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Review

The Complexities of Hepatic Drug Transport: Current Knowledge and Emerging Concepts

Priyamvada Chandra[1] and Kim L. R. Brouwer[1,2]

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Recently, hepatic transport processes have been recognized as important determinants of drug disposition. Therefore, it is not surprising that characterization of the hepatic transport and biliary excretion properties of potential drug candidates is an important part of the drug development process. Such information also is useful in understanding alterations in the hepatobiliary disposition of compounds due to drug interactions or disease states. Basolateral transport systems are responsible for translocating molecules across the sinusoidal membrane, whereas active canalicular transport systems are responsible for the biliary excretion of drugs and metabolites. Several transport proteins involved in basolateral transport have been identified including the Na^+-taurocholate co-transporting polypeptide [NTCP (*SLC10A1*)], organic anion transporting polypeptides [OATPs (*SLCO* family)], multidrug resistance–associated proteins [MRPs (*ABCC* family)], and organic anion and cation transporters [OATs, OCTs (*SLC22A* family)]. Canalicular transport is mediated predominantly via P-glycoprotein (*ABCB1*), MRP2 (*ABCC2*), the bile salt export pump [BSEP (*ABCB11*)], and the breast cancer resistance protein [BCRP (*ABCG2*)]. This review summarizes current knowledge regarding these hepatic basolateral and apical transport proteins in terms of substrate specificity, regulation by nuclear hormone receptors and intracellular signaling pathways, genetic differences, and role in drug interactions. Transport knockout models and other systems available for hepatobiliary transport studies also are discussed. This overview of hepatobiliary drug transport summarizes knowledge to date in this rapidly growing field and emphasizes the importance of understanding these fundamental processes in hepatic drug disposition.

KEY WORDS: ABC proteins; drug disposition; hepatic transport; hepatobiliary; SLC proteins; transporters.

SIGNIFICANCE OF HEPATIC TRANSPORT SYSTEMS IN DRUG DISPOSITION

Many endogenous and exogenous compounds, including drugs, are eliminated from the body by the liver via metabolism and/or excretion. Though the metabolic aspects of hepatic clearance have been the focus of research for several decades, the important role of hepatic transport systems in the hepatobiliary disposition of drugs and metabolites has been recognized only recently. Lipophilic molecules may move from plasma to hepatic cytosol by simple or facilitated diffusion. However, numerous transport proteins are available on the basolateral membrane of the hepatocyte to mediate uptake of amphipathic and polar organic compounds, as well as some lipophilic molecules, from sinusoidal plasma to hepatic cytosol. Hepatocellular protein binding and sequestration may influence the hepatobiliary disposition of some compounds. Hepatic transport proteins also play an important role in the excretion of drugs and metabolites from the hepatocyte. Uni- or bi-directional basolateral transport systems translocate polar molecules from hepatic cytosol into blood, whereas active canalicular transport systems are responsible for the biliary excretion of drugs and metabolites. During the past decade, there has been a surge of interest in the field of drug transport, and knowledge regarding hepatic transport systems has grown substantially. There is widespread interest in the hepatic transport of drugs and metabolites among pharmaceutical scientists, including medicinal chemists, pharmacologists, and clinicians, for several reasons:

1. Drug Design (Drug Delivery). Knowledge of structure-transport relationships for hepatic transport proteins would aid in the design of compounds with optimal transport properties. In some cases, extensive hepatic uptake or enhanced biliary excretion may be desirable characteristics for a potential drug candidate. In other situations, extensive hepatic uptake and biliary excretion may reduce systemic exposure and limit pharmacological activity, thus representing undesirable properties of the molecule.

2. Bioavailability. The liver is an important organ of first-pass elimination. Reduced or erratic systemic availability of drugs after oral administration may be related to dietary, disease, or drug-induced alterations in hepatic transport systems. For example, induction of a hepatic transport protein responsible for the hepatic uptake or biliary excretion of a drug could decrease systemic availability of that drug after

[1] Division of Drug Delivery and Disposition, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.
[2] To whom correspondence should be addressed. (e-mail: kbrouwer@unc.edu)

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oral administration. In addition to the liver, drug transport proteins that reside on the basolateral and apical membranes of the gastrointestinal epithelial cells also are crucial determinants of the bioavailability of many drug molecules.

3. Biliary Excretion. Compounds are excreted into bile by ATP-dependent canalicular transport proteins. The extent to which most drugs and metabolites undergo biliary excretion in humans is not readily appreciated due to the difficulties inherent in directly accessing bile drainage in healthy individuals. Many potentially useful therapeutic agents may be excluded in the early stages of drug development due to extensive biliary excretion that limits systemic exposure. Biliary excretion of drugs or metabolites may expose the intestinal epithelia to pharmacologically active or toxic species that can exert dose-limiting toxicities. Compounds excreted via bile into the intestine may be reabsorbed into the systemic circulation intact, metabolized with subsequent reabsorption, or may undergo elimination in the feces. Hepatic transport systems are crucial determinants of the enterohepatic recycling of compounds.

4. Interindividual Variability in Drug Pharmacokinetics and Pharmacodynamics. Disease-associated or genetic alterations in the expression and/or function of hepatic transport proteins may alter significantly the disposition of many endogenous and exogenous compounds, including drugs and metabolites. Although this field of research is still in its infancy, hepatic transport systems clearly are responsible for important variations in the disposition, pharmacological activity, and toxicity of some drugs. Elucidating mechanisms of interpatient variability in hepatic drug transport systems is prerequisite to achieving desirable therapeutic outcomes in diverse patient populations.

5. Drug/Nutrient-Transport Interactions. Drugs and nutrients may interact with hepatic transport proteins resulting in enhanced or impaired transport activity. Such interactions may be direct or indirect in nature and may involve alterations in expression as well as function of the transport protein. Elucidation of the clinical importance of hepatic transport interactions and development of methods to predict these interactions offer many exciting opportunities for research in the upcoming decades.

NOMENCLATURE

One of the more challenging aspects in the discipline of hepatic transport is the nomenclature. With the advent of molecular biology techniques, new proteins were rapidly identified and named. The early nomenclature ranged from descriptive but cumbersome (e.g., sister of P-glycoprotein, canalicular multispecific organic anion transporter) to duplicative (e.g., different names assigned to identical proteins, similar names assigned to non-orthologous gene products). In an effort to avoid further confusion, the gene nomenclature used in this review is based on the HUGO Gene Nomenclature committee (HGNC, http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html). Hepatic transport proteins discussed in this review belong to either the superfamily of sodium-independent transport systems designated "solute carriers" with the root gene symbol designated "*SLC*" ["*SLCO*" for family 21; (1)] or to the ATP-binding cassette superfamily with the root gene symbol designated "*ABC*." Ideally, the gene symbols should be italicized, with the protein symbols identical but non-italicized. However, because some journals do not allow this convention, the common protein nomenclature that is used most frequently in scientific discussions and in the literature was selected for this review. The text and figures include the italicized gene nomenclature in parentheses; in addition, the tables include the older nomenclature as well as less commonly used aliases. By convention, upper case refers to the human and lower case refers to the rodent genes and gene products.

DRUG TRANSPORT PROTEINS OF THE HEPATIC BASOLATERAL MEMBRANE

Hepatic elimination is generally a sequence of events involving uptake of xenobiotics from the sinusoidal blood followed by intracellular metabolism, and ultimately excretion. Molecules may be excreted from the hepatocyte across the canalicular membrane into bile, which is stored in the gallbladder in humans and is released periodically into the upper small intestine or across the basolateral membrane into sinusoidal blood with subsequent elimination by other organs (e.g., kidney). Flux of substrates through the hepatobiliary system is facilitated by the polarized nature of hepatocytes, which have distinct basolateral (sinusoidal and lateral) and apical (canalicular) membrane domains that differ in lipid and protein composition. The basolateral transport proteins, belonging to the gene superfamily of solute carriers (*SLC*), mediate the movement of compounds to and from the sinusoidal blood (Fig. 1). Lists of commonly identified substrates for the human transport proteins residing on the hepatic basolateral and canalicular membranes are provided in Tables I and II, respectively. It is interesting to note the degree of substrate overlap with many of the hepatic transport proteins.

