1549	35
	SUR2B	NM_020297	12p12.1	NP_064693	1549	36

Modified from Nies et al. (2007)

[a]Gene accession numbers and chromosomal localization compiled from "Gene" database at http://www.ncbi.nlm.nih.gov

[b]Protein accession number and amino acid number compiled from "Protein" database at http://www.ncbi.nlm.nih.gov and amino acid identity analyzed by the GAP tool of the HUSAR program package at http://genome.dkfz-heidelberg.de

cytoplasmic nucleotide-binding domains (NBDs) and, depending on the subfamily member, two or three membrane-spanning domains (MSDs). The most advanced structural information so far has been obtained for MRP1 (Rosenberg et al. 2010). Recent reviews on MRP1 and other members of the MRP subfamily include articles by Nies et al. (2007), Cole and Deeley (2006), Deeley et al. (2006), Kruh and Belinsky (2003), and Borst et al. (2000).

2 Tissue Distribution and Cellular Localization of Multidrug Resistance Proteins of the ABCC Subfamily

In this section we focus on tissue distribution and localization of MRP proteins in cellular membrane domains rather than on the levels of corresponding mRNAs, which need not be proportional to protein levels. MRP protein expression is often cell type specific, and the domain-specific localization of the transporters in distinct cell types is of major importance to understand the function of the MRP efflux pump under consideration. This may be exemplified by the apical localization of human MRP4 in kidney proximal tubules and brain capillary endothelial cells and its basolateral localization in prostate glandular epithelial cells and hepatocytes. The sorting and targeting of MRP subfamily members to distinct membrane domains in polarized cells is based on the interaction of the respective MRP protein with coupling and adaptor proteins such as radixin (Kikuchi et al. 2002), moesin, and NHERF1 (Hoque et al. 2009). The cell type-specific expression of an adaptor protein may thus determine the domain-specific localization of the respective MRP protein.

2.1 MRP1 Localization

The MRP1 protein is detectable in many human cell types and tissues with the highest levels in lung, testis, kidney, skeletal and cardiac muscles, placenta, and macrophages (Flens et al. 1996; Deeley et al. 2006). Normal human hepatocytes lack detectable amounts of MRP1 (Keppler et al. 2001). Immunohistochemical and immunofluorescence analyses indicate a predominant localization in cells of blood–tissue barriers. MRP1 is, for instance, present in the basolateral membrane of choroid plexus cells of the blood–cerebrospinal fluid barrier, in bronchial epithelium (Wright et al. 1998; Brechot et al. 1998), and in the apical syncytio-trophoblast membrane of the placenta (St-Pierre et al. 2000). Whether MRP1 contributes significantly to the function of the human blood–brain barrier is still controversial, although low levels of MRP1 protein were localized in the luminal membrane of human brain capillary endothelial cells (Nies et al. 2004).

2.2 MRP2 Localization

MRP2 represents the prototypical apically localized conjugate efflux pump of the MRP subfamily. It was first demonstrated in the apical (canalicular) membrane of rat and human hepatocytes (Keppler and Kartenbeck 1996; Büchler et al. 1996; Paulusma et al. 1997) and subsequently in the apical membrane of polarized cells of rat and human kidney proximal tubules (Schaub et al. 1997, 1999), human small intestine (Fromm et al. 2000), colon (Sandusky et al. 2002), gall bladder (Rost et al. 2001), segments of bronchi (Sandusky et al. 2002; König et al. 2003), and placenta (St-Pierre et al. 2000). MRP2 protein is absent or below current detection limits in several other normal human cell types and tissues, including endothelial cells of the blood–brain barrier (Nies 2007), exocrine pancreas (Sandusky et al. 2002; König et al. 2005), and skin (Sandusky et al. 2002). The exclusive apical localization of MRP2 is in line with its role in the excretion of many phase II conjugation products of drugs and endogenous substances into extracellular fluids such as bile, urine, and intestinal fluid.

2.3 MRP3 Localization

The MRP3 protein is expressed in many polarized cells and localized in the basolateral membrane domain. It was first localized in human and rat hepatocytes where it can mediate the efflux of organic anions into sinusoidal blood (König et al. 1999; Kool et al. 1999). Other cell types and tissues with MRP3 expression include cholangiocytes, gallbladder (Rost et al. 2001), pancreas (König et al. 2005), kidney distal tubules, enterocytes in the ileum and the colon, spleen, adrenal cortex, as well as several tumors (Scheffer et al. 2002b). MRP3 expression in rats and humans is inducible in some cell types, particularly in hepatocytes, but appears to be constitutive in other tissues. This was originally observed in Mrp2-deficient mutant rats with conjugated hyperbilirubinemia (Hirohashi et al. 1998), which are unable to secrete bilirubin glucuronosides into bile. In human liver, MRP3 levels may vary up to 80-fold among individuals (Lang et al. 2004), and several factors may play a role in hepatic MRP3 induction. Hereditary MRP2 deficiency in Dubin-Johnson syndrome (König et al. 1999) and various types of cholestatic liver disease may lead to increased MRP3 levels (Wagner et al. 2009). These hepatic disorders are often associated with elevated serum concentrations of bilirubin glucuronosides, which are normally secreted into bile by the apical conjugate efflux pump MRP2. The identification of MRP3 as an efflux pump transporting bilirubin glucuronosides (Lee et al. 2004), together with its localization in the hepatocyte basolateral membrane (König et al. 1999), supports the concept proposed earlier (Keppler and Kartenbeck 1996) that basolateral efflux accounts for the conjugated hyperbilirubinemia observed in many types of cholestatic hepatic disorders, and thereby has a compensatory role when the elimination of bilirubin glucuronosides via MRP2 in the canalicular membrane is impaired.

2.4 MRP4 Localization

Depending on the cell type, MRP4 is localized in the basolateral or in the apical membrane domain of a variety of polarized cells (reviewed by Russel et al. 2008). MRP4 was originally localized in the basolateral membrane of glandular epithelial cells of the prostate (Lee et al. 2000) and subsequently in the apical membrane of proximal tubule epithelial cells of kidney (van Aubel et al. 2002). In human and murine brain capillary endothelial cells, MRP4/Mrp4 is also localized in the apical (i.e. luminal) membrane (Nies et al. 2004; Leggas et al. 2004). A basolateral localization is observed in human, rat, and mouse hepatocytes (Rius et al. 2003), pancreatic duct epithelial cells (König et al. 2005), in choroid plexus epithelial cells (Leggas et al. 2004), and, interestingly, in polarized MDCKII cells (Lai and Tan 2002; Bartholomé et al. 2007). Additional cell types and tissues expressing the MRP4 protein include platelets (Jedlitschky et al. 2004; Rius et al. 2008; Niessen et al. 2010), erythrocytes (Klokouzas et al. 2003; Rius et al. 2008), astrocytes (Nies et al. 2004), adrenal glands (Zelcer et al. 2003a), epithelial cells of seminal vesicles (Rius et al. 2005), dendritic cells (van de Ven et al. 2008), and many cultured cell lines widely used for transfection studies, including HEK293, V79, U937, HL60, and HeLa (Rius et al. 2008).

2.5 MRP5 Localization

MRP5 protein has been detected in the epithelial cells of the urethra (Nies et al. 2002), smooth muscle cells and endothelial cells in the heart (Dazert et al. 2003), in the basal membrane of syncytiotrophoblasts, and in and around fetal vessels of placenta (Meyer zu Schwabedissen et al. 2005). In human brain, MRP5 was localized in astrocytes and pyramidal neurons (Nies et al. 2004) and in the blood–brain barrier, where it was found in the luminal (i.e., apical) membrane of brain capillary endothelial cells (Nies et al. 2004).

2.6 MRP6 Localization

MRP6 protein is strongly expressed in the basolateral membrane of human and rodent hepatocytes and kidney proximal tubule epithelial cells (Madon et al. 2000; Scheffer et al. 2002a; König et al. 2003; Beck et al. 2005). Genetic variants in the *MRP6 (ABCC6)* gene leading to a functional impairment of this efflux pump represent the molecular basis of the connective tissue disease pseudoxanthoma elasticum (PXE) (see the following).

2.7 MRP7–9 (ABCC10–12) Localization

Although it is assumed on the basis of mRNA analyses that MRP7–9 are widely expressed in human tissues (Kruh et al. 2007), only limited immunolocalization studies have been performed so far. MRP8 was localized to the axonal membrane of neurons in human cerebral cortex as well as to the apical membrane of HepG2 and MDCKII cells expressing recombinant MRP8 (Bortfeld et al. 2006). In tissue specimens containing ceruminous apocrine glands, MRP8 protein was expressed in the luminal membrane as well as in intracellular granules and large vacuoles of secretory cells including apocrine sweat glands (Toyoda et al. 2009; Martin et al. 2010).

3 Substrates of Multidrug Resistance Proteins of the ABCC Subfamily

Substrates of the members of the MRP subfamily of ATP-dependent efflux pumps are amphiphilic organic anions with an approximate molecular mass between 300 and 1,000 Da. However, some organic cations may become substrates in the presence of reduced glutathione (GSH), which is present in living cells at millimolar concentrations. This may be exemplified by cotransport of vincristine with GSH by MRP1 (Loe et al. 1996, 1998). Also some anionic compounds are only transported in the presence of GSH, as shown for MRP4-mediated cotransport of bile acids (Rius et al. 2003, 2006). Thus, the presence of GSH broadens the spectrum of substrates for some MRP subfamily members and this must be taken into consideration when the substrate specificity of these ATP-driven efflux pumps is characterized. This can be performed most precisely by the use of inside-out membrane vesicles containing the respective recombinant MRP protein (Keppler et al. 1998). The covalent glutathione *S*-conjugate leukotriene C_4 (LTC_4) was the first substrate for MRP1 identified in inside-out membrane vesicles, and transported with high affinity unidirectionally in the presence of ATP (Jedlitschky et al. 1994; Leier et al. 1994a). LTC_4 has remained the most characteristic substrate for most MRP subfamily members (see Table 2) and exhibits a high affinity for MRP1, MRP2, and MRP4. In addition to glutathione, other negatively charged moieties, such as glucuronate, sulfate, and phosphate, have been recognized as covalently bound moieties of MRP substrates. MRP subfamily members can often be considered as plasma membrane pumps responsible for the efflux of phase II conjugation products in drug metabolism and detoxification. In recent years, a large number of substrates for MRP subfamily members has been described and summarized (Cole and Deeley 2006; Nies et al. 2007; Nies and Keppler 2007; Borst et al. 2007; Kruh et al. 2007). Table 2 presents many of the important substrates for MRP subfamily members identified so far and underlines the broad substrate specificity of the MRP transporters.

Table 2 Substrates of human MRP transporters

Substrate[a]	K_m (μM)	References
MRP1		
Leukotriene C_4	0.1	Leier et al. (1994a)
Leukotriene D_4		Leier et al. (1994a)
S-Glutathionyl prostaglandin A_2		Evers et al. (1997)
Glutathionyl melphalan		Barnouin et al. (1998)
Bisglutathionyl chlorambucil		Barnouin et al. (1998)
Glutathione disulfide (GSSG)	93	Leier et al. (1996)
GSH (+verapamil)[b]	83	Loe et al. (2000)
Vincristine + GSH[c]		Loe et al. (1996, 1998)
Estrone 3-sulfate (+GSH)[b]	0.7	Qian et al. (2001)
Estrone 3-sulfate	4.2	Qian et al. (2001)
17β-Glucuronosyl estradiol	1.5	Jedlitschky et al. (1996)
6α-Glucuronosyl hyodeoxycholate		Jedlitschky et al. (1996)
Bisglucuronosyl bilirubin		Jedlitschky et al. (1997)
Lithocholyltaurine 3-sulfate		Jedlitschky et al. (1996)
Folate		Keppler et al. (1998)
Fluo-3	12	Keppler et al. (1999)
Cobalamin-OH	23	Beedholm-Ebsen et al. (2010)
MRP 2		
Leukotriene C_4	1	Cui et al. (1999)
Bisglucuronosyl bilirubin	0.7	Kamisako et al. (1999)
Monoglucuronosyl bilirubin	0.9	Kamisako et al. (1999)
17β-Glucuronosyl estradiol	7.2	Cui et al. (1999)
Ochratoxin A		Leier et al. (2000)
Cholecystokinin peptide	8.1	Letschert et al. (2005)
Estrone 3-sulfate		Kopplow et al. (2005)
Cholyl-L-lysyl-fluorescein	3.3	De Waart et al. (2010)
MRP3		
Leukotriene C_4	5.3	Zeng et al. (2000)
17β-Glucuronosyl estradiol	26	Zeng et al. (2000)
Bisglucuronosyl bilirubin		Lee et al. (2004)
Monoglucuronosyl bilirubin		Lee et al. (2004)
Dehydroepiandrosterone 3-sulfate	46	Lee et al. (2004)
Cholylglycine	248	Zeng et al. (2000)
MRP 4		
cGMP	9.7	Chen et al. (2001) and Van Aubel et al. (2002)
cAMP	44.5	Chen et al. (2001) and Van Aubel et al. (2002)
Leukotriene C_4	0.13	Rius et al. (2008)
Leukotriene B_4 (+GSH)[b]	5.2	Rius et al. (2008)
Prostaglandin E_2	3.5	Reid et al. (2003a) and Rius et al. (2005)
Prostaglandin $F_{2\alpha}$	12.6	Rius et al. (2005)
Thromboxane B_2	9.9	Rius et al. (2005)
Dehydroepiandrosterone 3-sulfate	2	Zelcer et al. (2003a)
17β-Glucuronosyl estradiol	30	Van Aubel et al. (2002) and Chen et al. (2001)

(*continued*)

Table 2 (continued)

Substrate[a]	K_m (μM)	References
Cholyltaurine (+GSH)[c]	7.7	Rius et al. (2003)
Cholyltaurine (+*S*-methyl-glutathione)[c]	3.8	Rius et al. (2003)
GSH (+ cholyltaurine)[c]	2,700	Rius et al. (2003)
S-methyl-glutathione (+cholyltaurine)[c]	1,200	Rius et al. (2003)
Cholate (+GSH)[c]	14.8	Rius et al. (2006)
Cholylglycine (+GSH)[c]	26	Rius et al. (2006)
Chenodeoxycholylglycine (+GSH)[c]	5.9	Rius et al. (2006)
Ursodeoxycholyltaurine (+GSH)[c]	7.8	Rius et al. (2006)
Folate	170	Chen et al. (2002)
Urate	1,500	Van Aubel et al. (2005)
ADP	170	Jedlitschky et al. (2004)
9-(2-Phosphonomethoxyethyl)adenine (PMEA)		Reid et al. (2003b)
Fluo-cAMP		Reichel et al. (2007)
MRP 5		
cGMP°		Jedlitschky et al. (2000)
cAMP°		Jedlitschky et al. (2000)
2′-Deoxyuridine 5′-monophosphate		Pratt et al. (2005)
Folate	1,000	Wielinga et al. (2005)
Methotrexate	1,300	Wielinga et al. (2005)
9-(2-Phosphonomethoxyethyl)adenine (PMEA)		Reid et al. (2003b)
MRP 6		
Leukotriene C_4	~0.6	Ilias et al. (2002) and Belinsky et al. (2002)
Cyclo(Trp-Asp-Pro-Val-Leu) (BQ123)		Ilias et al. (2002) and Belinsky et al. (2002)
S-Glutathionyl *N*-ethylmaleimide	~282	Ilias et al. (2002)
MRP 7		
17β-Glucuronosyl estradiol	58	Kruh et al. (2007) and Chen et al. (2003)
Leukotriene C_4		Chen et al. (2003)
MRP 8		
Dehydroepiandrosterone 3-sulfate	13	Chen et al. (2005) and Bortfeld et al. (2006)
Leukotriene C_4		Chen et al. (2005) and Kruh et al. (2007)
17β-Glucuronosyl estradiol	63	Chen et al. (2005)
cGMP°		Chen et al. (2005) and Kruh et al. (2007)
cAMP°		Chen et al. (2005)
Cholylglycine		Chen et al. (2005)
Folate		Chen et al. (2005)

Modified and based on Nies et al. (2007)
[a]Compounds listed have been identified as substrates by measurement of the ATP-dependent transport into inside-out membrane vesicles prepared from ABCC-expressing cells
[b]ATP-dependent stimulated transport of the first listed compound by the second listed compound
[c]ATP-dependent cotransport of both compounds; K_m values refer to the compound not in parenthesis

3.1 MRP1 Substrates

The cysteinyl leukotriene LTC_4 was identified as the first physiological substrate for MRP1 (Jedlitschky et al. 1994; Leier et al. 1994a). This finding was the result of the search for the molecular identity of the ATP-dependent efflux pump that mediates the release of LTC_4 from mastocytoma cells (Leier et al. 1994b). Several cell systems served in the identification of MRP1 substrates, including MRP1-overexpressing drug-selected cell lines, such as the human leukemia HL60/ADR cells (Jedlitschky et al. 1994), and transfected HeLa cells stably expressing MRP1 (Leier et al. 1994a). The discovery of the glutathione-containing LTC_4 as a high-affinity MRP1 substrate preceded the identification of many *S*-glutathionyl and glucuronosyl substrates for MRP1, as summarized in Table 2. These substrates share the property of being amphiphilic organic anions and conjugates with glutathione or glucuronate. Oxidized glutathione (GSSG) is a physiological substrate for MRP1 with a relatively low affinity, suggesting a role of MRP1 in the cellular defense against oxidative stress by decreasing concentrations of GSSG (Leier et al. 1996). GSH alone is not or only poorly transported by MRP1 (Leier et al. 1996; Loe et al. 1998). However, xenobiotics such as verapamil and several dietary flavonoids, including apigenin, stimulate GSH transport without being transported themselves (Loe et al. 2000; Leslie et al. 2003). Thus, GSH can play different roles in the MRP1-mediated transport: first as a cosubstrate together with hydrophobic compounds such as the Vinca alkaloids; second as a substrate, but only in the presence of xenobiotics such as verapamil and dietary flavonoids; and third as enhancer of MRP1-mediated transport of glucuronidated and sulfated conjugates, without being cotransported itself (Cole and Deeley 2006).

3.2 MRP2 Substrates

The substrate specificities of MRP2 and MRP1 (see Table 2) are quite similar, although differences in the kinetic properties have been established using inside-out membrane vesicles. For example, the K_m values of MRP2 for LTC_4 and 17β-glucuronosyl estradiol are ten and fivefold higher, respectively, than those for MRP1 (Cui et al. 1999), and MRP2 shows a higher affinity for mono- and bisglucuronosyl bilirubin than MRP1 (Kamisako et al. 1999). Furthermore, MRP2 mediates low-affinity transport of GSH and also of GSSG (Paulusma et al. 1999; Evers et al. 2000). The similar substrate specificity of human MRP2 and MRP1 was originally unexpected because the amino acid identity of both ABC transporters is only 50% (Table 1), but evidently the structural determinants for substrate binding of both proteins are much more alike than wrongly suggested by the linear amino acid sequences.

The comparison between human MRP2 and rat Mrp2, which share 78% amino acid identity, indicates a high similarity of their substrate specificity (for reviews

see König et al. 1999; Nies and Keppler 2007; Ito 2008). Prior to the molecular identification of rat Mrp2 and human MRP2 (Mayer et al. 1995; Paulusma et al. 1996; Büchler et al. 1996; Taniguchi et al. 1996; Ito et al. 1997), the function and substrate specificity of rat Mrp2 was studied by comparison of normal and hyperbilirubinemic mutant rats, termed Eisai hyperbilirubinemic (EHBR) rats and the GY/TR^{-} mutant rats (Jansen et al. 1985; Huber et al. 1987; Takikawa et al. 1991). These mutant rats are deficient in the secretion of various organic anions and anionic conjugates into bile because they lack a functional Mrp2 in the hepatocyte canalicular membrane (Paulusma et al. 1996; Büchler et al. 1996; Keppler and Kartenbeck 1996). These mutants contributed to the elucidation of the substrate specificity of Mrp2 and served as valuable controls in the cloning of Mrp2 (for reviews see König et al. 1999; Nies and Keppler 2007).

3.3 MRP3 Substrates

Like MRP1 and MRP2, human MRP3 transports a broad range of endogenous and xenobiotic, mostly conjugated, organic anions (Table 2). Glucuronidated compounds are important substrates for MRP3-mediated transport (reviewed by Borst et al. 2007), as exemplified by the efflux of mono- and bisglucuronosyl bilirubin across the basolateral membrane of hepatocytes back into sinusoidal blood (Lee et al. 2004). Human MRP3 also transports LTC_4 and *S*-(2,4-dinitrophenyl)glutathione with K_m values of 5.3 and 5.7 μM, respectively, in addition to methotrexate (Zeng et al. 2000). Although rat Mrp3 transports bile acids (e.g., cholyltaurine, cholylglycine, and sulfatolithocholyltaurine) with high affinity (Hirohashi et al. 2000). Human MRP3 mediates cholylglycine transport only with a low affinity, and cholyltaurine transport was below detectability (Akita et al. 2002; Zelcer et al. 2003b). This indicates significant species differences of MRP3/Mrp3 with regard to substrate specificity and affinity, particularly for bile acids (Akita et al. 2002).

3.4 MRP4 Substrates

MRP4 has been characterized as an ATP-dependent organic anion transporter of broad substrate specificity (Table 2). The first identified substrates of MRP4 were nucleoside monophosphate analogs used as antiretroviral drugs, particularly the nucleoside phosphonate analog 9-(2-phosphonylmethoxyethyl)adenine (PMEA) (Schuetz et al. 1999). The cyclic nucleotides, guanosine 3′,5′-monophosphate (cGMP), and adenosine 3′,5′-monophosphate (cAMP) represent important physiological substrates for MRP4 (Chen et al. 2001; Lai and Tan 2002; van Aubel et al. 2002). In addition to the cyclic nucleotides, MRP4 mediates ADP transport into

delta granules of human platelets and thus enables ADP accumulation in delta granules (Jedlitschky et al. 2004). However, MRP4 is also localized in platelet plasma membranes, which is a prerequisite for the release of LTC_4 from platelets during transcellular synthesis of this proinflammatory eicosanoid mediator (Rius et al. 2008). Other eicosanoids that are substrates for MRP4 include prostaglandins E_1 and E_2 (Reid et al. 2003a), prostaglandin $F_{2\alpha}$ and thromboxane B_2 (Rius et al. 2005), and the leukotrienes C_4 and B_4 (Rius et al. 2008). As indicated in Table 2, the presence of millimolar concentrations of GSH is an absolute requirement for LTB_4 transport by MRP4 (Rius et al. 2008).

Another group of physiologically and pathophysiologically important substrates comprises monoanionic bile acids, which are transported by MRP4 only together with GSH (Rius et al. 2003a; Rius et al. 2006). Thereby, MRP4 may contribute to the efflux of GSH and bile acids from hepatocytes into blood across the basolateral membrane and to bile acid transport across the apical (luminal) membrane of kidney proximal tubules.

3.5 MRP5 Substrates

The broad substrate specificity of MRP5 for organic anions comprises the anionic dye fluorescein diacetate (McAleer et al. 1999), the cyclic nucleotides cGMP and cAMP (Jedlitschky et al. 2000), a number of nucleoside monophosphate analogs, and some glutathione *S*-conjugates (Wijnholds et al. 2000). The finding that MRP5 functions as a cyclic nucleotide export pump (Jedlitschky et al. 2000) was subsequently confirmed in studies with intact cells (Wielinga et al. 2003). The ATP-dependent cyclic nucleotide transport by MRP5 was inhibited by several phosphodiesterase inhibitors, some of which are structurally closely related to cGMP and possibly able to enhance intracellular cyclic nucleotide concentrations by blocking their degradation as well as their export (Jedlitschky et al. 2000). Thus, MRP5, together with MRP4, may importantly contribute to the regulation of the tissue levels of cAMP and cGMP. However, the affinity of MRP5 to cAMP and cGMP and the relative contribution of phosphodiesterases to the control of tissue levels of cyclic nucleotides seem to vary depending on the cellular system (Nies et al. 2007).

3.6 MRP6 Substrates

The identification of the physiological substrates released by MRP6 across the basolateral membrane from hepatocytes and kidney proximal tubules into blood continues to be a challenge in MRP research because it should provide a decisive contribution to the understanding of the molecular pathogenesis of pseudoxanthoma

elasticum (PXE) (Vanakker et al. 2010). Inside-out membrane vesicles containing human MRP6 transported ATP dependently, the glutathione *S*-conjugates LTC_4 and, with low affinities, NEM-SG and BQ-123 (Table 2) (Ilias et al. 2002; Belinsky et al. 2002). The attractive hypothesis has been put forward that vitamin K, as acidic conjugate or in association with GSH, represents the substrate linking MRP6 and pseudoxanthoma elasticum (Borst et al. 2008).

3.7 Substrates for MRP7, MRP8, MRP9

The more recently identified MRP subfamily members, MRP7–9 (gene symbols ABCC10, ABCC11, ABCC12), have so far been characterized only in part (Table 2).

MRP7 mediates the transport of a number of physiological substrates including 17β-glucuronosyl estradiol and LTC_4 as determined with membrane vesicles from HEK293 cells expressing recombinant MRP7 (Kruh et al. 2007). MRP7 confers high levels of resistance to docetaxel, suggesting that it may be a substrate as well (Hopper-Borge et al. 2004).

MRP8 substrates (summarized in Table 2) include 17β-glucuronosyl estradiol, LTC_4, dehydroepiandrosterone 3-sulfate, cyclic nucleotides, cholylglycine, and folate (Chen et al. 2005). With regard to the established role of MRP8 in determining the earwax type, it is currently not clear which substrate transport is involved in earwax secretion by ceruminous apocrine glands (Yoshiura et al. 2006; Toyoda et al. 2009). MRP8 seems to be crucial for the secretion of odorants and their precursors from apocrine sweat glands (Martin et al. 2010). These compounds include N_{α}-3-methyl-3-hydroxy-hexanoyl-glutamine, which, based on its structure, may well be a direct MRP8 substrate (Martin et al. 2010).

MRP9 substrates have not been identified so far (Kruh et al. 2007).

4 Inhibitors of Multidrug Resistance Proteins of the ABCC Subfamily

Inhibition of transport mediated by members of the MRP subfamily has been based originally on structural analogs of the prototypic substrate LTC_4 and its cysteinyl-glycine derivative LTD_4 (Jedlitschky et al. 1994; Leier et al. 1994a). Potent LTD_4 receptor antagonists have been developed for the treatment of asthma, and the quinoline derivative MK571 represents an example for the early development of these drugs (Jones et al. 1989). MK571 has been used originally as a potent competitive inhibitor of ATP-dependent LTC_4 transport (Schaub et al. 1991; Leier et al. 1994b) and as a competitor for direct photoaffinity labeling of MRP1 with $[^3H]LTC_4$ (Jedlitschky et al. 1994; Leier et al. 1994a). MK571 has often been considered a potent and useful competitive inhibitor of MRP1-mediated transport;

however, it is also a competitive inhibitor of other MRP subfamily members that transport LTC_4, as shown, for example, for rat Mrp2 (Büchler et al. 1996), human MRP2 (Letschert et al. 2005), and MRP4 (Rius et al. 2003). Moreover, we observed inhibition of the hepatic uptake transporter OATP1B3 by MK571 with a K_i value of 0.6 μM (Letschert et al. 2005). Thus, MK571 as well as several other LTD_4 receptor antagonists (Keppler et al. 1998) are structural analogs of cysteinyl leukotrienes with a preferential high-affinity binding to LTD_4 receptors rather than selective inhibitors of MRP-mediated transport.

Cyclosporin A acts as a good but nonselective inhibitor of MRP1 (Leier et al. 1994a) and MRP2 (Kamisako et al. 1999), in addition to its more potent inhibition of MDR1 P-glycoprotein (Barrand et al. 1993), of the ATP-dependent bile salt export pump (Böhme et al. 1994; Byrne et al. 2002), and of the uptake transporters OATP1B1 and OATP1B3 (Shitara et al. 2003; Treiber et al. 2007).

The most advanced compounds among the published MRP inhibitors are several cyclohexyl-linked *tricyclic isoxazoles* that have been developed as potent and specific inhibitors of the MRP1-mediated ATP-dependent transport in membrane vesicles, in intact cells, and in vivo in mice (Norman et al. 2002, 2005; Qian et al. 2002; Mao et al. 2002). It is important to note that these tricyclic isoxazoles, as studied in detail with LY475776, depend in their action on the presence of millimolar concentrations of GSH, as present in living cells (Qian et al. 2002; Mao et al. 2002). Among MRP homologs the tricyclic isoxazole LY475776 seems to be highly specific for MRP1 (Norman et al. 2005).

Selective *inhibitors directed against MRP4* may be of considerable therapeutic interest to interfere with the cellular release of proinflammatory mediators such as prostaglandins (Reid et al. 2003a) and leukotrienes (Rius et al. 2008), or with the release of nucleotides in platelet function (Jedlitschky et al. 2004). However, potent, selective, as well as cell-specific inhibition of MRP4, has not yet been published. Useful inhibitors of MRP4 include several nonsteroidal anti-inflammatory agents such as indomethacin (Reid et al. 2003a) and sulindac sulfide in the presence of GSH (Rius et al. 2008), phosphodiesterase inhibitors such as dipyridamol, trequinsin, and sildenafil (Reid et al. 2003b), and LTD_4 receptor antagonists (see previous) such as MK571 (Rius et al. 2003) and montelukast (Rius et al. 2008). Useful inhibition of MRP4 in studies with inside-out membrane vesicles is also achieved by cholyltaurine (taurocholate) together with 5 mM GSH (Rius et al. 2008).

Selective *inhibition of MRP5* has not been achieved so far. Several phosphodiesterase inhibitors such as trequinsin, dipyridamol, zaprinast, and sildenafil show inhibitory potential (Jedlitschky et al. 2000), but the potency varies depending on the cell system and seems to be less (Reid et al. 2003b) than originally described (Jedlitschky et al. 2000). Weak and nonselective inhibitors include probenecid and benzbromarone (Jedlitschky et al. 2000; Reid et al. 2003b). Established CFTR inhibitors such as the arylaminobenzoates diphenylamine-2-carboxylate and 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB) are relatively potent inhibitors of MRP5 (Pratt et al. 2005). Interestingly, these compounds also inhibit *MRP8* (ABC11) (Bortfeld et al. 2006). *MRP7* confers resistance to docetaxel, nucleotide

analogs, and epothilone B, suggesting that these compounds or their metabolites interact with MRP7 (Hopper-Borge et al. 2009).

5 Genetic Variants, Knockout Animals, and Disease

Several thousand single-nucleotide genetic variants have been identified in the genes encoding the nine human *MRP (ABCC)* genes and are listed in the NCBI-SNP database (summarized by Nies et al. 2007). Whereas most sequence variants have no phenotypic consequences, some lead to impaired expression, localization, or function of the respective MRP protein. Unless compensated by alternative efflux pumps, the lack of a functional MRP transporter may cause alterations in elimination routes of drugs and endogenous compounds, as exemplified by hereditary MRP2 deficiency (reviewed by Nies and Keppler 2007), insufficient supply of compounds to other tissues, as hypothesized in MRP6 deficiency (Borst et al. 2008; Vanakker et al. 2010), or lack of a secretory product from apocrine glands in MRP8 deficiency associated with dry earwax syndrome and osmidrosis (Yoshiura et al. 2006; Toyoda et al. 2009).

