0031-6997/03/5503-425–461$7.00
PHARMACOLOGICAL REVIEWS
Vol. 55, No. 3
30301/1082282
Pharmacol Rev 55:425–461, 2003
Printed in U.S.A

Impact of Drug Transporter Studies on Drug Discovery and Development

NAOMI MIZUNO, TAKURO NIWA, YOSHIHISA YOTSUMOTO, AND YUICHI SUGIYAMA

Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan; and Pharmacokinetics Laboratory, Mitsubishi Pharma Corporation, Chiba, Japan

***Abstract*——Drug transporters are expressed in many tissues such as the intestine, liver, kidney, and brain, and play key roles in drug absorption, distribution, and excretion. The information on the functional characteristics of drug transporters provides important information to allow improvements in drug delivery or drug design by targeting specific transporter proteins. In this article we summarize the significant role played by drug transporters in drug disposition, focusing particularly on their potential use during the drug discovery and development process. The use of transporter function offers the possibility of delivering a drug to the target organ, avoiding distribution to other organs (thereby reducing the chance of toxic side effects), controlling the elimination process, and/or improving oral bioavailability. It is useful to select a lead compound that may or may not interact with transporters, depending on whether such an interaction is desirable. The expression system of transporters is an efficient tool for screening the activity of individual transport processes. The changes in pharmacokinetics due to genetic polymorphisms and drug-**

Address correspondence to: Dr. Yuichi Sugiyama, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: sugiyama@mol.f.u-tokyo.ac.jp

Article, publication date, and citation information can be found at http://pharmrev.aspetjournals.org.
DOI: 10.1124/pr.55.3.1.

drug interactions involving transporters can often have a direct and adverse effect on the therapeutic safety and efficacy of many important drugs. To obtain detailed information about these interindividual differences, the contribution made by transporters to drug absorption, distribution, and excretion needs to be taken into account throughout the drug discovery and development process.

I. Introduction

With publication of the complete human genome sequence in 2001 (International Human Genome Sequencing Consortium, 2001; Venter et al., 2001), this new information about all the human genes and their functions led to a change in drug research strategies and, in particular, the processes of drug discovery and development. Following the progress in genome-based drug discovery, international competition in drug discovery has become even keener. The particular strategy adopted for drug discovery will be a critical factor in determining whether a company is successful in its research and development operations. The strategy that target proteins are identified based on genomic information, so-called "genome-based drug discovery," is likely to become very popular. However, determination of the target protein alone is insufficient to allow the development of clinically significant drugs. It is also necessary to identify the lead compounds binding the target proteins by using combinatorial chemistry synthesis and high-throughput screening, optimize these lead compounds, and then select those with clinically effective pharmacological activity and minimize any side effects. A significant number of drug candidates entering clinical development are dropped at some stage due to unacceptable pharmacokinetic properties (White, 2000; Roberts, 2001). The pharmacokinetic profile should be a primary consideration in the selection of a drug candidate, ultimately contributing to its eventual clinical success or failure. It is now recognized that selection of a "robust" candidate requires a balance among efficacy, safety, and pharmacokinetic properties, and that the screening of these characteristics should be carried out as early as possible in the discovery process. Thus, many pharmaceutical companies are now carrying out rational high-throughput drug metabolism and pharmacokinetics screening systematically and establishing pharmacokinetic selection criteria (White, 2000; Roberts, 2001). For example, the high-throughput screening for absorption using Caco-2 cells and the screening for metabolic stability and metabolic enzyme inhibition using cytochrome P450 recombinant microsomes or human liver microsomes have become extremely popular. Attention is now being focused on optimizing the pharmacokinetic profiles of drug candidates using transporter function (Ayrton and Morgan, 2001; Mizuno and Sugiyama, 2002).

Many different drug transporters are expressed in various tissues, such as the epithelial cells of the intestine and kidney, hepatocytes, and brain capillary endothelial cells (Muller and Jansen, 1997; Koepsell, 1998; Meijer et al., 1999; Suzuki and Sugiyama, 1999; Inui et al., 2000a; van Aubel et al., 2000; Gao and Meier, 2001) (Table 1). In recent years, a number of important transporters have been cloned, and considerable progress has been made in understanding the molecular characteristics of individual transporters. It has now become clear that some of these are responsible for drug transport in various tissues, and they may be key determinants of the pharmacokinetic characteristics of a drug as far as its intestinal absorption, tissue distribution, and elimination are concerned (Oude Elferink et al., 1995; Zhang et al., 1998; Kim, 2000; Dresser et al., 2001; Kusuhara and Sugiyama, 2002; Russel et al., 2002). Studies of the functional characteristics, such as substrate specificity, and of the localization of cloned drug transporters could provide important information about the mechanisms of drug disposition. Transporters have been classified as primary, secondary, or tertiary active transporters. Secondary or tertiary active transporters, such as OAT[1], OATP, NTCP, OCT, OCTN, and PEPT, are driven by an exchange or cotransport of intracellular and/or extracellular ions (Burckhardt and Wolff, 2000; Dresser et al., 2001; Lee et al., 2001a). The driving force for primary active transporters like ATP-binding cassette transporters, such as MDR, MRP, and BCRP, is ATP hydrolysis

[1]Abbreviations: OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; NTCP, sodium taurocholate cotransporting peptide; OCT, organic cation transporter; PEPT, oligopeptide transporter; ASBT, apical sodium-dependent bile acid transporter; MDR, multidrug resistant (or resistance); MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein; SLC, solute carrier superfamily; ABC, ATP-binding cassette; BBB, blood-brain barrier; CNS, central nervous system; P-gp, P-glycoprotein; ACE, angiotensin-converting enzyme; AUC, area under concentration-time curve; TPGS, *d*-α-tocopheryl polyethylene glycol 1000 succinate; HIV, human immunodeficiency virus; PS, permeability-surface area; CL, clearance; E_2-17βG, estradiol-17β-glucuronide; PAH, *p*-aminohippurate; NSAID(s), nonsteroidal anti-inflammatory drug(s); CPT-11, irinotecan hydrochloride; CMV, canalicular membrane vesicle; BSEP, bile salt export pump; $CL_{int,bile}$, intrinsic clearance for the net biliary excretion from the blood; PS_1, hepatic uptake across the sinusoidal membrane; PS_2, efflux across the sinusoidal membrane from the liver; PS_3, excretion across the canalicular membrane; HIV-PI, HIV protease inhibitor; SXR, steroid xenobiotic receptor; PXR, pregnane X receptor; GS-X, glutathione *S*-conjugate export; FXR, farnesoid X-activated receptor; CAR, constitutive androstane receptor; HNF1, hepatocyte nuclear factor 1; CFTR, cystic fibrosis transmembrane conductance regulator; DJS, Dubin-Johnson syndrome; ER, endoplasmic reticulum; *Rdx*, radixin; SNP, single nucleotide polymorphism; K_p, tissue-to-plasma concentration ratio; LC/MS/MS, liquid chromatography/tandem mass spectrometry; P_{app}, apparent permeability; TCA, taurocholate; AM, acetoxymethyl ester.

TABLE 1
Major drug transporters expressed in intestine, kidney, liver, and brain of human or rat

Human		Rat		Human		Rat	
Name	Localization	Name	Localization	Name	Localization	Name	Localization
Intestine				**Liver**			
Peptide transporter				*Organic anion transporter*			
PEPT1	BBM	PepT1	BBM	NTCP	SM	Ntcp	SM
				OATP-C	SM	Oatp1	SM
Organic anion transporter				OATP8	SM	Oatp2	SM
OATP-B	ND[a]	Oatp3	BBM	OATP-B	SM	Oatp4	SM
OATP-D	ND			OAT2	SM	Oat2	SM
OATP-E	ND					Oat3	SM
ASBT	BBM	Asbt	BBM			NaPi-1/Npt1	SM
Organic cation transporter				*Organic cation transporter*			
		Oct1	BLM	OCT1	ND	Oct1	SM
		Oct3	ND			Oct1A	ND
		Octn1	ND	OCT3			
Primary active transporter						Octn1	*ND*
MDR1	BBM	Mdr1	BBM	*Primary active transporter*			
MRP2	BBM	Mrp2	BBM	MRP1	SM		
MRP3	BLM	Mrp3	BLM	MRP3	SM	Mrp3 (EHBR, TR$^-$)	SM
BCRP	BBM						
				MDR1	CM	Mdr1	CM
				MRP2	CM	Mrp2	CM
Kidney				BSEP/SPGP	CM	Bsep/Spgp	CM
Peptide transporter				BCRP	CM		
		PepT1	BBM				
PEPT2	ND	PepT2	BBM				
				Brain capillary endothelial cells			
Organic anion transporter				*Organic anion transporter*			
OAT1	BLM	Oat1	BLM	OATP-A	ND	Oat2	ALM, LM
OAT3	BLM	Oat3	BLM			Oat3	ND
OAT4	BBM			*Organic cation transporter*			
						Oct2	ND
		Oatp1	BBM	*Primary active transporter*			
		Oat-K1	BBM	MDR1	LM	Mdr1	LM
		Oat-K1	ND			Mrp1	ND
		NaPi-1/Npt1	BBM			Mrp5	ND
		Asbt	BBM				
Organic cation transporter							
		Oct1	BLM	**Choroid plexus**			
		Oct1A	ND	*Peptide transporter*			
OCT2	BLM	Oct2	BLM			PepT2	BBM
		Oct3	ND	*Organic anion transporter*			
						Oatp1	BBM
OCTN1	ND	Octn1	BBM			Oatp2	BLM
OCTN2	ND	Octn2	BBM			Oatp3	BBM
Primary active transporter				*Primary active transporter*			
						Oat3	BBM
MDR1	BBM	Mdr1	BBM			Mrp1	BLM
MRP2	BBM	Mrp2	BBM			Mdr1	BBM
MRP4	BBM	Mrp4	BBM				

SM, sinusoidal membrane; CM, canalicular membrane; BLM, basolateral membrane; BBM, brush border membrane; LM, luminal membrane, ALM, abluminal membrane.
[a] ND, not determined.