NTCP (*SLC10A1*)

The basolateral transporter responsible for sodium-dependent bile salt uptake is the Na^+-taurocholate co-transporting polypeptide [NTCP (*SLC10A1*)] (2). This protein transports taurocholate (TC) with Na^+ in a stoichiometry of 1:2 and is the predominant bile salt uptake system in rats (3). NTCP preferentially mediates Na^+-dependent transport of conjugated bile salts (TC, tauroursodeoxycholate, taurochenodeoxycholate), but also transports unconjugated bile salts (cholate) to a lesser extent. Dehydroepiandrosterone sulfate (DHEAS) (4), 3,3′,5-triiodo-L-thyronine (T_3), thyroxine (T_4) (5), bromosulfophthalein (BSP), and estrone-3-sulfate are non-bile salt substrates for this protein (3).

OATPs (*SLCO*; Previously *SLC21A*)

The organic anion transporting polypeptides (OATPs) represent a family of proteins that plays an important role in the hepatic clearance of many drugs. The OATP transport proteins are rather promiscuous with respect to substrate specificity, transporting a variety of organic anions, as well as some type II cations (bulky molecules with cationic groups located near the ring; e.g., quinidine) and neutral steroids. The OATP transporters are sodium-independent and may function as bi-directional transporters; hepatic uptake of substrates may be driven by countertransport of reduced

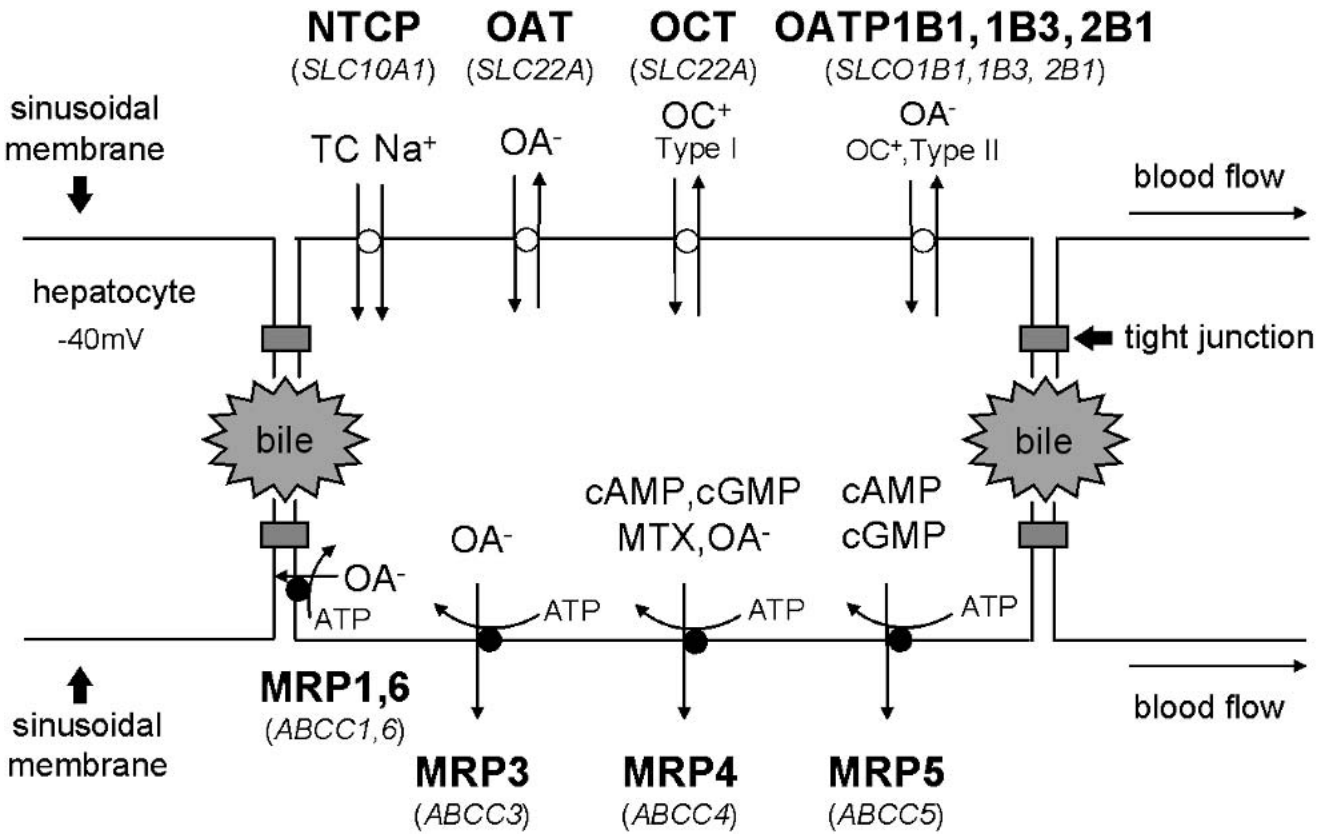

Fig. 1. Human hepatic basolateral transport proteins. Schematic representation of three adjacent hepatocytes with interconnecting canalicular spaces sealed by tight junctions. Sinuosoidal blood flowing through the liver bathes hepatocytes and delivers solutes to the basolateral hepatic membrane for uptake. Important basolateral transport proteins (protein name is in bold type with gene symbol listed below) are depicted with arrows denoting the direction of transport and ATP-dependent transporters designated by ●. For the OAT and OCT families, only mRNA have been detected in human liver. Typical substrates are listed (OA^-, organic anions; OC^+, organic cations; MTX, methotrexate; cAMP, adenosine 3′,5′-cyclic monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate).

glutathione which exists at high concentrations within the hepatocyte (6). Within this family, OATP1A2 (*SLCO1A2*), OATP1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*), and OATP2B1 (*SLCO2B1*), previously OATP-A, -C, 8, and -B, respectively, are the proteins predominantly expressed in human liver. OATP1B1 is the major Na^+-independent bile salt uptake system in human liver, with OATP1A2 and OATP1B3 playing a less extensive role; OATP2B1 does not transport bile salts (7). The cholephilic organic anion BSP, estrone-3-sulfate, and DHEAS are transported by all four human OATP proteins, although the extent of uptake of these substrates varies among the different proteins (7). In general, OATP1A2, OATP1B1, and OATP1B3 exhibit broad and overlapping substrate specificities; these proteins transport bile salts, numerous organic anions including estradiol-17β(β-D-glucuronide) ($E_2$17G) and anionic peptides ([D-penicillamine^{2,5}]-enkephalin (DPDPE) (3,8), BQ-123 [cyclo{D-Trp-D-Asp-L-Pro-D-Val-L-Leu}]). OATP1B3 exhibited the greatest uptake of these anionic peptides and was unique in transporting digoxin. The amphipathic organic cation *N*-methyl quinine is a specific OATP1A2 substrate (7). The transport properties of the rat Oatp and human OATP proteins cannot be predicted from the amino acid sequence identities, probably because most rat *Slco* gene products are not orthologs of the human OATP proteins (8). This is a major issue in drug development because the OATPs are involved in the hepatic uptake of many drugs. If hepatic uptake is the rate-limiting step in hepatic clearance of a compound, cross-species extrapolation to predict hepatic clearance or drug interactions in hepatic transport may be difficult considering that distinct proteins exhibiting different substrate specificities may be involved. Monovalent and sulfated bile salts, as well as sulfate, glucuronide, and glutathione conjugates, are substrates for rat Oatp1a1 (*Slco1a1*) [previously Oatp1 (*Slc21a1*)], whereas deltorphin II is a specific substrate for this protein (9,10). Rat Oatp1a4 (*Slco1a4*) [previously Oatp2 (*Slc21a5*)] shares this substrate specificity with the exception of sulfate conjugates, which are not high affinity substrates for this transporter. Digoxin, however, is a specific Oatp1a4 substrate (3). Oatp1b2 (*Slco1b2*) [previously Oatp4 (*Slc21a10*)], a liver-specific basolateral transporter, has high affinity for DHEAS, BSP, leukotriene C_4 (LTC_4), and anionic peptides (10).