Knockout mice with disrupted *Mrp* (*Abcc*) genes have been generated for most of the Mrp subfamily members, and the functional consequences have been characterized to a considerable extent (Table 3). Moreover, cross-breeding has enabled the generation of double- and triple-knockout animals. However, the kinetic differences between some Mrp transporters from mice and humans may be large and should be taken into consideration, as exemplified by the comparison between human MRP4 and mouse Mrp4 (de Wolf et al. 2007). Thus Mrp knockout mice are only in part indicative of a corresponding defect in humans.

The most extensively studied genetic variants of MRP transporters are those encoded in the *MRP2 (ABCC2)* gene (reviewed by Nies and Keppler 2007). These

Table 3 Knockout mice with disrupted *Mrp (Abcc)* genes

Deleted protein	Functional consequences	References
Mrp1	Increased sensitivity to anticancer drugs, impaired inflammatory response	Wijnholds et al. (1997) and Lorico et al. (1997)
Mrp2	Impaired bile canalicular secretion of organic anions (mild conjugated hyperbilirubinemia)	Chu et al. (2006), Nezasa et al. (2006) and Vlaming et al. (2006)
Mrp3	Impaired basolateral secretion of glucuronides	Belinsky et al. (2005) and Zamek-Gliszczynski et al. (2006)
Mrp4	Impaired secretion of organic anions, increased sensitivity to nucleoside analogs, accumulation of topotecan in mouse brain	Leggas et al. (2004) and Belinsky et al. (2007)
Mrp5	None detected (or fully compensated)	de Wolf et al. (2007)
Mrp6	Ectopic mineralization of connective tissue	Gorgels et al. (2005) and Klement et al. (2005)

studies were preceded by the early work on hyperbilirubinemic mutant rats (Jansen et al. 1985; Takikawa et al. 1991), now known to be selectively deficient in Mrp2 (Mayer et al. 1995; Paulusma et al. 1996; Büchler et al. 1996; Ito et al. 1997), and the corresponding defect in humans termed the *Dubin-Johnson syndrome* (Kartenbeck et al. 1996; Keppler and Kartenbeck 1996; Paulusma et al. 1997). A large number of nucleotide sequence variants in the human *MRP2 (ABCC2)* gene have been described, but only some of them lead to a loss of functional MRP2 in the plasma membrane (reviewed by Nies and Keppler 2007). The Dubin-Johnson syndrome was originally described in 1954 and characterized by its dark liver and by conjugated hyperbilirubinemia (Dubin and Johnson 1954; Sprinz and Nelson 1954). The disorder is inherited in an autosomal recessive mode and its incidence ranges from 1:1,300 among Iranian Jews (Shani et al. 1970) and 1:300,000 in the Japanese population (Wada et al. 1998; Kajihara et al. 1998). Conjugated hyperbilirubinemia is due to the efflux of bilirubin glucuronides from hepatocytes into blood via basolateral MRP3, which thereby compensates for the deficiency in MRP2-mediated elimination of the conjugates into bile. Immunofluorescence microscopy suggested an upregulation of MRP3 protein in the basolateral membrane of human hepatocytes in Dubin-Johnson syndrome (König et al. 1999). In Mrp2-deficient rats the upregulation of Mrp3 protein was more than 50-fold (Donner and Keppler 2001). The compensation of impaired MRP2 function in the hepatocyte canalicular membrane by basolateral MRP3-mediated efflux of substances serves as a hepatoprotective mechanism (Keppler and Kartenbeck 1996; Donner and Keppler 2001) and may apply also to drugs that would be hepatotoxic in cholestasis unless cleared by basolateral efflux into blood followed by renal excretion. This compensatory or protective basolateral efflux is not only mediated by MRP3 but also by other members of the MRP subfamily, particularly by MRP4 in the hepatocyte basolateral membrane (Rius et al. 2006). The diversion of hepatobiliary elimination to renal elimination under conditions of Mrp2 deficiency or in cholestasis has also been demonstrated noninvasively by positron emission tomography using ^{11}C-labeled *N*-acetyl leukotriene E_4 (Keppler et al. 1991; Guhlmann et al. 1995). The variants in the *MRP2 (ABCC2)* gene leading to Dubin-Johnson syndrome do not cause overt liver toxicity, rather, this is a well-compensated syndrome, and the mild conjugated hyperbilirubinemia is associated with a normal life span in humans and rats.

Many genetic variants in the *ABCC* genes (Saito et al. 2002; reviewed by Nies et al. 2007) encoding human *MRP3* (Lang et al. 2004; Lee et al. 2004), *MRP4* (Abla et al. 2008; Gradhand et al. 2008), and *MRP5* (Dazert et al. 2003; Stojic et al. 2007) have been identified, but no symptoms or diseases have been observed so far in individuals with such variants. However, challenge by drugs, particularly during cancer chemotherapy, may reveal signs of toxicity that were not previously linked to genetic variants in MRP3, MRP4, or MRP5. Variants affecting the protective function of MRP4 in the blood–brain barrier and in choroid plexus may be of particular interest, as suggested by the observations in Mrp4-deficient mice treated with topotecan (Leggas et al. 2004).

Loss of function genetic variants in the *ABCC6* gene encoding MRP6 are the cause of the autosomal recessive disease pseudoxanthoma elasticum (PXE), characterized by calcification of elastic fibers in the skin, arteries, cardiac tissue, and retina (Bergen et al. 2000; Le Saux et al. 2000; Ringpfeil et al. 2000). PXE must be discerned from PXE-like syndrome, which is genetically distinct but shares ectopic mineralization due to deficient protein carboxylation of vitamin K-dependent inhibitors of calcification (Vanakker et al. 2010). A large number of nonsense, missense, deletions, insertions, and splice site mutations in the *ABCC6* gene have been identified (reviewed by Bergen et al. 2007; Li et al. 2009; Uitto et al. 2010). Studies in Mrp6 knockout mice fully support the causative role of Mrp6 in PXE (Gorgels et al. 2005; Klement et al. 2005; LaRusso et al. 2008). As pointed out previously, the MRP6 protein is mainly expressed in the basolateral membrane of hepatocytes and in proximal tubule epithelial cells of the kidney cells (Keppler et al. 2001; Scheffer et al. 2002a; König et al. 2003). A better understanding of the pathogenesis of PXE and new approaches to its therapy await the identification of the decisive MRP6 substrate(s) released from hepatocytes and kidney proximal tubule epithelia into blood and contributing to the prevention of ectopic mineralization (Li et al. 2009; Vanakker et al. 2010; Uitto et al. 2010).

The single-nucleotide polymorphism 538G>A in the *ABCC11* gene, corresponding to the Gly180Arg variant in human *MRP8*, determines the earwax type (Yoshiura et al. 2006; Toyoda et al. 2009) and prevents the secretion of amino acid conjugates determining human axillary odor (Martin et al. 2010). The 538G>A variant reaches >95% in certain populations and may lead to positive selection in mate choice for low-odorant partners with a dysfunctional MRP8 (Martin et al. 2010). The Gly180Arg variant lacks *N*-linked glycosylation and is recognized as a misfolded protein undergoing proteasomal degradation (Toyoda et al. 2009). The endogenous compounds secreted by apocrine sweat glands include N_{α}-3-methyl-3-hydroxy-hexanoyl-glutamine, which may be a substrate for functional MRP8 (Martin et al. 2010).

Thus, genetic variants causing disorders (MRP2 and MRP8) or disease (MRP6) have been identified and characterized to a considerable extent in human MRP2 (Dubin-Johnson syndrome), MRP6 (pseudoxanthoma elasticum), and MRP8 (altered earwax and osmidrosis syndrome). Functionally relevant mutations in other members of the MRP subfamily have not yet been detected or they may be well compensated by other efflux pumps. Moreover, challenge of functionally deficient MRP transporters by drug exposure or drug–drug interaction may lead to unexpected side effects.

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In Vitro and In Vivo Evidence for the Importance of Breast Cancer Resistance Protein Transporters (BCRP/MXR/ABCP/ABCG2)

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Abstract The breast cancer resistance protein (BCRP/ABCG2) is a member of the G-subfamiliy of the ATP-binding cassette (ABC)-transporter superfamily. This half-transporter is assumed to function as an important mechanism limiting cellular accumulation of various compounds. In context of its tissue distribution with localization in the sinusoidal membrane of hepatocytes, and in the apical membrane of enterocytes ABCG2 is assumed to function as an important mechanism facilitating hepatobiliary excretion and limiting oral bioavailability, respectively. Indeed functional assessment performing mouse studies with genetic deletion or chemical inhibition of the transporter, or performing pharmacogenetic studies in humans

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support this assumption. Furthermore the efflux function of ABCG2 has been linked to sanctuary blood tissue barriers as described for placenta and the central nervous system. However, in lactating mammary glands ABCG2 increases the transfer of substrates into milk thereby increasing the exposure to potential noxes of a breastfed newborn. With regard to its broad substrate spectrum including various anticancer drugs and environmental carcinogens the function of ABCG2 has been associated with multidrug resistance and tumor development/progression. In terms of cancer biology current research is focusing on the expression and function of ABCG2 in immature stem cells. Recent findings support the notion that the physiological function of ABCG2 is involved in the elimination of uric acid resulting in higher risk for developing gout in male patients harboring genetic variants. Taken together ABCG2 is implicated in various pathophysiological and pharmacological processes.

Keywords ABC transporter · Efflux transporter · ABCG2 · BCRP · Breast cancer resistance protein · Pharmacokinetics · Mouse models · Feto-maternal transfer · Oral bioavailability · Hepatobiliary elimination · Blood–brain barrier · Secretion into milk · Multidrug resistance · Stem cells · Side population · Sulfasalazin · Fumitremorgin C · Ko143 · Elacridar · Uric acid · Gout

ABCG2 is a member of the ATP binding cassette (ABC)-transporter superfamily that summarizes a large number of membrane proteins mainly functioning as ATP-dependent efflux transporters. The transporter encoding *ABCG2* gene is located on chromosome 4q22, spans more than 66 kb, and consists of 16 exons ranging from 60 to 532 bp (Bailey-Dell et al. 2001). Due to its structure, which consists of only six transmembrane domains, ABCG2 is called a half-transporter, which is assumed to homodimerize to obtain functional activity. ABCG2 was first described by Doyle et al. as a major factor mediating drug resistance in a human breast cancer cell line selected for adriamycin resistance (MCF-7 AdrVp) and named breast cancer resistance protein (BCRP). The cellular multidrug-resistant phenotype resulting from an ATP-dependent reduction in the accumulation of anthracycline anticancer drugs was explained by overexpression of this newly identified efflux transporter (Doyle et al. 1998). At the same time Miyake et al. described this transporter as mechanism resulting in mitoxantrone resistance and named it MXR (mitoxantrone resistance factor) (Miyake et al. 1999). Whereas Allikmets et al. found the coding sequence as highly expressed in placenta (ABCP) (Allikmets et al. 1998). Several years later ABCG2 has evolved as established factor with profound impact on the pharmacological behavior of xenobiotics. Indeed, this transporter efficiently extrudes its substrates from cells, thereby mediating drug resistance and affecting pharmacokinetics.

In the following text we will give a comprehensive description on the multiple functions of ABCG2 in the human organism. First starting with insights into the basics of this transporter by giving a brief summary of its tissue distribution, its substrates (summarized in Table 1), and its inhibitors (summarized in Table 2), we will provide information on the current understanding of its *in vivo* effects by detailed

Table 1 Summary of nonchemotherapeutic substrates of ABCG2

Antivirals	
Abacavir	Pan et al. (2007)
Acyclovir	Jonker et al. (2005)
Lamivudine	Kim et al. (2007)
Zidovudine (AZT)	Pan et al. (2007) and Wang et al. (2003)
HMG-CoA reductase inhibitors (statins)	
Atorvastatin	Keskitalo et al. (2009a)
Cerivastatin	Matsushima et al. (2005)
Pravastatin	Matsushima et al. (2005)
Pitavastatin	Huang et al. (2006) and Fujino et al. (2005)
Rosuvastatin	Huang et al. (2006) and Kitamura et al. (2008)
Antibiotics	
Ciprofloxacin	Ando et al. (2007) and Merino et al. (2006)
Danofloxacin	Schrickx and Fink-Gremmels (2007)
Enrofloxacin	Pulido et al. (2006)
Gepafloxacin	Ando et al. (2007)
Nitrofurantoin	Merino et al. (2006)
Norfloxacin	Merino et al. (2006)
Ofloxacin	Ando et al. (2007) and Merino et al. (2006)
Ulifloxacin	Ando et al. (2007)
Calcium channel blockers	
Azidopine	Chearwae et al. (2006) and Shukla et al. (2006)
Dihydropyridine	Shukla et al. (2006)
Nitrendipine	Shukla et al. (2006)
Others	
Afluzosin	Zhao et al. (2009)
Albendazole sulfoxide	Merino et al. (2005a)
Cimetidine	Jonker et al. (2005), Pavek et al. (2005) and Zhao et al. (2009)
Diclofenac	Lagas et al. (2009a)
Dipyridamole	Zhang et al. (2005b) and Zhao et al. (2009)
Glyburide	Gedeon et al. (2006)
Leflunomide	Kis et al. (2009)
Sulfasalazine	van der Heijden et al. (2004)
Teriflunomide	Kis et al. (2009)
Oxfendazole	Merino et al. (2005a)
Endogenous substances	
Dehydroepiandrosterone Sulfate	Suzuki et al. (2003)
Estradiol-17β glucronide	Chen et al. (2003)
Estrone 3-sulfate	Suzuki et al. (2003)
Folic acid	Chen et al. (2003)
Vitamin K3	Shukla et al. (2007)
ProtoporphyrinIX	Jonker et al. (2002)
Riboflavin	van Herwaarden et al. (2007)
Uric acid	Woodward et al. (2009)

description of findings in animal models. Subsequently we will focus on the role of ABCG2 in development and progression of cancers summarizing findings in different tumor entities. It is assumed that expression of ABCG2 in immature cell subpopulations is an important contributor to multidrug resistance and tissue

Table 2 Summary of inhibitors of ABCG2

Inhibitor	IC_{50}	References	Inhibitor	IC_{50}	References
Abacavir	385 µM	Weiss et al. (2007)	Ko132	190–270 nM	Allen et al. (2002)
AG1478		Shi et al. (2009b) and Shi et al. (2009a)	Ko134	85–110 nM	Allen et al. (2002)
Amprenavir	181 µM	Weiss et al. (2007)	Ko143	23–26 nM	Allen et al. (2002)
Atazanavir	69 µM	Weiss et al. (2007)	Lopinavir	8 µM	Weiss et al. (2007)
Biricodar (VX-710)		Minderman et al. (2004)	Nicardipine	4.8 µM	Zhang et al. (2005b)
Cannabinol (CBN)	4.5 µM	Holland et al. (2007)	Nelfinavir	12.5–14 µM	Gupta et al. (2004) and Weiss et al. (2007)
Cannabidiol (CBD)	7.3 µM	Holland et al. (2007)	Novobiocin		Shiozawa et al. (2004) and Yang et al. (2003)
Ciclosporine A	1.4 µM	Gupta et al. (2006), Pawarode et al. (2007) and Xia et al. (2007)	Omeprazole	36 µM	Breedveld et al. (2004)
Chrysin	0.4–4.5 µM	Ahmed-Belkacem et al. (2005), Wang and Morris (2007) and Zhang et al. (2005a)	Pantoprazol	13 µM	Breedveld et al. (2004)
Curcumin I	~1.8 µM	Chearwae et al. (2006)	Phenylchrysin	0.29 µM	Ahmed-Belkacem et al. (2005)
Delavirdine	19 µM	Weiss et al. (2007)	Querceptin	0.28 µM	Yoshikawa et al. (2004)
Dipyridamole	6.4 µM	Zhang et al. (2005b)	Ritonavir	20 µM	Gupta et al. (2004)
Dofequidar fumarate		Katayama et al. (2009b)	Sirolimus	1.53 M	Gupta et al. (2006) and Pawarode et al. (2007)
Efavirenz	21 µM	Weiss et al. (2007)	Saquinavir	20–27 µM	Gupta et al. (2004) and Weiss et al. (2007)
Erlotinib		Shi et al. (2009a)	Tectochrysin	3.0 µM	Ahmed-Belkacem et al. (2005)
GF120918 (elacridar)	50–60 nM	Allen et al. (2002)	Tacrolimus	2.1 µM	Gupta et al. (2006) and Pawarode et al. (2007)
Fumitremorgin C			Delta 9-tetrahydrocannabinol	4.4 µM	Holland et al. 2007
Gefitinib	0.3 µM	Leggas et al. (2006)	Tetrahydrocurumin	6.9 µM	Limtrakul et al. (2007)
Imatinib			PZ-39		Peng et al. (2009)

regeneration; pathophysiological and physiological considerations on that aspect are depicted in detail. Finally, current findings on the role of ABCG2 in drug disposition are delineated in the context of genetic variants in the human organism.

1 ABCG2 Tissue Distribution

Maliepaard et al. were the first to describe localization and protein expression of ABCG2 in different nonmalignant transformed tissues including intestine, liver, kidney, testis, placenta, and the blood–brain barrier (Maliepaard et al. 2001a). Those findings set the stage for studying the implication of ABCG2 in drug absorption, distribution, and elimination. Especially the generation of an *Abcg2*$^{-/-}$ mouse model lacking the expression of the transporter and the identification of function impairing polymorphisms in humans provided important insights into the function of this transporter. Performing various in vivo studies that will be described in detail in the following, ABCG2 was demonstrated to not only enhance biliary excretion of substrate drugs but also to limit the uptake from the intestinal lumen after oral administration. Expression of ABCG2 in human intestinal samples has been extensively studied, suggesting maximal levels in duodenum, which decrease continuously down to the rectum. Importantly, no gender differences were observed in healthy volunteers (Gutmann et al. 2005). Furthermore, no influence of genetic variants on intestinal ABCG2 mRNA or protein expression, but significantly higher protein expression in female samples was reported by Zamber et al. (2003). Importantly they did not find a correlation between mRNA and protein expression in the human samples. This might explain the findings by Urquhart et al., who reported no difference in ABCG2 protein expression comparing biopsy samples obtained from terminal ileum and colon (Urquhart et al. 2008). Not only polymorphisms but also diseases might interfere with the activity of ABCG2. Indeed studies in patients with colitis ulcerosa and obstructive cholestasis demonstrated significantly reduced expression of ABCG2 in intestine (Englund et al. 2007; Zimmermann et al. 2006). In addition, ABCG2 has been demonstrated to function as an important determinant in the pharmacological sanctuary property of several blood–tissue barriers including the blood–brain, the blood–placental, and blood–testis barrier.

2 ABCG2 Substrates and Inhibitors

Since its discovery the list of substances interacting with ABCG2 has steadily increased. Interaction in this context means inhibition and/or direct transport. For most transporters the first line of evidence for a compound being a substrate is its inhibitory capacity, whereas interaction as a real substrate has to be further validated by direct transport assays. Importantly, identification as a transport inhibitor

does not mean the compound is a substrate per se. And a delay of direct transport assays can be caused by the lack of accessibility to sensitive methods for drug quantification.

However, in the case of cytotoxic compounds transporter-mediated efflux can be easily demonstrated by increased drug resistance in a cellular model. Using this approach a variety of anticancer drugs has been described to be transported by ABCG2 including the topoisomerase I inhibitors NB-506, edotecarin (J-10788), and becatecarin (Komatani et al. 2001; Robey et al. 2009), the topoisomerase II inhibitors etoposide and teniposide (Allen et al. 2003), and various camptothecin derivates such as topotecan (Maliepaard et al. 1999), irinotecan (CPT-11) (Maliepaard et al. 2001b), SN-38 (the active metabolite of irinotecan) (Kawabata et al. 2001), diflomotecan (BN80915), and 9-aminocamptothecin (Rajendra et al. 2003). Importantly, there are camptothecin derivates including karenitecin (BNP 1350), gimatecan, and exatecan (DX-891f), where ABCG2 is assumed to play a minor role in drug resistance (Gounder et al. 2008; Ishii et al. 2000; Marchetti et al. 2007; Van Hattum et al. 2002a, b). In addition, cellular overexpression of ABCG2 has been linked to decreased cytotoxicity of bisantrene, and anthracyclins such as daunorubicin, doxorubicin, and epirubicin (Chen et al. 1990; Litman et al. 2000; Robey et al. 2001b). Even if those compounds are often referred to as well-characterized substrates of ABCG2, they are no substrates of the wild-type transporter. Indeed, following studies revealed that drug-selected cancer cells used to identify ABCG2-mediated anthracyclin-resistance carry an acquired mutation of amino acid 482 from arginine to glycine or threonine that changed substrate specificity of the transporter (Honjo et al. 2001; Nakanishi et al. 2003a; Robey et al. 2003). Accordingly, treatment of cancer cells has been demonstrated to result in allele (R482G or R482T) specific gene amplification (Bram et al. 2007), explaining the observed cross-resistance pattern for ABCG2 in previous studies. A similar "false-positive" substrate description has been assumed for the anti-folate drugs methotrexate, ralitrexate (ZD 1694) and GW1843, but the published results on the impact of the acquired ABCG2-mutations (p.ABCG2 R482G, R482T) are not conclusive (Bram et al. 2006; Breedveld et al. 2007; Chen et al. 2003; Mitomo et al. 2003; Shafran et al. 2005; Volk and Schneider 2003). It seems noteworthy at this point, that recent findings in mice suggest an involvement of Abcg2 in the in vivo kinetics of methotrexate (Vlaming et al. 2009b, c). However this will have to be further elucidated.

Apart from the above described issue on mutated ABCG2, tyrosine kinase inhibitors are well characterized for their interaction with the transporter. Particularly, gefitinib an inhibitor of the EGFR tyrosine kinase has been extensively studied in this perspective. On the one hand this compound has been found to inhibit ABCG2 mediated drug resistance in various cancer models (Nakamura et al. 2005; Yanase et al. 2003). On the other hand resistance to gefitinib has been associated with ABCG2 expression (Elkind et al. 2005; Katayama et al. 2009a; Sugimoto et al. 2005), suggesting that gefitinib not only functions as inhibitor but also as substrate of the transporter (Noguchi et al. 2009). Based on the inhibitory capacity gefitinib has been evaluated as potential therapeutic strategy to modulate

transporter mediated changes in drug disposition. First results in mice showed that concomitant administration of gefitinib significantly increases oral bioavailability of the anti-cancer drug irinotecan (Stewart et al. 2004). However, gefitinib appears to function as dual ABCG2/ABCB1-inhibitor (Leggas et al. 2006), this should be part of the interpretation of those data. Similar to gefitinib, the EGFR inhibitor erlotinib was found to be transported by ABCG2 (Li et al. 2007; Shi et al. 2007; Shi et al. 2009a). The specific inhibitor of the bcr-able tyrosine kinase imatinib was first thought to only modulate ABCG2-meditated transport by inhibition (Houghton et al. 2004; Ozvegy-Laczka et al. 2004), leading to increased cytotoxicity of concomitantly given anti-cancer drugs (Liu et al. 2007; McDowell et al. 2007; Takigawa et al. 2007). However, following studies confirmed that ABCG2 directly transports imatinib in vitro and in vivo (Breedveld et al. 2005; Brendel et al. 2007; Burger et al. 2004; Gardner et al. 2009). Similarly the Pan ErbB-Inhibitor carnetinib (CI1033) and second-generation bcr-abl tyrosine kinase inhibitors nilotinib and desatinib were identified as inhibitors and substrates of ABCG2 (Brendel et al. 2007; Hegedus et al. 2009a; Hiwase et al. 2008; Tiwari et al. 2009). In contrast to the above mentioned the tyrosin kinase inhibitors bosutinib and lapatinib function as inhibitors, but are not transported by ABCG2 (Dai et al. 2008; Hegedus et al. 2009a). Similar results are shown for the multikinase inhibitor sunitinib (Dai et al. 2009; Shukla et al. 2009; Hu et al. 2009). However, pharmacogenomic pathway analysis identified mutations in ABCG2 (*ABCG2*-15622C/T and *ABCG2*c.1143C/T haplotype) as risk factors for sunitinib associated toxicities (van Erp et al. 2009). Similar contradictory results have been gathered concerning the multikinase inhibitor sorafenib. While in vivo experiments using knockout animals suggest that Abcg2 modulates brain penetration (Lagas et al. 2010), in vitro experiments on direct transport are not conclusive (Hu et al. 2009; Lagas et al. 2010), and need to be further evaluated. Including the potential influence of species related differences in substrate recognition.

In addition, to the above mentioned anti-cancer drugs several non-chemo-therapeutics have been identified as substrates of ABCG2, including antivirals, 3-Hydroxymethylglutary (HMG)-Coenzyme A (CoA) reductase inhibitors (statins), antibiotics, calcium channel blockers, and others (summarized in Table 1). ABCG2 has also been shown to transport several dietary constituents which in part exhibit carcinogenic effects including aflatoxin B, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP), benzo[a]pyrene-metabolites amino-3-methylimidazo[4,5-f]quinoline (IQ), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) (Ebert et al. 2005; Enokizono et al. 2008; van Herwaarden et al. 2003; van Herwaarden et al. 2006). In addition, fluorescent substrates such as Hoechst 33342 (Kim et al. 2002; Scharenberg et al. 2002), BODIPY FL prazosin (Robey et al. 2001a), DyeCycle Violet (Mathew et al. 2009; Telford et al. 2007) and photosensitizers pheophorbide a (PhA) (Henrich et al. 2006; Jonker et al. 2002; Robey et al. 2004; Tamura et al. 2008), pheophorbide a methyl ester and chlorine e6 (Robey et al. 2005) which are directly transported, represent important tools to measure ABCG2 transport activity in vitro.

Also important tools for studying ABCG2 are inhibitors of the transporter. The first fully characterized highly potent inhibitor of ABCG2 was the tremorgenic mycotoxin fumitremorgin C (FTC), that was originally isolated from *Aspergillus fumigatus* (Rabindran et al. 1998, 2000). Due to its neurotoxic potency FTC analogues with similar potency have been developed (van Loevezijn et al. 2001). Particularly the tetracyclic FTC analogues Ko132, and Ko134, and Ko143 appeared to be less neurotoxic, exhibited an ABCG2-specific inhibition profile (Allen et al. 2002), and are currently widely accepted as potent inhibitors. Since the description of FTC the list of ABCG2 inhibitors has been extended. Several compounds including butorylamides and synthetic analogs of butorylamide F (Takada et al. 2010), dimethoxyaurones (Sim et al. 2008), non-basic chalcone analogues (Han et al. 2008b), acridones (Boumendjel et al. 2007), ginsenosid metabolites (Jin et al. 2006), piperazinobenzopyranones, and phenalkylaminobenzopyranones (Boumendjel et al. 2005), several synthesized dihydropyridines (Zhou et al. 2005c), flavonoids (silymarin, hesperetin, quercetin, and daidzein) (Yoshikawa et al. 2004) and the stilbene resveratrol (Cooray et al. 2004) have been evaluated for their in vitro and/or in vivo inhibitory potency. In addition to those compounds several commonly used inhibitors are summarized in Table 2.

3 Abcg2 Function In Vivo: Data from Mouse Models

Mouse models are widely accepted as an important tool to identify the role of transporters in drug disposition. Even if species-related differences in substrate recognition have been described for several ABC transporters, so far there are no such data for the herein described ABCG2 transporter. However, for the better interpretation of the following section on ABCG2 function obtained by using genetic deletion or chemical inhibition of Abcg2 in vivo it should be mentioned that several studies point to significant species-related differences in expression of this particular transporter. First of all compared to humans, mice have been shown to express high levels of Abcg2 mRNA in kidney and only moderate levels in placenta (Allen et al. 1999). This suggests that findings on urinary excretion might be exaggerated, while the impact on feto-maternal transfer might be underestimated. Supporting this assumptions are findings comparing the influence of the ABCG2 inhibitor chrysin on pharmacokinetics of nitrofurantoin in mice and rats. In line with previous results showing higher mRNA expression in the small intestine in rats (Tanaka et al. 2005), these animals exhibited significantly higher susceptibility to the intestinal drug interaction compared to mice (Kawase et al. 2009). In the blood–brain barrier mRNA expression of ABCG2 appears lower in humans compared to commonly used animal models (Warren et al. 2009). However, data directly comparing the expression of ABCG2 in tissues of different species are rather limited. A recent study introducing absolute quantification of ABCG2 performing LC-MS/MS might have set the stage for similar studies in other tissues than liver. In the study by Li et al. ABCG2 protein expression was higher in rats and dogs

compared to human samples (Li et al. 2009b). However, these findings are in contrast to previous results comparing the phephorbide A efflux capacity in hepatocytes, showing highest efflux capacity isolated from humans and monkeys and very low Abcg2 associated efflux capacity in those isolated from rat and dog (Li et al. 2008).

3.1 Abcg2 and Oral Bioavailability

The first evidence pointing to additional efflux transporters limiting oral bioavailability were studies in *Mdr1a/Mdr1b*$^{-/-}$ mice that reported modulation of exposure using dual ABCB1/ABCG2 inhibitors. One example is the study by Jonker et al. showing that the dual ABCB1/ABCG2 inhibitor elacridar (GF120918) resulted in significantly increased oral bioavailability of topotecan in *Mdr1a/Mdr1b*$^{-/-}$ mice (Jonker et al. 2000). Data from this study indicated that the overall increase of topotecan exposure resulted from a combination of increased intestinal absorption and reduced hepatobiliar excretion. Similar results were obtained after oral coadministration of topotecan with the highly efficient ABCG2 inhibitor Ko143 (Allen et al. 2002). The role of Abcg2 as a limiting factor of oral topotecan uptake was further supported by ex vivo experiments demonstrating a twofold increase of the absorption rate in mice lacking Abcg2. In the same study the authors reported a 3.6-fold higher topotecan exposure in *Abcg2*$^{-/-}$ animals after oral administration of topotecan in vivo (Yamagata et al. 2007). Translation of those preclinical findings suggesting that inhibition of intestinal efflux transporters might result in enhanced bioavailability was conducted performing a clinical proof-of-concept study. In this study the participants received topotecan (1 mg/m^2) in combination with elacridar (1,000 mg), which enhanced oral bioavailability. However, in the human study elacridar only exhibited a moderate effect on the clearance of the anti-cancer drug (Kruijtzer et al. 2002).

Several other studies using the *Abcg2*$^{-/-}$ mouse model provided evidence for the role of Abcg2 in intestinal drug absorption. Increased intestinal uptake was reported for antibiotics (Merino et al. 2005b, 2006), ME3229, a prodrug of the hydrophilic glycoprotein IIb/IIIa antagonist ME3277 (Kondo et al. 2005), the anti-inflammatory drug sulfasalazine (Zaher et al. 2006), and the CDK inhibitor JNJ-7706621 (Seamon et al. 2006). In addition, exposure to a number of dietary constituents was identified to be influenced by Abcg2 including carcinogens such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (Jonker et al. 2005; van Herwaarden et al. 2003), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) (van Herwaarden et al. 2006).