(Lautier et al., 1996; Borst et al., 1999; Hooiveld et al., 2001; Lee et al., 2001a; Schinkel and Jonker, 2003). Most of the former transporters have a similar structure in that they have 12 putative membrane-spanning domains and their molecular mass is approximately 50 to 100 kDa. In contrast, the mean molecular weight of the latter transporters, involved in the cellular extrusion of xenobiotics, is comparatively high (150–200 kDa) and they all have two ATP-binding domains, except for BCRP. Furthermore, each gene family of transporters is composed of a multiplicity of members. Owing to the increase in the number of identified transporter genes, the Human Gene Nomenclature Committee has classified transporters using standardized names, such as the solute carrier superfamily (SLC) and ATP-binding cassette (ABC) transporters (http://www/gene.ucl.ac.ul/nomenclature/genefamily.shtml). These standardized names, accompanied by the conventional names, are shown in Table 2. The tissue distribution and elimination route of some drugs is determined by the degree of expression of each transporter subtype in each tissue and its corresponding substrate affinity and transport maximum. Thus, regulating the function of transporters should allow the highly efficient development of drugs with ideal pharmacokinetic profiles. As drug discovery involving the use of transport mechanisms increases, the need for an effective in vitro screening system for transporters will also increase. Accordingly, methods

allowing the rational prediction and extrapolation of in vivo drug disposition from in vitro data are urgently required. Although there has been intensive investigation of the functional analysis of the human genome, there are a large number of genes whose molecular protein function remains unknown (Venter et al., 2001). The human genome contains many genes that encode membrane transporters and related proteins (Table 3) (Venter et al., 2001). For drug discovery, development, and targeting one needs to know which transporters play a role in the disposition of a drug and its subsequent effects.

In this article, we summarize the key role played by drug transporters in drug disposition, and the strategic use of drug transporters in drug discovery and development is discussed. We also introduce possible strategies for drug discovery using transporters, including the transporter screening systems, methods for estimating the contribution of transporters to drug disposition, and the prediction of in vivo drug disposition from in vitro data.

II. Strategies for Drug Discovery Using Transporters

A. Drug Delivery to Target Tissues Using Transporters

One of the main goals is to develop pharmaceutical agents with no adverse effects. It is also desirable to develop drugs with a wide therapeutic spectrum of activity. Drug targeting is one effective approach both to increase the pharmacological activity of drugs and to reduce their side effects by enhancing delivery to the target site. Recent research has identified many types of transporters that are expressed selectively in the liver, kidney, and other organs and which, therefore, may be a promising target for drug delivery. Some instances of drug delivery to the liver or kidney are introduced here.

The most comprehensively documented case is pravastatin. Pravastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, undergoes enterohepatic circulation, which prolongs the exposures of the liver (target organ) to the drug and minimizes adverse side effects in the peripheral tissues. This enterohepatic circulation is mediated by transporters in every process from pravastatin gastrointestinal absorption to biliary transport. Pravastatin is taken up by the liver from the portal vein by OATP family proteins located on the sinusoidal (basolateral) membrane (Hsiang et al., 1999; Nakai et al., 2001; Sasaki et al., 2002). After exhibiting its pharmacological action in the liver, pravastatin is then excreted into the bile via MRP2 with only a minimum degree of metabolic conversion (Yamazaki et al., 1996). The fraction of the drug released into the duodenum is then reabsorbed by active transport (Tamai et al., 1995). Thus, efficient hepatobiliary transport by OATP and MRP2 plays an important role in the enterohepatic circulation, which is responsible for maintaining significant concentrations of this drug in the liver. Although the mechanisms governing the pharmacokinetic properties of this drug were identified after their development, attempts to design molecules during the drug discovery process will be required in the future.

It has been found that successful targeting of anticancer drugs can be achieved using oligopeptide transporter

TABLE 2
Abbreviations of human drug transporters

Abbreviation		Symbol[a]
MDR1/P-gp	Multidrug resistant gene/P-glycoprotein	*ABCB1*
BSEP/SPGP	Bile salt export pump/sister P-glycoprotein	*ABCB11*
MRP1	Multidrug resistance associated protein 1	*ABCC1*
MRP2/cMOAT	Multidrug resistance associated protein 2	*ABCC2*
MRP3	Multidrug resistance associated protein 3	*ABCC3*
MRP4	Multidrug resistance associated protein 4	*ABCC4*
BCRP	Breast cancer resistance protein	*ABCG2*
NTCP	Sodium taurocholate cotransporting peptide	*SLC10A1*
ASBT	Apical sodium-dependent bile acid transporter	*SLC10A2*
PEPT1	Oligopeptide transporter 1	*SLC15A1*
PEPT2	Oligopeptide transporter 2	*SLC15A2*
OATP-A	Organic anion transporting polypeptide-A	*SLC21A3*
OATP-C/OATP2/LST-1	Organic anion transporting polypeptide-C	*SLC21A6*
OATP8	Organic anion transporting polypeptide 8	*SLC21A8*
OATP-B	Organic anion transporting polypeptide-B	*SLC21A9*
OATP-D	Organic anion transporting polypeptide-D	*SLC21A11*
OATP-E	Organic anion transporting polypeptide-E	*SLC21A12*
OATP-F	Organic anion transporting polypeptide-F	*SLC21A14*
OCT1	Organic cation transporter 1	*SLC22A1*
OCT2	Organic cation transporter 2	*SLC22A2*
OCT3	Organic cation transporter 3	*SLC22A3*
OCTN1	Novel organic cation transporter 1	*SLC22A4*
OCTN2	Novel organic cation transporter 2	*SLC22A5*
OAT1	Organic anion transporter 1	*SLC22A6*
OAT2	Organic anion transporter 2	*SLC22A7*
OAT3	Organic anion transporter 3	*SLC22A8*
OAT4	Organic anion transporter 4	*SLC22A9*

[a] Standardized names classified by the Human Gene Nomenclature Committee.

TABLE 3
The putative molecular functions of 26,383 human genes (Venter et al., 2001)

	Number	%
Enzyme		
Hydrolase	1,227	4.0
Isomerase	163	0.5
Ligase	56	0.2
Lyase	117	0.4
Oxidoreductase	656	2.1
Synthase and synthetase	313	1.0
Transferase	610	2.0
Signal transduction		
Select regulatory molecule	988	3.2
Kinase	868	2.8
Receptor	1,543	5.0
Signaling molecule	376	1.2
Nucleic acid binding		
Nucleic acid enzyme	2,308	7.5
Transcription factor	1,850	6.0
None		
Transfer/carrier protein	203	0.7
Viral protein	100	0.3
Miscellaneous	1,318	4.3
Cell adhesion	577	1.9
Chaperone	159	0.5
Cytoskeletal structural protein	876	2.8
Extracellular matrix	437	1.4
Immunoglobulin	264	0.9
Ion channel	406	1.3
Motor	376	1.2
Structural protein of muscle	296	1.0
Protooncogene	902	2.9
Select calcium binding protein	34	0.1
Intracellular transporter	350	1.1
Transporter	533	1.7
Molecular function unknown	12,809	41.7

The functional predictions are based on similarity to sequences of known function.

PEPT1, expressed in tumors (Nakanishi et al., 1997, 2000). Some human cancer cell lines naturally express oligopeptide transport activity. The delivery of the peptide-mimetic anticancer drug, bestatin, a substrate of PEPT1, has been investigated. After i.v. administration of bestatin into nude mice-inoculated tumor cells, the bestatin concentration in PEPT1-transfected tumor was significantly greater than that in vector-transfected tumor (Nakanishi et al., 2000). Furthermore, repeated oral administration of bestatin specifically suppressed the growth of PEPT1-transfected tumors. It has been suggested that bestatin distributes to tumor tissues in a specific manner.

NTCP is the Na^+-bile acid cotransporting protein that mediates the hepatic uptake of bile acids (Hagenbuch et al., 1991). Since NTCP is exclusively expressed on the sinusoidal membrane of the liver (Meier, 1995), this transporter may be used as a target for drug delivery to that organ. Dominguez et al. have reported that coupling of drugs to the side chains of bile acids may be a useful strategy for specifically targeting liver tumor cells (Dominguez et al., 2001). A novel cisplatin-ursodeoxycholic derivative (Bamet-UD2) is efficiently transported by NTCP (Briz et al., 2002). The concentration of Bamet-UD2 in the liver was severalfold higher than that of cisplatin, while potentially toxic drug accumulation in other tissues, such as kidney, brain, and bone marrow, was significantly reduced (Dominguez et al., 2001). Thus, in mice, Bamet-UD2 exhibited strong antitumor activity without any side effects compared with cisplatin (Dominguez et al., 2001).

The targeting strategy should focus on the differential expression of transporters between the target organ and other organs, and it is essential to design molecules that are capable of being transported by a target organ-specific transporter. In particular, the use of blood-brain barrier (BBB)-specific influx transporters is expected to be an effective approach for the brain delivery of drugs acting on the central nervous system (CNS) because drug penetration into the brain is restricted under normal conditions.

B. Role of Brain Efflux Transporters

Brain capillary endothelial cells form the BBB and act as a self-defense mechanism, preventing xenobiotics from entering the brain. However, successful penetration of the blood-brain barrier is necessary if a drug is to reach the required concentration for a desired pharmacological effect. Efflux transport systems at such barriers provide protection for the CNS by removing drugs from the brain and transferring them to the systemic circulation. This is why the brain penetration of drugs is markedly restricted (Suzuki et al., 1997; Tsuji and Tamai, 1997; Fromm, 2000; Kusuhara and Sugiyama, 2001b; Lee et al., 2001a; Schinkel, 2001; Hagenbuch et al., 2002; Sun et al., 2003). Primary active transporters, such as P-gp encoded by MDR1 or MRP transporters, are responsible for the cellular extrusion of many kinds of drugs (Cole and Deeley, 1998; Borst et al., 1999; Kool et al., 1999; Kuwano et al., 1999). P-gp transports a wide variety of lipophilic, structurally diverse drugs, such as vinca alkaloids and anthracyclines. In general, the substrate specificities of efflux transporters are remarkably broad, and their affinities for substrates are much lower (of the order of micromolar to millimolar) than the affinities for pharmacological receptors (of the order of nanomolar to picomolar). Thus, these transporters are able to recognize a large number of xenobiotics with a wide variety of structures. In normal tissue, P-gp is expressed in the liver, kidney, small and large intestine, and brain capillary endothelial cells (Troutman et al., 2001) (Table 1). Thus, the brain penetration of drugs, which are substrates of this transporter, is extremely limited (Fromm, 2000; Tamai and Tsuji, 2000; Kusuhara and Sugiyama, 2001b; Schinkel, 2001). In mice that lack P-gp encoded by the mdr1a gene, the brain distribution of P-gp substrates is significantly increased compared with that in normal mice (Table 4). Clearly, these results demonstrate that P-gp plays a key role in the BBB. The transporter gene knockout mouse is a very important tool for investigating the role of transporters in drug disposition (Schinkel et al., 1994; Wijnholds et al., 1997, 2000; Jonker et al., 2001). Efflux transport should be taken

into consideration during drug development to improve brain penetration and to avoid drug-drug interactions involving these transporters and subsequent side effects. In general, BBB permeability increases with increasing lipophilicity because the passive entry of molecules into the brain increases. However, a number of highly lipophilic drugs demonstrate unexpectedly poor BBB penetration because they are effluxed by P-gp. An approach to increase lipophilicity is not always useful as far as improving brain penetration is concerned. Brain penetration of drugs that pharmacologically target the CNS could, therefore, be improved by modifying the drug so that it is not recognized by P-gp (Doan et al., 2002). Whether poor brain penetration of a drug is due to poor membrane permeability or active efflux should be investigated in detail at an early stage of drug development. The expression system of transporters is an efficient tool for screening transport activities. Human transporter gene transfected cells are especially important tools because human experimental systems are rather limited. Recent studies show that the transport activity of MDR1-transfected cells correlates well with the P-gp transport activity in vivo (Adachi et al., 2001; Yamazaki et al., 2001).