SLCO3A1 (previously *SLC21A11*) and *SLCO4A1* (previously *SLC21A12*) mRNA expression has been observed in liver; transport of estrone-3-sulfate was demonstrated by OATP1B1, 2B1, 3A1, and 4A1 [previously OATP-C, -B, -D, and –E, respectively (*SLC21A6, A9, A11,* and *A12*)] (8), whereas OATP4A1 also was capable of T_3, T_4, and taurocholate transport (11). OATP5A1 (previously OATPRP4) has been identified, yet little is known to date regarding its transport and biochemical properties (12).

Table I. Human Hepatic Basolateral Transport Proteins

Basolateral protein	Trivial names*	Gene symbol	Substrates†	References
NTCP		*SLC10A1*	BSP; cholate; estrone-3-sulfate; glycocholate	(3)
			taurochenodeoxycholate; tauroursodeoxycholate; TC	(3)
OATP1A2	OATP-A	*SLCO1A2 (*previously	Bile acids; BQ-123; BSP; DHEAS; DPDPE; $E_2$17G;	(7)
	OATP-1	*SLC21A3)*	estrone-3-sulfate; *n*-methyl quinine; ouabain; T_3; T_4	(7)
	OATP		Fexofenadine	(170)
OATP1B1	OATP-C	*SLCO1B1 (*previously	Bile acids; BQ-123; BSP; DHEAS; DPDPE; $E_2$17G;	(7)
	LST-1	*SLC21A6)*	estrone-3-sulfate; ouabain; T_3; T_4	(7)
	OATP2		Bilirubin; bilirubin glucuronides	(171)
			LTC_4; prostaglandin E_2	(172)
			Pravastatin	(173)
			Rifampin	(174)
OATP1B3	OATP-8	*SLCO1B3 (*previously	Bile acids; BQ-123; BSP; CCK-8; DHEAS; digoxin	(7)
	LST-2	*SCL21A8)*	DPDPE; $E_2$17G; estrone-3-sulfate; n-methyl quinine ouabain; T_3; T_4	(7)
			Monoglucuronosyl bilirubin	(100)
			Rifampin	(174)
OATP2B1	OATP-B	*SLCO2B1 (*previously	Benzylpenicillin	(8)
		SLC21A9)	BSP; DHEAS; estrone 3-sulfate	(7)
OAT2‡		*SLC22A7*	Prostaglandin E_2	(18)
			Prostaglandin $F_{2\alpha}$	(17)
			Salicylate	(20)
			Tetracycline	(19)
			Zidovudine	(21)
OAT4‡		*SLC22A11*	Bumetanide	(175)
			Estrone-3-sulfate	(17)
			Ketoprofen; salicylate	(20)
			MTX	(22)
			Ochratoxin A	(23)
			Prostaglandin E_2; prostaglandin $F_{2\alpha}$	(18)
			Tetracycline	(19)
			Zidovudine	(21)
OCT1‡		*SLC22A1*	Azidoprocainamide methoiodide; *n*-methyl-quinidine;	(176)
			n-methyl-quinine; tributylmethylammonium	(176)
			MPP^+; tetraethylammonium	(24,25,177)
OCT3‡	EMT	*SLC22A3*	Adrenaline; noradrenaline; tyramine	(178)
			Agmatine; MPP^+	(27,179)
MRP1	MRP, GS-X	*ABCC1*	Daunorubicin; doxorubicin; etoposide; vincristine	(180)
MRP3	MOAT-D	*ABCC3*	Acetaminophen glucuronide	(36)
	MLP2		$E_2$17G; monovalent and sulfated bile salts; MTX	(34,35)
	cMOAT2			
MRP4	MOAT-B	*ABCC4*	Azidothymidine	(43)
			cAMP; cGMP; PMEA	(39–41)
			MTX	(42)
MRP5	MOAT-C	*ABCC5*	cAMP; cGMP	(41)
	ABC11		PMEA	(39)
MRP6	MOAT-E	*ABCC6*	BQ-123	(46)
	MLP1			
MRP7‡		*ABCC10*	$E_2$17G; LTC_4	(49)
MRP8‡		*ABCC11*	cAMP; cGMP	(51)

* Abbreviations used: GS-X, glutathione S-conjugate pump; EMT, extraneuronal monoamine transporter; LST, liver-specific transporter; MLP, MRP-like protein; MOAT, multispecific organic anion transporter.

† Abbreviations used: BQ-123, [cyclo{D-Trp-D-Asp-L-Pro-D-Val-L-Leu}]; BSP, bromosulphophthalein; cAMP, adenosine 3′, 5′-cyclic monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; CCK-8, cholecystokinin-8; DHEAS, dehydroepiandrosterone; DPDPE, [D-penicillamine^{2,5}]-enkephalin; $E_2$17G, estradiol-17β(β-D-glucuronide); LTC_4, leukotriene C_4; MPP^+, 1-methyl-4-phenylpyridinium; MTX, methotrexate; PMEA, 9-(2-phosphonomethoxyethyl) adenine; TC, taurocholate; T_3, 3,3′,5-triiodo-L-thyronine; T_4, thyroxine.

‡ Only mRNA has been detected in human liver.

OATs (*SLC22A*)

The *Slc22* gene family includes the organic anion transporters (Oats), which were first cloned in kidney. Both Oat2 (*Slc22a7*) and Oat3 (*Slc22a8*) are expressed predominantly in rat liver and transport the prototypic anionic substrate para-aminohippurate (13,14). In addition, Oat2 transports dicarboxylates, indomethacin, methotrexate, salicylate, PGE_2, and nucleoside derivatives (13), whereas Oat3 transports the organic cation cimetidine, estrone-3-sulfate, and ochratoxin A

Table II. Human Hepatic Canalicular Transport Proteins

Canalicular protein	Trivial names*	Gene symbol	Substrates†	References
BSEP	Sister P-gp	*ABCB11*	Conjugated and unconjugated bile salts; TC	(53)
MRP2	CMOAT	*ABCC2*	Acetaminophen glucuronide; carboxydichlorofluorescein	(36)
	cMRP		Camptothecin; doxorubicin	(181)
			Cisplatin; vincristine	(182)
			Etoposide	(101)
			Glibenclamide; indomethacin; rifampin	(183)
			Glucuronide, glutathione, and sulfate conjugates; LTC_4	(56)
			MTX	(184)
			Pravastatin	(99)
MDR1	P-gp	*ABCB1*	Amprenavir; indinavir; nelfinavir; ritonavir; saquinavir	(185,186)
			Aldosterone; corticosterone; dexamethasone; digoxin	(187)
			Cyclosporin A; MX	(188)
			Debrisosoquine; erythromycin; lovastatin; terfenadine	(189)
			Digoxin; quinidine	(190)
			Doxorubicin; paclitaxel; rhodamine 123	(191,192)
			Etoposide	(193)
			Fexofenadine	(170)
			Losartan; vinblastine	(194)
			Tacrolimus	(195)
			Talinolol	(196)
MDR3	PFIC3	*ABCB4*	Phospholipids	(75)
	Phospholipid flippase			
BCRP	MXR, ABCP	*ABCG2*	Daunorubicin; doxorubicin; MX; sulfated conjugates	(77)

* Abbreviations used: MOAT, multispecific organic anion transporter; MXR, mitoxantrone resistance protein
† Abbreviations used: LTC_4, leukotriene C_4; MX, mitoxantrone; MTX, methotrexate; TC, taurocholate

(14). It has been hypothesized that Oat proteins function physiologically as basolateral excretion systems in the liver (15). The mRNA for OAT2, 4, and 5 have been detected in human liver (16). Overlapping substrates have been identified for OAT2 and 4 including prostaglandin $F_{2\alpha}$, tetracycline, salicylate, zidovudine, and prostaglandin E_2 (17–21), whereas OAT4 has the additional ability to transport methotrexate and ochratoxin A (22,23).