It seems noteworthy that there are a number of previously in vitro identified ABCG2 substrates, where pharmacokinetic analysis using the *Abcg2*$^{-/-}$ mouse models did not reveal modulation of bioavailability. Etoposide resistance has been repeatedly reported to be associated with ABCG2-mediated efflux (Chen et al. 1990; Kellner et al. 1997; Nakagawa et al. 1992); however, testing the influence of

elacridar on oral bioavailability in *Mdr1a/Mdr1b*$^{-/-}$ mice, which has previously proven to be an excellent indicator for the involvement of Abcg2, has failed to show an effect on oral bioavailability or clearance of this compound (Allen et al. 2003). Further examples are the HIV drugs abacavir (ABC) and zidovudine (AZT), which were transported by murine Abcg2 and inhibited human ABCG2 in vitro (Pan et al. 2007; Weiss et al. 2007). In accordance, with the assumption of ABCG2 playing a role in the cellular accumulation of at least ziduvodine was the finding that increased ABCG2 expression resulted in reduced antiviral activity in CD4^{+} T-cells that was reversed by concomitant treatment with FTC (Wang et al. 2003; Wang and Baba 2005). However, the following determination of pharmacokinetic parameters of abacavir and zidovudine in plasma and brain comparing wild-type and *Abcg2*$^{-/-}$ mice did not reveal any changes (Giri et al. 2008).

Similarly contradictory are the results of the tyrosine kinase inhibitor imatinib. Even if imatinib has been repeatedly reported as a substrate of Abcg2/ABCG2, comprehensive ADME analysis using *Abcg2*$^{-/-}$, *Mdr1a/Mdr1b*$^{-/-}$, and compound knockout animals revealed no significant influence on oral absorption of this substance. However, coadministration of the ABCG2 inhibitors pantoprazol and elacridar resulted in increased exposure in wild-type and knockout animals after oral administration, which has been explained by reduced clearance of imatinib. This phenomenon appears to be independent from Abcg2 and Mdr1a/Mdr1b (Breedveld et al. 2005; Oostendorp et al. 2009). However, another study reported that coadministration of the dual ABCB1/ABCG2 inhibitor tariquidar increased exposure to imatinib in mice (Gardner et al. 2009), which might also be caused by another mechanism. Performing a similar comprehensive ADME analysis again using *Abcg2*$^{-/-}$, *Mdr1a/Mdr1b*$^{-/-}$, and compound knockouts revealed that intestinal efflux of desatinib mainly depends on Mdr1a/Mdr1b even if this compound is transported by ABCG2 in vitro (Lagas et al. 2009b). In conclusion using the *Abcg2*$^{-/-}$ mouse model has provided important insights into the intestinal function of Abcg2, but the impact on oral bioavailability of a certain compound depends on its interaction with other intestinal efflux transporters such as Mdr1a/Mdr1b.

3.2 Abcg2 and Biliary Excretion

ABCG2 is expressed in the canalicular membrane of hepatocytes, and is therefore suggested to be part of the excretory machinery in liver. Indeed, numerous studies demonstrated the function of this transporter in the elimination of substrate drugs. Comparing biliary excretion in wild-type and *Abcg2*$^{-/-}$ animals revealed decreased clearance of methotrexate (Breedveld et al. 2004; Vlaming et al. 2009b), whereas no effect was seen for 7-OH-methotrexate (Vlaming et al. 2009b) and troglitazone sulphate (Enokizono et al. 2007b). Similar results were obtained for the hepatobiliary excretion of the HMG-CoA-reductase inhibitors pitavastatin (Hirano et al. 2005) and rosuvastatin (Kitamura et al. 2008) and several fluorchinolones (Ando

et al. 2007; Merino et al. 2006). Importantly, testing the expression of Abcg2 in mice revealed marked sex differences, suggesting higher activity of the transporter in male mice. Indeed, biliary excretion of known ABCG2 substrates including nitrofurantoin, PhiP, and cimetidine showed pronounced gender differences in *in vivo* studies supporting this notion (Merino et al. 2005c).

3.3 Abcg2 and the Blood–Brain Barrier

Although ABCG2 had been described in brain capillary endothelial and its apical localization had been suggested to modulate uptake of substrate drugs into the central nervous system (Cooray et al. 2002; Eisenblatter et al. 2003; Zhang et al. 2003), the current understanding of ABCG2 in the blood–brain barrier is still limited. In order to elucidate the impact of Abcg2 on cerebral drug accumulation various animal experiments have been performed. Although substrates exclusively transported by Abcg2 such as dantrolene or the phytoestrogens daidzein, genistein, and coumestrol exhibited remarkably increased brain accumulation after continuous infusion comparing wild-type and *Abcg2*$^{-/-}$ mice (approximately tenfold increase) (Enokizono et al. 2007b, 2008), the interpretation of data obtained for other Abcg2 substrates was often complicated by their affinity to ABCB1 (Mdr1a/Mdr1b) (Vlaming et al. 2009a).

Cisternino et al. were the first to suggest a function of Abcg2 in limiting the uptake of prazosin and mitoxantrone based on their findings of significantly increased brain accumulation in *Mdr1a*$^{-/-}$ mice treated with the known ABCB1/ABCG2 inhibitor elacridar (Cisternino et al. 2004). However, following experiments comparing the impact of elacridar on mitoxantrone accumulation performing in situ brain perfusion experiments employing *Abcg2*$^{-/-}$ and *Mdr1a/Mdr1b*$^{-/-}$ mice did not support this assumption (Lee et al. 2005) and suggested that ABCB1 plays the major role in limiting mitoxantrone uptake. Even if in vitro experiments suggested affinity of cimetidine, alfuzosin, dipyridamole, and LY2228820 to ABCG2, there was no detectible influence of the transporter on the cerebral accumulation of those substances (Zhao et al. 2009).

Even if no impact on oral bioavailability and biliary excretion was identified, clearly synergistic effects of Mdr1a/1b and Abcg2 were demonstrated for imatinib in the blood–brain barrier. This is in accordance with findings showing that this compound is a substrate of both transporters (Hamada et al. 2003; Ozvegy-Laczka et al. 2004). In detail, intravenous administration of imatinib resulted in increased brain penetration in *Abcg2*$^{-/-}$ (2.5-fold) and *Mdr1a/Mdr1b*$^{-/-}$ (3.6-fold) compared to wild-type animals. And the ABCG2 inhibitor pantoprazol increased accumulation in wild-type and *Mdr1a/Mdr1b*$^{-/-}$, but not in *Abcg2*$^{-/-}$ animals (Breedveld et al. 2005). Based on in situ brain perfusion experiments it has been suggested that in lower (<1 μM) concentrations Mdr1a mainly governs efflux of imatinib as no difference was observed at this concentration comparing wild-type and *Abcg2*$^{-/-}$ mice. However, deletion of the transporter significantly influences

cerebral accumulation at higher concentrations of imatinib (1 μM or ~20 μM). This phenomenon has been explained by saturation of Mdr1a/Mdr1b (Bihorel et al. 2007). A similar impression has been obtained for the dietary carcinogen PhiP (1-amino-1-methyl-6-phenylimidazp[4,5-b]pyridine). Although short-term in situ brain perfusion did not reveal differences between wild-type and *Abcg2*$^{-/-}$ mice, prolonged exposure with continuous infusion resulted in modest but detectible higher cerebral accumulation in animals lacking the transporter (Enokizono et al. 2008). In summary, findings on the influence of Abcg2 highly depend on the concentration, time of exposure, and certainly the affinity of the tested substrate to Mdr1a/Mdr1b.

Generation of a triple knockout mouse clearly demonstrated the role of Abcg2 in the blood–brain barrier. Indeed, treatment with topotecan resulted in modest elevation in cerebral drug disposition in either *Abcg2*$^{-/-}$ or *Mdr1a*/*Mdr1b*$^{-/-}$ mice (1.5-fold compared to wild-type mice), whereas the compound increased significantly in the Mdr1a/Mdr1b/Abcg2$^{-/-}$ mouse (12-fold increase), suggesting that both transporter function as compensatory mechanism in the case of absence of one at the blood–brain barrier (de Vries et al. 2007). Similar results were obtained for lapatinib (Polli et al. 2009), desatinib (Chen et al. 2009), and imatinib (Oostendorp et al. 2009). In contrast to those compounds, where Abcg2 appears to be the "secondary" mechanism, are findings for sorafenib. Performing experiments using the same animal models revealed increased cerebral accumulation in association with the loss of Abcg2 expression (4.3-fold increase), which dramatically increased in triple (9.3-fold increase) knockout animals, while no effect was seen in the *Mdr1a*/*Mdr1b*$^{-/-}$ mouse model (Lagas et al. 2010). Although Abcg2 and Mdr1a/Mdr1b are widely accepted as mechanisms reducing the uptake of substrates into the extracellular cerebral fluid, it is assumed that both transporters enhance accumulation in the ventricular cerebrospinal fluid. This has been first suggested by Zhuang et al. measuring topotecan levels in both cerebral compartments after coadministration of the dual ABCB1/ABCG2 inhibitor gefitinib (Zhuang et al. 2006). This assumption of different functions in brain compartments is supported by recent findings of Shen et al. reporting a 3.5-fold reduction of topotecan in the ventricular cerebrospinal fluid of triple knockout animals (Shen et al. 2009). The overall interpretation of the previously mentioned findings in murine models has been recently summarized by Lagas et al., who introduced another obviously important question concerning the expression levels of Abcb1 (Mdr1a/Mdr1b) and Abcg2 in the blood–brain barrier (Lagas et al. 2009c). It had been shown previously that mice exhibit threefold higher Abcb1 than Abcg2 protein levels in brain capillaries (Kamiie et al. 2008). Examining human brain capillaries obtained from patient samples (epilepsia/gliomas) revealed eightfold higher mRNA levels of ABCG2 compared to ABCB1. Excluding the possibility that those findings were related to the underlying disease or associated drug therapy it might be concluded that the contribution of ABCG2 in the human blood–brain barrier is underestimated extrapolating the function from animal studies.

3.4 Abcg2 in the Feto-Maternal Barrier

Abcg2 is highly expressed in the apical membrane of the syncytiotrophoblast the outer layer of the placental villi (Maliepaard et al. 2001a). Based on its localization and its efflux function it would be assumed that the transporter limits fetal exposure to substrate drugs. The first to demonstrate this function in placenta were Jonker et al. showing a twofold increase in the relative uptake of topotecan into the fetal circulation in *Mdr1a/Mdr1b*$^{-/-}$ mice treated with elacridar (Jonker et al. 2000). The implication of the transporter in the fetal protection system is further supported by ex vivo studies using dually perfused placental tissue. Chemical inhibition of Abcg2 has been demonstrated to significantly increase fetal recovery and reduce fetal elimination of cimetidine, BODY FL prazosin, and glyburide in rat placenta (Cygalova et al. 2009; Staud et al. 2006). Similar results were obtained for the antidiabetic drug glyburide and the food-derived carcinogens PhiP and nitrosodimethylamine (NDMA) in perfused human placenta (Annola et al. 2009; Kraemer et al. 2006; Myllynen et al. 2008; Pollex et al. 2008). In accordance are findings using pregnant *Abcg2*$^{-/-}$ mice, showing that the deletion of this transporter significantly increased maternal-to-fetal transfer of Abcg2 substrates such as topotecan, genistein, nitrofurantoin, and glyburide (Enokizono et al. 2007a; Jonker et al. 2002; Zhang et al. 2007; Zhou et al. 2008b).

Assessment for mechanisms modulating ABCG2 expression in human placenta revealed significantly lower levels associated with the naturally occurring genetic polymorphism *ABCG2* c.421C>A (Kobayashi et al. 2005). However, conflicting data exist on the impact of gestational age on the expression of ABCG2 (Mathias et al. 2005; Meyer zu Schwabedissen et al. 2006; Yasuda et al. 2005; Yeboah et al. 2006), and no impact was seen for smoking history of the mother (Kolwankar et al. 2005).

3.5 Abcg2 in the Lactating Mammary Gland

The efflux transporter has been detected in alveolar epithelial cells of the mammary gland. Importantly, apical expression of the transporter significantly increases during lactation, suggesting that this transporter might be involved in drug accumulation in breast milk (Alcorn et al. 2002; Jonker et al. 2005). Several substances had previously been reported to significantly accumulate in breast milk suggesting active transport to be involved in this process, including the known ABCG2 substrates acyclovir, cimetidine, nitrofurantoin, and PhIP resulting in plasma-to-milk ratios of 5:1, 29:3, 31:1, and 9:3, respectively (Alcorn and McNamara 2002; Jagerstad et al. 1994; McNamara et al. 1996; Oo et al. 2001). Assessment for the transfer of acyclovir, topotecan, nitrofurantoin, ciprofloxacin, PHiP and other dietary-derived carcinogens revealed significantly lower accumulation in breast milk of animals lacking Abcg2 (Jonker et al. 2005; Merino et al. 2005b, 2006;

van Herwaarden et al. 2006). In addition to the exposure of a breast-fed baby to drugs and carcinogens, Abcg2 has been shown to govern uptake of riboflavin (vitamin B2) into breast milk, resulting in 22-fold lower plasma-to-milk ratios in animals lacking the transporter (van Herwaarden et al. 2007).

4 ABCG2 a Contributor to Multidrug Resistance

As mentioned previously the evolution of ABCG2 as a determinant of drug disposition started with the identification of its involvement in multidrug resistance (MDR) of cancer cells. The phenomenon of developing global resistance to chemotherapy in clinical cancers has been a focus of extensive basic research to uncover the underlying biological causes. One of the enduring mechanisms of MDR is the efficient cellular efflux of anticancer drugs mediated by membrane-based transport proteins, and ABCG2 is assumed to be one of the contributors. Indeed, selection of cancer cells with antitumor drugs such as mitoxantrone, doxorubicin, topotecan, karenitecin (BNP1350), SN-38 (irinotecan), and flavopiridol results in significant induction of ABCG2, translating into lower cellular accumulation and prevention of anticancer drugs to reach their intracellular targets (Honjo et al. 2002; Kawabata et al. 2001; Maliepaard et al. 1999; Robey et al. 2001b; Schellens et al. 2000; Takara et al. 2009; Van Hattum et al. 2002b; Yang et al. 2000). Several mechanisms have been identified to be involved in developing ABCG2-mediated chemo-resistance including demethylation of CpG islets in the ABCG2 promoter resulting in increased gene transcription (Bram et al. 2009; Turner et al. 2006), gene amplification (Bram et al. 2007), and truncation at the 3′UTR of the ABCG2 mRNA, which is associated with a loss of the miRNA-159c binding site conferring higher mRNA stability (Apati et al. 2008; Sandberg et al. 2008; To et al. 2008, 2009).

In order to elucidate the influence of an efflux transporter on the overall outcome of cancer patients several groups have analyzed ABCG2 expression and its impact on clinical parameters. Particularly, leukemias have been focus of those studies. Without claiming completeness in mentioning all the studies conducted in this context, ABCG2 expression has been repeatedly reported to translate into poor response to chemotherapy or worse clinical prognosis in patients with acute myeloic leukemia (AML) (Benderra et al. 2004, 2005; Nakanishi et al. 2003b; Steinbach et al. 2002; Uggla et al. 2005; van den Heuvel-Eibrink et al. 2002). However, others did not see such an association in their clinical samples of AML patients (van der Kolk et al. 2002; van der Pol et al. 2003). In general AML blast cells consist of different subpopulation, and recent studies suggest that the more primitive blasts summarized in the $CD34^{+}CD38^{-}$ subpopulation exhibit significantly higher levels of ABCG2 compared to more mature cells (Ho et al. 2008; Shman et al. 2008; van den Heuvel-Eibrink et al. 2007). Considering this phenomenon suggests that the inconsistence in findings on clinical AML samples might be due to different amounts of primitive blasts in patient samples. It has been suggested that the more

primitive subpopulation is responsible for treatment failure due to its natural drug resistance and function as leukemia stem cells (Figueiredo-Pontes et al. 2008). However, in childhood acute lymphoid leukemia (ALL) a correlation of the transporter and clinical outcome has yet not been detected (Kourti et al. 2007; Sauerbrey et al. 2002; Stam et al. 2004; Swerts et al. 2006).

In addition to leukemias, ABCG2 expression has been studied in several solid tumors (recently summarized in Ross and Nakanishi 2010). Comparing tumor samples pre- and post treatment revealed increased expression in hepatoblastomas and colon adenocarcinomas (Candeil et al. 2004; Vander et al. 2008). Similar results were obtained in multiple myeloma (MM) patients treated with topotecan (Turner et al. 2006). However, in vitro studies using MM cell lines suggested that ABCG2 expression does not result in induced irinotecan resistance (Yano et al. 2008). It seems noteworthy at this point, that comparing expression of ABCG2 in cell lines commonly used in laboratories revealed considerable high expression independent of prior drug selection and the primary origin of the cancer (Imai et al. 2002).

Several groups have determined the role of ABCG2 in non small cell lung cancer (NSCLC), as it has been demonstrated that this transporter confers resistance to topoisomerase I inhibitors in NSCLC-derived cell lines (Bessho et al. 2006; Kawabata et al. 2003; Nagashima et al. 2006). Importantly, the resistance was reversible, culturing the cells in absence of the selecting agent and/or by concomitant treatment with ABCG2 inhibitors, suggesting a potential role of the efflux transporter in the response and outcome of NSCLC. Indeed, it has been reported that patients with ABCG2-positive NSC lung cancers (grade IIIB or IV) exhibited a lower response rate compared to those with ABCG2-negative biopsies (Yoh et al. 2004). However, following studies did not reveal a predictive value of ABCG2 expression on treatment outcome with platinum-based drugs (Li et al. 2009a; Ota et al. 2009; Surowiak et al. 2008). ABCG2 expression being no prognostic indicator has also been shown for ovarian cancer (Nakayama et al. 2002), locally advanced bladder cancer (Diestra et al. 2003), medulloblastomas and high-grade gliomas (Valera et al. 2007), and breast cancer (Faneyte et al. 2002). In contrast to that, ABCG2 expression has been identified as a prognostic factor for treatment outcome and progression-free survival in small cell lung cancers (Kim et al. 2009), poorer overall survival following surgery in esophageal squamosa cell carcinoma (Tsunoda et al. 2006).

Based on findings that ABCG2 might limit cellular accumulation of dietary-derived carcinogen and the identification of impaired function genetic variants, this transporter has been evaluated as a risk factor for tumor development and progression in several tumor entities including renal cell carcinoma (RCC), diffuse large B-cell lymphoma (DLBCL), androgen-independent prostate cancer (AIPC), and colorectal cancer (CRC). No effect of the frequently occurring *ABCG2* c.421C>A variant was seen for AIPC or CRC (Andersen et al. 2009; Gardner et al. 2008). However, in patients with RCC the frequency of the wild-type C allele was significantly higher compared to control subjects (age- and gender-adjusted OR = 1.96, 95% CI 1.32–2.93), but there were no associations between the c.421C>A

polymorphism and clinicopathologic factors (Korenaga et al. 2005). In contrast the 421A allele was associated with higher risk for DLBCL (OR = 1.49, 95% CI 1.02–2.17) especially in patients with younger age (below 50) (OR = 2.14, 95% CI 1.25–3.68), whereas the 421CC genotype was associated with poorer survival of DLBCL in patients younger at diagnosis (HR = 5.80, 95% CI 1.16–28.90) or with bulky tumor (HR = 4.36, 95% CI 1.04–18.31) (Hu et al. 2007).

5 ABCG2 a Marker of Cancer Stem Cells

In the section on ABCG2 as contributor to multidrug resistance it has been mentioned that this transporter has been identified to be highly expressed in a primitive subpopulation of AML blasts and that those cells are considered to be multidrug resistant leukemia stem cells (LSC). In general, it is hypothesized that tumors possess a minor proportion of so-called cancer stem/initiating cells. Those cells appear highly resistant to chemotherapy and are able to initiate disease recurrence and progression. LSC have been first described by Dick et al., who reported that the immature LSC of AML reside within the $CD34^{+}$ $CD38^{-}$ subpopulation; those cells are competent in engrafting in immunosuppressed mice, thereby transmitting AML. Those results are the basis for the cancer stem cell hypothesis, which postulates that tumors are maintained by a small minority of stem-like cancer cells, which possess the capacity for indefinite self-renewal (summarized by Misaghian et al. 2009). Several mechanisms are discussed to be involved in the drug resistance of LSCs. First the quiescent, noncycling state of LSCs, which remain most of the time in the G_0 phase of the cell cycle, induces resistance to conventional antitumor agents targeting the cell cycle machinery. Secondly it has been speculated that LSCs exhibit a highly active migration machinery that allows them to escape growth inhibition induced by the microenvironment in the stem cell niche. And third several transporters limiting the accumulation of anticancer agents have been shown to be highly expressed in cancer stem cells (CSCs) (Misaghian et al. 2009). One commonly used phenotypic marker (side population phenotype) of cancer stem cells is their capacity to extrude Hoechst 33342, a well-known substrate of ABCG2. In accordance with previous findings in AML blast cells, a primitive tumorigenic $ABCG2^{+}$ subpopulation has also been isolated from various tumor entities including NSCLC (Bertolini et al. 2009; Chen et al. 2008; Ho et al. 2007a), melanomas (Monzani et al. 2007), prostate cancer (Mathew et al. 2009), oral squamous cell carcinoma (Zhang et al. 2009), retinoblastomas (Balla et al. 2009; Seigel et al. 2005), ovarian carcinoma (Zhang et al. 2008), and neuroblastomas (Hirschmann-Jax et al. 2004). In addition to carcinoma tissue samples, CSCs have also been demonstrated in cancer cell lines derived from aerodigestive squamous cell carcinomas (Loebinger et al. 2008), hepatocarcinomas (Hu et al. 2008; Shi et al. 2008; Zhu et al. 2010), human bladder transitional cell cancers (Ning et al. 2009), pancreatic carcinomas (Wang et al. 2009; Zhou et al. 2008a), oral squamous cell carcinoma (Yajima et al. 2009), neuroblastoma (Mahller et al. 2009), osteosarcoma

(Tirino et al. 2008), esophageal carcinoma (Huang et al. 2009), breast carcinoma (Christgen et al. 2007), and head and neck squamous carcinomas (Chen et al. 2006), suggesting that this is not a tumor entity specific phenomenon. Chemical inhibition of ABCG2 is therefore assumed to be a possible therapeutic strategy to improve chemotherapeutic targeting of CSCs giving rise to studies focusing on inhibitors of the efflux transporters.

6 Side Population Phenotype in Stem Cells is Determined by ABCG2 Expression and Activity

In addition to cancer stem cells, ABCG2 expression and function is assumed to be a conserved feature of nonmalignant transformed stem cells summarized in the side population (SP). Those SP cells have been isolated from several tissues including myometrium (Ono et al. 2007), skeletal muscle (Uezumi et al. 2006), mammary gland (Alvi et al. 2003), heart (Oh et al. 2004), pancreas (Lechner et al. 2002), and liver (Hussain et al. 2005). However, the role of ABCG2 in these cells is currently not well understood. Hitherto, hematopoetic stem cells (HSC) as a highly active regenerative cell population have been focus of some studies concerning the function of ABCG2. Importantly, expression of ABCG2 appears to be regulated during the process of differentiation. Indeed, although bone marrow cells ($CD34^{-}$ c-Kit^{+}, Sca-1^{+}, Lin^{-}) have been shown to express high levels of ABCG2, those are significantly reduced in more mature $CD34^{+}$ cells (Scharenberg et al. 2002; Zhou et al. 2001). Then expression of ABCG2 is sharply upregulated again during erythroid differentiation, so that higher levels of the protein are detected in mature red blood cells (Zhou et al. 2005b). In general, it has been suggested that ABCG2 plays a role in the hematopoetic development and/or protection of HSCs against environmental noxes. Consistently with the latter hypothesis HSCs from *Abcg2*$^{-/-}$ mice have proven to be significantly more sensitive to mitoxantrone in drug-treated transplanted mice. But mice lacking *Abcg2* exhibit normal haematopoetic development and peripheral blood counts (Zhou et al. 2003), leading to the assumption that Abcg2 might not be essential for the differentiation itself. However, Jonker et al. found that *Abcg2*$^{-/-}$ mice displayed a previously unknown type of protoporphyria, where erythrocyte levels of protoporphyrin IX were increased about tenfold (Jonker et al. 2002). Consistently, erythroid cells engineered to express high levels of Abcg2 had significantly lower intracellular levels of this particular protoporphyrin (Zhou et al. 2005b). In addition, Abcg2 has been demonstrated to be linked to the survival of progenitor cells during hypoxia, which is closely linked to heme biosynthesis in those cells (Krishnamurthy et al. 2008). In conclusion it seems likely that ABCG2 plays a role in protection of stem cells against noxes. This needs to be considered in developing therapeutic strategies targeting chemoresistant cancer stem cells; however, the physiologic function of this transporter in progenitor cell-derived tissue regeneration needs to be further elucidated.

7 Pharmacogenomics of ABCG2

Single nucleotide polymorphisms (SNPs) of drug transporters, especially those resulting in amino acid exchanges, have been widely studied for their implication in drug disposition and therefore modification of drug response and susceptibility to drug-induced side effects. Considering the localization and function of ABCG2 as described in the previous sections, changes in transport activity would be expected to result in higher oral bioavailability due to its involvement in intestinal drug efflux, and increased plasma levels due to its implication in hepatobiliary secretion and its function in blood–tissue barriers.

The ABCG2 gene is located on chromosome 4q22 spans over 66 kb and consists of 16 exons, ranging from 60 to 532 bp (Bailey-Dell et al. 2001). Sequencing of the ABCG2 gene from human samples revealed over 80 naturally occurring sequence variations (Backstrom et al. 2003; Bosch et al. 2005; Honjo et al. 2002; Iida et al. 2002; Imai et al. 2002; Itoda et al. 2003; Kobayashi et al. 2005; Lee et al. 2007; Mizuarai et al. 2004; Zamber et al. 2003). To date there are 26 nonsynonymous, five synonymous (c.114T>C, c.369C>T, c.474C>T, c.1098G>A, and c.1425A>G) polymorphisms, three nonsense mutations (Q126X, E334X, and R575X), and one frameshift mutation (c.1515delC) described in healthy individuals or patients (compare Table 3). There is profound variability in the minor allele frequencies (AF) of those polymorphisms among populations of different ethnicities, and some of the polymorphisms have been described in single individuals only, such as the c.616A>C, p.I206L (Zamber et al. 2003), the c.2062G>A (p.D620N) (Honjo et al. 2002), and the frameshift mutation c.1515delC (p.AFFVM505-509 ASSLstop) (Itoda et al. 2003).

SNPs with low AF including the c.1291T>C (p.F431L, AF 0.006 in Japanese populations), or the c.1768A>T (p.N590Y, AF 0.001-0.003 in Caucasian populations) mutation have been studied for their activity in vitro showing reduced resistance toward known substrates such as SN-38, mitoxantrone, or topotecan, respectively (An et al. 2009; Itoda et al. 2003; Mizuarai et al. 2004; Tamura et al. 2006; Vethanayagam et al. 2005; Yoshioka et al. 2007; Zamber et al. 2003). Reduced transport activity was also demonstrated for the c.1322G>A (p.S441N, AF 0.001 in Japanese population) variant. This phenomenon was linked to reduced expression of the protein, which appears to be due to ubiquitin-mediated protein degradation in proteasomes (Itoda et al. 2003; Kondo et al. 2004; Nakagawa et al. 2008; Tamura et al. 2006, 2007). Similar results were obtained for the c.623T>C (p.F208S, AF not determined) variant (Nakagawa et al. 2008; Tamura et al. 2006). No effect on the in vitro transport activity was seen for the missense mutations c.445G>C (p.A149P; AF 0.01), c.458C>T (p.T153M; AF 0.033) c.496C>G (p.Q166E, AF not determined) c.616A>C (I206L AF not determined), c.488G>A (p.R163K AF 0.006), c.805C>T (p.P269S AF 0.006), and c.1711T>A (p.F571L, AF 0.005) (Kondo et al. 2004; Tamura et al. 2006).

Particularly the nonsynonymous polymorphism c.ABCG2 421C>A (p.141Q>K, rs2231142) that results in a glycine-to-lysine amino acid change at

Table 3 Summary on currently known polymorphisms located in the coding region of ABCG2

rs-Number	Position[a]	Location	NE	AAE	Frequency	In vitro effect
rs2231127	34	Exon 2	G>A	V12M	0.02–0.64	Lower expression in transfected cells, no effect on transport activity (Tamura et al. 2006)
	38	Exon 2	C>T	S13L	0.03 (Maekawa et al. 2006)	Not determined
rs12721640	114	Exon 4	T>C	S38S	n.d.	Not determined
rs2231139	123	Exon 4	C>T	Y123Y	n.d.	Not determined
rs72552713	376	Exon4	C>T	Q126Stop	0.01[c] (Kobayashi et al. 2005)	Not determined
rs2231142	421	Exon 5	C>A	Q141K	0.0–0.355 (Zamber et al. 2003)	Inconclusive data
	445	Exon 5	G>C	A149P	0.01 (Itoda et al. 2003)	No effect (Kondo et al. 2004)
	458	Exon5	C>T	T153M	0.033 (Mizuarai et al. 2004)	No effect (Tamura et al. 2006)
	479	Exon 5	G>A	R160Q	0.005[e] (Bosch et al. 2005)	Not determined
	488	Exon 5	G>A	R163K	0.006 (Itoda et al. 2003)	No effect (Kondo et al. 2004)
rs1061017	496	Exon 5	C>G	Q166E	n.d.	No effect (Kondo et al. 2004; Tamura et al. 2006)
rs3116439	564	Exon 6	A>G	G188G	n.d.	Not determined
rs12721643	616	Exon 6	A>C	I206L	One individual (Zamber et al. 2003)	No effect
rs1061018	623	Exon 6	T>C	F208S	None (Mathijssen et al. 2003)	Reduced transport of hematoporphyrin, drug resistance, and reduced expression (Tamura et al. 2006, 2007)
rs3116448	742	Exon 7	T>C	S248P	n.d.	Reduced transport (Tamura et al. 2006)
rs34678167	805	Exon 7	C>T	P269S	0.002	No effect (Kondo et al. 2004)
rs41282401	886	Exon 8	G>C	D296H	n.d.	Not determined
rs34881799	999	Exon 9	G>A	A333A		Not determined
rs3201997	1000	Exon 9	G>T	E334Stop	n.d.	Reduced transport (Tamura et al. 2006)
	1060	Exon 9	G>A	G354R	0.030 (Maekawa et al. 2006)	Not determined
rs35622453	1098	Exon 9	G>A	E366E	0.02 (Itoda et al. 2003)	Not determined
	1291	Exon 11	G>A	F431L	0.006–0.008 (Yoshioka et al. 2007)	Reduced resistance normal expression (Tamura et al. 2007; Yoshioka et al. 2007)
	1322	Exon 11		S441N	0.005[d] (Itoda et al. 2003)	Reduced E_1S, DHEAS, mitoxantrone, PAH, and hematoporphyrin transport activity and reduced expression (Kondo et al. 2004; Tamura et al. 2006)

(*continued*)

Table 3 (continued)

rs-Number	Position[a]	Location	NE	AAE	Frequency	In vitro effect
	1465	Exon 12	T>C	F489L	0.005–0.008[c] (Itoda et al. 2003; Kobayashi et al. 2005)	Reduced transport of mitoxantrone, no effect on hematoporphyrin, reduced resistance to SN38 and mitoxantrone (Tamura et al. 2006, 2007; Tamura et al. 2007)
rs58818712	1574	Exon 13	T>G	L525R	n.d.	Not determined
rs45605536	1582	Exon 13	G>A	T528A	n.d.	Not determined
rs9282571	1711	Exon14	T>A	F571L	0.005	No effect (Tamura et al. 2006)
	1723	Exon14	C>T	R575stop	0.005 (Bosch et al. 2005)[d]	not determined
	1768	Exon15	A>T	N590Y	0.01–0.03[b] (Mizuarai et al. 2004; Zamber et al. 2003)	Reduced activity (Vethanayagam et al. 2005)
	2062	Exon16	G>A	D620N	One individual (Honjo et al. 2002)	High protein expression and low activity (Vethanayagam et al. 2005)

[a]From start ATG, NE nucleotide exchange, AAE amino acid exchange
[b]Detected in Caucasian populations
[c]Detected in Japanese populations
[d]Dutch population summarizing 93% Caucasians

position 141 has been studied extensively. This polymorphism located in exon 5 has been described with moderate frequency in individuals of African-American (AF 0.02–0.05), European (AF 0.11–0.14), Hispanic (AF 0.10), or Middle Eastern (AF 0.13) descent, whereas higher frequencies were observed in populations of Asian descent namely Chinese (0.35) or Japanese (0.35) Asians (Zamber et al. 2003). Even if the in vitro evaluation in different cell types is not always consistent, this particular SNP has been linked to decreased plasma membrane expression (Imai et al. 2002), decreased drug transport (Lee et al. 2007; Morisaki et al. 2005), and reduced ATPase activity (Mizuarai et al. 2004). In addition, it has been suggested that this variant is associated with delicate changes in substrate specificity in vitro (Tamura et al. 2007) and affects stability of the ABCG2 protein due to enhanced susceptibility of the variant to ubiquitin-mediated proteasomal degradation (Furukawa et al. 2009). As mentioned previously no influence of this genetic variant on intestinal expression of the transporter has been observed (Urquhart et al. 2008; Zamber et al. 2003), whereas in placenta expression of ABCG2 was significantly decreased in samples harboring the homozygote mutant genotype (Kobayashi et al. 2005).