However, reducing brain penetration by efflux transporters is expected to enable the adverse CNS side effects to be avoided in some drugs with toxic CNS effects. Some fluoroquinolone antibacterials or anticancer agents exhibit low brain distribution, despite having a high lipophilicity, because they are prevented from entering the brain by P-gp (Tamai and Tsuji, 2000). This probably explains their relative lack of CNS side effects. The brain uptake of ivermectin is markedly increased (27-fold) in mdr1a knockout mice (Table 4). Mdr1a knockout mice are much more sensitive (100-fold) than normal mice to the neurotoxic effects caused by ivermectin (Schinkel et al., 1994). Drug design, which ensures that the compounds interact with brain efflux transporter, may be a successful way to avoid the CNS toxicity of drug candidates that exhibit such an effect. However, a drug-drug interaction involving P-gp or a genetic polymorphism of MDR1 may change the brain penetration of drugs and may affect safety if the drug candidate is a P-gp substrate. Thus, it is important to select a lead compound that has no intrinsic CNS toxic potential rather than targeting the brain efflux transporter. Drug-drug interactions via brain P-gp between loperamide, a substrate for P-gp, and quinidine, an inhibitor of P-gp, have been reported (Sadeque et al., 2000). Although the antidiarrheal agent loperamide is a potent opiate, it does not produce opioid CNS effects at usual doses in patients. When a 16-mg dose of loperamide alone was administered to eight healthy volunteers, loperamide produced no respiratory depression. However, respiratory depression occurred when loperamide (16 mg) was given with quinidine at a dose of 600 mg. These changes were not explained by increased plasma loperamide concentrations. Thus, inhibition of P-gp by quinidine increases the entry of loperamide into the CNS with resulting opiate-induced respiratory depression. The lack of respiratory depression produced by loperamide, which allows it to be safely used therapeutically, can be reversed by a drug-drug interaction mediated by P-gp, resulting in serious toxic and abuse potential.

In addition, some organic anion-transporting polypeptides (OATPs) are expressed in the brain. Many members of this transporter family mediate transport of a wide spectrum of amphipathic organic anions (Hagenbuch and Meier, 2003). Rat Oatp2 (*Slc21a5*) is localized on both the luminal and abluminal membranes of the rat BBB (Gao et al., 1999). In humans, immunohistochemical staining of brain tissue suggested that OATP-A is expressed in the brain endothelial cells, although its localization has not been confirmed (Gao et al., 2000). Because Oatp2 can mediate bidirectional transport, involvement of Oatp2 in the efflux transport across the BBB is possible (Asaba et al., 2000; Sugiyama et al., 2001). The recently characterized human OATP-F is a high-affinity thyroxine transporter and is selectively expressed in brain (Pizzagalli et al., 2002). OATP-F is possibly involved in the uptake of thyroxin from the blood into the CNS. Moreover, OAT3, OCT3, and OCTN2 appear to be expressed in the brain (Wu et al., 1998; Kusuhara et al., 1999; Wu et al., 1999; Cha et al., 2001; Ohtsuki et al., 2002). Their localization in the brain and physiological function remain to be elucidated. It is essential to identify most of the important transporters in the brain and to characterize their function to provide a basis for developing strategies to regulate drug disposition in the brain.

C. Role of Transporters in Drug Absorption

Various transporters are expressed in the brush-border membranes of intestinal epithelial cells (Table 1). They are involved in the efficient absorption of nutrients or endogenous compounds. The use of influx transporters expressed in the gut, such as PEPT1, ASBT, OATP-B, OATP-D, OATP-E, or rat Oatp3 (*Slc21a7*), will help improve drug absorption (Tsuji and Tamai, 1996; Oh et al., 1999; Tamai et al., 2000a; Walters et al., 2000; Kullak-Ublick et al., 2001; Lee et al., 2001b). Rat Oatp3, but not Oatp1 (*Slc21a1*) or Oatp2, is expressed in the small intestine, and is localized on the apical brush-border membrane of enterocytes (Walters et al., 2000). Because Oatp3 transports taurocholate, rat oatp3 is suggested to mediate the absorption of bile acids. PEPT1 mediates the transport of peptide-like drugs such as β-lactam antibiotics, angiotensin-converting enzyme (ACE) inhibitors and the dipeptide-like anticancer drug bestatin (Hori et al., 1993; Saito and Inui, 1995; Swaan et al., 1995; Terada et al., 1997; Inui et al., 2000b). Interestingly, valacyclovir, a valyl ester prodrug of the antiviral agent acyclovir, although it does not contain a peptide bond, is transported by PEPT1 (Balimane et al.,

TABLE 4
$K_{p,brain}$ values in mdr1a(−/−) and mdr1a(+/+) mice after intravenous injection of various drugs

	Time after administration	mdr1a(+/+)			mdr1a(−/−)			Ratio ($K_{p,brain,ko}$/$K_{p,brain,wt}$)	Reference
		Plasma Conc. (ng/ml)	Brain Conc. (ng/ml)	$K_{p,brain}$	Plasma Conc. (ng/ml)	Brain Conc. (ng/ml)	$K_{p,brain}$		
Asimadoline[a]	1 h	211	65	0.31	199	586	2.94	9.56	Jonker et al., 1999
Azasetron				0.119			0.833	7.0	Tamai and Tsuji, 2000
Carebastin				0.0403			0.319	7.9	Tamai and Tsuji, 2000
Cyclosporine	4 h	38	10.5	0.28	54	178	3.3	12	Schinkel et al., 1995b
Daunomycin	30 min			0.91			1.63	1.8	Adachi et al., 2001
Dexamethasone	4 h	17.2	4.9	0.28	17.2	12.1	0.7	2.5	Schinkel et al., 1995b
Diazepam	30 min			3.35			3.24	0.9	Adachi et al., 2001
Digoxin	4 h	669	55.5	0.08	1259	1939	1.5	19	Schinkel et al., 1995b
Digoxin	90 min	21.2	1.1	0.05	39.6	21.1	0.53	10.3	Mayer et al., 1997
Digoxin[a]	4 h	622	37.1	0.06	1775	1011	0.57	9.5	Schinkel et al., 1997
Doxorubicin	1 h			0.00077			0.0025	3.2	Kusuhara and Sugiyama, 2001a
Ebastine				0.114			0.846	7.4	Tamai and Tsuji, 2000
Grepafloxacin[a,b]	2 h			0.34			1	2.9	Tamai et al., 2000b
HSR903	2 h			0.38			2.85	7.5	Murata et al., 1999
Indinavir	4 h	19	1.6	0.08	21	17	0.81	10	Kim et al., 1998
Ivermectin[c]	4 h	22	0.9	0.041	70	41	0.586	14.3	Schinkel et al., 1995a
Ivermectin[c]	24 h	16	1.5	0.094	52	131	2.519	26.9	Schinkel et al., 1994
Loperamide[c]	4 h	13.3	4.1	0.31	26.7	55.3	2.1	6.7	Schinkel et al., 1996
Morphine	4 h	10.9	5.3	0.49	12.4	8.9	0.72	1.48	Schinkel et al., 1995b
Nelfinavir	4 h	14	1.2	0.09	17	45	2.65	31	Kim et al., 1998
Ondansetron	30 min	40.5	18.7	0.46	39.4	75.6	1.92	4.2	Schinkel et al., 1996
Progesterone	30 min			1.45			1.34	0.9	Adachi et al., 2001
Quinidine	10 min			0.17			4.64	28	Kusuhara et al., 1997
Rhodamine-123	4 h	2.55	0.54	0.21	2.84	2.81	0.99	4.66	de Lange et al., 1998
Saquinavir	4 h	31	4.1	0.13	34	30	0.88	6.67	Kim et al., 1998
Sparfloxacin[a,b]	2 h			0.14			0.54	3.9	Tamai et al., 2000b
Tacrolimus	5 h			2.73			16.4	6.1	Yokogawa et al., 1999
Valspodar	4 h			0.6			1.2	2	Desrayaud et al., 1998
Verapamil	1 h	35	15	0.42	43	142	3.3	7.9	Hendrikse et al., 1998
Vinblastine	4 h	3	5	1.7	6	112	18.7	11	Schinkel et al., 1994
Vincristine				0.027			0.066	2.4	Tamai and Tsuji, 2000

Drugs were administered intravenously to both wild-type and mdr1a or mdr1a/1b double knockout mice. Plasma and brain concentrations were determined at the time of death. $K_{p,brain}$ was obtained by dividing the brain concentration by the plasma concentration.
[a] Mdr1a/mdr1b double knockout mice were used for this experiment.
[b] $K_{p,brain}$ values of grepafloxacin and sparfloxacin were evaluated using plasma unbound concentration.
[c] Oral administration.

1998; Sawada et al., 1999). From the viewpoint of drug delivery, L-valyl esterification of poorly absorbed drugs has been suggested as a useful strategy for improving their bioavailability and therapeutic efficacy.

However, primary active efflux transporters, such as P-gp, MRP2, or BCRP, are expressed on the brush-border membrane of enterocytes (Table 1) and excrete their substrates into the lumen, resulting in a potential limitation of net absorption (Gotoh et al., 2000; Hirohashi et al., 2000a; Jonker et al., 2000; Taipalensuu et al., 2001). Active secretion of absorbed drug is now becoming recognized as a significant factor in oral drug bioavailability (Wacher et al., 2001; Zhang and Benet, 2001). P-gp contributes to the absorption of many drugs because of its broad substrate specificity (Borst et al., 1999; Fromm, 2000; Troutman et al., 2001). The intestinal P-gp content correlates with the AUC after oral administration of digoxin, a P-gp substrate, in humans (Greiner et al., 1999). This result suggests that P-gp in the epithelium of the gut wall determines the plasma concentration of orally administered digoxin. Another report involving a patient undergoing a small bowel transplant has also demonstrated that the plasma concentration of orally administered tacrolimus, a substrate of both P-gp and CYP3A4, correlated well with the mRNA expression of intestinal MDR1, but not CYP3A4 (Masuda et al., 2000). These results suggest that intestinal P-gp, rather than CYP3A4, is a good probe to predict intraindividual variations in tacrolimus pharmacokinetics. Furthermore, high levels of MDR1 are strongly associated with reductions in survival rates after living-donor liver transplantation and subsequent immunosuppressive therapy with tacrolimus (Hashida et al., 2001). Intestinal MDR1 is also a powerful prognostic indicator of living-donor liver transplantation outcomes.