OCTs and OCTNs (*SLC22A*)

In contrast to type II cations, the hepatic uptake of smaller type I organic cations (e.g., tetraethylammonium, azidoprocainamide methoiodide) is mediated by the electrogenic organic cation transporter [OCT1 (*SLC22A1*)] (24,25). Oct1 is expressed in the basolateral membrane of rat hepatocytes (26). Another member of this gene family is OCT3 (*SLC22A3*); the membrane localization and function of this protein remain to be determined in human liver, although expression and transport of 1-methyl-4-phenylpuridinium iodide in the HepG2 hepatoma cell line have been documented (27). OCTN1 (*SLC22A4*) and OCTN2 (*SLC22A5*) are novel organic cation transporters containing a nucleotide binding site sequence motif (28,29) that also belong to the *SLC22* gene family. The precise membrane localization and substrate specificity of these novel organic cation transporters in rat and human liver remain to be determined.

MRP1 (*ABCC1*)

In addition to the *SLCO* and *SLC22* gene products, basolateral excretion of organic anions from the human hepatocyte to sinusoidal blood may be mediated by members of the ATP-dependent multidrug resistance–associated protein [MRP, (*ABCC*)] subfamily. There are currently nine members in this subfamily, seven of which play a major role in the hepatic excretion of organic anions (30). MRP1 (*ABCC1*) is expressed at low levels on the lateral membrane (31) and is stored primarily in intracellular vesicles in human hepatocytes (32). Intracellular GSH has been shown to be required for MRP1 transport (33), although it may not be needed for the transport of conjugated drugs.

MRP3 (*ABCC3*)

Mrp3 (*Abcc3*) is an inducible basolateral organic anion transporter that mediates the hepatic excretion of monovalent (e.g., taurocholate and glycocholate) and sulfated bile salts, as well as other organic anions such as $E_2$17G, methotrexate (34,35), and acetaminophen glucuronide (36). Glucuronide conjugates are considerably higher affinity substrates than glutathione conjugates for this protein (35). The expression level of Mrp3 is very low in normal rats but is induced by phenobarbital and cholestatic conditions (37). MRP3/Mrp3 also is induced in humans and animals that exhibit naturally occurring hereditary defects in biliary excretion of organic anions (38). Up-regulation of this basolateral transport protein appears to compensate for the diminished ability to excrete organic anions into bile. Mrp3 also is postulated to play an important role in the enterohepatic circulation of bile salts (35).

MRP4 (*ABCC4*) and MRP5 (*ABCC5*)

The basolateral MRP4 (*ABCC4*) and MRP5 (*ABCC5*) proteins possess the unusual ability to transport the cyclic nucleotides adenosine 3′, 5′-cyclic monophosphate (cAMP)

and guanosine 3′, 5′-cyclic monophosphate (cGMP) (39). However, discrepancies in the K_m determinations for their transport by MRP4 and MRP5 have been reported in the literature, possibly due to the use of differing *in vitro* systems (40,41). Non-nucleotide substrates also exist including methotrexate (42), the antiviral agent 9-(2-phosphonomethoxyethyl) adenine, and the reverse transcriptase inhibitor azidothymidine (43); however, the importance of these transporters as drug/metabolite carriers awaits further investigation. In addition, sulfated bile acids and steroids have been shown to competitively inhibit MRP4 transport (44). Interestingly, however, chronic elevation of bile acid levels caused by downregulation of Bsep [bile salt export pump (*Abcb11*)], resulted in increased expression of Mrp4 (45).

MRP6 (*ABCC6*)

Mrp6 (*Abcc6*) has been characterized as both a lateral and canalicular transporter in rat hepatocytes (46). This protein does not appear to be important in hepatic excretion of phase II biotransformation products (e.g., glucuronide, sulfate, and glutathione conjugates) because it does not transport typical anionic substrates, with the exception of the cyclopentapeptide BQ-123 (46). Although MRP6 mRNA has been found in high levels in human liver and kidney, its role in drug transport remains to be elucidated (47).

MRP7 (*ABCC10*) and MRP8 (*ABCC11*)

mRNA for MRP7 (*ABCC10*) has been shown to be expressed in several tissues including liver (48), while HEK293 cells transfected with MRP7 demonstrated transport of $E_2$17G and LTC_4 (49). mRNA for a new member of the MRP family, MRP8 (*ABCC11*), is present in the liver at very low levels with higher expression in breast and testes (50). MRP8 is highly homologous with MRP5 and therefore, not surprisingly, also transports cyclic nucleotides (51).

DRUG TRANSPORT PROTEINS OF THE HEPATIC CANALICULAR MEMBRANE

BSEP (*ABCB11*)

The biliary excretion of xenobiotics and metabolites occurs predominantly by unidirectional ATP-dependent export pumps that transport substrates across the canalicular membrane into bile (Fig. 2). These canalicular transport proteins belong to the ABC superfamily of transporters (52). Bsep, the bile salt export pump [sister gene of P-glycoprotein (*Abcb11*)], is responsible for excretion of conjugated and unconjugated bile salts into the canalicular space (53). In patients with progressive familial intrahepatic cholestasis type 2 (PFIC2), biliary bile salt concentrations are <1% of normal because the *ABCB11* gene is mutated and BSEP is absent from the canalicular membrane (54,55). Though this protein does not appear to play a key role in hepatic excretion of xenobiotics, it may be an important site of drug interactions resulting in hepatotoxicity, as discussed below.

MRP2 (*ABCC2*)

The multidrug resistance–associated protein 2 [MRP2 (*ABCC2*)], the most widely studied apical member of the