The clinical impact of the frequently occurring ABCG2 polymorphisms (c.421C>A; c.34G>A) has been focus of several independent studies (compare Table 4). Particularly the influence on disposition and efficacy of campthotecin derivates has been addressed. Even if irinotecan and its active metabolite SN-38 are widely accepted substrates of ABCG2, studies concerning the impact of genetic variants on pharmacokinetics rather suggest that the multiplicity of PK-related genes involved in metabolism and transport of this particular compound limits the detection of strong associations between impaired function variants and pharmacokinetic parameters. Indeed, no significant effect of the c.421C>A or c.34G>A SNPs on pharmacokinetic parameters of irinotecan, its active metabolite SN-38, or SN-38 glucuronide (SN-38G) was observed in 156 Asian patients with nonsmall cell lung cancer (NSCLC) (Han et al. 2007). Similar results were obtained by de Jong et al. in 88 Caucasian cancer patients (de Jong et al. 2004). However, in 45 Asian cancer patients genotyped for c.421C>A Jada et al. noted higher exposure to SN-38G and significantly higher relative extend of glucuronidation, whereas no effect was reported for the parent compound or its active metabolite SN-38 (Jada et al. 2007). It seems noteworthy at this point, that a study in patients with NSCLC suggested an association of the c.*ABCG2* 34G>A and 421C>A polymorphisms with a higher incidence of grade 3 diarrhea in a combination therapy of irinotecan and cisplatin, even if no effect on kinetic parameters of SN-38 was evident (Han et al. 2008a). This is supported by recent findings revealing a moderate association between the *ABCG2**IIB haplotype (c.421C>A and IVS12 + 49G>T) and the incidence of irinotecan-induced neutropenia (Sai et al. 2010). Cha et al. identified an association between the intronic ABCG2 polymorphism rs2622604 and grade 3–4 myelosuppression (OR 14.56) in patients treated with irinotecan (Cha et al. 2009). The notion that this particular

SNP might result in changes of ABCG2 activity is supported by findings of Poonkuzhali et al. who provided evidence that rs2622604 was associated with lower mRNA expression of ABCG2 (Poonkuzhali et al. 2008). The importance of noncoding regions for ABCG2 functions is further supported by a report from Zhou et al. showing that the deletion of CTCA in the promoter of ABCG2 (–19572–19569delCTCA, rs4148162) was associated with an impaired relative extent of conversion (REC) of irinotecan to SN-38. The higher REC of irinotecan to SN-38 in wild-type patients was associated with an approximately 39% increase in AUC of SN-38. Importantly, in accordance with previous findings the same study did not find any phenotypic association between the ABCG2 c.34G>A or c.421C>A (Zhou et al. 2005a). As mentioned previously the multiplicity of genes involved in irinotecan metabolism and transport translates into difficulties confirming a role of ABCG2 in clinical studies, most of the so far performed genotype–phenotype association studies on irinotecan are based on small patient cohorts. Therefore to definitely confirm a role of ABCG2 in disposition and efficacy of irinotecan in humans requires large patient data sets and the consequent analysis of haplotypes as well as individual polymorphisms. However, studies addressing the impact of the ABCG2 genotype on pharmacokinetic parameters of the camptothecin derivates diflomotecan and 9-aminocampthotecin have shown a significant influence. Indeed, Zamboni et al. demonstrated changes in pharmacokinetic parameters of 9-aminocamptothecin (9-AC), the lactone metabolite of rubitecan (9-nitrocamptothecin, 9-NC, RFS2000), whereas no significant effect was seen for 9-NC itself (Zamboni et al. 2006). This finding is in line with previous in vitro reports showing that 9-AC but not 9-NC is a substrate of ABCG2 (Rajendra et al. 2003). Similar results were obtained for diflomotecan (Sparreboom et al. 2004).

Studies on genetic variants of ABCG2 and their effect on pharmacokinetic parameters of tyrosine kinase inhibitors (TKIs) are still limited; however, it has been reported that the c.421C>A polymorphism results in significantly higher gefitinib accumulation in patients harboring the impaired function allele (Li et al. 2007). Accordingly it has been hypothesized that the increased steady state exposure to gefitinib could increase the risk of gefitinib-induced adverse drug reactions. Indeed, Cusatis et al. found in 124 patients treated with gefinitib a significant association between diarrhea and ABCG2 genotype. A total of 44% of patients with the 421A allele developed diarrhea compared to only 12% of those who were homozygous carriers of the wild type (Cusatis et al. 2006). Although no influence of the c.421C>A variant on the PK of imatinib was noted by Gardner et al. (Gardner et al. 2006), Poonkuzhali et al. found a trend for an increased apparent oral clearance in patients carrying at least on variant allele in the promoter region (−15994C>T; rs7699188) (Poonkuzhali et al. 2008). This is in line with their finding that this polymorphism is associated with higher expression of the transporter. However, Rudin et al. did not find an effect of this particular polymorphism on the PK of another TKI, namely erlotinib, but they were able to report that

the following polymorphisms, c.1143C>T and −15622C>T, resulted in changes of erlotinib exposure. Even though, there was no definitive effect on experiencing toxic side effects such as diarrhea or skin rash among the 80 patients included in this study (Rudin et al. 2008). Similar results were obtained by van Erp et al. showing that patients harboring at least one copy of the c.1143C>T/−15622C>T haplotype experienced more toxic side effects when treated with sunitinib (van Erp et al. 2009).

Even if some of the previously mentioned studies revealed contradictory results on the influence of ABCG2 genetics on substrate drug disposition, there are several reports that consistently demonstrated an important contribution of this transporter to interindividual variability of pharmacokinetics (Keskitalo et al. 2009a, b; Sparreboom et al. 2005; Tomlinson et al. 2010; Zhang et al. 2006). The characterization of ABCG2 genotype-related modifications of drug disposition has been challenged by the availability of specific and nontoxic substrates of ABCG2 (Xia et al. 2005). Therefore identification of the anti-inflammatory drug sulfasalazine as a specific substrate of ABCG2 by Zaher et al. was an important milestone (Zaher et al. 2006). Following studies in humans revealed a significant impact of ABCG2 polymorphisms on the oral bioavailability of sulfasalazine. Indeed, Yamasaki et al. reported significantly increased exposure and reduced clearance in patients harboring the variant allele (Yamasaki et al. 2008). Similar results were obtained by Urquhart et al., suggesting sulfasalazine as a potential probe drug for intestinal ABCG2 function (Urquhart et al. 2008). However, a recent report by Adkison et al. showed no influence of the ABCG2 genotype on sulfasalazine disposition. It seems noteworthy that in this study the volunteers received enteric-coated tablets (Adkison et al. 2010). Even if sulfasalazine as an ABCB1 inert and nontoxic ABCG2 substrate opened the possibility to study the implication of ABCG2 and its genetic variants in intestinal drug absorption, further studies and potentially substrates are warranted to elucidate the impact of ABCG2 on hepatobiliary elimination (Table 4).

8 ABCG2 a Risk Factor for Gout

Genetic risk factors for defined phenotypes, especially diseases, are currently under investigation performing genome-wide association studies. It seems noteworthy that the nonsynonymous polymorphism c.421C>A (rs2231142) has been one of the genetic markers of uric acid levels in population based cohorts (Dehghan et al. 2008; Kolz et al. 2009). Consistent with these findings are following clinical evaluations showing that this polymorphism is predictive for developing gout (Stark et al. 2009; Wang et al. 2010). In a following study by Woodword et al. the function of ABCG2 as urate transporter was verified, thereby providing the rationale for that finding (Woodward et al. 2009).

Table 4 Summary of pharmacogenetic studies focussing on genetic variants of ABCG2

Study	Polymorphisms analysed	Substrate	Effect	References
de Jong et al.	421C>A	Irinotecan	No significant effect on irinotecan or SN-38 kinetic paramters	de Jong et al. (2004)
Han et al.	421C>A and 34G>A	Irinotecan[a]	No significant effect on irinotecan, SN-38, or SN-38-glucuronide disposition	Han et al. (2007)
Han et al.	421C>A and 34G>A	Irinotecan	No significant effect on SN-38 kinetics, but predictive factor for grade 3 diarrhea	Han et al. (2009)
Jada et al.	421C>A	Irinotecan	No effect on irinotecan or SN-38 AUC (normalized to dose and body surface), higher SN-38G AUC, and higher relative extend of glucuronidation (REG; AUC SN-38G/AUC SN-38) and biliary index (BI; AUC irinotecan/REG)	Jada et al. (2007)
Cha et al.	rs2622604 (intonic)	Irinotecan	Association with irinotecan-induced severe myelosuppression (grades 3 and 4)	Cha et al. (2009)
Zhou et al.	−19572 to 19569bp CTCAdel	Irinotecan	Reduced relative extend of conversion (REC) of irinotecan to SN-38 with 30% reduction in SN-38 AUC	Zhou et al. (2005a)
	c.34G>A		Trend to reduced C_{max} ($p = 0.056$)	
Sai et al.	421C>A and IVS12+49G>T	Irinotecan	Moderate association with irinotecan induced neutropenia	Sai et al. (2010)
Sparreboom et al.	421C>A	Diflomotecan	2.9-fold increase in AUC	Sparreboom et al. (2004)
Zamboni et al.	421C>A	9-Aminocam-tothecin	3.5-fold increase in 9-AC lactone AUC/dose	Zamboni et al. (2006)
Li et al.	421C>A	Gefitinib	1.3-fold higher accumulation at steady state in heterocygotes	Li et al. (2007)

(*continued*)

Table 4 (continued)

Study	Polymorphisms analysed	Substrate	Effect	References
Cusatis et al.	421C>A	Gefitinib	Higher incidence of drug-induced diarrhea no effect on skin toxicity	Cusatis et al. (2006)
Poonkuzhali et al.	−15994C>T (rs7699188)	Imatinib	increased expression of ABCG2 in multiple tissues associated with higher imatinib clearance	Poonkuzhali et al. (2008)
Gardner et al.	421C>A	Imatinib	No effect on AUC for 1 dosing interval, the apparent oral clearance, and the average steady-state plasma concentration (Css).	Gardner et al. (2006)
Thomas et al.	421C>A	Erlotinib	24% reduced erlotinib clearance in carriers of at least one 421A allele	Thomas et al. (2009)
Rudin et al.	−15622C/T and c.1143C/T haplotype	Erlotinib	1.3-fold increase in Cmax and AUC no effect of 421C>A, 34G>A or −15994C>T (rs7699188), respectively	Rudin et al. (2008)
van Erp et al.	−15622C/T and c.1143C/T haplotype	Sunitinib	Carriers of one copy of the TT haplotype had higher incidence of any toxicity higher than grade 2	van Erp et al. (2009)
Adkison et al.	421C>A	Sulfasalazine (enteric coated)	No significant association between genotype and sulfasalazine and metabolite kinetics, no influence of pantoprazole and famotidine as inhibitors	Adkison et al. (2010)
Yamasaki et al.	421C>A	Sulfasalazine (immediate release)	3.4-fold increased AUC (0–48), 2.5-fold higher Cmax, and 3.7-fold decreased CL in individuals homozygote for AA	Yamasaki et al. (2008)

(*continued*)

Table 4 (continued)

Study	Polymorphisms analysed	Substrate	Effect	References
Urquhart et al.	421C>A and 34G>A	Sulfasalazine (suspension)	2.3-fold increased AUC (0–∞), 1.7-fold higher Cmax in patients with CA genotype	Urquhart et al. (2008)
Colombo et al.	421C>A	Nelfinavir	None on pharmacokinetics	Colombo et al. (2005)
Kim et al.	421C>A and 34 G>A and 376C>T	Lamivudine	None on pharmacokinetics	Kim et al. (2007)
Marsh et al.	421C>A	Paclitaxel	None on pharmacokinetics or progression-free survival	Marsh et al. (2007)
Ieri et al.	421C>A	Pitavastatin	None on pharmacokinetics	(Ieiri et al. 2007)
Zhang et al.	421C>A	Rosuvastatin	1.8-fold increase in AUC, 1.9-fold increase in C max, oral elimination decreased to 57%	Zhang et al. (2006)
Tomlinson et al.	c.421C>A	Rosuvastatin	421AA is associated with 6.9% higher LDL-cholesterol reduction (comparable to doubling the dose)	Tomlinson et al. (2010)
Keskitalo et al.	c.421C>A	Rosuvastatin	421AA 144% increase in AUC (0–∞) (vs. 421CC) and 131% increase in Cmax (vs. 421CC)	Keskitalo et al. (2009b)
Keskitalo et al.	c.421C>A	Atorvastatin	421AA 72% increase in AUC (0–∞)	Keskitalo et al. (2009b)
Keskitalo et al.	c.421C>A	fluvastatin	421AA 72% increase in AUC (0–∞) (vs. 421CC)	Keskitalo et al. (2009a)
Keskitalo et al.	c.421C>A	Simvastatin lactone	421AA 111% increase in AUC (0–∞) and 46% decrease in simvastatin acid/lactone ratio (vs. 421CC) no effect on simvastatin acid	Keskitalo et al. (2009a)
Keskitalo et al.	c.421C>A	Pravastatin	None on pharmacokinetics	Keskitalo et al. (2009a)
Ho et al.	c.421C>A	Pravastatin	None on pharmacokinetics	Ho et al. (2007b)
Lal et al.	421C>A	Doxorubicin	None on pharmacokinetics	Lal et al. (2008)

(*continued*)

Table 4 (continued)

Study	Polymorphisms analysed	Substrate	Effect	References
Sparreboom et al.	421C>A	Topotecan	1.34-fold increase oral bioavailability	Sparreboom et al. (2005)
Adkison et al.	421C>A	Nitrofurantoin	No effect on plasma and urine pharmacokinetic parameters	Adkison et al. (2008)
Warren et al.	rs17731538 and rs13120400	Methotrexate	Associated with clinical MTX response in psoriasis patients, no effect on toxicity	Warren et al. (2008)
Gardner et al.	421C>A	No specific drug	No association with the prevalence of prostate cancer, Longer survival in patients with the wild-type variant	Gardner et al. (2008)
Kim et al.	421C>A	Rituximab plus cyclophos-phamide/ doxorubicin/ vincristin/ prednisone	Association with grade I–IV diarrhea, no effect on response, survival, or hematology toxicity profiles in patients with DLBCL	Kim et al. (2008)
Erdélyi et al.	421C>A	ALL BMF 95 protocol	None on toxicity	Erdelyi et al. (2006)

[a]Coadministered with cisplatin

9 Summary

We intended to give a broad overview on functions fulfilled by the efflux transporter ABCG2. Besides its role in drug disposition we have summarized the current understanding of its implication in pathophysiology. However, some aspects of ABCG2 were only touched in this chapter, therefore in order to obtain additional information we recommend to the reader some current reviews published on this transporter (Cascorbi et al. 2001; Ding et al. 2010; Doyle and Ross 2003; Gandhi and Morris 2009; Gradhand and Kim 2008; Hegedus et al. 2009b; Ishikawa and Nakagawa 2009; Krishnamurthy and Schuetz 2006).

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Molecular Mechanisms of Drug Transporter Regulation

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Abstract Interindividual differences in drug transporter expression can result in variability in drug response. This variation in gene expression is determined, in part, by the actions of nuclear hormone receptors that act as xenobiotic- and endobiotic-sensing transcription factors. Among the ligand-activated nuclear receptors, signaling through the Pregnane X Receptor (PXR), Constitutive Androstane Receptor (CAR), Farnesoid X Receptor (FXR), and Vitamin D Receptor (VDR) constitute major pathways regulating drug transporter expression in tissues. Hence, these endobiotic- and xenobiotic-sensing nuclear receptors are intrinsically involved in environmental influences of drug response. Moreover, because nuclear receptor genes are polymorphic, these transcription factors are also thought to contribute to heritability of variable drug action. In this chapter, the molecular

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aspects of drug transporter gene regulation by ligand-activated nuclear receptors will be reviewed including their clinical relevance.

Keywords Gene expression · Nuclear receptors · Transcription factors · Variable drug response

Abbreviations

AhR	Aryl hydrocarbon receptor
BCRP	Breast cancer resistance protein
BSEP	Bile salt export pump
CAR	Constitutive androstane receptor
CYP	Cytochrome P450
FXR	Farnesoid X receptor
MATE1	Multidrug and toxin extrusion transporter member 1
MDR1	Multidrug resistance protein 1
MRP	Multidrug resistance-associated protein
Nrf2	Nuclear factor-E2-p45-related factor 2
NTCP	Sodium-taurocholate cotransporting polypeptide
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
OSTα/β	Organic solute transporter α/β
P-gp	P-glycoprotein
PPARγ	Peroxisome proliferator-activated receptor γ
PXR	Pregnane X receptor
RXR	9-*cis* retinoic acid receptor
SHP-1	Small heterodimer partner 1
SNP	Single nucleotide polymorphism
SULT	Sulfotransferase
UGT	UDP-glucuronosyltransferase
VDR	Vitamin D receptor

1 Introduction

In the study of clinical drug response, it is appreciated that differences in the expression of drug disposition genes among patients contribute greatly to the interindividual variation observed. For example, there is significant interindividual variability in the expression and activity of the major human oxidative drug metabolizing enzyme, cytochrome P450 3A4 (CYP3A4) (Wilkinson 2005). In healthy subjects

without concomitant interacting medications and dietary confounders (e.g., grapefruit juice), there was an 11-fold variation in intravenous midazolam clearance, indicative of significant baseline variability in hepatic CYP3A4 expression and function (Lin et al. 2001). Results from this type of functional assessment of drug metabolism agree well with high variability (33-fold) found in hepatic CYP3A4 protein expression in a cohort of human livers (Westlind-Johnsson et al. 2003). The relevance of interindividual variation in drug transporter expression to pharmacokinetics is less understood than that for drug metabolism. This is largely due to the complex influence of drug transporters on pharmacokinetic parameters such as clearance and volume of distribution (Grover and Benet 2009), as well as a lack of valid substrates that can be used as specific in vivo probes (Fenner et al. 2009). Despite the current deficiencies in establishing quantitative drug transporter phenotypes in vivo to better clarify the importance of variation in drug transporter expression, there has been significant progress in our understanding of the molecular mechanisms that determine drug transporter expression.

Similar to drug metabolizing enzymes, there is ample evidence to demonstrate that the tissue protein expression of drug transporters differs widely among individuals (Table 1). For instance, the expression of the important drug efflux transporter P-glycoprotein (P-gp), encoded by the Multidrug Resistance Protein 1 gene (MDR1) has 20- to 55-fold variability in human liver (Meier et al. 2006; Owen et al. 2005; Schuetz et al. 1995) and about three- to sixfold variability in the small intestine (Durr et al. 2000; Simon et al. 2007). This degree of variation is similarly observed in liver and intestine for other drug efflux transporters such as Multidrug Resistance-Associated Protein 2 (MRP2), Bile Salt Export Pump (BSEP), and Breast Cancer Resistance Protein (BCRP) (see Table 1). With respect to drug

Table 1 Studies examining interindividual variability in drug transporter protein expression

Transporter	Tissue	Interindividual variation in expression	Number of individuals examined	References
P-gp	Intestine	Sixfold	44	Simon et al. (2007)
P-gp	Intestine	Threefold	8	Durr et al. (2000)
P-gp	Liver	55-fold	41	Schuetz et al. (1995)
P-gp	Liver	20.5-fold	110	Meier et al. (2006)
Pgp	Liver	20-fold	26	Owen et al. (2005)
MRP2	Intestine	11-fold	44	Simon et al. (2007)
MRP2	Liver	365-fold	110	Meier et al. (2006)
BSEP	Liver	Sevenfold	9	Schuetz et al. (2001)
BSEP	Liver	31-fold	15	Ho et al. (2010)
BSEP	Liver	18.5-fold	110	Meier et al. (2006)
BCRP	Intestine	1.8-fold	13	Urquhart et al. (2008)
BCRP	Intestine	"Highly variable"	32	Zamber et al. (2003)
OATP1B1	Liver	21-fold	21	Ho et al. (2006)
OATP1B3	Liver	6.7-fold	21	Ho et al. (2006)
OAT2	Liver	Tenfold	34	Shin et al. (2010)
OCT1	Liver	83-fold	136	Nies et al. (2009)

uptake transporters, interindividual variation in hepatic expression is also evident (Table 1). For example, the protein expression of Organic Anion Transporting Polypeptide 1B1 (OATP1B1) varies 21-fold (Ho et al. 2006), whereas that for Organic Cation Transporter 1 (OCT1) can differ by 83-fold (Nies et al. 2009). For some drug transporters, protein expression is altered by the presence of disease-causing genetic mutations. For example, mutations in both BSEP that cause intra-hepatic cholestasis (Strautnieks et al. 1998, 2008) and MRP2 in Dubin-Johnson syndrome (Paulusma et al. 1997) result in outright loss of transporter expression. However, these mutations are rare and do not explain the variation in drug transporter expression present in the general population.

Other genetic contributors have been considered to determine the degree of transporter expression. Single nucleotide polymorphisms (SNPs) in the drug transporter proximal promoters are highly prevalent (Hesselson et al. 2009). Indeed, promoter polymorphisms in OATP2B1 (Aoki et al. 2009) and Multidrug and Toxin Extrusion Transporter Member 1 (MATE1) (Ha Choi et al. 2009) are associated with decreased hepatic and renal expression of these transporters, respectively. There is also some evidence to suggest that epigenetic factors need to be considered. This appears to be the case for Organic Anion Transporter 3 (OAT3), whose expression is influenced by promoter methylation (Kikuchi et al. 2006). For a number of transporters, SNPs in the coding region of their respective genes do not affect overall protein expression. Rather, these SNPs often influence cellular trafficking and plasma membrane distribution or protein stability. To name a few, loss of function SNPs in OATP1B1 (Tirona et al. 2001), MATE1 (Chen et al. 2009), and BCRP (Mizuarai et al. 2004) are thought to result from membrane trafficking defects.

A more attractive mechanism that likely determines a significant proportion of the overall variation of drug transporter expression in tissues relates to the actions of nuclear hormone receptors and their transcriptional regulation of gene expression. It has been long known that an adaptive biological response is present that aims to minimize the potential toxic effects of xenobiotics that one may be exposed to. Early studies demonstrated that phenobarbital treatment decreases the plasma concentration of the antiseizure medication phenytoin and coumarin anticoagulants in humans (Cucinell et al. 1963), likely by upregulating the drug metabolism machinery (Remmer et al. 1973; Schoene et al. 1972). With the notion that induction of hepatic drug metabolism by exposure to certain drugs was due to a transcriptional mechanism (Adesnik et al. 1981), it followed that xenobiotic sensing and response systems were involved. The molecular mechanisms of the adaptive response to drug exposure were clarified with the discovery of the ligand-activated nuclear receptors Pregnane X Receptor (PXR) (Bertilsson et al. 1998; Blumberg et al. 1998; Goodwin et al. 1999; Kliewer et al. 1998; Lehmann et al. 1998), the Consitutive Androstane Receptor (CAR) (Baes et al. 1994; Forman et al. 1998; Honkakoski et al. 1998; Sueyoshi et al. 1999) and the Farnesoid X Receptor (FXR) (Makishima et al. 1999; Parks et al. 1999; Wang et al. 1999). In addition, the receptor for the hormone 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3], Vitamin D Receptor (VDR) (Baker et al. 1988) was subsequently shown important in regulating intestinal drug metabolism (Makishima et al. 2002). Another well-established

Table 2 Human drug transporter regulated by or implicated to be regulated by ligand-activated nuclear receptors

Gene	Nuclear receptors	References
Uptake transporters		
OATP1A2	PXR	Meyer zu Schwabedissen et al. (2008), Miki et al. (2006) and Oscarson et al. (2006)
OATP1B1	FXR, PXR	Jung et al. (2007) and Marzolini et al. (2007)
OATP1B3	FXR	Jung et al. (2002) and Ohtsuka et al. (2006)
OATP4C1	AhR	Toyohara et al. (2009)
OSTα/β	FXR	Boyer et al. 2006 and Landrier et al. (2006)
NTCP	FXR	Denson et al. (2001)
Efflux transporters		
P-gp (MDR1)	PXR, CAR, VDR	Geick et al. (2001) and Saeki et al. (2008)
MRP2	PXR, CAR, FXR	Kast et al. (2002)
MRP3	PXR, CAR, VDR	Jiang et al. (2009), McCarthy et al. (2005) and Teng et al. (2003)
MRP4	CAR	Assem et al. (2004)
BCRP	PXR, CAR	Jigorel et al. (2006b) and Oscarson et al. 2006)
BSEP	FXR	Ananthanarayanan et al. (2001), Plass et al. (2002) and Schuetz et al. (2001)

signaling pathway that regulates drug metabolism genes involves the tetrachlorodibenzo-p-dioxin or the arylhydrocarbon (Ah) receptor (AhR) (Burbach et al. 1992; Hoffman et al. 1991; Poland et al. 1976). More recently, oxidative stress was shown to trigger an adaptive response involving the Nuclear Factor-E2-p45-Related Factor 2 (Nrf2) signaling pathway and induction of select drug detoxication genes (Hayashi et al. 2003; Kang et al. 2004; Kwak et al. 2003; Nguyen et al. 2000; Venugopal and Jaiswal 1996; Wakabayashi et al. 2004). Although these xenosensing pathways are commonly considered to regulate the expression Phase I and Phase II drug metabolism genes (Tirona and Kim 2005), there is now considerable evidence that a number of important drug transporter genes form a part of nuclear receptor gene batteries.

In this chapter, we will review transcriptional regulation of drug transporter gene expression. We will focus on the pharmacology of nuclear receptor signaling through PXR, CAR, FXR, and VDR because these represent key pathways involved in drug transporter expression (Table 2). In addition, evidence of the emerging roles of nuclear receptors in interindividual variation in drug transporters will be detailed.

2 Transcriptional Regulation of Drug Transporters

2.1 Nuclear Receptor Signaling

A system of xenobiotic sensing and hormonal regulation of gene expression is mediated through the actions of the superfamily of nuclear receptor genes (Chawla et al. 2001; Mangelsdorf and Evans 1995). Typically, these nuclear receptors are resident in the cellular cytoplasm and upon binding to ligands, a common signaling

mechanism is elicited whereby the bound receptor translocates to the nucleus, heterodimerizes with the 9-*cis* retinoic acid receptor (RXR) and binding of the complex to regulatory regions of target genes. Upon release of corepressor proteins and the recruitment of coactivators, there is a stimulation of the general transcriptional machinery. Ligand binding to each nuclear receptor within the heterodimer causes differential allosteric communication that results in permissive, conditional, or nonpermissive transcriptional activation (Shulman et al. 2004).

2.1.1 Pregnane X Receptor

PXR can be considered the most important nuclear receptor involved in regulating drug disposition genes. It is expressed in a number of tissues and cells including hepatocytes (Kliewer et al. 1998), enterocytes (Kliewer et al. 1998), lymphocytes (Albermann et al. 2005), brain (Lamba et al. 2004b), and endothelial cells of the blood–brain barrier (Bauer et al. 2004). It is a "promiscuous" receptor that is bound and activated by chemically diverse compounds owing to a large and flexible ligand-binding cavity (Watkins et al. 2001, 2003a, b) (Table 3). Signaling through PXR has broad downstream effects on target gene expression as a number of oxidative

Table 3 Selected activators and inhibitors of PXR

Compound	References
Activators	
Rifampin	Luo et al. (2002)
Hyperforin	Moore et al. (2000a)
Clotrimazole	Luo et al. (2002)
Phenytoin	Luo et al. (2002)
Phenobarbital	Luo et al. (2002)
Dexamethasone	Pascussi et al. (2001)
Flucloxacillin	Huwyler et al. (2006)
Artemisinin	Burk et al. (2005b) and Huang et al. (2004)
Nicotine	Lamba et al. (2004b)
Nifedipine	Drocourt et al. (2001)
Omeprazole	Drocourt et al. 2001 and Raucy et al. (2002)
Paclitaxel	Mani et al. (2005)
Carbamazepine	Luo et al. (2002)
Topotecan	Schuetz et al. (2002)
Etoposide	Schuetz et al. (2002)
Sulfinpyrazone	Luo et al. (2002)
Ritonavir	Luo et al. (2002)
Nafcillin	Yasuda et al. (2008)
Inhibitors	
Ketoconazole	Huang et al. (2007)
Trabectedin (ET-742)	Synold et al. (2001)
Sulforaphane	Zhou et al. (2007)
Stigmasterol	Carter et al. (2007)
Coumestrol	Wang et al. (2008a)
A-792611	Healan-Greenberg et al. (2008)

enzymes such as CYP2A6 (Itoh et al. 2006), CYP2B6 (Goodwin et al. 2001; Wang et al. 2003), CYP2C9 (Chen et al. 2004; Ferguson et al. 2002; Gerbal-Chaloin et al. 2002), CYP2C19 (Chen et al. 2003; Gerbal-Chaloin et al. 2001), CYP3A4 (Goodwin et al. 1999), CYP3A7 (Bertilsson et al. 2001; Pascussi et al. 1999), and carboxylesterase 2 (Yang and Yan 2007) are target genes. Other genes regulated by PXR include conjugative enzymes such as UDP-glucuronosyltransferse 1A1 (UGT1A1) (Sugatani et al. 2001, 2004), UGT1A3 (Gardner-Stephen et al. 2004), UGT1A4 (Gardner-Stephen et al. 2004), UGT2B7 (Gallicano et al. 1999), and sulfotransferase 2A1 (SULT2A1) (Echchgadda et al. 2007; Fang et al. 2007). Among those genes regulated by PXR signaling, CYP3A4 expression is the most affected of all enzymes when examined by microarray analysis of inducer-treated cultured human hepatocytes (Healan-Greenberg et al. 2008). It should be noted that there are clear differences in the activation of PXR orthologs between species that account for variability in CYP3A inducibility by various drugs (Kocarek et al. 1995). For example, pregnenolone carbonitrile induces the expression of rodent Cyp3a genes more powerfully than human CYP3A4, whereas rifampin upregulates human CYP3A4 much more strongly than Cyp3a in rodents (Tirona et al. 2004; Xie et al. 2000).