BCRP is a multidrug-resistance protein that is a new member of the ATP-binding cassette transporter family (Doyle et al., 1998). BCRP has only one ATP-binding cassette and six putative transmembrane domains (Rocchi et al., 2000), suggesting that BCRP is a half-transporter, which may function as a homo- or heterodimer. BCRP plays a role in the secretion of clinically important drugs such as topotecan (Jonker et al., 2000). When both topotecan, a substrate of BCRP, and GF120918, an inhibitor of both BCRP and P-gp, were administered orally, the bioavailability of topotecan was increased in P-gp-deficient mice (over 6-fold) compared with mice

given vehicle alone (Jonker et al., 2000) (Table 5). Thus, BCRP appears to be a major determinant of the bioavailability of topotecan following oral administration. Because GF120918 inhibits both P-gp and BCRP, P-gp-deficient mice have been used to exclude any confounding effects of P-gp inhibition. Topotecan is a weak-to-moderate substrate of P-gp, thus P-gp also appears to play a role in the bioavailability of topotecan. BCRP is expressed not only in the intestine, but also in the bile canalicular membrane and placenta (Maliepaard et al., 2001). Thus, treatment with GF120918 reduced the plasma clearance and hepatobiliary excretion of topotecan (Table 5). Furthermore, in pregnant GF120918-treated, P-gp-deficient mice, the fetal penetration of topotecan was 2-fold higher than that in pregnant mice given vehicle alone (Maliepaard et al., 2001). These results indicate that BCRP plays an important role in protecting the fetus from topotecan. The bioavailability of topotecan in humans is moderate, with a high interpatient variability (30 ± 8%) (Schellens et al., 1996). By combining oral topotecan with an effective BCRP inhibitor, the bioavailability of topotecan might be markedly improved and the interindividual variability might be reduced (de Bruin et al., 1999). Although GF120918 inhibits not only BCRP but also P-gp, fumitremorgin C, an extract of *Aspergillus fumigatus,* has been shown to potently inhibit BCRP but not P-gp or MRP, suggesting that fumitremorgin C is a highly selective inhibitor of BCRP (Rabindran et al., 1998; Ozvegy et al., 2001). So, the strategic application of BCRP inhibitors may lead to more effective oral chemotherapy with topotecan or other drugs that are BCRP substrates.

In principle, the inhibition of intestinal efflux transporters is a useful way to improve the oral bioavailability of a coadministered drug (Sikic et al., 2000). It has been shown that treatment with water-soluble vitamin E (*d*-α-tocopheryl polyethylene glycol 1000 succinate [TPGS]) enhances the absorption of cyclosporine in healthy volunteers or liver transplant recipients (Sokol et al., 1991; Chang et al., 1996). Another report has demonstrated that TPGS also increased the solubility of amprenavir, an HIV protease inhibitor, and inhibited the efflux transport systems and enhanced the permeability of amprenavir through Caco-2 cell monolayers (Yu et al., 1999). Overall, TPGS enhances the absorption flux of amprenavir by increasing its solubility and permeability. This improvement is very significant since the bioavailability of amprenavir in conventional capsule formulations is almost zero, and the softgel formulation containing vitamin E-TPGS is 69% bioavailable for dogs (Yu et al., 1999). Surfactants, such as Cremophor EL or Tween 80, have been found to be potent inhibitors of P-gp (Lo et al., 1998; van Zuylen et al., 2001). Both are used as formulation vehicles for a variety of poorly water-soluble drugs, including the anticancer agents paclitaxel and docetaxel. The use of these surfactants may increase the intestinal absorption of some drugs through P-gp inhibition and, thus, improve the drug bioavailability of P-gp substrates.

Inhibition studies using P-gp inhibitors in Caco-2 cell monolayers are simple to perform and are widely used to evaluate the contribution of P-gp to the absorption of a drug candidate. However, there are few studies describing any quantitative investigations or the theoretical aspects involved. Table 6 shows the effect of P-gp inhibitors on the apical-to-basal or basal-to-apical flux of P-gp substrates across Caco-2 cell monolayers. As a result, the changes in the flux of P-gp substrates can be classified into three types (Fig. 1). In the first type, the basal-to-apical flux scarcely changes and the apical-to-basal flux increases markedly in the presence of a P-gp inhibitor (Fig. 1A). In the second case, both fluxes are changed but the degree of change in the apical-to-basal flux is greater than that in the basal-to-apical flux in the presence of a P-gp inhibitor (Fig. 1B). In the third case, both fluxes are changed but the degree of change in the basal-to-apical flux is greater than that in the apical-to-basal flux in the presence of a P-gp inhibitor (Fig. 1C). An example of the first case is grepafloxacin, the second case is illustrated by saquinavir and indinavir, while examples of the third case include Rhodamine 123, cyclosporine, vinblastine, and digoxin (Table 6). Figure 2 shows a schematic diagram illustrating the transcellular transport of P-gp substrates in Caco-2 cell monolayers. PS_1 and PS_2 represent the permeability-surface area (PS) products for the influx and non-P-gp-mediated efflux across the apical membrane of Caco-2 cell monolayers, respectively; PS_3 and PS_4 represent the PS products for the efflux and influx across the basal membrane of Caco-2 cell monolayers, respectively; and $PS_{P\text{-}gp}$ represents the PS product for P-gp-mediated efflux across the apical membrane. $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$ represent the steady-state transport clearances in the apical-to-basal direction and the basal-to-apical direction, respectively. Supposing a steady-state flux (constant velocity of tran-

TABLE 5
Effect of BCRP inhibitor GF120918 on pharmacokinetics of topotecan in mdr1a/1b(−/−) mice (Jonker et al., 2000)

	Vehicle-Treated	GF120918-Treated	Ratio
Oral administration of topotecan (1 mg/kg)[a]			
AUC (mg · h/l)	96	596	×6.2
Intravenous administration of topotecan (1 mg/kg)[a]			
AUC (mg · h/l)	200	406	×2.0
Biliary excretion[b] (%)	14.7	5.5	×0.4
Intestinal content of [^{14}C]topotecan-derived radioactivity[c]			
Intestinal (%)	31.8	10.2	×0.3
Plasma (ng/ml)	40	102	×2.6

[a] Mdr1a/1b(−/−) mice were given an oral dose of GF120918 (50 mg/kg) or vehicle 15 min before an oral or intravenous dose of topotecan (1 mg/kg).
[b] Cumulative biliary excretion of unchanged topotecan for 11 min.
[c] GF120918 (50 mg/kg) was administered orally; 15 min later, [^{14}C]topotecan (1 mg/kg) was administered intravenously; 60 min after administration of [^{14}C]topotecan, the intestinal content of [^{14}C] was determined.

TABLE 6
The effects of P-gp inhibitors on transcellular transport of P-gp substrates in Caco-2 cell monolayers

Substrate (μM)	Inhibitor (μM)	−Inhibitor[a]		R_{caco}(−I)	+Inhibitor[a]		R_{caco} (+I)	Ratio(+I/cont)		Type[b]	Reference
		A-B	B-A	B-A/A-B	A-B	B-A	B-A/A-B	A-B	B-A		
Grepafloxacin (5)	Grepafloxacin 1000	0.6	2.1	3.4	1.7	1.6	0.9	2.77	0.76	A	Yamaguchi et al., 2000
Saquinavir (5)	PSC-833 1	2.3	22.8	9.8	8.8	12.9	1.5	3.78	0.57	B	Kim et al., 1998
Indinavir (5)	PSC-833 1	1.7	18.4	11	7.0	10.6	1.5	4.20	0.58	B	Kim et al., 1998
Rhodamine-123 (5)	Verapamil 100	0.1	3.7	34	0.3	0.3	1.3	2.46	0.09	C	Takano et al., 1998
Cyclosporine (1)	Verapamil 100	3.5	25.4	7.2	6.4	7.5	1.2	1.80	0.30	C	Alsenz et al., 1998
Vinblastine (0)	GF120918 10	3.8	12.9	3.4	6.2	5.9	1.0	1.61	0.46	C	Lentz et al., 2000
Digoxin (5)	CP114416 1	1.6	12.5	7.7	4.2	3.5	0.8	2.57	0.28	C	Wandel et al., 1999

[a] Papp ($\times 10^{-6}$ cm/s).
[b] Classification types for the effect of P-gp inhibitors on the transcellular transport of P-gp substrates shown in Fig. 1.

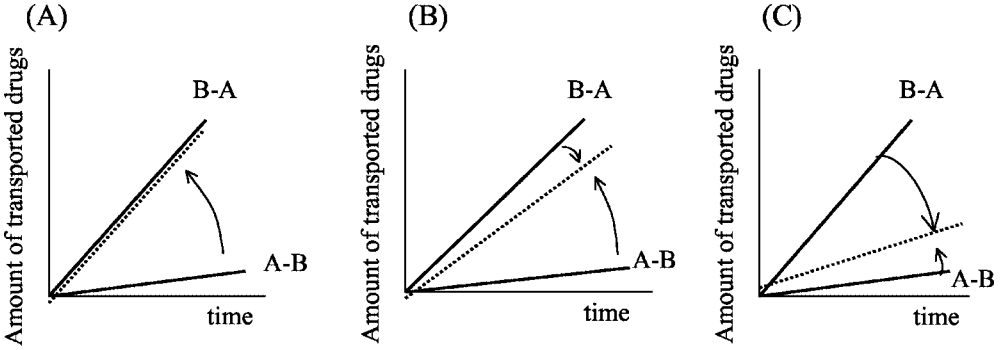
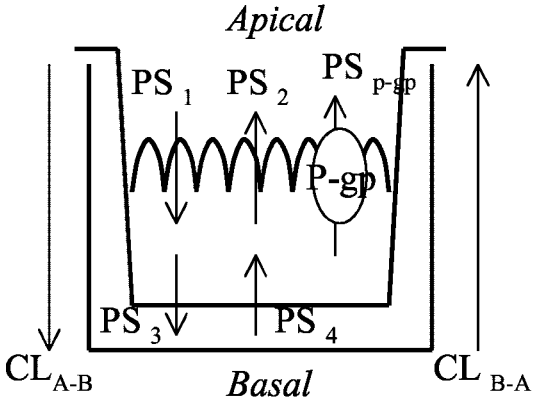