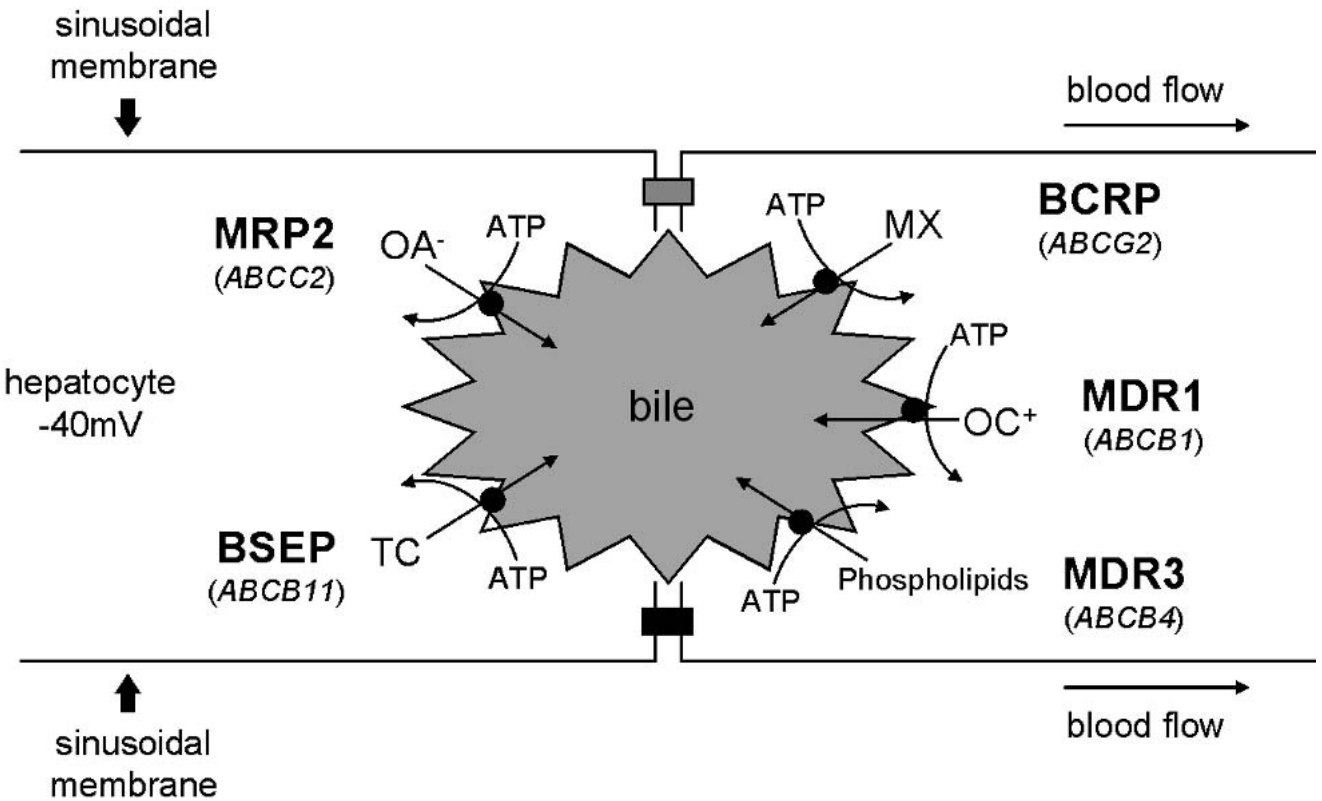

Fig. 2. Human hepatic canalicular transport proteins. Schematic representation of two adjacent hepatocytes as described in Fig. 1. Important canalicular transport proteins (protein name is in bold type with gene symbol listed below) are depicted with arrows denoting the direction of transport and ATP-dependent transporters designated by ●. Typical substrates are listed (OA^-, organic anions; OC^+, organic cations; TC, taurocholate; MX, mitoxantrone).

MRP/Mrp family, and originally designated as the canalicular multispecific organic anion transporter (cMOAT), is responsible for the biliary excretion of organic anions including LTC_4, divalent bile salts, and glutathione, glucuronide, and sulfate conjugates (56). A more complete list of substrates is presented in Table II. Absence of this protein on the canalicular membrane of hepatocytes is the basis for the defect in biliary excretion of organic anions in Groningen Yellow/Transport-deficient Wistar rats (GY/TR^-) (57,58) and Eisai hyperbilirubinemic Sprague-Dawley rats (EHBR) (59), as well as in patients with Dubin-Johnson syndrome (38). To compensate for a deficiency in biliary excretion of organic anions, including conjugated bile acids, levels of MRP3 (*ABCC3*), a hepatic basolateral member of this subfamily, are increased in patients with Dubin-Johnson syndrome; Mrp3 (*Abcc3*) also is upregulated in the naturally occurring Mrp2-deficient rats (60). This observation supports the hypothesis that the affinity of a compound for canalicular and basolateral excretory transporters, as well as the activity of the respective transporters, may determine whether a compound is excreted predominantly in bile or urine. Alterations in transport activity due to drug or nutrient interactions or patient-specific factors (e.g., disease, genetics) may influence significantly the route of hepatic excretion of compounds.

MDR1 (*ABCB1*)

The most widely recognized canalicular transporter is MDR1, the multidrug resistance protein [P-glycoprotein (*ABCB1*)]. Overexpression of this protein is one mechanism by which cancer cells develop resistance to an array of chemotherapeutic agents that exhibit a wide range of structures and mechanisms of action (61). MDR1 primarily mediates the transport of hydrophobic cations. The typical MDR1 substrate exhibits one, or preferably more, planar aromatic rings that enable interaction with a hypothesized "flat" hydrophobic region of the MDR1 drug-binding domain, preferentially a cationic charge at physiological pH, a bulky structure (e.g., molecular weight >400) (62), and a log partition coefficient >1 and preferentially >2 (63). More recent studies using *Abcb1* gene knockout mice demonstrated that Mdr1 also is important in the distribution and elimination of relatively small, aliphatic and aromatic, permanently charged cationic molecules (64,65). Classic MDR1/Mdr1 substrates include chemotherapeutic agents (daunorubicin, doxorubicin, etoposide, paclitaxel, vinblastine, vincristine), cardiac glycosides (digoxin), narcotic analgesics (methadone, morphine), rhodamine 123, cyclosporin A, and a host of other therapeutic and diagnostic agents (66). Three-dimensional quantitative structure-activity relationship (QSAR) models for MDR1 have been developed in an attempt to rank order substrates, predict compounds that may modulate binding sites, and identify structural requirements of modulators (67–69). Clearly, MDR1 plays a major role in the hepatic excretion of a vast number of endogenous and exogenous compounds, including many drugs and metabolites.

Factors altering expression levels of this transporter also have been investigated. Mdr1 levels and activity (V_{max}) have been shown to be decreased in rat canalicular plasma membrane vesicles by ~22% and ~35%, respectively, by protein-calorie malnutrition (70). A positive correlation has been demonstrated between COX-2 overexpression and *Abcb1* mRNA and Mdr1 protein levels in renal rat mesangial cells. This translated into increased Mdr1 activity as measured by rhodamine 123 efflux (71). In addition, ultraviolet irradiation and heat shock increased Mdr1 expression, whereas a down-regulation of *Abcb1* has resulted from lipopolysaccharide-induced endotoxemia in rodents (72,73). Cytokines also have been implicated in the regulation of this transport protein as interleukin-1β and -6 exposure both have been shown to decrease Mdr1 levels (73). In addition, Annaert *et al.* demonstrated increased Mdr1 protein and function over time in sandwich-cultured rat hepatocytes (74). These examples demonstrate that Mdr1 may be regulated by a variety of factors.

MDR3 (*ABCB4*)

Another multidrug resistance protein, MDR3 (*ABCB4*) and its rodent ortholog Mdr2, serve primarily as phosphatidylcholine translocases. MDR3 and Mdr2 play a crucial role in biliary phospholipid secretion and basic liver physiology in humans and rodents, respectively. Patients classified with PFIC type 3 cholestasis exhibit mutations in the *ABCB4* gene. The physiologic role of MDR3 as a drug transporter remains to be established (75).

BCRP (*ABCG2*)

Breast cancer resistance protein [BCRP (*ABCG2*)], a 72-kDa ABC half-transporter, was detected after RNA fingerprinting in a multidrug-resistant human breast cancer subline (76). This protein dimerizes in the plasma membrane and confers resistance to compounds such as mitoxantrone, doxorubicin, daunorubicin, and sulfated conjugates (77). BCRP is distributed in several tissues: placenta, small intestine, colon, hepatic canalicular membrane, breast, and venous and capillary endothelium (78). BCRP may play an important role in the biliary excretion of the sulfated conjugates of steroids and xenobiotics.

MODEL SYSTEMS FOR HEPATOBILIARY DRUG TRANSPORT

A major limitation in the field of hepatobiliary transport has been the lack of suitable model systems that retain hepatic architecture, hepatocyte function, and bile formation. The advantages of *in vivo* and isolated perfused liver techniques, with respect to reflecting the true physiologic state of the liver, are offset by the inefficiency (in terms of time and animal consumption) and the species limitations (predominantly rodents) inherent in these approaches. As previously discussed, important differences in hepatic transport proteins and substrate specificity may exist between rodents and humans. Furthermore, the complexities of the whole organ limit the ability to study individual hepatic uptake and excretion mechanisms. The lack of specific and potent inhibitors for the transport proteins primarily involved in hepatobiliary drug disposition limits the ability to probe the influence of individual transporters on overall disposition.