2.1.2 Constitutive Androstane Receptor

The nuclear receptor CAR is activated by fewer compounds than PXR (Table 3) with phenobarbital being the prototypical activator. It is expressed in the liver (Choi et al. 1997), small intestine (Burk et al. 2005a), kidney (Lamba et al. 2004a), adrenals (Lamba et al. 2004a), testis (Lamba et al. 2004a), brain (Lamba et al. 2004a), and lymphocytes (Siest et al. 2008). This receptor regulates similar genes as PXR, albeit with some differences with respect to relative magnitude of target gene expression. For example, in human hepatocytes, CYP2B6 rather than CYP3A4 is most sensitive to the inductive effects of a CAR activator (Finkelstein et al. 2006). Interestingly, phenobarbital causes inductive responses through CAR signaling despite that it is not a direct ligand of the receptor (Moore et al. 2000b). Recent reports have demonstrated an important role of kinase activation in the nonligand activation of CAR (Hosseinpour et al. 2007; Inoue and Negishi 2008; Sueyoshi et al. 2008). Moreover, structural studies have shed insight into the molecular mechanisms that determine the constitutive activity of this receptor (Suino et al. 2004; Xu et al. 2004). Twenty-two splice variants of CAR are present in tissues (Auerbach et al. 2003; Lamba et al. 2003) and many display constitutive, ligand-independent activation in vitro. However, one splice variant (CAR3) is ligand activated and transactivates the CYP3A4 and CYP2B6 genes (Auerbach et al. 2005).

2.1.3 Farnesoid X Receptor

FXR is a bile acid receptor important in the maintenance of enterohepatic bile acid, glucose, and lipid homeostasis (Makishima et al. 1999; Parks et al. 1999). This

receptor is expressed in several tissues including the liver, small intestine, kidney, and adrenals (Houten et al. 2007). Although it functions as a bile acid rather than a drug receptor, FXR activation has become a relevant nuclear signaling pathway regulating drug metabolism and transport. Studies have identified functional FXR response elements or regulation by FXR for drug metabolizing enzyme genes such as CYP3A4 (Gnerre et al. 2004), SULT2A1 (Echchgadda et al. 2004; Miyata et al. 2006), UGT2B isoforms (Barbier et al. 2003; Kaeding et al. 2008) suggesting an important role in determining the pharmacokinetics of numerous drugs. Although bile acids are endogenous ligands for FXR, there have been no reports to date that indicate that prescription drugs are functional FXR agonists. However, a number of FXR agonists are under drug development (Dussault et al. 2003a; Hartman et al. 2009) or have been isolated from natural products such as coffee (e.g., cafestol) (Ricketts et al. 2007). Interestingly, the thiozolidinedione compound troglitazone, which is an agonist of the nuclear receptor Peroxisome Proliferator Activated Receptor γ (PPARγ), is a partial FXR agonist (Hanley et al. 1997). However, the clinically used drugs pioglitazone and rosiglitazone appear devoid of FXR agonist activity (Hanley et al. 1997). A number of FXR antagonists have been identified including the gugulipid constituent guggulsterone (Urizar et al. 2002) and the soy lipid stigmasterol (Carter et al. 2007). Hence, there exists the possibility that food–drug interactions may occur due to interactions at the level of FXR.

2.1.4 Vitamin D Receptor

Although the ubiquitously expressed VDR is known largely as a 1,25(OH)2D3 receptor, it is also activated by the bile acid lithocholate (Makishima et al. 2002). Aside from vitamin D analogs, there are no reports to date to indicate that VDR is activated by drugs in clinical use. Interest in VDR as a determinant of drug disposition was initially piqued when vitamin D was shown to induce CYP3A4 in the intestinal Caco-2 cell line (Schmiedlin-Ren et al. 1997). It was later demonstrated that VDR regulated intestinal CYP3A4 expression and first-pass intestinal drug metabolism (Thummel et al. 2001). Similarly, in cultured human cells, VDR regulates CYP2B6 and CYP2C9 (Drocourt et al. 2002) as well as SULT2A1 (Echchgadda et al. 2004).

2.2 Drug Transporters Regulated by Nuclear Receptors

2.2.1 MDR1 P-gp

Exposure of drugs such as rifampin and phenobarbital were first found to co-ordinately induce the expression of both CYP3A4 and MDR1 (P-gp) in human colon adenocarcinoma cells (LS180) (Schuetz et al. 1996a). Subsequently, the pharmacokinetic drug–drug interaction between rifampin and the P-gp substrate

drug digoxin was associated with increased intestinal expression of P-gp (Greiner et al. 1999). With the discovery of xenosensors, it soon became apparent that the MDR1 gene was directly regulated by this PXR and CAR through a response element 8 kb upstream of the transcription start site (Burk et al. 2005a, b; Geick et al. 2001). Furthermore, VDR was shown to transactivate the MDR1 upstream enhancer at multiple response elements (Saeki et al. 2008), providing a mechanism for enhanced P-gp expression in colon adenocarcinoma cells (Caco-2) treated with 1,25(OH)2D3 (Schmiedlin-Ren et al. 1997).

2.2.2 Multidrug Resistance-Associated Proteins

Studies in humans first showed that rifampin treatment upregulated MRP2 in enterocytes (Fromm et al. 2000), suggesting a role for PXR in the regulation of this efflux transporter. It was later discovered that PXR and CAR transactivated a nuclear receptor response element in the MRP2 proximal promoter and that MRP2 induction was evident in cultured human hepatocytes treated with rifampin or phenobarbital (Kast et al. 2002). MRP2 expression in duodenal biopsies correlated with the carbamazepine dose, further confirming intestinal regulation of this transporter by PXR/CAR ligands (Simon et al. 2007). Moreover, transcriptional profiling in carbamazepine-treated patients demonstrated higher levels of hepatic MRP2 than in control subjects, indicating that liver MRP2 can also be regulated in vivo through PXR/CAR signaling (Oscarson et al. 2006). Interestingly, the MRP2 gene is transactivated by FXR through the same proximal response element as PXR/CAR and induction of transporter was observed in cultured human hepatocytes treated with the FXR agonist chenodeoxycholate (Kast et al. 2002). MRP3 appears regulated by PXR as treatment of human liver cell lines with PXR activators induces transporter expression (Teng et al. 2003). Furthermore, MRP3 expression correlates well with PXR expression in normal and cancerous colon tissue and MRP3 is induced in human colon cancer cells by rifampin in vitro (Jiang et al. 2009). A role for VDR in controlling intestinal MRP3 expression is indicated by the upregulation of MRP3 in human colon cancer cell lines by active vitamin D and identification of the *cis*-acting VDR response element in the mouse Mrp3 gene (McCarthy et al. 2005). Lastly, CAR is a regulator of MRP4 expression because the transporter is upregulated in human liver cancer cell lines by overexpression of CAR and upregulation of MRP4 mRNA in a cultured human liver cell line treated with phenobarbital (Assem et al. 2004).

2.2.3 Breast Cancer Resistance Protein

There is indirect evidence that hepatic BCRP expression is regulated by nuclear receptors. Studies in cultured human hepatocytes revealed increased BCRP mRNA and protein levels when cells were treated with phenobarbital and rifampin, suggesting that CAR and PXR are involved (Jigorel et al. 2006a). This notion is

corroborated by elevated hepatic BCRP mRNA expression in patients treated with carbamazepine (Oscarson et al. 2006).

2.2.4 Bile Salt Export Pump

Because BSEP expression is highly affected by bile acid flux through the hepatocyte, it became evident that such feedback responses would be related to FXR signaling. Indeed, FXR transactivates the BSEP proximal promoter (Ananthanarayanan et al. 2001; Plass et al. 2002; Schuetz et al. 2001) and the FXR ligand chenodeoxycholate induces BSEP protein expression in cultured human hepatocytes (Schuetz et al. 2001). It is noteworthy that BSEP expression in cultured human hepatocytes is downregulated by treatment with the PXR/CAR activators rifampin and phenobarbital, although the mechanisms involved remain unknown (Jigorel et al. 2006a).

2.2.5 Organic Anion Transporting Polypeptides

Several members of the OATP gene family of drug uptake transporters appear to be regulated by nuclear receptors. OATP1A2 expression in cultured breast cancer cells can be upregulated when treated with rifampin, and this effect is PXR dependent (Meyer zu Schwabedissen et al. 2008; Miki et al. 2006). The mechanism of gene regulation was further clarified with the identification of the PXR response element in the OATP1A2 distal upstream enhancer (Meyer zu Schwabedissen et al. 2008). This signaling process is present in liver as OATP1A2 expression, probably in cholangiocytes, upregulated in patients treated with carbamazepine (Oscarson et al. 2006). Hepatic OATP1B3 is positively regulated by FXR as evidenced by the identification of a proximal FXR response element in the transporter gene (Jung et al. 2002) and induction of protein in human liver cell lines (Jung et al. 2002) and cultured human hepatocytes (Ohtsuka et al. 2006). Furthermore, decreased hepatic OATP1B3 expression was observed in livers harboring a loss of function genetic polymorphism in FXR (*1b) (Marzolini et al. 2007). For OATP1B1, there is a current absence of data to demonstrate that this transporter is directly regulated by nuclear receptors. However, OATP1B1 expression was upregulated in cultured human hepatocytes implicating a role for PXR signaling (Jigorel et al. 2006a). This is also supported by data from patients treated with the PXR activator carbamazepine where hepatic OATP1B1 mRNA expression was elevated when compared to control patients (Oscarson et al. 2006). Additionally, OATP1B1 expression in livers of individuals with the FXR*1b polymorphism is decreased, suggesting that FXR may regulate this gene (Marzolini et al. 2007). The kidney OATP4C1 transporter was recently found to be induced by 3-methylcholanthrene and HMG-CoA reductase inhibitors in vitro (Toyohara et al. 2009). Upregulation of this transporter appears to be a result of ligand activation of the AhR, which transactivates a response element in the OATP4C1 proximal promoter (Toyohara et al. 2009).

2.2.6 Sodium-Taurocholate Cotransporting Polypeptide

In the face of high-circulating bile acids, sodium-taurocholate cotransporting polypeptide (NTCP) expression in liver is downregulated in an attempt to protect against toxic bile acid hepatotoxicity (Gartung et al. 1996). Here, bile acids activate liver FXR, which in turn upregulates the expression of the atypical nuclear receptor, small heterodimer partner 1 (SHP-1) (Goodwin et al. 2000). SHP-1 acts as a negative regulator of RXR transactivation of the Ntcp promoter leading to transporter downregulation (Denson et al. 2001).

2.2.7 Organic Solute Transporter α/β

The expression of OSTα/β is positively regulated by the actions of FXR in enterocytes and hepatocytes. Patients with cholestatic liver disease have higher OSTα and OSTβ mRNA and protein levels than those with normal liver (Boyer et al. 2006). In addition, patients treated with chenodeoxycholate have elevated OSTα and OSTβ mRNA expression in ileum (Landrier et al. 2006). FXR was found to transactivate response elements in both OSTα and OSTβ gene promoters (Landrier et al. 2006).

2.3 Nuclear Receptor Splice Variants and Drug Transporter Expression

Splice variants of nuclear receptors are commonly observed in tissues. With PXR, at least ten splice variants have been detected (Fukuen et al. 2002), with the most commonly found forms being the wild-type (WT), an alternative exon 1B (PAR-2) version with an additional 29 amino acid N-terminus (Bertilsson et al. 1998) and a form that lacks 37 amino acids of the ligand binding domain (PXR.2) (Dotzlaw et al. 1999). Each splice variant can be detected in individual livers or enterocyte samples but to varying relative magnitudes of expression (Gardner-Stephen et al. 2004; Lamba et al. 2004b). The PXR.2 variant has been found to have reduced or absent transactivation activity (Gardner-Stephen et al. 2004; Hustert et al. 2001) and in certain models shown to have dominant negative effects on WT PXR activation of target gene promoters (Lin et al. 2009). It has been considered that the levels of the PXR.2 variant are too low relative to WT PXR in liver to cause effects on target gene expression. However, the possibility exists that there are, in certain individuals, higher amounts of PXR.2 relative to WT PXR in liver (Lin et al. 2009) or intestine (Gardner-Stephen et al. 2004) to exert downstream effects on drug transporter expression. The PAR-2 splice variant has similar transactivation activity as WT PXR (Bertilsson et al. 1998). Recently, it has been shown that a genetic polymorphism involving a 6-bp deletion in the PAR-2 promoter was

associated with reduced MDR1 (P-gp) and CYP3A4 expression in human livers (Liu et al. 2009). The relevance of the PXR splice variant-specific genetic polymorphism on P-gp-mediated hepatic drug clearance or intestinal absorption remains to be determined.

2.4 Nuclear Receptor Antagonism and Impact on Drug Transporters

There has been interest in inhibitors of nuclear receptors for use as therapeutic agents or as chemical tools to explore gene expression pathways (Tables 3 and 4). PXR antagonists such as ketonconazole are being considered as adjunct treatment to counteract cancer chemotherapeutic drug resistance by acting to suppress the tumoral expression of drug transporters such as P-gp (Huang et al. 2007; Wang et al. 2007). In breast cancer, PXR is highly expressed and regulates the expression of the estrogen uptake transporter OATP1A2 (Meyer zu Schwabedissen et al. 2008; Miki et al. 2006). Blockade of PXR activity by treatment with the HIV protease inhibitor A-792611 (Healan-Greenberg et al. 2008) inhibits estrogen-mediated breast cancer cell proliferation in vitro (Meyer zu Schwabedissen et al. 2008). Although a number of PXR antagonists have been identified, there is a lack of clinical studies to demonstrate that blockade of this nuclear receptor would have desired effects.

2.5 In Vitro and Animal Models of Drug Transporter Transcriptional Regulation

A number of experimental systems are commonly employed to study nuclear receptor-mediated regulation of drug transporter expression in humans. Although these models are often used to understand mechanisms of gene expression, they

Table 4 Selected modulators of CAR

Compound	References
Activators	
Artemisinin	Burk et al. (2005b)
Phenobarbital	Kawamoto et al. (1999)
CITCO	Maglich et al. (2003)
Oltipraz	Merrell et al. (2008)
Phenytoin	Wang et al. (2004)
Fluvastatin	Kobayashi et al. (2005)
Simvastatin	Kobayashi et al. (2005)
Atorvastatin	Kobayashi et al. (2005)
Inhibitors	
PK1119	Li et al. (2008)

may also be used to predict drug–drug interactions that involve transporter induction. The most frequently utilized systems include cultured primary human hepatocytes and liver slices, humanized mouse models, transformed hepatocytes or cancer cell lines, reporter-gene assays, coactivator recruitment assays and receptor-binding assays. Each system differs in the degree of biological complexity and experimental ease. Examination of gene expression changes in cultures of primary human hepatocytes after drug challenge is considered the "gold standard" approach by the drug regulatory agencies in the prediction of drug–drug interactions. Although results from this model relate well to metabolic drug–drug interactions in vivo, there is little information that provides evidence that induction of transporters in cultured human hepatocytes has relevant effects on the pharmacokinetics of drugs that are transporter substrates. The human liver slice model has been considered more attractive than primary hepatocytes because tissue architecture is preserved allowing for natural interaction between hepatocytes, other cell types, and extracellular matrix. Indeed, drug transporter expression is sensitive to exposure to nuclear receptor agonists in human liver slices (Olinga et al. 2008). Because of well-known species differences in gene regulation by nuclear receptors, a number of "humanized" mouse models have been described, each with differing variations in nuclear receptor composition (PXR/CAR) (Huang et al. 2004; Scheer et al. 2008; Xie et al. 2000). With the high cost and limited availability of primary human hepatocytes, various transformed cell systems have been developed to mimic the adaptive response in liver. These include the Fa2N-4 immortalized human hepatocyte clone (Hariparsad et al. 2008; Mills et al. 2004; Ripp et al. 2006) as well as the HepaRG human hepatoma cell line (Aninat et al. 2006; Kanebratt and Andersson 2008; Lambert et al. 2009a, b; McGinnity et al. 2009). Reporter gene assays in which cell lines are stably or transiently transfected with luciferase reporters and nuclear receptors are often used in mechanistic studies to examine *cis*- and *trans*-acting factors in regulatory regions of genes (Goodwin et al. 1999). The advantages of this system are its technical simplicity and predictability of in vivo effects.

The PXR reporter gene assay predicts well the magnitude of CYP3A4 induction in vivo as assessed in high-quality clinical drug–drug interaction studies (Tirona and Kim 2009). The ratio of maximal plasma drug concentration of the inducing drug (C_{max}) to in vitro EC_{50} for PXR activation appears to be a useful metric to predict the propensity for a compound to elicit an inductive response on a PXR target gene such as CYP3A4. When the C_{max}/EC_{50} breaks a threshold of 0.1, there is good likelihood that PXR is activated in vivo and CYP3A4 is induced (Tirona and Kim 2009). However, it remains to be determined whether this metric would predict drug transporter induction in vivo and if reporter assays using other trans-acting factors (e.g., CAR or FXR) have utility.

In some instances, however, the plasma drug concentrations (C_{max}) do not serve as good surrogate measures of the intracellular levels at the site of nuclear receptors. This has implications for the concentrations of drugs used during in vitro assays of drug transporter regulation. One should be cognizant that intracellular drug accumulation and hence the concentration exposed to nuclear receptors is

modulated by the actions of drug uptake and efflux transporters. For example, overexpression of the rifampin uptake transporter OATP1B1 enhanced rifampin-mediated PXR activation in a cell-based reporter assay (Tirona et al. 2003). In another model, induction of Cyp3a in the livers of mice deficient in P-gp was greater than that in wild-type mice when both were treated with rifampin (Schuetz et al. 1996b). This was consistent with elevated levels of rifampin in livers of P-gp knockout compared to wild-type mice presumably because P-pg transports rifampin.

3 Therapeutic Aspects of Drug Transporter Regulation

3.1 Drug–Drug Interactions Involving Drug Transporter Regulation

Although the list of compounds that activate the nuclear receptors PXR, CAR, and FXR has rapidly grown over the last decade, it is interesting to note that from a therapeutic perspective relatively few prescription drugs are known to cause clinically relevant induction-type drug interactions. The number of such clinically relevant inducers amounts to roughly 20 drugs, and these are found in only five major therapeutic categories: anticonvulsants, antibiotics, human immunodeficiency virus protease inhibitors, nonnucleoside reverse transcriptase inhibitors, and miscellaneous additional drugs (Table 5). It can be appreciated that within each category, not all drugs are inducers, and therefore a “class-effect” for inductive drug interactions does not exist. Although most of these inducing agents have been described to upregulate drug metabolizing enzymes (e.g., CYP3A4), there are considerably fewer examples of drugs causing clinically relevant induction of drug transporters (Table 6). For the most part, the clinical studies involve induction of P-gp by various PXR agonists such as rifampin or St. John’s wort, resulting in decreased drug levels of substrate drugs. There is also reason to consider that MRP2 induction in vivo may have clinical relevance because the pharmacokinetics of MRP2 substrate drugs such as ezetimibe-glucuronide and mycophenolate can be significantly affected.

3.2 Nuclear Receptor Pharmacogenetics and Drug Transporter Expression

Polymorphisms in the NR1I2 (PXR) gene have been extensively studied (Koyano et al. 2002; Zhang et al. 2001). Few, rare nonsynonymous polymorphisms in PXR are demonstrated to have reduced transcriptional activity (Hustert et al. 2001; Lim et al. 2005). Other single nucleotide polymorphisms (SNP) in PXR are located in

Table 5 Prescription and herbal medicines known to cause clinically relevant induction-type drug–drug interactions

Drug	References
Anticonvulsants	
Phenobarbital	Conney et al. (1965) and Kawamoto et al. (1999)
Carbamazepine	Luo et al. (2002)
Oxcarbazepine	Lloyd et al. (1994)
Phenytoin	Conney et al. (1965)
Valproic acid	DeVane (2003)
Lamotrigine	Benedetti (2000)
Topiramate	Benedetti (2000)
Felbamate	Benedetti (2000)
Antibiotics	
Nafcillin	Qureshi et al. (1984)
Rifampin	Acocella (1978)
Rifabutin	Finch et al. (2002)
HIV protease inhibitors	
Ritonavir	[a]
Nelfinavir	[a]
Lopinavir	[a]
Tipranavir	King and Acosta (2006)
Amprenavir	Justesen et al. (2003)
Atazanavir	Perloff et al. (2005)
Nonnucleoside reverse Transcriptase inhibitors	
Efavirenz	Mouly et al. (2002)
Nevirapine	Murphy et al. (1999)
Other	
Bosentan	van Giersbergen et al. (2002)
St. John's Wort	Ruschitzka et al. (2000), Durr et al. (2000), Johne et al. (1999), Durr et al. (2000) and Johne et al. (1999)

[a]Product monograph

regulatory regions or introns of the gene and are linked with variation in hepatic CYP3A4 content (Lamba et al. 2008). It remains challenging to determine the relevance of PXR polymorphisms to drug transporter expression because induction of transporters occurs in co-ordination with drug metabolism enzymes and that drug disposition is often determined by both transport (for example P-gp) and metabolism (CYP3A4). With these considerations, an influence of PXR genetics was also observed for doxorubicin pharmacokinetics in breast cancer patients, whereby the PXR*1b haplotype was associated with decreased drug clearance (Sandanaraj et al. 2008). Moreover, a PXR SNP is associated with reduced and subtherapeutic levels of the HIV protease inhibitor atazanavir (Siccardi et al. 2008). In addition a PXR polymorphism is associated with altered pharmacokinetics of the P-gp substrate drug prednisolone (Miura et al. 2008).

FXR gene (NR1I4) polymorphisms have become associated with a number of phenotypes. The relatively common polymorphism within the Kozak sequence (FXR*1b) shows reduced function in vitro and is linked with decreased hepatic gene expression (e.g., OATP1B1 and OATP1B3) (Marzolini et al. 2007). At present,

Table 6 Drug–drug interactions involving transporter induction

"Perpetrator" drug	"Victim" drug	Effect on "Victim" drug pharmacokinetics[a]	Induced transporter	Implicated nuclear receptor	References
Rifampin	Digoxin	↓AUC 30%	P-gp	PXR	Greiner et al. (1999)
St. John's Wort	Digoxin	↓AUC 25%	P-gp	PXR	Johne et al. (1999)
St. John's Wort	Talinolol	↓AUC 31%	P-gp	PXR	Schwarz et al. (2007)
St. John's Wort	Fexofenadine	↓AUC 48%	P-gp	PXR	
Rifampin	Ezetimibe	↓AUC 57% Ezetimibe ↓AUC 65% Ezetimibe-glucuronide	P-gp, MRP2	PXR	Oswald et al. (2006)
Rifampin	Mycophenolate	↓AUC 18%	MRP2	PXR	Naesens et al. (2006)
Rifampin	Atorvastatin	↓AUC 80% Atorvastatin ↓AUC 93% Atorvastatin lactone	P-gp	PXR	Backman et al. (2005)
Rifampin	Carvediolol	↓AUC 41–50%	P-gp, MRP2	PXR	Giessmann et al. (2004)

[a]*AUC* area under the plasma concentration time curve

there is no information to indicate that FXR polymorphisms relate to interindividual differences in drug clearance. However, the FXR*1b genotype is associated with an increased risk for intrahepatic cholestasis of pregnancy (Van Mil et al. 2007) and for cholesterol gallstone disease (Kovacs et al. 2008).

3.3 Xenobiotic Receptors as Drug Targets: Implications for Drug Transporter Expression

The nuclear receptors that regulate drug transporters are being considered potential novel drug targets. This is not a new concept, as activating CAR by treatment with phenobarbital has long been used in the treatment of neonatal hyperbilirubinemia to upregulate the expression of bilirubin glucuronidation (UGT1A1) and biliary conjugate excretion (MRP2) (Huang et al. 2003, 2004; Sugatani et al. 2001). Recent interest in target therapeutics on CAR relate to its physiological role in energy homeostasis and potential role in hyperlipidemia (Maglich et al. 2009) and nonalcoholic steatohepatitis (Yamazaki et al. 2007).

For PXR, antagonists to circumvent drug resistance in various cancers are being considered as a way to suppress drug metabolism and transport in tumors and hence

multidrug resistance. Moreover, it appears that PXR has an antiapoptotic effect in colon cancer unrelated to regulation of drug metabolism but due to a combination of upregulation of antiapoptotic genes and downregulation of proapoptotic genes (Zhou et al. 2008). Similar to CAR and FXR, PXR is involved in lipid and cholesterol homeostasis, and therefore targeting of this receptor may have utility in the prevention or treatment of cardiovascular disease. Indeed, PXR activation has complex effects on serum lipoproteins (de Haan et al. 2009; Hoekstra et al. 2009; Masson et al. 2005; Ricketts et al. 2007; Sporstol et al. 2005). The potential for therapeutic modulation of PXR activity for other diseases of lipid excess have also been suggested for cerebrotendinous xanthomatosis (Dussault et al. 2003b) and Niemann-Pick type C1 disease (Langmade et al. 2006). Genetic polymorphisms in PXR have also been linked to increased risk for inflammatory bowel diseases such as Crohn’s disease and ulcerative colitis (Dring et al. 2006; Langmann et al. 2004) and are associated with increased disease severity in nonalcoholic fatty liver disease (Sookoian et al. 2010). Evidently, mice with genetic deficiency in PXR show signs of intestinal inflammation (Shah et al. 2007). The mechanism for control of gut inflammation appears to result from mutual inhibition of PXR and NF-κB signaling (Shah et al. 2007; Zhou et al. 2006). It is therefore interesting that the drug rifaximin, a PXR agonist, is being used in the treatment of inflammatory bowel disease (Ma et al. 2007).

FXR agonists have received attention recently because compounds like INT-747 (Mencarelli et al. 2009) and WAY-362450 (Evans et al. 2009; Flatt et al. 2009; Hartman et al. 2009) have compelling preclinical data to demonstrate antiatherosclerotic activities. Moreover, FXR activation results in hepatic and vascular anti-inflammatory responses (Li et al. 2007; Wang et al. 2008b; Zhang et al. 2009), which may augment other cardiovascular benefits. FXR agonism may also be a novel strategy in the treatment or prevention of cholesterol gallstone disease by increasing phospholipid and bile acid concentrations in the maintenance of cholesterol solubility in bile (Moschetta et al. 2004). Furthermore, there is experimental evidence that activation of FXR may be of benefit in the treatment of nonalcoholic fatty liver disease (Figge et al. 2004; Kong et al. 2009).

The important question remains whether targeting PXR, CAR, or FXR is safe given that among other things, modulation of these nuclear receptors could have undesired effects on drug transporter expression leading to drug–drug interactions.

4 Perspectives

The molecular mechanisms involved in the regulation of drug transporter gene expression have been better clarified with the discoveries in ligand-activated nuclear receptor signaling. However, further study is needed to translate these mechanisms to improve the therapeutic use of drugs. For instance, although there is greater appreciation for the high variability in drug transporter expression in tissues (Table 1), the precise contribution of environmental factors and nuclear

receptor signaling to that variability remains to be clarified. Furthermore, despite that we can observe regulation of drug transporter expression in various (in vitro) experimental models, there is a current inability to quantitatively translate that information to predict the impact on drug transporter substrate pharmacokinetics. In this regard, the field would benefit from the validation of specific drug transporter substrates as in vivo probes of transporter activity.

Exciting progress is being made in understanding the role of drug transporter regulation at the blood–brain barrier on the central nervous system distribution of drugs. Currently, it is known that PXR is expressed in brain capillaries to regulate the expression of drug transporters (Bauer et al. 2004, 2006, 2008; Narang et al. 2008; Zastre et al. 2009). Recent developments in positron-emission tomography-based brain imaging in humans have provided compelling evidence for a significant role of P-gp as a component of the blood–brain barrier (Kreisl et al. 2010; Lee et al. 2006; Sasongko et al. 2005; Toornvliet et al. 2006). It will be interesting to learn if modulation of PXR activity in brain capillary endothelial cells will confer altered central drug action or decreased risk for side effects in humans. Moreover, it has recently been demonstrated that brain amyloid-β efflux mediated by P-gp can be enhanced in a mouse model of Alzheimer's disease by treatment with a PXR agonist (Hartz et al. 2010). Hence, the benefits of modulation of the blood–brain barrier through nuclear receptor signaling need not only apply to small molecule drugs.

In conclusion, the expression of drug transporters is importantly determined by transcriptional signaling by several ligand-activated nuclear receptors. It can be expected that additional molecular insights to gene expression together with translational studies that apply transporter phenotyping will provide a basis for strategies to improve drug therapy.

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In Vivo Probes of Drug Transport: Commonly Used Probe Drugs to Assess Function of Intestinal P-glycoprotein (ABCB1) in Humans

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Abstract Intestinal P-glycoprotein (P-gp, ABCB1) may significantly influence drug absorption and elimination. Its expression and function is highly variable, regio-selective and influenced by genetic polymorphisms, drug interactions and intestinal diseases. An in vivo probe drug for intestinal P-gp should a registered, safe and well tolerated nonmetabolized selective substrate with low protein binding for which P-gp is rate-limiting during absorption. Other P-gp dependent processes should be of minor influence. The mechanism(s) and kinetics of intestinal uptake must be identified and quantified. Moreover, the release properties of the dosage form should be known. So far, the cardiac glycoside digoxin and the $ß_1$-selective blocker talinolol have been used in mechanistic clinical studies, because they meet most of these criteria. Digoxin and talinolol are suitable in vivo probe drugs for intestinal P-gp under the precondition, that they are used as tools in carefully designed pharmacokinetic studies with adequate biometrically planning of the sample size and that several limitations are considered in interpreting and discussion of the study results.