FIG. 1. Schematic diagram illustrating the effect of P-gp inhibitors on the apical-to-basal (A-B) or basal-to-apical (B-A) flux of P-gp substrates across Caco-2 cell monolayers. The changes in the flux of P-gp substrates can be classified into three types (A–C). Solid lines or dotted lines represent the flux across monolayers without or with P-gp inhibitors, respectively. Three classification types can be interpreted according to eqs. 1–5 shown in Fig. 2



$$CL_{A\text{-}B} = \frac{PS_1 \times PS_3}{(PS_2 + PS_{P\text{-}gp}) + PS_3} \quad \text{(Eq. 1)}$$

$$CL_{B\text{-}A} = \frac{PS_4 \times (PS_2 + PS_{P\text{-}gp})}{(PS_2 + PS_{P\text{-}gp}) + PS_3} \quad \text{(Eq. 2)}$$

$$R_{caco} = \frac{CL_{B\text{-}A}}{CL_{A\text{-}B}} = \frac{PS_4 \times (PS_2 + PS_{P\text{-}gp})}{PS_1 \times PS_3} \quad \text{(Eq. 3)}$$

$$\frac{CL_{A\text{-}B}(+I)}{CL_{A\text{-}B}(cont)} = \frac{(PS_2 + PS_{P\text{-}gp}) + PS_3}{PS_2 + PS_3} \quad \text{(Eq. 4)}$$

$$\frac{CL_{B\text{-}A}(+I)}{CL_{B\text{-}A}(cont)} = \frac{PS_2 \times ((PS_2 + PS_{P\text{-}gp}) + PS_3)}{(PS_2 + PS_3)(PS_2 + PS_{P\text{-}gp})} \quad \text{(Eq. 5)}$$

FIG. 2. Schematic diagram illustrating transcellular transport of P-gp substrates in Caco-2 cell monolayers. PS_1 and PS_2 represent the permeability-surface area (PS) products for the influx and non-P-gp-mediated efflux across the apical membrane of the cell monolayers, respectively. PS_3 and PS_4 represent the PS products for the efflux and influx across the basal membrane of the cell monolayers, respectively. $PS_{P\text{-}gp}$ represents the PS product for P-gp-mediated efflux across the apical membrane. $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$ represent the steady-state clearances across the monolayers in the apical-to-basal direction and the basal-to-apical direction, respectively. $CL_{A\text{-}B}(+I)$ and $CL_{B\text{-}A}(+I)$ represent the steady-state transport clearances when P-gp is completely inhibited by a P-gp inhibitor.

scellular transport) and "sink" conditions (constant concentration gradients), $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$ are given by eqs. 1 and 2 of Fig. 2, respectively. The flux ratio across the monolayer (R_{caco}), defined as the ratio of $CL_{A\text{-}B}$ to $CL_{B\text{-}A}$, is given by eq. 3 of Fig. 2. The degree of change in $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$, when P-gp in completely inhibited by P-gp inhibitor, is given by eqs. 4 and 5 of Fig. 2, respectively. $CL_{A\text{-}B}(+I)$ and $CL_{B\text{-}A}(+I)$ represent the clearances when

P-gp is completely inhibited by a P-gp inhibitor. The experimental data in Table 6 can be interpreted using eqs. 3 to 5 of Fig. 2, where it has been estimated that $PS_2 \gg PS_3$ in the first case (Fig. 1A), $PS_2 \fallingdotseq PS_3$ in the second case (Fig. 1B), and $PS_2 \ll PS_3$ in the third case (Fig. 1C). Furthermore, it has been estimated that the value of $PS_{P\text{-}gp}$ is 2-fold greater in the case of grepafloxacin, 6- to 8-fold greater in the case of saquinavir and indinavir, and 6- to 21-fold greater in case of Rhodamine 123, cyclosporine, vinblastine, and digoxin compared with the non-P-gp-mediated efflux clearance (PS_2). In some cases, it has been found that the basal-to-apical flux is still greater than the apical-to-basal flux with P-gp inhibitors. The reason for this phenomenon may be not only that the inhibitor concentration is insufficient, but also that the efflux transporters on the apical membrane, other than P-gp, also play a role in the efflux of these drugs.

D. Control of Elimination by Drug Transporters (Uptake and Efflux Transporters in the Liver and Kidney)

Multispecific transporters are expressed in the liver and kidney and play an important role in the elimination of many xenobiotics, acting as a detoxification system. Many drugs are excreted into the urine via organic anion and cation transport systems, expressed on brush-border and basolateral membranes of renal tubular cells (Table 1) (Burckhardt and Wolff, 2000; Inui et al., 2000a; Sekine et al., 2000; van Aubel et al., 2000; Dresser et al., 2001; Masereeuw and Russel, 2001; Russel et al., 2002). As far as the liver is concerned, a wide variety of transporter families are known to be present at the sinusoidal and canalicular membranes and play a significant role in hepatobiliary excretion (Table 1) (Oude Elferink et al., 1995; Yamazaki et al., 1996; Muller and Jansen, 1997; Keppler and Konig, 2000; Kullak-Ublick et al., 2000; Faber et al., 2003). Secondary active transporters expressed on the sinusoidal membrane are responsible for the uptake of drugs from the blood into hepatocytes (Meier et al., 1997). Primary active transporters expressed on the canalicular membrane are involved in the biliary excretion of both parent drugs and their metabolites (Kusuhara et al., 1998; Hooiveld et al., 2001). Since some transporters are specifically expressed on hepatocytes or renal tubular cells, they can be used as a target for drug delivery to the liver or kidney, possibly resulting in direct control of the elimination process. The transporters expressed in the liver and kidney are introduced here.

1. Organic Anion Transporting Polypeptide (SLC21A) Family. Organic anion transporting polypeptides (OATPs) have been isolated from rats, at first as candidates for the sodium-independent uptake system in the liver (Meier et al., 1997; Muller and Jansen, 1997). OATPs form a growing gene superfamily and mediate transport of a wide spectrum of amphipathic organic anions such as bromosulfophthalein, estradiol-17β-glucuronide (E_2-17βG), bile acids, thyroid hormones, and drugs such as pravastatin, temocaprilat, and BQ-123 (Meier et al., 1997; Muller and Jansen, 1997; Ishizuka et al., 1998; Kakyo et al., 1999; Reichel et al., 1999; Abu-Zahra et al., 2000). Although some important members of this transporter superfamily are selectively expressed in rodent and human livers, most OATPs are expressed in multiple tissues including the BBB, choroid plexus, lung, heart, intestine, kidney, placenta, and testes (Hagenbuch and Meier, 2003). A human OATP-C (also referred to as OATP2 and LST-1) is predominantly expressed in the liver (Abe et al., 1999; Hsiang et al., 1999; Konig et al., 2000a; Tamai et al., 2000a). Due to its broad substrate specificity, OATP-C is considered to play a major role in the hepatic uptake of organic anions. Recently, Cui et al. demonstrated that OATP-C transports bilirubin and its mono- and diglucuronide, suggesting that OATP-C is important from a physiological point of view (Cui et al., 2001b). In addition to OATP-C, OATP-B and OATP8 are also localized on the sinusoidal membrane of hepatocytes (Table 1) (Konig et al., 2000b; Kullak-Ublick et al., 2001). The tissue distribution of OATP-B is much broader than that of liver specific OATP-C. Although the expression of OATP-B is most abundant in human liver, it is also present in the pancreas, lung, gut, ovary, testes, and spleen. (Tamai et al., 2000a; Kullak-Ublick et al., 2001; St-Pierre et al., 2002). OATP-B transports sulfate conjugates of steroids, but not glucuronide conjugates and bile salts, whereas OATP-C transports both types of steroid conjugates (Kullak-Ublick et al., 2001; Tamai et al., 2001). OATP8 is exclusively expressed on the basolateral membrane of hepatocytes (Konig et al., 2000b). Although OATP-C and OATP8 exhibit broad overlapping substrate specificities, OATP8 is unique in transporting digoxin and exhibits an especially high transport activity for anionic peptides [D-penicillamine(2,5)] enkephalin (opioid-receptor agonist), BQ-123 (endothelin-receptor antagonist), and cholecystokinin-8 (gastrointestinal peptide hormone 8) (Ismair et al., 2001; Kullak-Ublick et al., 2001). The bile salts, substrates for OATP-C, are reportedly not transported by OATP8 (Konig et al., 2000b). Because OATP-C, OATP-B, and OATP8 are localized on the same membrane domain with overlapping substrate specificity, the contribution of OATP-C, OATP-B, and OATP8 to the total hepatic uptake of each ligand needs to be clarified.

2. Organic Anion Transporter (SLC22A) Family. OAT1 and OAT3 are mainly expressed in the kidney and localized on the basolateral membrane of the proximal tubules (Table 1) (Sekine et al., 1997; Hosoyamada et al., 1999; Kusuhara et al., 1999; Sekine et al., 2000; van Aubel et al., 2000; Dresser et al., 2001; Kojima et al., 2002). Their substrates include relatively small and hydrophilic organic anions, such as *p*-aminohippurate (PAH), methotrexate, β-lactam antibiotics, nonste-

roidal anti-inflammatory drugs (NSAIDs), and antiviral nucleoside analogs (Uwai et al., 1998; Apiwattanakul et al., 1999; Cihlar et al., 1999; Jariyawat et al., 1999; Wada et al., 2000). Recently, Oat3 knockout mice have been developed, and Oat3$^{-/-}$ mice exhibit impaired organic anion transport function in renal and choroid plexus epithelia but not in the liver (Sweet et al., 2002). This indicates that Oat3 plays an essential role in renal, but not hepatic, organic anion uptake.

Generally, amphipathic organic anions with a relatively high molecular weight, such as OATP substrates, are eliminated from the liver by metabolism and/or biliary excretion, while small and hydrophilic organic anions, such as OAT substrates, are excreted into the urine. The tissue distribution and elimination pathways of drugs can be explained by similarities and differences in the substrate recognition by these transporters expressed in the liver and kidney. Thus, modifying the drug so that it is recognized by OATP or OAT may lead to liver or kidney organotropism. Although, in general, OAT families are mainly expressed in the kidney, OAT2 is abundantly expressed in the liver and, to a lesser extent, in the kidney, and localized to the basolateral membrane of the liver (Simonson et al., 1994; Sekine et al., 1998). OAT2 transports relatively small and hydrophilic organic anions, such as indomethacin and salicylate, and may be involved in the hepatic uptake of these drugs (Morita et al., 2001). However, the OATP family is supposed to be responsible for the hepatic uptake of amphipathic organic anions.