Hepatocytes exhibit less complexity than the intact organ while retaining important liver-specific cellular functions that may be lacking in other *in vitro* systems (i.e., plasma membrane vesicles; transport proteins transfected in non-mammalian cells) and may be used to examine specific transport functions at the membrane level. Hepatocytes isolated

from human liver tissue avoid potential issues with respect to species differences in hepatobiliary disposition (79,80). Isolated hepatocytes have been used extensively to investigate hepatic uptake mechanisms, although redistribution of canalicular membrane proteins and loss of cell polarity limit the utility of freshly isolated hepatocytes for studying drug excretion (81). Hepatocyte-derived cell lines (WIF-B, HepG2) that form "excretory domains" similar to bile canaliculi have been used in transport protein trafficking and regulation studies (82–85). Canalicular secretion of substrates has been studied in hepatocyte couplets (cell pairs that did not separate during collagenase treatment and in which the bile canaliculus is conserved) (86–88). Hepatocytes cultured in a conventional configuration (on a rigid substratum) are not suitable for studying hepatobiliary transport due to the rapid loss of normal hepatocyte morphology and liver-specific functions, including hepatic transport properties, and failure to reestablish normal canalicular networks (89–91). Primary cultures of hepatocytes maintained between two layers of gelled collagen (sandwich-culture configuration) that develop intact canalicular networks, maintain hepatic transport protein expression and function, and reestablish polarized excretory function, are a useful *in vitro* model system to study hepatobiliary disposition of compounds (91–93). Immunohistochemical localization of transport proteins, as well as direct measurement of transporter function, in sandwich-cultured hepatocytes avoid artifacts that may arise when examining freshly isolated hepatocytes or separating canalicular and basolateral membranes from native tissue.

The development of techniques to isolate separately hepatic basolateral (bLPM) and canalicular (cLPM) liver plasma membranes and form intact vesicles led to extensive use of this *in vitro* methodology to investigate electrogenicity, energy and ion dependency, and inhibitors of hepatic transport systems (94,95). Though bLPM and cLPM vesicles are useful model systems for mechanistic studies and have been used to a limited extent with human tissue (96,97), isolation of highly purified fractions with proper orientation of the vesicles is not trivial. *In vitro-in vivo* correlations with these relatively artificial systems have been conducted (98). Most recently, the cloning and expression of transport proteins in non-mammalian and mammalian cells has become invaluable in the functional characterization of hepatic transporters, as well as in identifying driving forces, substrate specificity, and specific inhibitors (99–101). However, the relative contribution of individual proteins in the hepatic uptake and excretion of substrates *in vivo* will be difficult to establish using these techniques. Though genetic models of transport protein deficiencies have been used to address this issue, it is clear that hepatocytes deficient in a crucial transport system develop adaptive mechanisms (e.g., up-regulation of a compensatory transporter) or do not survive. Thus, data from knockout models must be interpreted with caution. Use of small interfering RNA to transiently knock-down the expression of a specific transport protein *in vivo* or *in vitro* may be a useful approach to study the expression, regulation, and/or function of hepatic drug transport proteins (102). Undoubtedly, a combination of sophisticated techniques will be required to elucidate the complex processes involved in the hepatobiliary disposition of drugs and metabolites.

The model systems discussed above may be used at various stages in the drug development process to assess the hepatobiliary disposition of new chemical entities. Cell lines expressing one or more hepatic transport proteins may be useful in determining the affinity of a lead compound, as well as structural analogs, for a specific transport protein. Though these relatively artificial systems are extremely efficient at determining whether a compound is transported by a specific protein, they fall short of elucidating the role that a specific transport protein may play in overall hepatobiliary disposition of a compound when multiple transport systems are present. To address this fundamental question, hepatocytes or the intact organ is required. Due to low throughput and high compound requirements for perfused organ and *in vivo* studies, these more labor-intensive methods typically are reserved for development candidates. Sandwich-cultured hepatocytes, with intact canalicular networks, properly localized transport proteins, and functional metabolic systems, offer significant advantages in both the early stages of candidate selection and at later stages in the drug development process. At the early stages of lead optimization and candidate selection, sandwich-cultured hepatocytes can be used to identify analogs with specific transport properties (limited or enhanced hepatobiliary uptake or efflux) that make them suitable for further development. At later stages of drug development, these systems also may be useful to assess the relevance of transport inhibition and/or identify mechanisms of hepatotoxicity.

NEW DEVELOPMENTS IN HEPATOBILIARY DRUG TRANSPORT

Regulation of Hepatobiliary Drug Transport Activities

Understanding the basic regulation of hepatic transport proteins will increase knowledge of hepatic transport biology and facilitate predictions of how drugs and/or disease states that affect intracellular regulatory mechanisms may alter hepatic transport of endogenous and exogenous compounds. Mechanisms for regulation of membrane transport generally involve alterations in transporter function or changes in the number of transport molecules in the membrane. Long-term modulation of transport protein expression can occur at several different levels: transcription, translation, and post-translation. Transcription factors play an important role in the regulation of transporter gene expression in hepatocytes (103). Nuclear hormone receptors comprise a large superfamily of ligand-activated transcription factors that mediate developmental as well as physiological responses to both endogenous and exogenous compounds. Xenobiotics interact with these receptors, and the resulting complexes bind to the regulatory region of the gene to modulate expression. Once a ligand binds to the receptor, the complex proceeds to bind to the heterodimeric partner retinoic acid X receptor (RXR) and this dimer then is able to initiate transcription. To date, several nuclear hormone receptor types have been reported to be involved in the transcriptional regulation of hepatic transport proteins: pregnane X receptor (PXR), peroxisome proliferator-activated receptor α (PPARα), farnesoid X receptor (FXR), liver X receptor (LXR), and the constitutive androstane receptor (CAR). The identification of ligands for these nuclear hormone receptors, and the specific roles that they play in transcriptional control of transporter gene expression, comprise an important area of ongoing research that is reviewed briefly in this section according to

transporter type. A summary of this information is provided in Table III.

Studies of transporters expressed at the basolateral membrane have shown that bile acid treatment of primary rat hepatocytes and transfected HepG2 cells resulted in down-regulation of *Slc10a1* via an Fxr-mediated induction of shp (small heterodimeric protein) (104). Cytokine-mediated inflammatory cholestasis has been attributed to transcriptional down-regulation of *Slc10a1* and *Abcc2* gene expression by the retinoic acid receptor and RXR (105). FXR has been implicated in the regulation of *SLCO1B3* as treatment of HepG2 cells with chenodeoxycholic acid resulted in an increase in *SLCO1B3* mRNA (106). Additionally, studies in PXR-null mice demonstrated that *Slco1a4* regulation occurs via a PXR-dependent pathway (107). Treatment of wild-type mice with the PXR ligand, pregnenolone-16α-carbonitrile, resulted in a large increase in *Abcc3* mRNA, whereas no activation was observed in the PXR-knockout mice (107). Studies using Wistar Kyoto rats demonstrated that CAR does not play a key role in phenobarbital-mediated induction of Mrp3 (108).

Transcriptional regulation of several canalicular transporters also has been investigated. *ABCB11* was found to be transcriptionally regulated by the bile acid chenodeoxycholic acid via the FXR/RXRα heterodimer (109). In a study by Kauffman *et al.*, redox-active compounds caused induction of *ABCC1* and *ABCC2* gene expression; however, the role of PXR in mediating this phenomenon was unclear (110). In rodents however, *Abcc2* mRNA induction occurred in a PXR, CAR, and FXR-dependent manner as demonstrated by incubating rat hepatocytes and *Abcc2* promotor-transfected HepG2 cells with agonists of these nuclear hormone receptors (111). PXR was first reported to be involved in the transcriptional regulation of cytochrome P450 (CYP) 3A4 (112). However, recent studies have shown that PXR also is involved in mediating induction of MDR1 (113,114). Induction of the phospholipid flippase MDR3 by fibrates is mediated by PPARα as demonstrated using wild-type and PPARα-knockout mice (115). A concise review on regulation of hepatic drug and bile salt transporters has been published by Kullak-Ublick and Becker (116).

Recent work also has elucidated some mechanisms of short-term regulation and trafficking of hepatic transporters (85,117–119). Short-term regulatory effects usually occur rapidly and do not involve increased transcription or translation. Membrane proteins often reside in intracellular vesicles that serve as a compartment from which transporters may be recruited or stored (120,121). Transporter insertion or recruitment into the membrane may be a response to several different cellular stimuli. For example, disruption of cell-cell contacts in hepatocytes results in marked internalization of Mrp2 (122). Hyperosmotic conditions cause more Mrp2 molecules to reside in hepatic intracellular vesicles, whereas hypoosmolarity increases Mrp2 content in the canalicular membrane (123).