Keywords P-glycoprotein · ABCB1 · Digoxin · Talinolol · Intestinal absorption

1 Introduction

1.1 Expression, Function and Variability of Intestinal P-glycoprotein in Man

There is substantial evidence that human gut mucosa acts as an invasion barrier for numerous small hydrophobic amphophilic xenobiotic compounds by means of both biotransformation and active secretion, the latter being governed by adenosine triphosphate (ATP)-binding cassette (ABC)-type transporters. The most important ABC transport protein is P-glycoprotein, a 170 kD protein. It was originally identified as an important element in the modulation of the resistance against chemotherapy of cancer. P-glycoprotein acts as an efflux-transporter localized at the apical membranes of various cells with excretory function, like enterocytes, hepatic canalicular cells, proximal tubular cells in kidney, endothelial cells of brain capillaries, or syncytiotrophoblasts in the placenta. It is a major part of the physiological functions of organ barriers (e.g., intestinal absorption barrier, blood–brain barrier, placenta barrier) and excretory organs (liver, kidney, intestine) (Cordon-Cardo et al. 1990; Sugawara et al. 1988; Thiebaut et al. 1987). The potential role for intestinal P-glycoprotein in limiting drug absorption and thereby increasing prehepatic elimination as well as its meaning for drug elimination has been addressed by numerous groups (Chan et al. 2004; Ho and Kim 2005; Murakami and Takano 2008; Takano et al. 2006).

Intestinal expression of P-glycoprotein in humans is highly variable and regioselective. *ABCB1*mRNA and P-glycoprotein content in the duodenum vary in

healthy white subjects by a factor of three to ten (Bernsdorf et al. 2006; Greiner et al. 1999; Lown et al. 1997; Oswald et al. 2006; Schwarz et al. 2007; Siegmund et al. 2002b). However, the expression level is low in the duodenum and proximal jejunum and in the ascending colon. Increasingly higher expression has been observed along more distal regions (distal jejunum, ileum) (Englund et al. 2006; Mouly and Paine 2003; Seithel et al. 2006; Thorn et al. 2005; Zimmermann et al. 2005). The basal expression and function of intestinal P-glycoprotein does not seem to be related to age and gender (Larsen et al. 2007; Schwartz 2003).

There is contradictory information whether the expression of intestinal P-glycoprotein in noninduced subjects is influenced by *ABCB1*-gene polymorphisms. For the first time, Hoffmeyer et al. observed in 21 healthy white subjects, that duodenal P-glycoprotein-content (Western blot analysis) tends to be lower in carriers of the synonymous C3435T-polymorphism in exon 26. The difference observed between carriers of 3435CC and 3435TT was approximately twofold (Hoffmeyer et al. 2000). This phenotype of C3435T was recently confirmed by Larsen et al. (2007). Furthermore, it was found that *ABCB1*mRNA expression was significantly decreased among subjects carrying at least one variant allele for C1236T in exon 12 and G2677GT in exon 21 whereas C3435T was without marked influence (Schwarz et al. 2007). In contradiction to these results, significant correlation between the *ABCB1* genotype and the duodenal *ABCB1*mRNA and P-glycoprotein content could not be observed in a larger group of 37 healthy white subjects. (Siegmund et al. 2002b) Genetic influence on intestinal P-glycoprotein expression was also not found in Japanese recipients of living-donor liver transplantation (Goto et al. 2002; Hosohata et al. 2009). In a small group of 13 healthy Japanese subjects, the variant T allele introduced even higher expression of *ABCB1*mRNA (not significant) (Moriya et al. 2002; Nakamura et al. 2002). It is important to recognize that there is a need for phenotyping P-glycoprotein function, because synonymous single-nucleotide polymorphisms that do not alter coding sequences and expression levels may affect functions of the transport protein by the timing of cotranslational folding and insertion into the membrane, thereby altering the structure of substrate and inhibitor interaction sites (Kimchi-Sarfaty et al. 2007; Schaefer et al. 2006). In conclusion, P-glycoprotein expression and function cannot be predicted by genotyping.

The expression of intestinal P-glycoprotein is highly influenced by drug interactions. Like CYP3A4, P-glycoprotein is regulated by the nuclear pregnane-X receptor (PXR) and the constitutive androstane receptor (CAR) (Burk et al. 2005; Geick et al. 2001). Therefore, expression of intestinal P-glycoprotein increases by about 1.5-fold to 8.3-fold after induction with rifampicin, 3.5-fold (mRNA-level) after carbamazepine, and 1.6-fold after St John's wort (SJW) (Giessmann et al. 2004a, b; Greiner et al. 1999; Oswald et al. 2006; Schwarz et al. 2007; Westphal et al. 2000b). The magnitude of induction by rifampicin and SJW seems to be influenced by the *ABCB1* gene polymorphism (Hoffmeyer et al. 2000; Schwarz et al. 2007).

Many substances were identified as inhibitors of intestinal P-glycoprotein function; e.g. verapamil, quinidine, macrolide antibiotics, valspodar (PSC833),

HIV-protease inhibitors, immunosuppressants or constituents of grapefruit juice (Ho and Kim 2005; Marzolini et al. 2004; Takano et al. 2006).

It is currently unknown whether intestinal P-glycoprotein is influenced by pathological factors. There is some evidence that intestinal P-glycoproteinis is increased in patients with active Crohn's disease to an extent that is twice the content in healthy subjects and decreased in patients with persistent diarrhea (Buchman et al. 2005; Lemahieu et al. 2004). In healthy subjects with experimental subclinical hypothyreosis, duodenal *ABCB1*mRNA expression and immunoreactive P-glycoprotein increased 1.4-fold and 3.8-fold, respectively, after treatment with levothyroxine (200 μg for 17 days) (Siegmund et al. 2002a).

In the enterocytes, P-glycoprotein is embedded in a very complex environment equipped with many other apical and basolateral efflux carriers of the ABC-superfamily and with uptake transporters of the organic anion transporting polypeptide (OATP), organic cation transporter (OCT), and peptide transporter (PEPT) families (Ho and Kim 2005). Little is known so far about the interplay between efflux and uptake carriers to mediate unidirectional transcellular fluxes, which is the precondition for substance absorption and intestinal secretion. It should be recognized that there is ample experimental evidence for the existence of complex adaptation processes in multidrug transporter expression in the intestine (and in other organs) in case of bowel surgery or genetic deficiency of transporters (Cisler and Buchman 2005; Drozdowski and Thomson 2006; Glaeser and Fromm 2008; Johnson et al. 2006; Klaassen and Lu 2008; Oswald et al. 2006b; Severijnen et al. 2004; Weale et al. 2005).

1.2 Criteria for an In Vivo Probe Drug of Intestinal P-glycoprotein

An in vivo probe drug for intestinal P-glycoprotein should be suitable to measure the following variability in the efflux transport function:

- Basal variability in noninduced subjects
- Regio-selective differences along the small intestine
- Influence of ABCB1 gene polymorphisms, gender, age and other physiological conditions
- Influence of potential inducers and inhibitors of P-glycoprotein
- Influence of pathological factors (e.g. gastrointestinal disease, systemic inflammations and auto-immune states, hormonal influences, etc.)
- Adaptation processes to bowel surgery and genetic deficiencies of drug metabolizing enzymes and other multidrug transporter proteins.

In selection of a probe drug for intestinal P-glycoprotein, it is important to know that many substrates of P-glycoprotein also interact with other transport proteins such as ABCC1, ABCC2, ABCC3, or ABCG2 and many are subjected to drug

biotransformation with only a few exceptions (Chan et al. 2004; Kim et al. 1999; Murakami and Takano 2008; Schuetz et al. 1996; Tran et al. 2002; Wacher et al. 1995). Furthermore, it must be considered that inducers of P-glycoprotein also may influence other transport proteins and drug metabolizing enzymes and that most of the inhibitors of P-glycoprotein are also inhibitors of drug metabolizing enzymes (Ho and Kim 2005; Urquhart et al. 2007). Due to the overlapping properties of drug transporters and metabolizing enzymes (substrate selectivity, inhibition, induction), only a few drugs with minor metabolism are candidates for the determination of intestinal P-glycoprotein function in man. In the US Food and Drug Administration (FDA) draft guidance for drug interaction studies (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf), digoxin, loperamide, quinidine, vinblastine and talinolol are listed as acceptable in vitro P-glycoprotein substrates. These examples were selected according to the criteria (1) selectivity, (2) low to moderate membrane permeability, (3) minor metabolism, (4) commercially availability, and (5) suitability as an in vivo probe drug. However, the applicability of a candidate to be an in vivo probe drug in man is dependent on several additional conditions. From our point of view, a probe drug for the quantification of intestinal P-glycoprotein in human beings should meet the following requirements:

- The drug is a selective substrate for P-glycoprotein
- Intestinal efflux via P-glycoprotein is the rate-limiting process in absorption
- Other P-glycoprotein dependent pharmacokinetic processes (e.g. organ distribution, hepatic excretion, renal excretion) are of minor influence
- The mechanism(s) and kinetics of intestinal uptake are identified and quantified
- The release properties of the dosage form are known (e.g. site of disintegration) considering regio-selective expression of P-glycoprotein and variability of intestinal transit
- The drug is not significantly metabolized and has low plasma protein binding
- The drug is safe and well tolerated by healthy subjects
- The drug is registered for use in man and is available for intravenous and oral administration
- The drug is rapidly absorbed and has a short half-life to avoid long clinical study periods and sequence bias
- Validated assays are available to quantify the drug (and metabolites) in blood, urine and feces as recommended for studies on bioavailability, bioequivalence and pharmacokinetics (Viswanathan et al. 2007).

Unfortunately, none of the candidates mentioned above meet all of these criteria. Loperamide influences intestinal motility and quinidine and vinblastine are not suitable for routine clinical studies in healthy subjects because of safety concerns. So far, the cardiac glycoside digoxin and the $ß_1$-selective blocker talinolol have been widely used in many mechanistic clinical studies, because they are nearly not metabolized and are well tolerated. Therefore, this review will focus on suitability and limitations of digoxin and talinolol to measure function of intestinal P-glycoprotein in man.

2 Digoxin

2.1 *Safety, Physicochemical Properties and Pharmacokinetics*

Digoxin is a secondary glycoside from the common foxglove (*Digitalis lanata*) being used for treatment of heart failure and cardiac arrhythmias. It has a narrow therapeutic window and even small elevations of the plasma levels may cause severe side effects. Common adverse reactions may just occur at therapeutic levels of 0.5–2.0 ng/ml such as cardiac arrhythmias, nausea and vomiting and central nervous effects (Bauman et al. 2006; Piergies et al. 1994; Tuncok et al. 1997).

Digoxin (molecular weight 780.95 Da) provides low water solubility (0.065 g/l) and intermediate, pH-independent lipid solubility (logP = 1.67). It is a class II compound with low solubility but high permeability according to the biopharmaceutics classification system (BCS) (Amidon et al. 1995; Wu and Benet 2005). Other authors classified digoxin as a class I and even class IV compound, considering obviously the standard dose of 0.25 mg being soluble in 240 ml water (class I) or to support the conception that so far unknown intestinal uptake transporters are involved in absorption of class IV compounds (Lindenberg et al. 2004; Shugarts and Benet 2009).

Digoxin is rapidly absorbed from the gastrointestinal tract; peak plasma concentrations are reached within 3 h. Digoxin solution is nearly completely absorbed whereas the bioavailability of elixirs and immediate release tablets is incomplete and accounts for 60–80% and 70–85%, respectively. The drug is widely distributed (volume of distribution, 5–8 l/kg); major distribution compartments are skeletal muscles (65%), liver (13%) and heart (4%). Approximately 25% are bound to plasma proteins. Digoxin has a long terminal half-life of 1.5–2 days (Aronson 1980; Iisalo 1977). Approximately 10% of the dose are metabolized by hydrolysis, oxidation and conjugation; major metabolites are 3β-digoxigenin, 3-keto-digoxigenin and their glucuronides and sulfate conjugates (Hinderling and Hartmann 1991; Watson et al. 1973). Sixty to eighty percent of intravenous digoxin are excreted into urine unchanged, predominantly by glomerular filtration (Aronson 1980; Iisalo 1977). Renal handling of digoxin also involves tubular secretion and tubular reabsorption (Doherty et al. 1969; Rengelshausen et al. 2003; Steiness 1974). Approximately 20–40% of an intravenous and oral dose are eliminated by hepatic and intestinal secretion (Table 1) (Caldwell and Cline 1976; Hinderling and Hartmann 1991; Sumner and Russell 1976).

2.2 *Affinity to P-glycoprotein In Vitro and in Animal Studies*

The first in vitro evidence that digoxin might be a substrate of ABCB1 came from Tanigawara et al. (1992). In this study, it was demonstrated that the transepithelial transport of digoxin (100 nM) in *MDR1*-transfected LLC-PK1 cells, a porcine

Table 1 Major physicochemical and pharmacokinetic characteristics of digoxin and talinolol (Aronson 1980; Atkinson and Begg 1988; Caldwell and Cline 1976; Doherty et al. 1969; Drescher et al. 2003; Giessmann et al. 2004a; Greiner et al. 1999; Hinderling and Hartmann 1991; Iisalo 1977; Kurata et al. 2002; Rengelshausen et al. 2003; Schwarz et al. 2007; Siegmund et al. 2002a; Steiness 1974; Sumner and Russell 1976; Watson et al. 1973; Westphal et al. 2000a, b)

	Digoxin	Talinolol
pKa	13.5	9.16
logP (pH 7.4)	1.67	1.08
Water solubility (g/l)	0.065	5.1
Bioavailability (%)	60–80	55–70
Distribution volume (l/kg)	5–8	3–6
Plasma protein binding (%)	20–30	50–70
Renal clearance (ml/min)	140–160	150–190
Nonrenal clearance (ml/min)	80–100	120–180
Metabolite excretion (%)	5–15	<1
Half-life (h)	30–50	10–17

kidney tubular cell line, was nearly eightfold higher in the basal-to-apical (b → a) direction compared to the apical-to-basal (a → b) direction and could be inhibited by the known ABCB1 inhibitors vinblastine, quinidine, and verapamil (all 20 μM). The same group found that also cyclosporine inhibits (1–10 μM) the secretory net transport of digoxin (55 nM), whereas digoxin (100 μM) did not influence the transport of cyclosporine (85.5 nM) (Okamura et al. 1993). Ito et al. found that the digoxin transport (200 nM) across renal tubular cells is a saturable (K_m = 14.1± 1.6 μM), energy dependent process, because 2,4-dinitrophenol (1 mM) and sodium azide (10 mM) significantly reduced the b → a flux (Ito et al. 1993a). Moreover, inhibitors of P-glycoprotein (quinidine, verapamil and vincristine) significantly increased the a → b flux of digoxin by 132–175% and inhibited the b → a secretion by 49–55%. Schinkel et al. later on confirmed the affinity of digoxin to P-glycoprotein using the LLC-PK1 cells transfected with human and murine *ABCB1*/*Abcb1a* (Schinkel et al. 1995). The transepithelial transfer of radiolabeled digoxin (2 μM) was considerably higher in the secretory direction than in the absorptive direction without reaching saturation (K_m > 2 μM). Several other researches confirmed that digoxin is a high affinity substrate for P-glycoprotein in studies with Caco-2, LLC-PK1 and *MDR1*-MDCKII cells. The efflux ratios (apparent permeability P_{app} b → a/a → b) ranged in Caco-2 cells from 4 to 8 and in *ABCB1*-transfected cells from 26 to 52 (Table 2) (Keogh and Kunta 2006; Neuhoff et al. 2003; Pauli-Magnus et al. 2001; Rautio et al. 2006; Shirasaka et al. 2006; Taub et al. 2005).

Digoxin seems not to be an inhibitor of P-glycoprotein. Although being highly transported across monolayers of MDR1-transfected cells with high efflux ratio, digoxin showed no effects on b → a permeability of calcein-AM, vinblastine, colchicin, prazosin, and cyclosporine (Okamura et al. 1993; Rautio et al. 2006).

In parallel to the first in vitro experiments using cell models, it was demonstrated in dogs that cyclosporine and quinidine influence the renal excretion of digoxin. After intravenous administration of cyclosporine in cremophor EL (0.5–3.5 μM) as

Table 2 In vitro studies in which was shown that digoxin is a substrate of P-glycoprotein

Cell model	Apical-to-basal (a → b) and basal-to-apical (b → a) transport via cell monolayer	References
Caco-2	In native cells (0.1 μM digoxin): a → b: $1.35 \pm 0.13 \times 10^{-6}$ cm/s, b → a: $5.73 \pm 0.19 \times 10^{-6}$ cm/s, ratio: 4.24 In induced cells (10 nM vincristine): a → b: $0.82 \pm 0.13 \times 10^{-6}$ cm/s, b → a: $8.27 \pm 0.20 \times 10^{-6}$ cm/s, ratio: 10.1	Shirasaka et al. (2006)
L-MDR1, Caco-2	b → a net transport (% of added digoxin, 5 μM): 16.0 ± 4.4% in Caco-2, 11.7 ± 3.6% in L-MDR1 and 3.3 ± 1.1% in LLC-PK1 cells. No b → a/ a → b differences in the presence of PSC833 (1 μM)	Pauli-Magnus et al. (2001)
L-MDR1	[^{3}H]digoxin (100 nM), b → a/a → b ratio: 8, inhibited by vinblastine, quinidine or verapamil (all 20 μM)	Tanigawara et al. (1992)
L-MDR1	[^{3}H]digoxin (55 nM), b → a > a → b (no efflux ratio given), inhibited by cyclosporine (1, 5, 10 μM)	Okamura et al. (1993)
L-MDR1	[^{3}H]digoxin (2 μM), substantially higher b → a versus a → b, K_m > 2 μM	Schinkel et al. (1995)
MDCKII	[3H]digoxin (20 nM), a → b: $0.79 \pm 0.53 \times 10^{-6}$ cm/s, b → a: $4.07 \pm 0.25 \times 10^{-6}$ cm/s, ratio: 5.2 (2.0 in presence of 200 μM verapamil)	Taub et al. (2005)
LLC-PK1	[^{3}H]digoxin (200 μM), b → a: 254.5 ± 4.2, a → b: 188.2 ± 22.9 fmol/cm^2/h, ratio 3.8 (normalized to mannitol) Saturable b → a transport: K_m 14.1 ± 1.6 μM, V_{max}: 184.5 ± 38.0 pmol/cm^2/h In presence of quinidine (20 μM), verapamil (20 μM), vincristine (20 μM): b → a (10 nM digoxin) decrease by −51%, −45%, −59%; a → b, increase by 75%, 32%, 46%	Ito et al. (1993a)
MDR1-MDCKII	b → a/a → b ratio for digoxin (0.05–10 μM): 33.6 ± 3.2 (1.28 ± 0.13 in presence of 2 μM elacridar) IC_{50} for b → a inhibition: elacridar = 0.18 μM, itraconazole = 0.95 μM, quinidine = 9.4 μM, verapamil = 8.1 μM	Keogh and Kunta (2006)
MDR1-MDCKII	Digoxin (43 nM), P_{app} a → b 3.07 ± 0.30 nm/s, P_{app} b → a: 159 ± 17 nm/s, b → a/a → b ratio: 51.8	Rautio et al. (2006)
MDR1-MDCKII	[3H]digoxin (20 nM), a → b: $0.32 \pm 0.10 \times 10^{-6}$ cm/s, b → a: $8.15 \pm 0.91 \times 10^{-6}$ cm/s, ratio: 25.5 (1.3 in presence of 200 μM verapamil)	Taub et al. (2005)

well as after infusion of quinidine (37.5 μg/kg min), the urinary recovery of digoxin was reduced by about 50% without change of renal blood flow or urine flow (both $p < 0.001$). The authors concluded that there is a luminally localized secretory system for digoxin in the kidneys (de Lannoy et al. 1992). In a landmark study, Schinkel et al. observed that digoxin concentrated 35.3-fold in brain tissue of *Abcb1a*(−/−) knockout mice 4 h after intravenous injection of 1 mg/kg

compared to wild-type animals (Schinkel et al. 1995). In another study with digoxin (0.2 mg/kg), the same group found that genetic deficiency of P-glycoprotein decreased fecal excretion of digoxin significantly by 75% and 48% but increased renal elimination by 120% and 240% after intravenous and oral administration, respectively (Mayer et al. 1996). Wild-type mice with a cannulated gallbladder showed a substantial intestinal secretion of intravenously given digoxin (16.4% of dose); biliary and intestinal excretion in *Abcb1a* knockout mice accounted for only 66% and 13.4%, respectively, of the rates in wild-type animals. Digoxin significantly accumulated 66-fold in brain tissue of the deficient mice (after 8 h). Maximum brain levels were measured 72 h after bolus injection; at that time, the drug was not detectable in plasma anymore. The results obtained with *Abcb1a*(−/−) mice were confirmed later on by the same group in studies using *Abcb1a*/*b*(−/−) knockout mice (Schinkel et al. 1997). Finally, Kawahara et al. compared pharmacokinetics of digoxin in *Abcb1a*(−/−) and wild-type mice. In deficient animals, $AUC_{0–24h}$ was increased nearly threefold and mean residence time 1.3-fold whereas renal and biliary clearance was reduced to one third of the values in wild-type animals (Kawahara et al. 1999).

Mayer et al. measured the influence of the specific P-glycooprotein inhibitor PSC833 (valspodar) on pharmacokinetics of digoxin in wild-type and *Abcb1a*/*b*(−/−) mice (Mayer et al. 1997). In *Abcb1a*/*b*(−/−) mice, the brain-to-plasma ratio was increased 27.8-fold and 10-fold 4 h and 24 h, respectively, after oral and intravenous administration of digoxin (0.05 mg/kg). The fecal excretion was reduced nearly by half whereas urinary excretion was doubled. Concomitant oral administration of PSC833 (50 mg/kg) caused pharmacokinetic changes in disposition of intravenous digoxin in wild-type mice which were similar to the changes in untreated knockout mice. In the *Abcb1a*/*b*(−/−) mice, presence of PSC833 unexpectedly increased urinary excretion and reduced fecal excretion of digoxin; biliary excretion was also significantly lowered. Similar unexpected evidence came from Fromm et al. who studied the effects of quinidine on digoxin disposition in wild-type and *Abcb1a*(−/−) mice. In wild-type animals, coadministration of quinidine (100 mg/kg) increased the concentrations of digoxin (0.5 mg intravenously) in plasma, brain, liver, kidney and intestine 1.4- to 2-fold. Quinidine comedication, however, also increased the plasma and tissue levels by 110–180% in *Abcb1a*(−/−) animals but reduced concentration of digoxin in brain tissue significantly (Fromm et al. 1999). These findings suggest that PSC833 and quinidine are very likely to be also inhibitors of other – currently unknown – excretory and/or uptake systems that are important for the distribution and elimination of digoxin.

2.3 Evidence from Mechanistic Clinical Studies

Convincing evidence for digoxin to be secreted by intestinal P-glycoprotein was obtained in mechanistic clinical studies using an intestinal multilumen perfusion catheter (Drescher et al. 2003; Glaeser et al. 2002; Igel et al. 2007). The authors

confirmed that intestinal P-glycoprotein is involved in digoxin elimination after intravenous administration from the blood into the gut lumen and prevents systemic exposure with luminally administered digoxin (eight male healthy subjects). After intravenous administration of 1.0 mg digoxin, 0.45 ± 0.24% of the dose appeared in the perfusate of a jejunal segment. Assuming the overall length of the small intestine of 3–5 m and that P-glycoprotein expression is constant along the small intestine, the authors calculated an average digoxin elimination of more than 11% within 3 h. Perfusion with quinidine reduced the intestinal digoxin secretion by 50%. The concentration of quinidine in the segment (104 ± 43.4 μM) was nearly 50-fold higher than the IC_{50} for inhibition of digoxin transport in Caco-2 cells (2.2 μM) (Wandel et al. 1999). After pretreatment of the healthy subjects with rifampicin (600 mg daily, 10 days), secretion of digoxin into the perfusion segment in the proximal jejunum increased by about 80%. Nonrenal clearance increased by 88% and the renal excretion decreased by 18% whereas renal clearance and half-life remained unchanged by transporter induction with rifampicin. 53% of the $AUC_{0–96h}$ were predicted by P-glycoprotein expression in shed enterocytes (Drescher et al. 2003).

Oral bioavailability of 0.5 mg digoxin was studied in seven healthy male subjects using the same technique; about 22% of the dose were absorbed into the systemic circulation via the 20-cm jejunal perfusion segment. In the presence of quinidine (116 ± 69 μM), bioavailability increased by about 150% and the amount excreted into urine by 250%. 1.26% of the absorbed digoxin dose were secreted back into the adjacent segment (Igel et al. 2007).

2.4 Digoxin Disposition and Induction of Intestinal P-glycoprotein

Greiner et al. (1999) used for the first time digoxin as a probe drug for intestinal P-glycoprotein in man. In order to induce P-glycoprotein they treated eight healthy male subjects with 600 mg rifampicin once daily for 10 days (Greiner et al. 1999). Rifampicin induced duodenal P-glycoprotein by 1.4-fold (immunohistochemistry) and 3.5-fold (Western blot), respectively. This up-regulation of P-glycoprotein was associated with 3.2-fold increase of the nonrenal clearance of digoxin and decrease of bioavailability by 21%. Urinary excretion of digoxin after oral and intravenous dosing lowered by 31% and 17%, respectively, whereas renal clearances and half-lives remained unchanged. More than 50% of the $AUC_{0–144h}$ were predicted by intestinal P glycoprotein expression. A borderline correlation was also observed between plasma AUC of digoxin and intestinal CYP3A4 levels. Basal, noninduced P-glycoprotein content correlated not to AUC of digoxin. However, this clinical study confirmed convincingly that intestinal protein content of P-glycoprotein predicts absorption of digoxin in man, and in turn, that digoxin is a suitable probe drug for the function of intestinal P-glycoprotein.

Similar indirect evidence for the value of digoxin as a probe drug for intestinal P-glycoprotein function was obtained in drug interaction studies with SJW (Gurley et al. 2008; Johne et al. 1999; Mueller et al. 2004). Ingredients of SJW act like rifampicin as ligands of the nuclear PXR and induce intestinal P-glycoprotein in man (Dresser et al. 2003; Durr et al. 2000; Fromm et al. 2000; Geick et al. 2001; Giessmann et al. 2004b; Greiner et al. 1999; Luo et al. 2002; Moore et al. 2000; Schwarz et al. 2007; Wentworth et al. 2000; Westphal et al. 2000b). In a single-blind, placebo-controlled, parallel-group study, 25 healthy subjects were treated with 0.25 mg digoxin for 15 days (Johne et al. 1999). After loading for 5 days, SJW ($N = 13$) or placebo ($N = 12$) were coadministered for 10 days. Single dose SJW was without any effect on digoxin disposition. After multiple-dose comedication, $AUC_{0–24h}$, C_{max} and C_{trough} decreased by 20–26% whereas half-lives remained unchanged. Interestingly, the digoxin trough levels decreased in dependence on the duration of SJW treatment; a significant decrease from the placebo group occurred after 7 days. The pharmacokinetic changes resulted most likely from increasingly reduced absorption of digoxin following induction of intestinal P-glycoprotein. In another placebo-controlled, parallel-group study in 96 healthy subjects, it was shown later, that the interaction of SJW with digoxin varies with the SJW preparation and seems to be correlated to the dose, particularly to the ingredient hyperforin (Mueller et al. 2004). The chronic effects of rifampicin (300 mg, twice daily, 7 days) and SJW (300 mg three times daily) on digoxin pharmacokinetics (0.25 mg) were confirmed by the results of a recent study in 18 healthy subjects. The $AUC_{0–24h}$ and C_{max} of digoxin decreased by about 25% and about 37%, respectively, after both kinds of induction. Half-lives also remained unchanged in this study (Gurley et al. 2008).

2.5 Digoxin Disposition and Inhibition of Intestinal P-glycoprotein

In the US Food and Drug Administration (FDA) draft guidance for drug interaction studies, digoxin is the recommended probe drug for the investigation of potential substrates/inhibitors or inducers of P-glycoprotein (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf). The suitability of digoxin to evaluate interactions with intestinal P-glycoprotein has been confirmed in numerous clinical trials. Some of the most important studies are addressed in the following (Table 3).