The reported toxicity of some drugs is occasionally caused by concentrative tissue distribution due to active transport. The OAT1-mediated transport of ochratoxin A, a potent nephrotoxin, has been reported, suggesting that accumulation of the toxin via OAT1 in proximal tubules may be the primary event in the development of ochratoxin A-induced nephrotoxicity (Tsuda et al., 1999; Jung et al., 2001b). A similar effect has been proposed for adefovir, cephalosporin antibiotics, and β-lactam antibiotics, which accumulate extensively in the tubules (Cihlar et al., 1999; Jariyawat et al., 1999; Takeda et al., 1999, 2002a). Active transport processes may increase the intracellular concentration and appear to be directly related the development of tubular injury. Thus, designing a drug that is not transported by OAT1 or coadministering OAT1 inhibitors may be an effective way of reducing the nephrotoxicity of these compounds (Cihlar et al., 2001). A fluorescence assay to screen for novel human OAT1 inhibitors has been developed (Cihlar and Ho, 2000) and it has been suggested that NSAIDs may reduce adefovir nephrotoxicity since they efficiently inhibit the human OAT1-specific transport of adefovir at clinically relevant concentrations (Apiwattanakul et al., 1999; Mulato et al., 2000).

3. Organic Cation Transporter (SLC22A) Family. The OCT family of proteins is involved in the uptake of organic cations into the liver or kidney from blood. OCT1 and OCT2 are expressed in epithelial cells of the kidney, liver, and intestine, and appear to be localized to the basolateral membranes of the cells (Table 1) (Meyer-Wentrup et al., 1998; Urakami et al., 1998). These transporters mediate the uptake of a variety of organic cations, such as dopamine, choline, 1-methyl-4-phenylpyridinium (MPP$^+$), N^1-methylnicotinamide, TEA, and cimetidine (Martel et al., 1996; Zhang et al., 1997; Breidert et al., 1998; Koepsell, 1998; Urakami et al., 1998; Zhang et al., 1998). Rat Oct1 is expressed in both the liver and kidney, although its human counterpart is expressed predominantly in the liver, while human and rat Oct2 are present mainly in kidney and brain (Grundemann et al., 1994; Gorboulev et al., 1997). Rat Oct3 mRNA has been found to be most abundant in the placenta, with a moderate presence in the intestine, heart, and brain (Kekuda et al., 1998).

Recently, the pharmacological and physiologic role of Oct1 has been investigated using Oct1 knockout (Oct1$^{-/-}$) mice (Jonker et al., 2001). The distribution and excretion of the model substrate TEA after intravenous administration has been compared in wild-type and Oct1$^{-/-}$ mice. In Oct1$^{-/-}$ mice, accumulation of TEA in liver was 4- to 6-fold lower than in wild-type mice, indicating that for TEA, Oct1 is the main uptake system in the liver (Table 7). In addition, direct intestinal excretion of TEA was reduced about 2-fold, showing that Oct1 also mediates basolateral uptake of TEA into enterocytes (Table 7). Excretion of TEA into urine over 1 h accounted for 53% of the dose in wild-type mice compared with 80% in knockout mice, probably because in Oct1$^{-/-}$ mice less TEA accumulates in the liver and thus more is available for rapid excretion by the kidney (Table 7).

Similarly, the distribution of metformin, a biguanide, to the liver and intestine in Oct1$^{-/-}$ mice was significantly lower than that in wild-type mice, whereas distribution to the kidney and the urinary excretion profile showed only minimal differences (Wang et al., 2002). Oct1 is responsible for hepatic uptake as well as playing a role in the intestinal uptake (via basolateral membrane) of metformin, while the renal distribution and excretion are mainly governed by other transport mechanisms. Biguanides are oral antihyperglycemic agents used for the treatment of type 2 diabetes mellitus, but they are associated with lactic acidosis, a potentially fatal side effect. Following the administration of metformin, the blood lactate concentration significantly increased in wild-type mice, whereas only a slight increase was observed in Oct1$^{-/-}$ mice (Wang et al., 2003). The hepatic concentration of metformin in Oct1$^{-/-}$ mice was markedly reduced, whereas its plasma concentration time profile was similar in wild-type and Oct1$^{-/-}$ mice. These results indicate that the Oct1-mediated hepatic uptake of biguanides plays an important role in lactic acidosis.

TABLE 7
Levels of radioactivity in female wild-type and Oct1$^{-/-}$ mice with a cannulated gallbladder at 60 min after i.v. injection of [^{14}C]TEA (0.2 mg/kg) (Jonker et al., 2001)

	^{14}C concn (ng−eq g^{-1} or ml^{-1}) ± SD (n = 4)		Excretion (%)[c]		Ratio (Oct1$^{-/-}$/wt)
	wt[a]	Oct1$^{-/-}$	wt	Oct1$^{-/-}$	
Plasma	23.8 ± 4.9	17.1 ± 3.0*			0.72
Brain	1.6 ± 0.3	1.9 ± 0.4			1.22
Spleen	35 ± 5	42 ± 2*			1.21
Kidney	441 ± 152	236 ± 20*			0.53
Liver	1225 ± 26 (25.3±0.8[b])	283 ± 44**(5.8±1.0**)			0.23
Bile			0.35 ± 0.09	0.14 ± 0.01*	0.41
Small intestine			1.31 ± 0.19	0.67 ± 0.09**	0.51
Cecum			0.12 ± 0.02	0.09 ± 0.04*	0.72
Colon			0.03 ± 0.01	0.04 ± 0.01	1.23
Urine			53.3 ± 16.8	80.0 ± 15.6*	1.50

* $P < 0.05$; ** $P < 0.01$.
[a] wt, wild type.
[b] Mean percentage of administered dose ± SD (n = 4).
[c] Total TEA found in the contents of small intestine, cecum, and colon. Urine was collected from the bladder.

4. Multidrug Resistance-Associated Protein 2 (ABCC2). MRP2, located on the bile canalicular membrane, is involved in the biliary excretion of clinically important anionic drugs as well as the intracellularly formed glucuronide- and glutathione-conjugates of many drugs (Paulusma et al., 1996; Ito et al., 1997; Keppler et al., 1997; Ito et al., 1998b; Konig et al., 1999a). In the liver, xenobiotics are metabolized by the so-called phase I and II enzymes, which are mainly cytochrome P450 and conjugating enzymes, respectively. After these enzymatic reactions, the conjugated metabolites produced are pumped out from hepatocytes into the bile. This ATP-dependent efflux transporter plays a physiologically important role as the "phase III" xenobiotic detoxification system (Ishikawa, 1992). In addition, MRP2 mediates the biliary excretion of not only conjugated metabolites, but also unchanged organic anions, such as grepafloxacin (a new fluoroquinolone antibiotics) or cefodizime and ceftriaxone (β-lactam antibiotics) (Sathirakul et al., 1993; Kusuhara et al., 1998; Sasabe et al., 1998). These antibiotics have been shown to be effective in the treatment of inflammatory conditions affecting the biliary tract because they are efficiently excreted into the bile (Suzuki and Sugiyama, 1999). The biliary excretion of these antibiotics gives these drugs a pharmacological advantage due to the target organ.

However, in some cases there is a major accumulation of drugs in the bile duct via MRP2 expressed on the bile canalicular membrane, which results in toxic effects on bile epithelial cells or gastrointestinal cells. It is supposed that the reactive glucuronide of the NSAID diclofenac is selectively transported into bile via MRP2, where it exhibits toxic effects on the bile canalicular membrane (Seitz et al., 1998). Similarly, methotrexate is concentrated in bile compared with plasma and undergoes enterohepatic circulation, resulting in adverse effects in the intestine. It has been reported that the biliary excretion of methotrexate is mediated by MRP2 (Masuda et al., 1997). A structural modification of such drugs to reduce their biliary excretion would be useful.

Although CPT-11 is an effective anticancer drug, its clinical use is frequently limited by a form of gastrointestinal toxicity, severe diarrhea (Rowinsky et al., 1994). Such severe diarrhea exhibits a large degree of interpatient variability. The action of its active metabolite, SN-38, on gastrointestinal cells is believed to be responsible for this toxicity (Araki et al., 1993). The biliary excretion of SN-38 and SN-38 glucuronide and subsequent uptake by gastrointestinal epithelial cells may be associated with this diarrhea (Kaneda and Yokokura, 1990). It has been shown that MRP2 is involved in the biliary excretion of SN-38 and SN-38 glucuronide (Chu et al., 1997a,b), and there is a large degree of interindividual variability in the transport activity of SN-38 via MRP2, as shown by a study using human bile canalicular membrane vesicles (CMVs) (Chu et al., 1998). Thus, the biliary excretion of its metabolites mediated by MRP2 has been proposed to be linked to its unpredictable gastrointestinal toxicity. An attempt to prevent this toxicity using potent MRP2 inhibitors has been investigated. Probenecid is a potential candidate, which can be used clinically to inhibit the biliary excretion of CPT-11 metabolites (Horikawa et al., 2002b). In actual fact, it has been shown that coadministration of probenecid markedly reduces both SN-38 exposure and CPT-11-induced late-onset toxicity in the gastrointestinal tissues of rats, possibly by inhibiting the biliary excretion of CPT-11 and/or its metabolites (Horikawa et al., 2002a). It is expected that this agent will be used clinically to prevent toxicity. Approaches using intentional drug-drug interactions (positive drug interactions) like this case may become more important in the future.

Control of the elimination route, such as biliary or urinary excretion, is also one of the strategies used to avoid potentially toxic effects. In some cases, transporters expressed in the liver or kidney may determine the elimination route affecting the systemic plasma concentrations of drugs. Many ACE inhibitors are actually administered as prodrugs and are metabolized to their active forms, such as enalaprilat, captoprilat, cilazaprilat, ramiprilat, and spirapprilat. They are excreted predominantly into the urine. In contrast, temocaprilat is excreted via both bile and urine (Oguchi et al., 1993).

The presence of an excretion route other than the urinary one confers a pharmacokinetic advantage, particularly in the treatment of patients with renal failure. In such patients, the AUC and C_{max} values of captopril and enalapril are markedly increased because these ACE inhibitors are eliminated primarily via renal excretion (Oguchi et al., 1993). In contrast, alterations in these pharmacokinetic parameters are minimal for temocaprilat because of the presence of the biliary excretion pathway (Oguchi et al., 1993). A multiple elimination pathway will result in a relatively stable pharmacokinetic profile compared with only a single elimination pathway. Although the biliary excretion of temocaprilat is governed by MRP2 at the canalicular membrane, it has been suggested that other ACE inhibitors are not good substrates of MRP2 (Ishizuka et al., 1997, 1999). The affinity for MRP2 is expected to be the predominant factor in determining the biliary excretion of any series of ACE inhibitors. Drugs that are excreted into both the bile and urine to the same degree may be expected to exhibit minimal interindividual differences in their pharmacokinetics.