Transporter translocation to and from the canalicular membrane has been shown to be dependent on signaling pathways. Gatmaitan *et al.* (124) demonstrated that Mdr1, Mdr2, Bsep, and Mrp2 protein content in canalicular membrane vesicles increased 1.5-fold by TC and 3-fold by 2′-*O*-dibutyryl adenosine 3′, 5′-cyclic monophosphate (DBcAMP), a cell-permeable cAMP analog. In addition, pretreatment with colchicine (a microtubule inhibitor) entirely blocked the effect of TC and partially blocked the effect of DBcAMP, suggesting that trafficking of these transporters to the canalicular membrane occurs partly via microtubules.

The short-term regulation of hepatic basolateral transporters also has been investigated. Cellular fractionation studies revealed that cAMP treatment caused Ntcp to be trafficked from endosomes to the basolateral membrane (118). This pathway involved phosphoinositide 3-kinase and protein kinase B and was sensitive to cytochalasin D, an actin filament formation inhibitor. In addition to stimulating trafficking of proteins from endosomal compartments to their respective membrane, cAMP also may affect protein activity via direct phosphorylation of the transporter. For example, loss of transport activity of Oatp1a1 has been attributed to increased phosphorylation with no internalization of the protein (125). These examples demonstrate the possibility of modulating intracellular cAMP levels to therapeutically regulate hepatocyte function in liver disease (i.e., cholestasis). Translocation of proteins is a highly regulated process that may influence hepatic transport and, ultimately, the hepatobililary disposition and systemic exposure to drugs and metabolites.

Drug Interactions in Hepatobiliary Transport

Numerous drug interactions in hepatic transport have been reported. For example, cyclosporin A inhibits the up-

Table III. Regulation of Hepatic Transport Proteins

Transport protein	Suppression (↓) activation (↑)	Nuclear receptor*	Ligand	References
NTCP	↑	RARα	Retinoids	(105)
	↓	SHP	Activation by FXR	(105)
OATP1B1	↓	SHP	Activation by FXR	(197)
OATP1B3	↑	FXR	Bile Acids	(106)
MRP3	↑	CAR	Phenobarbital	(198)
BSEP	↑	FXR	Bile Acids	(45,109)
MRP2	↑	PXR, CAR, FXR	Xenobiotics	(111)
MDR1	↑	PXR	Xenobiotics	(113,114)
MDR3/Mdr2	↑	PPARα	Fatty acids, fibrates, DHEAS	(115)

* Abbreviations used: CAR, constitutive androstane receptor; FXR, farnesoid X receptor; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; SHP, small heterodimer partner.

take of cerivastatin in human hepatocytes due, in part, to inhibition of OATP1B1 (126). This interaction may partially explain the 3- to 4-fold elevations in the plasma AUC and maximum plasma concentrations of cerivastatin in kidney transplant patients administered cyclosporin A (127). Angelin *et al.* demonstrated that quinidine administration to normal healthy volunteers decreased the biliary clearance of digoxin by 42% (128); quinidine inhibits the MDR1-mediated transport of digoxin (129). The hepatobiliary disposition of the Mdr1 substrate doxorubicin was evaluated in the isolated perfused rat liver. GF120918, a second-generation MDR1/Mdr1 inhibitor, decreased the cumulative biliary excretion of doxorubicin and its major metabolite, doxorubicinol, by 84% and 72%, respectively, relative to control (130). This transport interaction was verified *in vivo* with bile-duct cannulated *Abcb1a*(–/–) mice, in which the cumulative biliary excretion of doxorubicin and doxorubicinol was reduced by 82% and 62%, respectively, relative to wild-type mice (131). In addition to inhibition interactions, induction of hepatic transport proteins also may result in increased biliary clearance of substrates. Rats pretreated for 12 days with tamoxifen, an Mdr1 substrate and inducer, exhibited a ~12-fold increase in hepatic *Abcb1b* mRNA; the biliary excretion of tamoxifen and metabolites also was increased from 8–51% of the administered dose (132). Drug-induced hepatotoxicity also may be attributed to hepatic transport interactions. Bosentan, the first orally active endothelin receptor antagonist, inhibits Bsep resulting in intracellular accumulation of cytotoxic bile salts which may cause bile salt-induced liver damage; this interaction has been implicated as one mechanism in the development of cholestatic liver injury (133). Elucidation of mechanisms responsible for drug interactions in hepatobiliary transport is a topic of ongoing research in many laboratories.

Relationships Between Hepatic Drug Transport and Metabolic Systems

During the past decade, it was noted that MDR1 and CYP3A have substantial overlap in substrate specificities and common inducers (134). The HIV protease inhibitors amprenavir and nelfinavir induced both intestinal Mdr1 and hepatic CYP3A levels significantly (135). Schuetz *et al.* demonstrated that both proteins were up-regulated by rifampin, reserpine, phenobarbital, and clotrimazole in a human colon carcinoma cell line expressing MDR1 and CYP3A (136). The functional relationship of these two systems serves to detoxify and eliminate xenobiotics, and altered levels of these proteins can affect the concentration-time profile and therapeutic effects of many drugs. Interestingly, MDR1 plays a role in modulating the expression of CYP3A by influencing the intracellular concentrations of substrates that induce CYP enzymes (137). This relationship can have serious consequences for effective drug therapy. One example that demonstrates the complexities of drug metabolism-transport interactions is the vincristine-doxorubicin-dexamethasone (VAD) combination that is used to treat multiple myeloma. Because dexamethasone is a potent inducer of both CYP3A and MDR1, the intracellular concentrations of vincristine and doxorubicin are expected to decrease due to increased MDR1 activity (134); vincristine concentrations will be further reduced due to increased metabolism by CYP3A. In modulation studies in male and female rats, only dexamethasone treatment resulted in induced levels of both Mdr1 and CYP3A, whereas other modulators revealed a lack of coordinated regulation of these two proteins (138). In contrast, CYP3A inhibitors also have been identified as MDR1 inhibitors. Azole antifungals, ergot alkaloids, and macrolide antibiotics, all known CYP3A inhibitors, simultaneously inhibited MDR1 function to varying degrees (139). Another study supporting the idea of an interplay between CYP3A and Mdr1 demonstrated that when the latter was inhibited by GF120918 in isolated perfused rat livers, the extent of hepatic metabolism of a common substrate, tacrolimus, was increased due to the increased availability to the metabolizing enzymes (140). Schuetz *et al.* found that under certain circumstances, hepatic CYP3A protein levels were elevated in *Abcb1b*(–/–) mice compared to the gene competent counterparts suggesting some level of coordinated regulation of both these proteins (141). Functionally, this effect was demonstrated by Lan *et al.*; Mdr1 efflux influenced the extent of metabolism of erythromycin by CYP3A using the erythromycin breath test (142). The average metabolite AUCs were 1.9- and 1.5-fold higher in the *Abcb1b*(–/–) and *Abcb1a*(–/–) mice, respectively, compared to controls. Thus the absence of efflux by Mdr1 increased erythromycin availability to the metabolizing enzyme and hence resulted in higher metabolite exposure.

Although PXR is a key regulator of *CYP3A* gene expression in mammalian liver (112,143), numerous PXR ligands are substrates of MDR1. Recently, a distinct PXR binding site (DR4 nuclear response element) in the 5′-upstream region was identified and found to be essential for MDR1 induction by rifampin (113). In addition, FXR nullizygous mice, which have decreased levels of Bsep and thus elevated hepatic bile acid concentrations, also possessed an up-regulation of CYP3A and CYP2B drug-metabolizing enzymes (45). The coordinate regulation of hepatic drug metabolizing enzymes and hepatic transporters by PXR, as well as other nuclear hormone receptors, is currently under investigation. Clearly, cellular models with intact hepatic transport and metabolic systems will be required to elucidate the mechanisms of these complex interactions.