The influence of verapamil (240 mg/day, t.i.d.), an unspecific and moderate modulator of P-glycoprotein, on the steady-state pharmacokinetics of digoxin (0.25 mg twice daily, 2 weeks) was evaluated by Rodin et al. in ten healthy subjects (Rodin et al. 1988). Verapamil increased the AUC of digoxin by 50% and the C_{max} by 40%. Renal clearance remained unchanged. These results are explained by increase of digoxin bioavailability as caused by inhibition of intestinal P-glycoprotein, given that the volume of distribution was not influenced by

Table 3 Single dose (SD) and multiple dose (MD) drug interaction studies with digoxin and talinolol after oral (po) and/or intravenous (iv) administration

Inhibitor	Design	AUC ratio	C_{max} ratio	CL_R ratio	IC_{50} (μM)	Peak plasma concentration (μM)	Gut concentration (μM)	Reference(s)
Studies with digoxin								
Talinolol	SD, po	1.23*	1.45*	1.04	198	0.73	1,100	Collett et al. (2005) and Westphal et al. (2000a)
Itraconazole	SD, po	1.52*	1.34	0.80*	1.3	0.53	1,134	Jalava et al. (1997) and Keogh and Kunta (2006)
Clarithromycin	SD, po	1.64*	1.83*	0.83	~300	1.63	1,337	Rengelshausen et al. (2003) and Wakasugi et al. (1998)
Clarithromycin	SD, iv	1.19	–	–	~300	1.63	1,337	Rengelshausen et al. (2003) and Wakasugi et al. (1998)
Clarithromycin	SD, po	1.47*	1.75*	–	~300	1.63	1,337	Gurley et al. 2008, Rengelshausen et al. (2003) and Wakasugi et al. (1998)
Grapefruit juice	SD, po	1.10*	1.23	1.0	–	–	–	Becquemont et al. (2001)
Ritonavir	SD, po	1.86*	–	0.65*	28.2	19.6	1,665	Ding et al. (2004) and Keogh and Kunta (2006)
Verapamil	MD, po	1.50*	1.44*	1.04	10/5.9	0.13	704	Fenner et al. (2009), Keogh and Kunta (2006) and Rodin et al. (1988)
Verapamil	MD, po	–	1.50*	1.13	10/5.9	0.13	704	Fenner et al. (2009), Hedman et al. (1991) and Keogh and Kunta (2006)
Quinidine	MD, po	1.77*	1.75*	–	14.1/21	8.4	–	Fenner et al. (2009), Keogh and Kunta (2006) and Pedersen et al. (1983)
Quinidine	MD, po	–	1.55	0.71*	14.1/21	4.5	3,083	Fenner et al. (2009), Hedman et al. (1990) and Keogh and Kunta (2006)
Valspodar[a]	MD, po	1.74*	1.74*	0.35*	0.1	1.56	1,317	Kovarik et al. (1999) and Song et al. (1999)

Valspodar[b]	MD, po	3.05*	2.44*	0.25*	0.1	1.49	659	Kovarik et al. (1999) and Song et al. (1999)
Carvedilol[c]	MD, po	1.56*	1.38*	–	4	65.2	61.5	Baris et al. (2006), Fenner et al. (2009) and Tenero et al. (2000)
Carvedilol[d]	MD, po	1.24	1.0	–	4	65.2	61.5	Baris et al. (2006), Fenner et al. (2009) and Tenero et al. (2000)
Carvedilol	SD, po	1.19*	1.60*	–	4	265	246	De Mey et al. (1990), Fenner et al. (2009) and Tenero et al. (2000)
Carvedilol	SD, iv	0.96	1.05	–	4	265	246	De Mey et al. (1990), Fenner et al. (2009) and Tenero et al. (2000)
Studies with talinolol								
Erythromycin	SD, po	1.52*	1.26*	–	>100	17.1	10,900	Josefsson et al. (1982), Keogh and Kunta (2006) and Schwarz et al. (2000)
TPGS[e]	SD, po	1.20*	1.36*	–	–	–	–	Bogman et al. (2005)
silymarin	MD, po	1.30*	1.27*	–	–	–	–	Han et al. (2009)
Ginkgo biloba extract	MD, po	1.22*	1.26*	–	–	–	–	Fan et al. (2009b)
Schisandra chinensis extract	MD, po	1.52*	1.51*	–	–	–	–	Fan et al. (2009a)

The ratios for AUC, C_{max} and CL_R indicate the pharmacokinetic changes in the presence of the potential P-glycoprotein inhibitor. Furthermore, IC_{50} values and peak plasma concentrations from the studies or from literature and apparent gut concentrations (I_2, dose/250 mL administration volume) of the inhibitors are given

*p at least <0.05 compared to control

[a]Valspodar was given single-dose orally (400 mg)

[b]Valspodar was given repeatedly twice daily (a 200 mg)

[c]Study was performed in males ($N = 12$)

[d]Study was performed in females ($N = 12$)

[e]Tocopheryl polyethylene glycol 1000 succinate (0.04%)

verapamil. This conclusion is supported by the results of a clinical investigation in six patients with chronic atrial fibrillation receiving oral digoxin (0.25–0.5 mg/day) and verapamil (240 mg/day) for at least 4 weeks (Hedman et al. 1991). Verapamil significantly elevated steady-state plasma concentrations of digoxin by 44%, whereas renal clearance was not influenced. Hepatic and/or intestinal secretion of digoxin as assessed by duodenal-marker-perfusion technique was reduced by 43% in the presence of verapamil, which corroborates the conception that this effect is caused by inhibition of intestinal P-glycoprotein. Inhibition of intestinal P-glycoprotein also seems to be the reason for an increase in digoxin bioavailability by 18% after comedicaton of 100 mg talinolol as it was observed in ten healthy subjects (Westphal et al. 2000a).

However, there are many other clinical studies, in which the increase of the digoxin AUC in presence of a P-glycoprotein substrate/inhibitor was more than 43%, e.g. after administration of PSC833 (valspodar), itraconazole or clarithromycin (Gurley et al. 2008; Jalava et al. 1997; Kovarik et al. 1999; Rengelshausen et al. 2003). Increase of AUC-values by 43% is the maximum elevation that can be solely explained by complete blockade of the presystemic elimination of digoxin via intestinal P-glycoprotein efflux assuming that the bioavailability of commonly used immediate release digoxin tablets is 70%. This upper limit of the "AUC-calibration range" ensures that inhibitory potency of clinical relevance can be reliably measured with digoxin if taken in mind that the world-widely accepted equivalence range for primary outcome characteristics for decisions in drug interaction studies is 0.80–1.25 (http://www.emea.europa.eu/pdfs/human/ewp/056095en.pdf, www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070124.pdf). If AUC-values are elevated by more than about 40% in presence of an inhibitor, influences on distribution or elimination routes for digoxin, e.g. by the intestine or kidneys, have to be taken into account.

Many efforts have been spent to predict pharmacokinetic interactions between digoxin and potential substrates and/or inhibitors of P-glycoprotein in order to facilitate the decision whether a clinical trial is needed. The FDA draft guidance document recommends that a drug interaction study should be performed if the ratio of maximum systemic inhibitor concentration at steady state $[I]$ over inhibitory potency measures (inhibitor constant, K_i or concentration to inhibit 50% P-glycoprotein activity, IC_{50}) is above 0.1. As recently mentioned by Fenner et al., it appears that the FDA guidance has applied the ratio introduced by Rowland et al., who showed that for a single route of clearance, the increase in the AUC in the presence of an inhibitor can be represented as $1 + [I]/K_i$. When $[I]/K_i$ is 0.1, the predicted increase in AUC ratio will be 10% (Fenner et al. 2009; Rowland et al. 1973). However, there are many doubts whether the inhibitor concentrations (K_i/IC_{50}) for interaction with P-glycoprotein in tumor cells or transfected cells are similar to the concentrations necessary for inhibition of a transporter embedded in the apical membrane of highly differentiated human cells (enterocytes, tubular cells in kidneys and liver). Furthermore, it is unknown whether plasma levels of an inhibitor are identical with the concentrations on P-glycoprotein receptor site in the opposite, apical cell membrane.

To predict interactions with P-glycoprotein that occur during absorption, the ratio $[I_2]/\mathrm{IC}_{50} > 10$ has been recommended with $[I_2]$ being the inhibitor gut concentrations (dose divided by administration volume). However, the real concentrations in the apical membrane after oral administration of the inhibitor are dependent on many factors, which are not to predict easily (disintegration and dissolution rate of the test and reference drug, availability of luminal water, rate of intestinal water absorption, etc.). Fenner et al. recently reevaluated 123 digoxin drug interaction trials. Taking AUC-ratios and C_{max}-ratios as measures for systemic exposure of digoxin at steady-state and the in vitro characteristics of $[I]/\mathrm{IC}_{50}$ and $[I_2]/\mathrm{IC}_{50}$, it appeared that $[I]/\mathrm{IC}_{50} > 0.1$ is predictive for a P-glycoprotein related interaction with digoxin and that, however, to a limited extent, $[I_2]/\mathrm{IC}_{50} < 10$ is predictive for lack of interactions. Nevertheless, the authors observed a high number of false-negative predictions with $[I]/\mathrm{IC}_{50} < 1$ and false-positive predictions with $[I_2]/\mathrm{IC}_{50} > 10$ (Fenner et al. 2009).

2.6 *Regioselective Absorption of Digoxin*

Intestinal absorption of digoxin seems to be dependent on the intestinal site of disintegration of the digoxin dosage form and dissolution of the drug substance. This is in agreement with the regio-selective expression of intestinal P-glycoprotein; with the transporter being higher expressed in distal regions (Englund et al. 2006; Wilding et al. 1992; Zimmermann et al. 2005). Concomitant administration of the anticholinergic drug propantheline (10 mg t.i.d., 10 days) which is known to inhibit intestinal transit causes as much as a threefold increase in serum concentrations in patients on maintenance therapy with a slowly dissolving brand of digoxin tablets (Manninen et al. 1973b). In contrast, metoclopramide (10 mg t.i.d., 10 days), which increases gastrointestinal transit rate produced a marked decrease in digoxin concentrations (Manninen et al. 1973a). Propantheline had no effect when digoxin was administered in solution (eight healthy subjects) (Manninen et al. 1973a).

2.7 *Digoxin as a Probe Drug for Genetic Polymorphisms of P-glycoprotein*

The database on the influence of *ABCB1* gene polymorphisms on digoxin disposition in man is currently as contradictory as the data on genetic variability of intestinal P-glycoprotein expression (Table 4). Unfortunately, most of the pharmacogenetic studies with digoxin were not performed in a prospective manner with adequate primary endpoints and sufficient statistical power. The study samples commonly comprised the control groups from previous drug interactions studies.

Table 4 Gene polymorphisms of ABCB1 and changes in the pharmacokinetics of digoxin and talinolol

Ethnic group	Genotype/ haplotype number of carriers	Dose	Changes observed	References
Digoxin				
Caucasians	C3435T 7 CC, 7 TT	0.25 mg tablet steady-state	↑ 38% C_{max} for TT	Hoffmeyer et al. (2000)
Caucasians	C3435T 8 CC, 8 CT, 8 TT	0.5 mg, oral steady-state	↑ 21% $AUC_{0–4h}$, ↔ $AUC_{0–24h}$, ↑ 23% C_{max}, ↑ 36% C_{trough}, ↑ 20% A_e for TT	Johne et al. (2002)
25 Caucasians, 6 Africans, 1 Asian	C3435T 14CC, 8CT, 10TT		↑ 20% $AUC_{0–48h}$ for TT	Verstuyft et al. (2003)
Caucasians	C3435T 12CC, 25CT, 13TT	1.0 mg tablet single dose	No difference of $AUC_{0–4h}$	Gerloff et al. (2002)
25 Caucasians, 6 Africans, 1 Asian	G2677/A 12GG, 11GT, 7TT, 1GA, 1AA	0.5 mg tablet single dose	No difference of $AUC_{0–48h}$	Verstuyft et al. (2003)
Caucasians	G2677/A 12GG, 21GT, 7TT, 7GA, 3AA	1.0 mg tablet single dose	No difference of $AUC_{0–4h}$	Gerloff et al. (2002)
Japanese	C3435T 5CC, 4CT, 6TT	0.25 mg tablet single dose	↓ 20% $AUC_{0–4h}$, ↓ 35% $AUC_{0–24h}$ (ns)	Sakaeda et al. (2001)
Japanese	C3435T 5CC, 6TT	0.25 mg tablet 0.25 mg/5 ml solution	Tablet: not difference Solution sprinckled over duodenal mucosa: ↓ 25% $AUC_{0–4h}$, ↓ 55% C_{max}	Morita et al. (2003)
Japanese	G2677T/A/ C3435T 5GC/GC, 5GC/ TT, 5TT/TT	0.5 mg, oral-iv, single dose	↑ 30% F, ↑ 25% AUC_{iv}, ↑ 70% AUC_{po}, ↓ 32–40% CL_R, ↓ 32–40% CL_{NR} for TT/TT	Kurata et al. (2002)
Chinese	C1236T/G2677T/ A/C3435T 7TTT/TTT, 5TGC/CGC	0.25 mg, oral, single dose	↑ 45% $AUC_{0–4h}$, ↑ 80% C_{max} for TTT/TTT	Xu et al. (2008)
Caucasians	C1236T/G2677T/ A/C3435T 38 CGT, 33 TTT	0.57 mg (DDD)	↑ C_{trough} by 0.18–0.21 ng/ml per additional T allele; 4.3-fold higher risk for digoxin levels >2.0 mg/ml for TTT	Aarnoudse et al. (2008)
Talinolol				
Caucasians	C3435T 13CC, 29 CT, 13 TT	30 mg iv, 100 mg po, single dose	No difference for TT	Siegmund et al. (2002b)

(*continued*)

Table 4 (continued)

Ethnic group	Genotype/ haplotype number of carriers	Dose	Changes observed	References
Caucasians	G2677T/A 19GG, 22GT, 9TT, 2GA, 3TA	30 mg iv, 100 mg po, single dose	↑ AUC in TT/TA	Siegmund et al. (2002b)
Chinese	C3435T 6CC, 6CT, 6TT	100 mg po, single dose	No difference for TT	Han et al. (2009)
Caucasians	C1236T/G2677T/ A/C3435T 5TTT, 4CGC	30 mg iv, 50 mg po, single dose	↑ Inductive response in TTT	Schwarz et al. (2007)

ns not significant, *DDD* defined daily dose, *po* per os, *iv* intravenous

Primary pharmacokinetic parameters that are suitable to describe intestinal P-glycoprotein activity are bioavailability and nonrenal clearance or intestinal (fecal) clearance of intravenous digoxin. Instead, week surrogate parameters were commonly measured as $AUC_{0–4h}$, C_{max}, C_{trough} (at steady state) or t_{max}. A pharmacogenetic study on digoxin pharmacokinetics in healthy subjects with adequate sample size is still missing.

The *ABCB1* 3435T allele seems to be associated with higher digoxin bioavailability and higher plasma exposure as shown in retrospective studies with small sample size (5–14 per group) and digoxin doses of 0.5 and 1.0 mg (single doses) and 0.25 mg (steady-state) (Johne et al. 2002; Kurata et al. 2002; Verstuyft et al. 2003). These findings are in agreement with probably lower expression of intestinal P-glycoprotein in carriers with the 3435T allele (Hoffmeyer et al. 2000). Xu et al. observed markedly higher digoxin plasma levels in subjects with the TTT–TTT in comparison to the TGC–CGC haplotype in subjects comprising the *ABCB1* C1236T, G2677T/A and C3435T single nucleotide polymorphisms (Xu et al. 2008). This observation agrees well with the haplotype dependent expression of intestinal P-glycoprotein as described by Schwarz et al. (2007).

In a prospective population-based cohort study (Rotterdam study), digoxin serum levels and DNA to genotype *ABCB1* C1236T, G2677T/A and C3435T were available from 195 participants (Aarnoudse et al. 2008). All *ABCB1* variants were significantly associated with increased serum digoxin concentration by 0.18–0.21 ng/ml per additional *T* allele. Up to 11.5% of the variability in digoxin concentrations is explained by the *TTT* haplotype. It was concluded from the obtained data, that *TTT* carriers of the combined C1236T-G2677T/A-C3435T are also at higher risk for toxic digoxin serum concentrations above 2.0 ng/ml (OR 4.3; 95% CI 1.4–13.4).

These results were contradicted by Gerloff et al. (2002), Sakaeda et al. (2001) and Kurzawski et al. (2007). Gerloff et al. (1.0 mg digoxin per os) observed no influence of *ABCB1* G2677T/A and C3435T on digoxin plasma concentrations (Gerloff et al. 2002). Sakaeda et al. (0.25 mg digoxin per os) measured even

lower plasma levels in carriers of 3435TT. (Sakaeda et al. 2001) Kurzawa et al. found no influence of *ABCB1* G2677T/A and C3435T on steady-state digoxin plasma levels (0.25 mg digoxin) in small groups of Polish patients (Kurzawski et al. 2007).

2.8 Limitations of Digoxin

Pharmacokinetic studies with digoxin require sampling (plasma, urine, feces) for at least 7 days because of the long terminal half-life of digoxin. Therefore, controlled studies with digoxin are of long duration and of high risk for dropping out and sequence effects. Furthermore, analytical problems are likely because of the small range between the average C_{max}-values of 1–5 ng/ml after single oral doses of 0.25–1.0 mg and the limit of quantification of 0.1–0.2 ng/ml provided by immunological assays; e.g. (Greiner et al. 1999; Gurley et al. 2000a). As a consequence, it is likely that the terminal elimination rate of digoxin has been underestimated in many pharmacokinetic studies, particularly after single administration of low doses. Analytical problems may be also the reason for the lack of data on fecal excretion of digoxin in clinical studies. However, the availability of highly sensitive mass-spectrometric assays will overcome these limitations in future studies (Hashimoto et al. 2008; Kirby et al. 2008; Ni et al. 2008).

Digoxin has low water solubility and high permeability and belong to the Class 2 drugs of the BCS system if the low therapeutic dose and the existence of intestinal uptake transporters for digoxin are ignored (Amidon et al. 1995; Lindenberg et al. 2004; Shugarts and Benet 2009; Wu and Benet 2005). According to the current hypothetical conception, high permeability of digoxin will allow rapid transfer into apical gut membranes but its low solubility in water will limit concentration in enterocytes, thereby preventing saturation of the efflux transporter (Wu and Benet 2005). Therefore, the suitability of digoxin to be a probe drug of P-glycoprotein must be highly influenced by the properties of the dosage form. Digoxin solution administered as soft gelatin capsules seems therefore inapplicable for mechanistic studies because its bioavailability is close to 100%, i.e., the uptake of digoxin solution into the systemic circulation by which mechanisms, however, it is mediated cannot be rate limited by the function of intestinal P-glycoprotein. Morita et al. have recently shown, that digoxin is better and more rapidly absorbed when the dose of 0.25 mg is sprinkled in 5 ml solution directly over the surface of the duodenum using an endoscope compared to swallowing a conventional tablet with 200 ml tap water (Morita et al. 2003). Obviously, intestinal P-glycoprotein is a major variable in digoxin absorption only when it appears in the apical membrane in low concentrations at which the balance between the rate of uptake can be measurably limited by the P-glycoprotein mediated efflux. However, the real concentration of digoxin at the place of absorption is unknown because it is dependent on several luminal factors as disintegration/dissolution of the dosage form, availability of water for dissolution, gastric emptying or small intestinal transit time.

So far, it is unknown what is behind high permeability of digoxin; simple nonionic diffusion or coordinate interplay of apical uptake transporters with basolateral efflux carriers, which function is dependent on expression level and drug affinity. For other organs, uptake carriers for digoxin are already discovered. Digoxin is an unique substrate of the human uptake transporter OATP1B3 (Kullak-Ublick et al. 2001; Noe et al. 1997). Therefore, variability of cellular uptake may influence the suitability of digoxin to be a probe for intestinal P-glycoprotein. For instance, digoxin uptake in rat hepatocytes and Oatp2-expressing *Xenopus oocytes* significantly decreases in the presence of amiodarone (10 μM). The same concentration of the inhibitor had only a slight inhibitory effect on the P-glycoprotein-mediated digoxin transfer via LLC-PK_1 cell monolayers (Kodawara et al. 2002). Therefore, the known increase of digoxin plasma levels after comedication of amiodarone in man may result, at least in part, from competition with the hepatic uptake of digoxin (Holt et al. 1983; Nademanee et al. 1984). For the strong OATP-inhibitor rifampicin, it was shown, that it reduces hepatic uptake and exposure of digoxin to hepatic biotransformation in rats (perfusion models), whereas quinidine increases liver cell exposure by inhibition of the canalicular P-glycoprotein (Lau et al. 2004; Shitara et al. 2002; Weiss et al. 2008). Single dose administration of rifampicin in rats may even mask the inductive effects of dexamethasone on digoxin disposition (Lam et al. 2006). However, digoxin is extensively metabolized by cytochrome P4503A in rats (>70% of an i.p. dose) (Harrison and Gibaldi 1976; Schmoldt and Ahsendorf 1980; Shitara et al. 2002). Contrary to the situation in rats, there is no convincing information from clinical studies in man, whether inhibitors of intestinal P-glycoprotein may influence hepatic uptake of digoxin. Triscari et al. investigated the interaction of digoxin (0.2 mg) and of the OATP1B1 substrate pravastatin (20 mg) at steady-state (9 days) in 18 healthy male subjects. In the presence of pravastatin, AUC, C_{max}, t_{max} and urinary excretion of digoxin were unchanged (Triscari et al. 1993). Therefore, OATP1B1-mediated hepatic uptake seems to be a minor variable in disposition of digoxin. However, some recent evidence point to an insert variant allele of the OATP1B3 gene to be associated with higher digoxin plasma concentrations in Japanese patients with terminal renal failure (Tsujimoto et al. 2008). Future clinical studies should focus on whether drug induced variability in hepatic uptake of digoxin is really a negligible limitation in drug interaction studies with digoxin in healthy subjects, even though the drug is nearly unmetabolized and only a small dose portion enters the entero-hepatic circle (less than 10%).

Another problem arises in multiple-dose studies with digoxin, because continuous exposure to the drug might induce P-glycoprotein expression in concentration-dependent manner as shown in Caco-2 cells (Takara et al. 2002a, b). Taken into mind that the magnitude of P-glycoprotein induction seems to be lower in carriers of variant ABCB1 alleles, the genotype related differences at steady-state as described by Hoffmeyer et al., Johne et al., and Aarnoudse et al. may have been overestimated or they result entirely from genotype-related extent of transporter up-regulation (Aarnoudse et al. 2008; Hoffmeyer et al. 2000; Johne et al. 2002).

Distribution volume is another critical P-glycoprotein-related variable in digoxin disposition. Carriers of the *ABCB1* 3435TT genotype have a lower apparent

distribution volume for digoxin compared to carriers of the 3435CC allele as recently demonstrated using nonlinear mixed-effect model simulations. That means, that the genotype related AUC differences in the study of Verstuyft et al. resulted entirely from changes in distribution volume rather than from difference in digoxin absorption (Comets et al. 2007; Verstuyft et al. 2003). It is also to consider that the half-life depends on the distribution volume and is not a suitable measure for intestinal P-glycoprotein function. Therefore, subtle pharmacokinetic phenotyping with digoxin needs a study arm with intravenous digoxin to exclude P-glycoprotein-related variations in distribution volume.

The major elimination route for digoxin from the systemic circulation is glomerular filtration. Up to 50% of the excretion of digoxin accounts to renal tubular secretion as the glomerular filtation rate in healthy subjects is approximately 120 ml/min compared to the renal clearance of 140–160 ml/min and considering the unbound fraction in plasma being 70–80%. Two active transporter in renal tubular cells were identified so far; the apical efflux carrier P-glycoprotein and the recently discovered organic anion transporter OATP4C1, which serves as a basolateral uptake transporter for cardiac glycosides (digoxin $k_m = 7.7$ μM; ouabain $k_m = 0.38$ μM) at the proximal tubule cells in the human kidney (de Lannoy and Silverman 1992; Ito et al. 1993b; Mikkaichi et al. 2004; Tanigawara et al. 1992). Renal P-glycoprotein can be influenced by the known P-glycoprotein inhibitors quinidine, verapamil, vinca alkaloids, cyclosporine, clarithromycin or ritonavir as shown by in vitro studies using kidney epithelial cell lines and by various animal models (de Lannoy et al. 1992; Hori et al. 1993; Ito et al. 1993b; Okamura et al. 1993; Tanigawara et al. 1992; Wakasugi et al. 1998). It is unknown so far, whether P-glycoprotein inhibitors modulate the basolateral transporter OATP4C1.

Inhibition of renal P-glycoprotein has to be assumed if the AUC-values of conventional digoxin tablets increase by more than 43% after comedication of inhibitors. Digoxin AUC increases after clarithromycin (250 mg, b.i.d., 3 days) by 64%, after intraconazole (200 mg once daily, 5 days) by 67%, and after valspodar (PSC833) by 74% (400 mg, single dose) and 200% (200 mg twice daily, 4 days), respectively (Jalava et al. 1997; Kovarik et al. 1999; Rengelshausen et al. 2003). In order to differentiate, whether interaction with intestinal or renal P-glycoprotein or interaction with both has caused higher digoxin systemic exposure, a careful pharmacokinetic evaluation is required which includes determination of bioavailability, renal and nonrenal clearance and the amount excreted into urine. For example, in presence of itraconazole, the amount of digoxin excreted into the urine significantly increased by 22% although renal clearance significantly decreased by 20% (Jalava et al. 1997). Itraconazole obviously influenced both intestinal and renal P-glycoprotein. Therefore, inhibition of intestinal P-glycoprotein is the reason for higher AUC-values only if changes of renal clearance are excluded by the interaction study. It is also important to recognize that many drugs given concomitantly with digoxin may influence renal blood flow and renal digoxin clearance independent of whether they interact with P-glycoprotein as suggested for nitropusside, hydralazine or captopril (Cleland et al. 1986; Cogan et al. 1981; Mujais et al. 1984).

Another, more or less theoretical limitation for digoxin as a probe drug for intestinal P-glycoprotein results from its biotransformation because about 15% of an oral dose are metabolized by sugar cleavage and glucuronidation, a significant part by presystemic processes (Hinderling and Hartmann 1991; Lacarelle et al. 1991). A borderline correlation was observed between plasma AUC of oral digoxin and intestinal CYP3A content ($r = -0.54$, $p < 0.05$) in the interaction study with rifampicin mentioned above (Greiner et al. 1999). This correlation may have reflected coregulation of intestinal P-glycoprotein and CYP3A4 by rifampicin (Urquhart et al. 2007). So far, there is no evidence from literature that metabolism of digoxin is significantly influenced by inducers and/or inhibitors of P-glycoprotein in man.

It has been reported that digoxin undergoes colonic bacterial degradation by *Eubacterium lentum*. This may result in increased digoxin exposure in case of comedication with antimicrobial drugs (Dobkin et al. 1982; Lindenbaum et al. 1981). It seems however unlikely that eradication of *Eubacterium lentum* may influence bioavailability of immediate release digoxin, because the germ is rarely found in the jejunum and ileum of healthy subjects (Simon and Gorbach 1984).

3 Talinolol

3.1 Safety, Physicochemical Properties and Pharmacokinetics

Talinolol (Cordanum®) is a selective postsynaptic $ß_1$-adrenoceptor antagonist without partial agonistic activity which was launched on the German market for treatment of arterial hypertension and coronary heart disease. The oral standard dose of 100 mg is safe and well tolerated. There are no reports on drug related adverse events being probably or likely related to the study medication in single-dose and repeated-dose studies in healthy subjects applying doses of 50–100 mg talinolol (Giessmann et al. 2004a; Siegmund et al. 2002a; Siegmund et al. 2003; Westphal et al. 2000a, b). Even single doses of 400 mg were well tolerated (De Mey et al. 1995).

Talinolol is highly soluble in water (pH 7.4: 1.24 g/l; pH 7.0: 4.5 g/l; 37°C) and has a pH-dependent partition coefficient (pH 7.4: logP 1.08; 37°C). Accordingly, it belongs to the class III (high solubility, low permeability) of the BCS classification system (Amidon et al. 1995; Le Petit 1985). The drug is erratically and incompletely absorbed from the gastrointestinal tract. The absolute bioavailability is about 55–70%. It is widely distributed ($V_d = 3$–6 l/kg) and 50–70% are bound to plasma proteins (Giessmann et al. 2004a; Siegmund et al. 2002a; Trausch et al. 1995; Westphal et al. 2000a, b). A characteristic finding in talinolol pharmacokinetics after oral administration is the double-peak phenomenon which is probably caused by an intestinal "storage pathway" beyond the gut lumen (Weitschies et al. 2005). After repeated-dose administration of 100 mg talinolol (5–7 days), the

minimum (trough) serum concentrations are approximately 45–60 ng/ml, maximum (peak) concentrations are about 300 ng/ml and the AUC_{0-24h} are in the range of 3,000 ng h/ml at steady-state (Giessmann et al. 2004a; Westphal et al. 2000b). Talinolol is nearly not metabolized (Giessmann et al. 2004a; Siegmund et al. 2002a; Westphal et al. 2000a, b). Small amounts (below 1% of the dose) of 4-*trans* and 3-*cis* hydroxytalinolol are generated by CYP450 dependent hydroxylation of the cyclohexyl ring; 2-*trans* and 3-*trans* isomers have in some cases also been detected. Hydroxylation of the phenyl ring, degradation of the side chains or conjugation of talinolol and the metabolites do not occur (Oertel et al. 1994; Schupke et al. 1996). Significant chiral differences in the metabolism were not observed (Wetterich et al. 1996; Zschiesche et al. 2002). Pretreatment with rifampicin significantly increases the metabolic clearance of talinolol in healthy subjects; however, metabolism still accounts for less than 1% of the total body clearance. The elimination half-life of talinolol is between 10 and 17 h (Giessmann et al. 2004a; Siegmund et al. 2002a, b; Trausch et al. 1995; Westphal et al. 2000a, b). 3.7–25% (median: 9.3%) of intravenous talinolol (30 mg) is excreted into the bile (six cholecystectomized patients) with slight preference of the S(−)-enantiomer. The biliary concentrations are up to 90-fold above the serum levels (Terhaag et al. 1989). After intravenous administration (30 mg, 18 healthy subjects), about 43% of the dose are excreted with the urine, and about 22% is recovered in the feces. After oral administration (100 mg), about 30% are excreted with the urine and another 30% appear in the feces (Bernsdorf et al. 2006). Renal clearance is about 150–190 ml/min which is higher than the product of nonprotein bound talinolol ($f_u \sim 0.4$) and filtration rate in healthy subjects (~120 ml/min) (Bernsdorf et al. 2006; Giessmann et al. 2004a; Siegmund et al. 2002a; Westphal et al. 1996, 2000b). Therefore, active tubular secretion must contribute to the renal elimination. There is a small intrasubject variability but high intersubject variability of all talinolol pharmacokinetic characteristics as shown in a bioavailability study with four bioequivalent tablet formulations (Siegmund et al. 2003).