Mrp2-deficient rats, such as transport-deficient rats and Eisai hyperbilirubinemic rats, exhibit hyperbilirubinemia such as the Dubin-Johnson syndrome due to a defect in the biliary excretion of bilirubin glucuronides. Mrp3 is induced on the hepatic basolateral membrane of Mrp2-deficient animals (Hirohashi et al., 1998; Donner and Keppler, 2001; Soroka et al., 2001) and is able to excrete glucuronide conjugates of xenobiotics (Hirohashi et al., 1999, 2000b). Thus, these results are consistent with the hypothesis that Mrp3 may be involved in the sinusoidal efflux of glucuronide conjugates in these mutants. It is plausible that in the cholestatic liver, bilirubin glucuronides are effluxed from the liver into the blood via sinusoidal Mrp3, resulting in jaundice. Moreover, immunohistochemical studies have indicated that MRP3 is induced in the sinusoidal membrane of patients suffering from Dubin-Johnson syndrome (Konig et al., 1999b).

5. Bile Salt Export Pump (ABCB11). Intrahepatic cholestasis can be induced by interference with the secretion of biliary constituents resulting in an intracellular accumulation of bile salts and other toxic bile constituents within hepatocytes. BSEP, located on the canalicular membrane, mediates the transport of bile acids such as taurocholic acid (Gerloff et al., 1998; Kullak-Ublick et al., 2000). Cholestasis induced by some drugs is mediated, at least in part, by inhibition of BSEP, resulting in intracellular accumulation of cytotoxic bile salts. The immunosuppressant, cyclosporine, has been shown to produce *cis*-inhibition of BSEP-mediated bile salt transport (Stieger et al., 2000). A similar mechanism has been postulated for rifampicin and glibenclamide (Stieger et al., 2000). In contrast, the cholestatic estrogen metabolite, E_2-17βG, causes *trans*-inhibition of BSEP-mediated bile salt transport and, therefore, exerts its cholestatic action only after its excretion by MRP2 into the canalicular lumen (Stieger et al., 2000). In addition, some other MRP2 substrates cause *trans*-inhibition of the BSEP-mediated transport of bile acids (Akita et al., 2001). Horikawa et al. have reported the inhibition potential of a series of therapeutic drugs, producing clinical cholestasis, on BSEP and MRP2 (Horikawa et al., 2003). Although most of the drugs have only a minimal inhibitory effect on Bsep- and Mrp2-mediated transport in rat CMVs, cloxacillin inhibited BSEP-mediated transport in both rat and human CMVs. Since the inhibitory effect on BSEP-mediated transport by cloxacillin was more marked in human CMVs than in rat CMVs, species differences in inhibitory potential need to be considered (Horikawa et al., 2003). Troglitazone is a thiazolidinedione insulin-sensitizing agent for the treatment of noninsulin-dependent diabetes mellitus, but it was withdrawn from the market because of liver toxicity (Funk et al., 2001a). Although the mechanism underlying this troglitazone-associated hepatotoxicity is at present unclear, it has been suggested that a cholestatic mechanism is involved (Funk et al., 2001a). Troglitazone and, to a much greater extent troglitazone sulfate, the main troglitazone metabolite eliminated into bile, competitively inhibit ATP-dependent taurocholate transport via BSEP (Funk et al., 2001a,b). This inhibition of the hepatobiliary export of bile salts by troglitazone and troglitazone sulfate may lead to a drug-induced intrahepatic cholestasis in humans, possibly contributing to the hepatotoxicity of troglitazone. One should consider the possibility that drugs which inhibit BSEP may cause cholestasis. The evaluation of BSEP inhibition will play an important role in the identification of compounds that could be a potential cause of cholestasis.

Obtaining more data on the substrate specificities and expression level of each human transporter will be of great help in improving drug design by targeting specific transporters and controlling their elimination. The route of elimination may be controlled by using transporters that are expressed selectively in either the liver or kidney.

III. Clinical Implications of Transporter-Mediated Drug Interactions

A. Drug-Drug Interactions Involving Elimination

Drug-drug interactions involving membrane transport can be classified into two categories: one caused by competition for the substrate binding sites of the transporters, and the other caused by a change in the expression level of the transporters. Due to the broad substrate specificity of P-gp, drug-drug interactions involving P-gp are very likely (Lin, 2003). Table 8 gives an overview of the known drug interactions that involve, at least in part, P-gp. This gives an indication of the interactions that one may expect during combination therapy. P-gp

TABLE 8
Example of the possible involvement of P-gp with clinical drug-drug interactions

Inhibitor or Inducer				Inhibited or Induced Drug							Possible Mechanisms of Drug-Drug Interactions	Reference
	IC_{50} or K_i in vitro (μM)[a]		Dose (mg)		AUC	C_{max}	BA	CL	$T_{1/2}$	Others		
	Mean	Range[b]										
Inhibition					*−fold*							
Valspodar	0.182	0.022–0.41	400	Digoxin				CL_r −62%			A	Kovarik et al., 1999
				Paclitaxel	2.5							Sikic et al., 2000
			400	Dexamethasone	1.24							Kovarik et al., 1998
Erythromycin	105	10–200	500	Atrovastatin	1.32	1.38			NS		B	Siedlik et al., 1999
			1500	Digoxin		2.04					A	Maxwell et al., 1989
			2000	Talinolol	1.34				NS		A	Schwarz et al., 2000
				Fexofenadine	2.09	1.82					A	Davit et al., 1999
			1000	Saquinavir	1.99	2.06					B	Grub et al., 2001
			1000	Cyclosporine	1.97	2.13		CL −38%	NS	Met% in plasma −49%	B	Freeman et al., 1987
			2000	Cyclosporine	2.15	2.8	36→60%		NS		B	Gupta et al., 1988
			2000	Cyclosporine	2.15		36→60%	CL −13%			B	Gupta et al., 1989
Verapamil	83.1	3–236	240	Digoxin		1.44		CL_{bile} −43%, CL_r, NS			A	Hedman et al., 1991
			160	Digoxin		1.4					A	Mackstaller and Alpert, 1997
			240	Digoxin		1.6–1.8					A	Cobbe, 1997
Itraconazole			200	Digoxin	1.5			CL_r −20%			A	Jalava et al., 1997
			200	Quinidine	2.4	1.6		CL_r −50%	×1.6	M/P(AUC) −50%	B	Kaukonen et al., 1997
Cyclosporine	7.24	3.4–17	1050	Docetaxel	11.2		8→90%				B	Malingre et al., 2001b
			1050	Paclitaxel	8			×8			B	Meerum Terwogt et al., 1999
			180–540	Paclitaxel	1	1						Britten et al., 2000
			280	Sirolimus	1.45	1.71					B	Kaplan et al., 1998
			16 mg/kg infusion	Doxorubicin	1.8			CL −37%	NS	M/P(AUC) −75%	B	Rushing et al., 1994
			5–21 mg/kg	Etoposide	1.59			CL_{tot} −35%	×1.73			Lum et al., 1992
GF120918			1000	Paclitaxel	7		×7				B	Malingre et al., 2001a
			800	Doxorubicin	1			CL_r NS				Sparreboom et al., 1999
Quinidine	62.8	1.5–157		Digoxin		2		CL_{tot} −50%			A	Yu, 1999
			800	Digoxin		1.54		CL_{bile} −42%, CL_r −29%			A	Hedman et al., 1990
Quinine	280		750	Digoxin		1.1		CL_{bile} −35%			A	Hedman et al., 1990
Ketoconazole	18.8	1.5–36		Fexofenadine	2.64	2.35					A	Davit et al., 1999
			400	Saquinavir	2.9	2.71					B	Grub et al., 2001
			200	Saquinavir	1.69	1.36					B	Grub et al., 2001
			200	Tacrolimus	2		14→30%	CL, F_h, NS			B	Floren et al., 1997

TABLE 8
Continued

Inhibitor or Inducer			Inhibited or Induced Drug						Possible Mechanisms of Drug-Drug Interactions	Reference
	IC_{50} or K_i in vitro (μM)[a]			AUC	C_{max}	BA	CL	$T_{1/2}$	Others	
	Mean	Range[b]	Dose (mg)							
Propafenone			600	Digoxin	*−fold*		CL_{tot} −31%, CL_r −32%			A	Calvo et al., 1989
Clarithromycin				Digoxin			CL_r −48%			A	Wakasugi et al., 1998
Vitamin E				Cyclosporine	1.61	1.37			M/P(AUC) NS	C	Chang et al., 1996
Talinolol			100	Digoxin	1.23	1.45	CL_r NS	NS		A	Westphal et al., 2000a
Ritonavir			300	Saquinavir	58	33				B	Merry et al., 1997
Nelfinavir			2250	Azithromycin	2	2					Amsden et al., 2000
Diltiazem			30	Tacrolimus		4.3				B	Hebert and Lam, 1999
Atrovastatin			80	Digoxin	1.15	1.2				A	Boyd et al., 2000
			10	Digoxin		1					Boyd et al., 2000
Induction											
Rifampin			600	Fexofenadine	0.43	0.59		CL_r NS	NS	A	Hamman et al., 2001
			600	Talinolol	0.65			P-gp ×4.2		A	Westphal et al., 2000b
			600	Tacrolimus			14→7%	CL_{tot} +47%			Hebert et al., 1999
			600	Digoxin	0.42			CL_r NS	NS	P-gp ×3.5	A	Greiner et al., 1999
			600	Glyburide	0.78				−15%		C	Niemi et al., 2001
			600	Glipizide					−36%		C	Niemi et al., 2001
			600	Saquinavir	0.35							Grub et al., 2001
			600	Saquinavir	0.57							Grub et al., 2001
St. John's wort				Digoxin	0.82					P-gp ×1.4	A	Durr et al., 2000
				Digoxin	0.75	0.74					A	Johne et al., 1999
				Cyclosporine	Trough conc. −64%							Barone et al., 2000

BA, bioavailability; CL_r, renal clearance; CL_{tot}, total clearance; CL_{bile}, biliary clearance; NS, not significant; M/P(AUC), the ratio of AUC of metabolites to that of parent drugs; A, involvement of P-gp is suggested to be obvious; B, the cases cannot be distinguished between involvement of P-gp and CYP3A4; C, the cases cannot discriminate among other possibilities.