Genetic Differences in Hepatobiliary Transport

Variations in expression levels and activity of hepatic transport proteins due to genetic polymorphisms may result in altered disposition and pharmacokinetics of some drugs and may impact therapeutic efficacy. Recent studies are beginning to elucidate the mechanisms involved in regulation of transport proteins at this level. Although polymorphisms in some transport proteins have been shown to result in altered transport capacity in *in vitro* systems, very few have demonstrated significant alterations in transport kinetics in humans.

Single nucleotide polymorphisms (SNPs) have been identified in the *SLCO* family; however, few have been shown to have an effect *in vivo*. Several SNPs were observed in the *SLCO1A2* and *SLCO1B3* genes in 48 Japanese individuals; however, no functional studies were performed (144). In a study investigating the genetic polymorphisms of *SLCO2B1* and *SLCO1B1* in 267 healthy Japanese subjects, several SNPs were identified and each was expressed in HEK293 cells for functional analysis. The V_{max} of one OATP2B1 mutant was decreased to 42.5% of control levels; however, the physiological effects *in vivo* are unknown (145). The effects of polymor-

phisms in the liver-specific *SLCO1B1* gene on expression and function have been studied extensively. In a study by Michalski *et al.*, 81 human livers were analyzed for OATP1B1 by immunoblotting, and a new naturally occurring mutation was identified (146). After introduction of this mutation into the MDCKII cell line, the protein was retained intracellularly with diminished transport function. Functional analysis of several SNPs in *SLCO1B1* identified in European- and African-Americans also revealed reduced transport and decreased expression in the plasma membrane (147). Nishizato *et al.* studied a group of 120 healthy individuals and found 5 non-synonymous variants in the *SLCO1B1* gene and 1 non-synonymous variant in the *SLCO1B3* gene (148). Subjects with the OATP1B1 variants exhibited altered pravastatin kinetics.

Conrad *et al.* observed a low-frequency (<1%) naturally occurring mutation in MRP1 that resulted in an amino acid substitution (149). When the mutated protein was expressed in transfected HEK293 cells, LTC_4 transport was reduced 2-fold. In another study of 48 healthy Japanese subjects, 16 mutations in MRP1 were detected, 4 of which resulted in amino acid substitutions (150).

Genetic polymorphisms in the MRP6 transporter have been studied, and although no effects on transport have been noted thus far, the polymorphism has been linked to pseudoxanthoma elasticum, an inherited systemic disorder of connective tissue (151).

Different mutations resulting in nonfunctional MRP2 protein in humans is associated with the hyperbilirubinemic condition known as Dubin-Johnson syndrome (152). Liver biopsies from these patients revealed an absence of MRP2 protein (153). Different mutations in the *MRP2* gene may result in impaired maturation and/or trafficking/localization of the protein (154,155) or impaired ATP-hydrolysis (156). A recent study in Japanese subjects identified six mutations in the *MRP2* gene, four of which were associated with amino acid substitutions (150). The functional consequences of these alterations were not determined.

Although no direct correlations have been made between genetic polymorphisms in *ABCB1* and hepatic expression, the interested reader is directed to several excellent reviews on this topic (157–159). Several studies have identified a correlation between the SNP in exon 26 (C3435T) and MDR1 function. Hoffmeyer *et al.* analyzed the *ABCB1* sequence in 21 healthy volunteers and found that this polymorphism resulted in increased digoxin plasma concentrations and decreased MDR1 expression levels (160). This silent mutation is the only one thus far identified that results in altered expression in humans. However, conflicting reports regarding the functional effects of this polymorphism exist as Sakaeda *et al.* reported that a single oral dose of digoxin resulted in lower serum concentrations compared to subjects possessing the wild-type allele (161). In another study performed in three ethnic Asian populations (Chinese, Malays, and Indians), three SNPs were found to be polymorphic (located on exons 12, 21, 26); however, no effects on function were examined (162). Kimchi-Sarfaty *et al.* characterized the substrate specificity and cell surface expression of the five most common MDR1 mutants in a vaccinia virus-based transient expression system and concluded that cell surface distribution, expression, and function were not different from wild-type MDR1 (163).

Mitomo *et al.* performed site-directed mutagenesis and expressed three variant forms of BCRP in HEK293 cells to examine substrate specificity (164). No transport activity was detected with two of the mutants while the third demonstrated that Arg 482 is critical for the transport of methotrexate.

Elucidation of genetic polymorphisms in hepatic transport genes, determination of their functional significance, and development of tests to identify patients exhibiting clinically significant polymorphisms is an important area of ongoing research that may have a major impact in the therapeutic use of drugs that are substrates for transporters.

Knockout Models

As discussed above, numerous hereditary defects in hepatobiliary transport (e.g., EHBR and GY/TR$^-$ rats and patients with Dubin-Johnson syndrome that do not express Mrp2/MRP2 on their canalicular membrane) exist. These naturally occurring mutants have been useful in characterizing the role of specific proteins in hepatic transport of endogenous and exogenous compounds, including drugs and metabolites.

Abcb1 knockout mouse models, both single gene knockout [*Abcb1a*(–/–), *Abcb1b*(–/–)] and double gene knockout strains [*Abcb1a/1b*(–/–)] (165) have been used to examine the influence of Mdr1 in drug disposition. However, data generated from knockout models need to be interpreted cautiously because other physiological alterations may exist in these mutants. In addition to the potential for compensatory upregulation of other transporter genes, as previously discussed, alterations in metabolism or binding may confound data interpretation. For example, Schuetz *et al.* noted altered expression of hepatic CYP3A in *Abcb1* knockout mice (141). In addition, the hyperbilirubinemia in TR$^-$ rat strains has been shown to alter protein binding of some drugs due to the substantial binding of bilirubin to albumin (166).

Knockout mice with disrupted *Abcc5* alleles recently have been developed, but the role of the Mrp5 protein products in hepatic drug transport remains to be elucidated (167). *Abcc5* gene-knockout mice are healthy but deficient in nucleotide analog transport. Mrp1-deficient mice, which exhibit increased sensitivity to anticancer drugs and have increased glutathione levels, are being used to determine the transport characteristics of Mrp1 (168,169). Despite potential limitations, transgenic technology has provided a powerful tool that can be used to examine the role of gene products (transport proteins) in hepatobiliary transport.

THE EXCITING FUTURE OF HEPATOBILIARY DRUG TRANSPORT RESEARCH

This overview of hepatobiliary drug transport, although far from comprehensive, highlights the major hepatic transport systems identified to date that mediate hepatic uptake, excretion, and/or interactions with xenobiotics, including drugs and metabolites. Though our knowledge of hepatic transport from a physiologic, pharmacological, and clinical perspective has increased substantially during the past decade, we clearly lack a complete understanding of the complex processes involved in hepatobiliary drug disposition. Many important questions remain unanswered. How do xe-

nobiotics move through the hepatocyte from the basolateral domain to metabolic sites, and from sites of metabolism to the basolateral or canalicular domains? Why are some metabolites excreted preferentially into bile whereas others translocate across the basolateral membrane into sinusoidal blood? Fundamental issues regarding the regulation of hepatic transport processes in normal and diseased liver need to be addressed. Ongoing and future research undoubtedly will enhance our current understanding of hepatobiliary drug transport by characterizing the molecular basis of membrane translocation, elucidating the substrate binding site(s) of these proteins, and defining the mechanisms by which molecules inhibit and induce hepatic transport proteins. Application of this knowledge in the drug development process represents a new and exciting aspect of this discipline. On the horizon is the development of moderate/high-throughput screening methods to rapidly identify new chemical entities that are substrates for specific hepatic transport systems. Data generated with these screening techniques will allow systematic characterization of structure-transport relationships for both animal and human hepatic transport proteins. The development of *in vitro* techniques to examine hepatic drug transport processes in human liver will provide important insights regarding hepatobiliary drug disposition in humans. Elucidating the mechanisms involved in hepatic drug transport, defining patient-specific factors that affect transporter function, and characterizing how xenobiotic interactions may alter these processes, are fundamental to our knowledge of how the liver disposes of endogenous and exogenous compounds and are prerequisites to exploiting these processes to achieve desirable clinical outcomes.

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