3.2 *Affinity to P-glycoprotein In Vitro and in Animal Studies*

Talinolol shares the typical physicochemical properties and in vitro findings of common P-glycoprotein substrates (Tables 1 and 5). In transport experiments using Caco-2 cell monolayers, the transport rates of talinolol were 2-fold to 26-fold higher in the b → a as compared to the a → b direction. The differences are significantly decreased or abolished in the presence of strong inhibitors of P-glycoprotein as verapamil, LY335984, constituents of grapefruit juice or surfactants (Bogman et al. 2005; de Castro et al. 2007; El Ela et al. 2004; Hayeshi et al. 2008; Ingels et al. 2004; Ofer et al. 2005). Permeability in both directions is not stereoselective (Wetterich et al. 1996). The affinity of talinolol to P-glycoprotein was also confirmed in monolayer studies using Caco-2 and LLC-PK1 cells transfected with human *ABCB1*. In Caco-2 cells, the b → a permeability for R(+)

Table 5 In-vitro studies with talinolol and comparative in vitro studies with digoxin and talinolol to show affinity to P-glycoprotein

Cell model	Apical-to-basal (a → b) and basal-to-apical (b → a) transport via cell monolayer		References
Caco-2	S(−) talinolol (200 μM)	a → b: 181 ± 118 ng/cm^2 h, b → a: 1,736 ± 314 ng/cm^2 h, ratio: 9.6	Wetterich et al. (1996)
	R(+) talinolol (200 μM)	a → b: 200 ± 109 ng/cm^2 h, b → a: 1,737 ± 352 ng/cm^2 h, ratio: 8.7	
	Presence of verapamil (0.5 mM)	S(−) talinolol, a → b: 457 ± 0.5 ng/cm^2 h, b → a: 645 ± 17.6 ng/cm^2 h, ratio: 1.41 R(+) talinolol, a → b: 459 ± 14.9 ng/cm^2 h, b → a: 639 ± 58.8 ng/cm^2 h, ratio: 1.39	
Caco-2	rac. Talinolol (1 mM)	a → b: 0.17 × 10^{-6} cm/s, b → a: 6.1 × 10^{-6} cm/s, ratio: 36 a → b: 1.93 × 10^{-6} cm/s (in presence of 0.5 mM verapamil) a → b: 0.66 × 10^{-6} cm/s (in presence of 50% grapefruit juice)	Spahn-Langguth and Langguth (2001)
Caco-2	rac. Talinolol (100 μM)	a → b: 1.9 × 10^{-7} cm/s, b → a: 14 × 10^{-7} cm/s, ratio: 7.4	de Castro et al. (2007)
	IC_{50} for inhibition b → a	Verapamil 28 μM, grapefruit juice 0.6%, naringin 2,409 μM, naringenin 236 μM, dihydroxybergmottin 34 μM, epoxybergamottin 0.7 μM	
Caco-2	Efflux ratios for talinolol (30 μM) between 2.05 and 25.6 (bach 1) and 1.03 and 13.05 (bach 2) from 10 laboratories		Hayeshi et al. (2008)
ABCB1-Caco-2	S(−) talinolol (250 μM)	a → b: 23 ± 8.9 pM/cm^2 h, b → a: 1,282 ± 73 pM/cm^2 h, ratio: 55	Doppenschmitt et al. (1999)
	R(+) talinolol (250 μM)	a → b: 18.1 ± 8.4 pM/cm^2 h, b → a: 1,253 ± 60 pM/cm^2 h, ratio: 69	
ABCB1-LLC-PK1	rac. Talinolol (10 mM)	a → b: 1.09 × 10^{-7} cm/s, b → a: 5.33 × 10^{-7} cm/s, ratio: 4.89 a → b: 1.57 × 10^{-7} cm/s, b → a: 4.08 × 10^{-7} cm/s, ratio: 2.60 (with 10 μM cyclosporine)	Shirasaka et al. (2010)
Caco-2	Digoxin (2 nM)	a → b: 4.8 ± 0.2 × 10^{-6} cm/s, b → a: 40.4 ± 2.6 × 10^{-6} cm/s, ratio: 8.4	Neuhoff et al. (2003)
	Talinolol (10 μM)	a → b: 4.1 ± 0.4 × 10^{-6} cm/s, b → a: 24.8 ± 1.9 × 10^{-6} cm/s, ratio: 6.0	
Caco-2	Digoxin	a → b: K_m = 1,150 ± 179 μM, J = 718 ± 2.38 pM/min, efflux activity 10.5±1.70 × 10^{-6} cm/s b → a: K_m = 177 ± 9.2 μM, J = 434 ± 97.4 pM/min, efflux activity 40.9 ± 11.0 × 10^{-6} cm/s	Troutman and Thakker (2003)
	Talinolol	a → b: K_m = 414 ± 60.4 μM, J = 212 ± 19.7 pM/min, efflux activity 8.62 ± 0.5 × 10^{-6} cm/s b → a: K_m = 103 ± 5.1 μM, J = 200 ± 20.5 pM/min, efflux activity 32.5 ± 5.1 × 10^{-6} cm/s	

talinolol and S(−) talinolol was about 55-fold to 70-fold higher than the a → b permeability (Doppenschmitt et al. 1999). In LLC-PK1 cells, the b → a permeability was 4.9-fold higher than the a → b permeability; the ratio decreased to 2.60 in presence of strong P-glycoprotein inhibitor cyclosporine A (10 μM) (Shirasaka et al. 2009). In 2003, Neuhoff et al. compared the transport properties of talinolol, quinidine and digoxin across Caco-2 monolayers in dependence of the pH (Neuhoff et al. 2003). The transport characteristics of talinolol and digoxin at pH 7.4 were very similar: Talinolol, P_{app} (a → b) 4.13 ± 0.4 × 10^{-6} cm/s, P_{app} (b → a) 24.8 ± 1.9 × 10^{-6} cm/s, efflux ratio 6.0; digoxin, P_{app} (a → b) 4.8 ± 0.2 × 10^{-6} cm/s, P_{app} P_{app} (b → a) 40.4 ± 2.6 × 10^{-6} cm/s, efflux ratio 8.4. In another comparative in vitro study with Caco-2 monolayers including digoxin and talinolol, the apparent K_m, flux rates (J) and the intrinsic ABCB1-mediated efflux activity for the absorptive and secretory transport was determined. The K_m-values for digoxin and talinolol in the a → b direction and b → a direction were in the same order of magnitude (Troutman and Thakker 2003).

Talinolol seems to be a week inhibitor of P-glycoprotein. It increased digoxin a → b transport (10 μM) about twofold with IC_{50} of 198 ± 11 μM in Caco-2 cells (Collett et al. 2005). In vinblastine-induced Caco-2 cells using a radioligand assay with verapamil, the IC_{50} value for racemic talinolol was 2,271 ± 335 μM compared to 2.11 ± 0.47 μM for racemic verapamil (Neuhoff et al. 2000). In another study, the IC_{50} for talinolol was 1,064 ± 105 μM compared to 303 ± 26 μM for quinidine, 34 ± 1.2 μM for vinblastine and 2.5 ± 0.3 μM for verapamil (Doppenschmitt et al. 1999). There seems to be also a binding site with higher affinity for talinolol (Doppenschmitt et al. 1998; Doppenschmitt et al. 1999).

The following observations support the hypothesis that talinolol is a substrate of intestinal P-glycoprotein in animals:

1. In everted sacs of rat ileum, the serosal-to-mucosal permeability of talinolol exceeds the mucosal-to-serosal permeability. The differences are abolished after incubation with verapamil (Spahn-Langguth et al. 1998).
2. Transporter induction with rifampicin results in lower intestinal permeability of talinolol in the duodenum, jejunum and colon of rats (in situ perfusion) (Hanafy et al. 2001). In the contrary, the small instinal permeability of talinolol (100 μM) is fivefold increased in the presence of verapamil (150 μM) both in Wistar rats and in NMR1 mice (Mols et al. 2009). Furthermore, bioavailability of oral talinolol in rats (10 mg/kg) is significantly increased after comedication of grapefruit juice or some of its constituents like the P-glycoprotein inhibitors narigin or bergamottin (de Castro et al. 2007, 2008; Spahn-Langguth and Langguth 2001).
3. In *Abcb1a/1b*(−/−) knock-out mice, the apparent permeability in perfused intestinal tissue is sevenfold increased for talinolol but not for atenolol or metoprolol (Mols et al. 2009). The plasma levels of talinolol in knock-out mice after oral administration exceeded nearly threefold the levels in wildtype mice (Schwarz et al. 2001).

3.3 Evidence from Mechanistic Clinical Studies

Absorption of talinolol in doses between 25 mg and 50 mg is controlled by a capacity-limited process, as shown in a dose-escalation study with 25, 50, 100 and 400 mg talinolol in 12 healthy subjects. Total body clearance of talinolol decreased in the range 25 mg > 50 mg > 100 mg = 400 mg while terminal half-life remained unchanged. It was hypothesized by the authors that P-glycoprotein mediated efflux transport was the underlying intestinal process that saturates in subtherapeutic doses of talinolol (Wetterich et al. 1996). The disposition of talinolol in single oral doses above 100 mg is not dose-dependent as confirmed by a bioequivalence study with doses of 100 mg and 200 mg (Siegmund et al. 2003). Accordingly, for pharmacokinetic studies in healthy subjects, immediate release talinolol in doses of 50–100 mg seems to be the adequate study medication under standard conditions (overnight fasting, upright position, 240 ml water) to provide substrate concentrations on the receptor site which just saturates the efflux transporter.

Gramatté and coworkers have been the first proving active intestinal secretion of talinolol in man using an intestinal steady-state perfusion method (triple lumen tubing technique) (Gramatte et al. 1996; Gramatte and Oertel 1999). The appearance of talinolol in the upper jejunum (110–130 cm beyond the teeth) was measured after intravenous infusion of 25 mg within 140 min in six healthy subjects (Gramatte and Oertel 1999). During all intestinal perfusions, the mean intraluminal talinolol concentrations were 2.4-fold to 7.5-fold (median, 5.5-fold) higher than the corresponding serum concentrations; i.e., talinolol was secreted into the gut lumen against a steep concentration gradient. The intestinal secretion rate ranged from 1.94 to 6.62 μg/min per 30 cm jejunum. The luminal concentration could not be explained by base-trapping; the expected concentration gradient at the measured intraluminal pH 7.14 is 1.9. Therefore, intestinal secretion of talinolol must have been caused by an active process that is susceptible to inhibition by R-verapamil, a strong inhibitor of P-glycoprotein (Haussermann et al. 1991; Noviello et al. 1997). This was concluded from the finding that the intestinal secretion rates decreased by 44–71% when R-verapamil (565 μM) was perfused into the jejunal lumen by the tube system. The intestinal secretion of talinolol was not influenced by fluid secretion (solvent drag); talinolol was secreted even at high fluid absorption rates.

Active intestinal secretion was also confirmed for talinolol administered directly into the gut lumen in six healthy subjects (steady-state perfusion with triple lumen tubes) (Gramatte et al. 1996). To evaluate regional differences, 625 μg talinolol per minute were infused for 160 min while the test segment of the tube was located between 95 and 115 cm and, in a second study period, between 160 and 235 cm beyond the teeth. In both positions, the talinolol transport rates were in linear correlation to the amount of talinolol perfused per time unit (intestinal perfusion rate). During each distal perfusion, however, there was a shift of this relationship towards higher perfusion rates; that means, to achieve the same talinolol absorption rate in the more distal intestinal regions, a higher perfusion rate was needed.

The authors concluded from the regional differences, that there is site-dependent transepithelial transport of talinolol along the small intestine of humans. Interestingly, in about 20% of the measurements, the amount of talinolol leaving the intestinal test segment was greater than the amount that had entered it. At perfusion rates below 600 μg/min, already absorbed talinolol was back-secreted (negative transport rate values) into the gut lumen against a steep concentrations gradient of about 4,200. Extrapolating the secretion rates in the test segment to the length of the small intestine (3–5 m) and ignoring regio-selective expression of P-glycoprotein, about 30–40 mg talinolol have been secreted per hour from the systemic circulation into the small intestinal tract. Regio-selective absorption of talinolol was also confirmed by the data on systemic availability of talinolol that was simultaneously measured. AUC and C_{max} decreased with increasing distance of the perfusion port from the teeth by up to 85%. The results of both perfusion studies reflected the findings on regio-selective expression of intestinal P-glycoprotein published several years later (Englund et al. 2006; Zimmermann et al. 2005).

3.4 Talinolol Disposition and Induction of Intestinal P-glycoprotein

Further clinical evidence for the applicability of talinolol as a probe drug for intestinal P-glycoprotein came from interaction studies with rifampicin and SJW (Schwarz et al. 2007; Westphal et al. 2000b). The effect of rifampicin induction (600 mg, 9 days) on the pharmacokinetics of talinolol after intravenous (30 mg) and repeated oral administration (100 mg, 14 days) was investigated in eight male healthy volunteers (Westphal et al. 2000b). After up-regulation, higher systemic clearance, shorter half-life and somewhat higher nonrenal clearance (not significant) after intravenous administration were observed. Furthermore, up-regulation of P-glyoprotein was associated with lower bioavailability by 21%. The amount of oral talinolol excreted into urine decreased by 25%. Renal clearance remained unchanged. About 50% of the systemic clearance of intravenous talinolol was predicted by the content of duodenal P-glycoprotein. As the renal clearance of talinolol was not influenced by rifampicin induction, the increase in systemic clearance has to be attributed to the intestinal clearance. The effects of rifampicin were even more pronounced after oral administration. Cmax, AUC and bioavailability were reduced by 38%, 35% and 20%. Despite markedly lowered serum concentrations, the ß-blocking effect as assessed by bicycle exercise was not significantly decreased. The effect of transporter induction with SJW (900 mg, 12 days) on disposition of intravenous (30 mg) and oral talinolol (50 mg) was evaluated in nine male healthy subjects (Schwarz et al. 2007). After SJW, nonrenal clearance of intravenous talinolol increased by 35%. Bioavailability was reduced by 25% and the amount excreted into urine by 44%. Nonrenal clearance tended to increase by 36%. Although there was no correlation between pharmacokinetic parameters of talinolol and intestinal *ABCB1*mRNA levels, the pattern of

pharmacokinetic changes pointed to induction of intestinal P-glycoprotein to be the rationale behind the effects after consumption of SJW.

3.5 Talinolol Disposition and Inhibition of Intestinal P-glycoprotein

The impact of P-glycoprotein inhibition on pharmacokinetics of oral talinolol (50 mg) was investigated in a study with erythromycin (2.0 g) (Eberl et al. 2007; Schwarz et al. 2000). In the presence of erythromycin, the AUC_{0-24h}, the C_{max} and the amount of talinolol excreted into urine were increased by 52%, 26% and 36%, respectively. Renal clearance remained unchanged. T_{max} and half-life were reduced by 46% and 16%, respectively, most likely as result of faster talinolol absorption. The higher serum concentrations (AUC_{0-24h}) and the higher amount of talinolol in urine are most likely caused by a strong, possibly even complete, inhibition of intestinal P-gp and, consequently, complete absorption of talinolol from gut lumen. The faster absorption of talinolol results obviously from faster gastric emptying as cause by the prokinetic effect of erythromycin (Keshavarzian and Isaac 1993).

A comparable influence on the extent of oral talinolol absorption was observed in nine healthy subjects when talinolol (50 mg) was swallowed with the surfactant D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS), an inhibitor of P-glycoprotein in vitro. In the presence of TPGS, the $AUC_{0-\infty}$ and the C_{max} of talinolol increased (20% and 36%, respectively). The elimination half-life remained unchanged. Quite similar changes in pharmacokinetics of talinolol (increased $AUC_{0-\infty}$ and C_{max}, unchanged half-life) were obtained in studies with healthy Chinese subjects after treatment (14 days) with silymarin (420 mg), a purified extract from the seeds of milk thistle, and with extracts of *Schisandra chenensis* (600 mg) and *Ginkgo biloba* (360 mg) that were shown to inhibit P-glycoprotein in vitro (Fan et al. 2009a, b; Han et al. 2009; Wan et al. 2006; Wang et al. 2005; Zhang and Morris 2003a, b).

3.6 Regioselective Absorption of Talinolol

Regio-selective absorption from the gastrointestinal tract is a characteristic feature of substrates of intestinal P-glycoprotein. Such a behavior was also shown for talinolol (100 mg) prepared in immediate release hard gelatin capsules and enteric-coated sustained release hard gelatin capsules (Weitschies et al. 2005). Enteric-coated capsules are known to disintegrate predominantly in the distal jejunum or the ileum where the expression of P-glycoprotein is increased (Englund et al. 2006; Wilding et al. 1992; Zimmermann et al. 2005). The capsules contained additionally 100 mg paracetamol to assess the intestinal site of capsule disintegration; paracetamol is rapidly and completely absorbed from all parts of the intestine (Naslund et al. 2000). Enteric-

coated paracetamol appeared in serum with a lag-time of about 3 h and reached maximum concentrations after about 4 h; uncoated paracetamol appeared after 1 h. In accordance with the conception that expression of P-glycoprotein is increased along the small intestine, talinolol absorption from enteric-coated capsules was reduced by about 50% compared to absorption from immediate release capsules, whereas the absorption of paracetamol remained unchanged.

3.7 Talinolol as a Probe Drug for Genetic Polymorphisms of P-glycoprotein

In 37 healthy Caucasian subject, no impact of nine *ABCB1* polymorphisms including C1236T, G2677T/A, and C3435T on duodenal expression of P-glycoprotein was observed. Despite larger interindividual variability in expression of ABCB1 in the noninduced subjects, the AUC-values of talinolol were significantly higher in subjects with the 2677TT/TA genotype compared to carriers with at least one wild-type allele (Siegmund et al. 2002b). Interestingly, in an interaction study with SJW, the *ABCB1* genotype appeared to be associated with lower basal expression of intestinal P-glycoprotein and lower magnitude of induction by SJW. Subjects with the combined *ABCB1* genotype comprising C1236T, G2677T/A, and C3435T polymorphisms had a lower basal intestinal mRNA content and were less inducible by SJW. Individuals that are heterozygous for the synonymous C1236T in exon 12 showed a significantly lower decrease in the $AUC_{0-\infty}$ of oral talinolol compared to wild-type subjects. Subjects that are heterozygous for the nonsynonymous G2677T/A had a lower decline in bioavailability after SJW compared with wild-type (Schwarz et al. 2007).

3.8 Limitations of the Application of Talinolol as a Probe Drug

Soon after publication of convincing evidence on active intestinal secretion of talinolol in man, the same group presented some unexpected pharmacokinetic observations that challenge the conception that talinolol is a selective probe-drug for intestinal P-glycoprotein (Gramatte et al. 1996; Gramatte and Oertel 1999; Schwarz et al. 1999). After concomitant oral administration of talinolol (50 mg) and R-verapamil (120 mg) in nine subjects, a significantly reduced AUC_{0-24h}, a lower renal excretion (both by 24%) was observed, whereas half-live and renal clearance were not changed. The anticipated effect in the presence of R-verapamil was an increase rather than a decrease of talinolol bioavailability because R-verapamil is an inhibitor of P-glycoprotein (Haussermann et al. 1991; Noviello et al. 1997). Interestingly, t_{max} was shorter in the presence of verapamil (1.5 ± 1.0 h vs. 3.2 ± 0.8 h) and one distinct early concentration maximum appeared instead of the characteristic second peak after 3–5 h. Talinolol was

obviously better absorbed in proximal regions of the small intestine, probably by inhibition of intestinal P-glycoprotein in the presence of verapamil. Verapamil was very rapidly absorbed and reached maximum concentrations just at the time when the initial talinolol peak appeared. At that time, the authors could not explain the absence of the expected delayed absorption peak of talinolol after verapamil comedication. However, lower instead of higher talinolol absorption in the presence of verapamil was also measured in *Abcb1a/b*(−/−) knock-out mice (Schwarz et al. 2001). As expected, the plasma levels of talinolol in the deficient animals were about threefold higher than in wild-type animals. Surprisingly, coadministration of verapamil (16 mg/kg) resulted in significantly lower talinolol concentrations in both groups and not, as expected after "chemical Abcb1 knock-out", in better absorption and higher plasma levels in wild-type rats, similar to the situation in nonpretreated *Abcb1a/b*(−/−) knock-out mice. The authors concluded that inhibition of an intestinal uptake transport may overshadow the effects of verapamil on P-glycoprotein.

Further evidence for the existence of an unknown uptake transporter for talinolol came from a clinical study with talinolol (50 mg) in 24 healthy subjects after single (300 ml) and repeated ingestion (900 ml/day for 6 days) of grapefruit juice. Under both conditions, $AUC_{0-\infty}$, C_{max} and urinary excretion of talinolol were lowered by more than 50% whereas half-life and renal clearance remained unchanged. These findings were contrary to the study hypothesis to which the authors had expected higher bioavailability of talinolol because grapefruit juice and its constituents inhibit the unidirectional talinolol transport in Caco-2 cells and increase talinolol absorption in rats (de Castro et al. 2007, 2008; Spahn-Langguth and Langguth 2001). Otherwise, grapefruit juice is a potent inhibitor of human OATP1A2 (competition assay with fexofenadine uptake in HeLa cells) at concentrations (0.5–5%) that had no effect on P-glycoprotein (competition assay with vinblastine in L-MDR1 and LLC-PK1 cells) (Dresser et al. 2002). One active ingredient of grapefruit juice seems to be narigin that inhibits fexofenadine uptake in HeLa cells transfected with human OATP1A2 nearly as potent as verapamil (IC_{50} 3.6 μM versus 2.6 μM) (Bailey et al. 2007). Because human OATP1A2 seems to be the only intestinal uptake transporter for fexofenadine and grapefruit juice and narigin decrease the $AUC_{0-\infty}$ and C_{max} of fexofenadine in healthy subjects by at least 40%, it can be speculated that OATP1A2 is also an intestinal uptake transporter for talinolol in man (Bailey et al. 2007; Dresser et al. 2002, 2005; Glaeser et al. 2007). With regard to the double-peak phenomenon of talinolol, it should be mentioned that OATP1A2 is highly expressed in the ileum but low in the jejunum (Meier et al. 2007).

This conception is supported by recent in vitro studies using *Xenopus laevis* oocytes expressing rat Oatp1a5 which is the closest equivalent to human OATP1A2. (Shirasaka et al. 2010). Talinolol was taken up into the oocytes by the saturable Oatp1a5 ($K_m = 2$ mM). Naringin was a strong inhibitor of the uptake ($IC_{50} = 12.7$ μM). In contrast, the narigin IC_{50} for inhibition of the P-glycoprotein mediated b → a permeability of talinolol in Caco-2 cells was much higher (2 mM) (de Castro et al. 2008). In line with these data, naringin in low concentrations (200 μM) lowered the permeability of talinolol in rat small intestine (in situ closed loop method) by

about 40% (inhibition of Oatp1a5). In the presence of high naringin concentrations (2 mM), permeability increased by 75% (inhibition of P-glycoprotein). The effect of naringin on talinolol (10 mg/kg) absorption in rats was also concentration dependent; 20 μM naringin were without effect, 50 μM naringin lowered the $AUC_{0–6h}$ by 57%, but 200 μM and 2,000 μM naringin increased talinolol absorption by approximately 90% (Shirasaka et al. 2009). It can be concluded from these rat experiments, that intestinal absorption of talinolol is obviously influenced by an uptake transporter of the OATP-family, which is more sensitive to inhibition by constituents of grapefruit juice than the intestinal P-glycoprotein transporter. The species differences in the effects of grapefruit juice on absorption of talinolol in man and rats results most likely from the species differences in the affinity of naringin to OATPs and P-glycoprotein (Shirasaka et al. 2009). Very recently, Bolger et al. have simulated nonlinear talinolol absorption using the ACAT model in the GastroPlus software using data on regio-selective expression of P-glycoprotein and OATP1A2 as variables (Bolger et al. 2009; Tubic et al. 2006).

Further candidates for intestinal talinolol uptake may be member(s) of the multidrug organic cation transporter (OCT) family for which other ß-adrenergic antagonists are substrates and verapamil is an inhibitor (Dudley et al. 2000; Zhang et al. 1998). Talinolol was found to be an inhibitor of the [^{14}C]tetraethylammoniumbromide (TEA) uptake in LLC-PK_1 cells expressing OCT2. The IC_{50} value was 150 μM compared to 18 μM, 38 μM and >5,000 μM for verapamil, naringenin and naringin, respectively. However, little is known on expression and functional meaning of OCTs in the human intestine. Therefore, it is entirely speculative to conclude from the in vitro date in LLC-PK1 cells on the role of OCTs in talinolol absorption and whether the unexpected decrease of talinolol absorption after concomitant administration of verapamil and grapefruit juice have resulted (at least in part) from inhibition of intestinal uptake carriers of the OCT multidrug transporter family.

There is evidence from a pharmacokinetic study in *Abcc2*-deficients rats (GY-TR^-) that talinolol is a substrate of the efflux transporter ABCC2 (MRP2) (Bernsdorf et al. 2003). Absorption of talinolol was also shown to be influenced by *ABCC2* gene polymorphisms (Haenisch et al. 2008). However, there was also shown to be a wide overlapping of substrate spectrum of P-glycoprotein and MRP2, intestinal P-glycoprotein and MRP2 are coregulated via the same nuclear receptor signal pathway and most of the P-glycoprotein inhibitors are also inhibitors of MRP2 (Fromm et al. 2000; Giessmann et al. 2004b; Urquhart et al. 2007).

4 Conclusions and Recommendations

4.1 Selectivity for Intestinal P-glycoprotein

Digoxin and talinolol are substrates of P-glycoprotein as confirmed in different cell models (Caco-2, LLC-PK1, L-MDR1, MDR1-MDCKII) and in *Abcb1* knock-out mice. Digoxin and talinolol have similar affinity to P-glycoprotein as shown in

transport studies using Caco-2 cells monolayers. Digoxin seems not to be an inhibitor of P-glycoprotein; talinolol has low inhibitory potency compared to standard inhibitors of P-glycoprotein (verapamil, vinblastine, quinidine and valspodar). Digoxin is also a substrate of the hepatic uptake transporter OATP1B3 and of the basolateral uptake transporter OATP4C1 in the proximal tubule cells in the human kidney. There is evidence, that talinolol is also a substrate of ABCC2, OATPs (e.g. OATP1A2) and OCTs (e.g. OCT2).

Intestinal transfer of digoxin and talinolol in animals is significantly influenced by the function of P-glycoprotein; this was shown by competition studies using in situ intestinal perfusion models. In human beings, digoxin and talinolol are secreted against steep concentrations gradients into the lumen of the small intestine by a mechanism that can be influenced by inhibitors and inducers of P-glycoprotein as experimentally confirmed by intestinal perfusion methods. Intestinal P-glycoprotein is a rate-limiting process in the pharmacokinetics of digoxin and talinolol; approximately 50% of the AUC of oral digoxin and of intravenous talinolol can be predicted by expression of P-glycoprotein in the duodenum. Inhibition of intestinal P-glycoprotein (e.g. verapamil, erythromycin) leads to higher bioavailability whereas up-regulation of the intestinal efflux transporter (e.g. by rifampicin, SJW) results in lower bioavailability and increased intestinal (nonrenal) excretion of digoxin and talinolol.

The function of P-glycoprotein in other organs may influence distribution volume of the probe drugs, as hypothesized at least for digoxin. Major influence on disposition of digoxin, however, comes from P-glycoprotein function in the kidneys as confirmed in drug interactions studies with valspodar, ritonavir, or quinidine. For talinolol, variability of renal P-glycoprotein function might be of minor influence.

Digoxin and talinolol are nearly not metabolized and moderately bound to plasma protein. Therefore, the function of hepatic uptake transporters (e.g. OATP1B1, OATP1B3), hepatic P-glycoprotein and drug metabolizing enzymes and changes in protein binding may not significantly limit the suitability of digoxin and talinolol as a measure of intestinal P-glycoprotein.

4.2 Limitations Resulting from Intestinal Uptake Mechanisms

The net uptake of digoxin and talinolol obviously results from the difference of the intestinal uptake capacity minus the P-glycoprotein mediated efflux capacity. The intestinal uptake mechanism(s) for digoxin and talinolol, however, are not identified so far. For digoxin, there must be a so far unknown low-affinity high-capacity uptake transporter system which dominates the intestinal net absorption at concentrations that can be reached in the apical membrane after administration a single therapeutic dose of digoxin in solution; in that scenario, digoxin is nearly completely absorbed. The intestinal P-glycoprotein transporter can constrain the net uptake by approximately 30%, if the membrane concentrations of the slowly and poorly water soluble digoxin are relatively lower, e.g. after administration of

conventional 0.25 mg tablets. In that alternative scenario, the uptake transporter is activated to a lower extent leading to significant reduction of overall uptake caused by P-glycoprotein to which digoxin has obviously higher affinity than to the uptake transport system. So far, there is no information whether factors that influence P-glycoprotein function (e.g. inhibitors or inducers) may also influence the digoxin uptake mechanism(s).

In case of talinolol, intestinal P-glycoprotein seems to be just saturated by test doses of 50–100 mg in solution or immediate release tablets which bioavailability is about 55–70%; lower doses are of lower bioavailability. There is strong evidence that intestinal uptake of talinolol is mediated by a member(s) of the OAPT-family (e.g. OATP1A2) which is susceptible to inhibition by substances known to be also inhibitors of P-glycoprotein just at common doses (e.g. R-verapamil, constituents of grapefruit juice). Therefore, inhibition of the intestinal uptake transporter(s) leading to lower bioavailability may overshadow the functional outcome of P-glycoprotein inhibition, which is increased bioavailability of talinolol. This major limitation must be definitely considered in planning drug interaction studies with talinolol. However, affinity of talinolol to efflux and uptake carriers may be a useful property in mechanistic studies to evaluate the complex interplay between efflux and uptake transporters in the intestinal tract.

4.3 Safety and Methodological Issues

Single dose studies with digoxin (0.25–0.5 mg, intravenous and per os) and talinolol (30 mg intravenous, 50–100 mg per os) are safe and severe adverse reactions are not expected. Both drugs are commercially available. The experimental conditions in probe drug studies with digoxin and talinolol must be strictly standardized (adequate dosage form, upright position during administration, intake of water and meal, etc.). The discrepancies in study results may origin, at least in part, from the experimental conditions. Investigators have to consider, that the "barrier function" of P-glycoprotein in digoxin absorption depends on the properties of the dosage form, that the expression of intestinal P-glycoprotein is regio-selective ("absorption window" in the proximal jejunum), that the existence of a regio-selective intestinal uptake for digoxin and talinolol cannot be excluded, and that meal and coadministration of drugs (interaction studies) may influence intestinal transit and dissolution of the probe drugs. Repeated-dose studies are not recommended because up-regulation of P-glycoprotein during the study periods cannot be excluded.

Mechanistic clinical studies on function of intestinal P-glycoprotein with digoxin and talinolol as in vivo probe drugs should be biometrically planned according to the international recommendations for bioequivalence studies and drug interaction studies.

To confirm absence of a P-glycoprotein-mediated influence, e.g. by potential inhibitors or inducers, $AUC_{0-\infty}$ and C_{max} may be primary pharmacokinetic characteristics as in other bioequivalence studies using an intrasubject, cross-over

design. The sample size should be at least 20 in studies with digoxin and 12 in studies with talinolol, assuming (1) intrasubject coefficients of variation of the $AUC_{0-\infty}$ of 8–20% for digoxin and 14% talinolol, (2) the standard equivalence range of 0.80–1.25, (3) a significance level of 0.05 (alpha error), and (4) a power of 80% of two one-sided *t*-tests (log-scale) (Siegmund et al. 2003; Steinijans et al. 1995). If the power is set to 90%, 26 and 14 subjects are needed in studies with digoxin and talinolol, respectively (nQuery 5.0, StatSol, Cork, Ireland).

To evaluate P-glycoprotein related differences as caused for instance by drug interactions, genetic polymorphisms, gender, age, or gastrointestinal diseases, a parallel-group study design is needed. In such mechanistic studies, primary study characteristics should be (1) oral bioavailability to measure the intestinal "absorption barrier function" and (2) intestinal clearance after intravenous administration to measure the "excretory function" of intestinal P-glycoprotein. Nonrenal clearance might be used as a surrogate for intestinal clearance. Secondary characteristics to be measured are volume of distribution, renal clearance and half-life to discuss nonintestinal P-glycoprotein related variability and influence by additional factors (e.g. organ perfusion). The mean intersubject coefficients of variation of for oral bioavailability of digoxin and talinolol are 10.7% and 25.3% and for nonrenal clearance are 28.1% and 29.5%, respectively (Drescher et al. 2003; Giessmann et al. 2004a; Greiner et al. 1999; Kurata et al. 2002; Rengelshausen et al. 2003; Schwarz et al. 2007; Siegmund et al. 2002a; Westphal et al. 2000b; Westphal et al. 2000a). To confirm a 20% difference in bioavailability from control with statistical power of 80% and significance level of 0.05 (Mann-Whitney rank-sum test), eight subjects per group are needed in digoxin studies and 30 subjects in talinolol studies. To confirm 20% difference in nonrenal clearance, 36 subjects and 39 subjects, respectively, should be included.

Because of the recommendations for blood sampling to cover at least 80% of the $AUC_{0-\infty}$ by data points (AUC_{0-t}) and to measure cumulative urinary and fecal excretion of the probe drugs completely (a precondition to assess clearance values), sampling periods of at least 7–9 days are necessary in single dose studies with digoxin and of 5 days with talinolol. Further preconditions are ambitious study participants and staff members, in particular to quantify fecal excretion of the probe drugs; for digoxin, highly sensitive mass-spectrometric assays must be developed.

Digoxin and talinolol are suitable in vivo probe drugs for intestinal P-glycoprotein under the precondition, that they are used as tools in carefully designed pharmacokinetic studies with adequate biometrically planning of the sample size and that several limitations are considered in interpreting and discussion of the study results.

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