[a] IC_{50} or K_i values in vitro are cited from Wu et al., 2000; Kim et al., 1999; Kawahara et al., 2000; Zhang and Benet, 1998; Yumoto et al., 1999; Gao et al., 2001; Tiberghien and Loor, 1996.

[b] Represents minimal and maximal values reported by several authors.

inhibitors, such as quinidine, valspodar, and verapamil, are known to increase plasma concentrations of digoxin, a cardiac glycoside, because they block its biliary and/or urinary excretion via P-gp (Table 8) (Hedman et al., 1990, 1991; Kovarik et al., 1999). Since the therapeutic range of digoxin is small, changes in its plasma concentration are potentially very serious.

Since OAT1 is responsible for the urinary excretion of a wide variety of anionic agents, it may be involved in renal drug-drug interactions. Furosemide undergoes tubular secretion before reaching its target site, the loop of Henle. Coadministration of probenecid significantly inhibits the tubular secretion of furosemide and, therefore, its diuretic effect is markedly reduced (Inui et al., 2000a; Uwai et al., 2000). It is well known that probenecid inhibits the renal secretion of many other anionic drugs via organic anion transport systems. The renal excretion of ciprofloxacin, benzylpenicillin, and acyclovir is reduced by coadministration of probenecid (Overbosch et al., 1988; Tsuji et al., 1990; Jaehde et al., 1995). OAT1 is a candidate for the transporter responsible for these interactions on the renal basolateral membrane because probenecid is able to inhibit OAT1 (Hosoyamada et al., 1999). In addition, fatal interactions have been reported between methotrexate and NSAIDs (Tracy et al., 1992; Kremer and Hamilton, 1995). NSAIDs significantly reduced methotrexate renal clearance This interaction seems to be linked to severe adverse effects after chemotherapy (Thyss et al., 1986). Methotrexate is specifically taken up by OAT1- or OAT3-expressing cells, and NSAIDs inhibit the methotrexate uptake mediated by these transporters (Takeda et al., 2002b). These results suggest that the basolateral membrane OAT1 and/or OAT3 is involved in the methotrexate-NSAID interaction in the kidney.

In addition, hepatic drug-drug interaction via OATP-C has been reported. In kidney transplant recipients treated with cyclosporine, the AUC of cerivastatin was 3.8-fold higher than that in healthy volunteers who were not given cyclosporine (Fig. 3) (Muck et al., 1999). The mild-to-moderate reduction in renal function in kidney transplant recipients compared with healthy controls is unlikely to be responsible for the observed pharmacokinetic effects, because the renal clearance of cerivastatin is negligible (Muck et al., 1997). Shitara et al. have examined the effect of cyclosporine on the uptake of cerivastatin into human hepatocytes to investigate the mechanism of their drug-drug interaction (Shitara et al., 2003). As a result, cyclosporine was found to inhibit transporter-mediated cerivastatin uptake in human hepatocytes with K_i values of 0.28 to 0.69 μM. In addition, the uptake of cerivastatin was examined in human OATP-C-expressing MDCK II cells and cerivastatin was shown to be a substrate of human OATP-C, like pravastatin (Hsiang et al., 1999; Nakai et al., 2001). OATP-C-mediated uptake of cerivastatin was also inhibited by cyclosporine with a K_i value of 0.2 μM in transfected cells. These results suggest that the drug-drug interaction between cerivastatin and cyclosporine can be explained by inhibition of the transporter-mediated hepatic uptake of cerivastatin and, at least in part, its OATP-C-mediated uptake. Recently, a severe drug-drug interaction between cerivastatin and gemfibrozil was reported and, in the United States, 31 deaths from severe rhabdomyolysis were reported in patients taking cerivastatin, 12 of whom were taking concomitant gemfibrozil (Charatan, 2001). This resulted in the withdrawal of cerivastatin from the market (Alexandridis et al., 2000; SoRelle, 2001). It is possible that the transporter in the sinusoidal membrane of the liver is involved in this drug-drug interaction, and the mechanism governing this needs to be investigated.

B. Drug-Drug Interactions Involving Absorption

There are some instances where intestinal P-gp may be involved in human drug-drug interactions associated with absorption (Table 8). For example, coadministration of erythromycin, a macrolide antibiotic, enhances the AUC of the orally administered β-blocker, talinolol, by 34% (Schwarz et al., 2000). A P-gp substrate, talinolol is eliminated mainly via the urine without any significant systemic metabolism. The renal clearance of talinolol is unaffected by coadministration of erythromycin, hence its intestinal absorption appears to be altered by coadministration of erythromycin (Schwarz et al., 2000). Therefore, it appears that the increase in oral bioavailability of talinolol after concomitant administration of erythromycin is caused by an increased net intestinal absorption due to P-gp inhibition by the latter. The metabolism by CYP3A in the human small intestine is a major factor limiting oral bioavailability, accompanied by P-gp-mediated efflux (Wacher et al., 2001; Zhang and Benet, 2001). Talinolol or digoxin is a good substrate of P-gp, but not of CYP3A. Thus, the role of P-gp in intestinal secretion is directly confirmed by these examples. Since the substrate specificities of CYP3A and P-gp overlap (Wacher et al., 1995), many drugs may be substrates of both. In such cases it is difficult to distinguish between the contribution of CYP3A and that of P-gp to the increased oral bioavailability.

Recently, an interesting report has appeared describing an interaction between fexofenadine and grapefruit, orange, and apple juice (Dresser et al., 2002). Unmetabolized fexofenadine is a substrate of P-gp, and it is known that grapefruit juice may inhibit P-gp activity (Spahn-Langguth and Langguth, 2001). Thus, it can be predicted that the plasma concentration of orally administered fexofenadine will be increased by coadministration of grapefruit juice if the latter inhibits intestinal P-gp. Nevertheless, grapefruit juice produced markedly lower plasma fexofenadine concentrations in healthy volunteers (Table 9) (Dresser et al., 2002). The fexofenadine AUC and C_{max} values following the administration of grapefruit juice were reduced to approximately 30% of

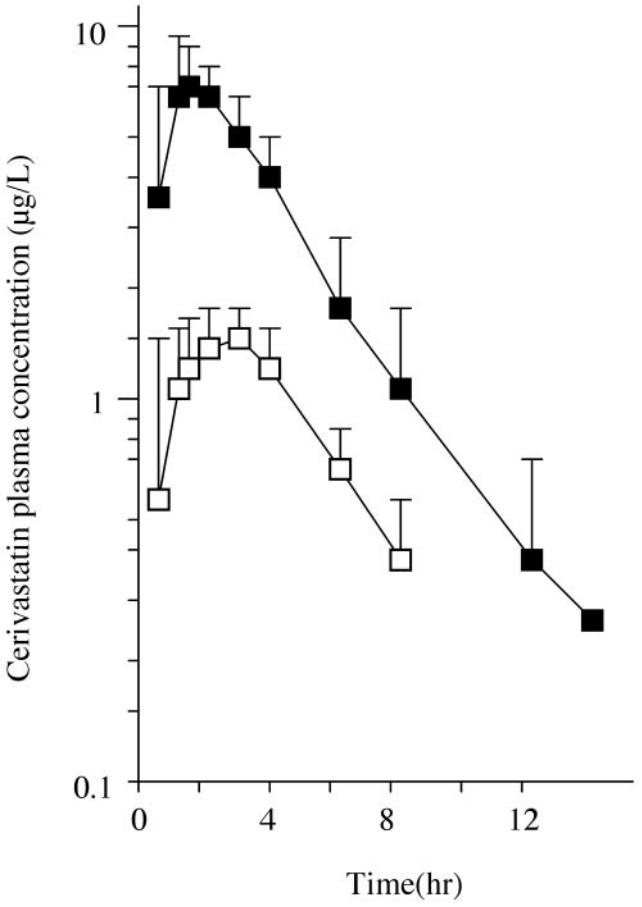


FIG. 3. Change in the disposition of cerivastatin caused by cyclosporine treatment. Cerivastatin plasma concentration-time profiles after oral administration of 0.2 mg cerivastatin in kidney transplant recipients receiving stable individual cyclosporine treatment (■) compared with healthy subjects without cyclosporine intake (□) are shown (Muck et al., 1999).

those following administration of water, while the fexofenadine elimination half-life and renal clearance remained unaffected. Similar phenomena have been reported following the coadministration of orange or apple juice. Fexofenadine is actually the substrate of not only P-gp, but also the drug uptake transporter, OATP (Cvetkovic et al., 1999). In addition, grapefruit juice produces a modest inhibition of P-gp-mediated digoxin efflux transport in cell lines expressing P-gp. In contrast, grapefruit, orange, and apple juices caused marked inhibition of OATP-mediated fexofenadine uptake in cell lines expressing OATPs. In view of the above results, it appears that OATP-mediated fexofenadine uptake is actually inhibited. Since inhibition of P-gp and OATPs in the liver would reduce the biliary secretion and increase plasma fexofenadine concentrations, it appears that the fexofenadine-juice interaction is primarily the result of reduced fexofenadine absorption from the gastrointestinal tract. Fruit juices are more potent inhibitors of OATPs than P-gp, which can reduce oral drug bioavailability.

Induction of transporter represents a new type of drug-drug interaction. Greiner et al. have reported a rifampin-digoxin interaction involving the induction of intestinal P-gp in humans (Greiner et al., 1999). The AUC value of oral digoxin is significantly lower during rifampicin treatment (600 mg/day for 10 days) but the effect is less pronounced after intravenous administration of digoxin (Greiner et al., 1999) (Table 10). The renal clearance and half-life of digoxin are unaltered by rifampin. However, rifampin treatment increases the intestinal P-gp content 3.5-fold, which correlates with the AUC value after oral but not intravenous administration of digoxin (Fig. 4). P-gp is a determinant of the disposition of digoxin and the rifampin-digoxin interaction appears to occur largely at the level of the intestinal P-gp expression. Since rifampin also induces intestinal MRP2, coadministration of rifampin is expected to increase the secretion into the lumen of MRP2 substrates, such as glutathione or glucuronide conjugates (Fromm et al., 2000). St. John's wort is one of the most commonly used over-the-counter herbal medicines in the United States and is widely used in the treatment of mild depression. However, the U.S. Food and Drug Administration alerted health professionals to the risk of drug interactions with St. John's wort (Henney, 2000). Coadministration of St. John's wort and the HIV protease inhibitor indinavir reduced the latter's exposure by 57% in healthy volunteers (Piscitelli et al., 2000). The induction of intestinal P-gp by St. John's wort is assumed to play a role in these phenomena. In addition, the administration of St. John's wort to healthy volunteers over 14 days resulted in an 18% reduction in digoxin exposure after a single dose of digoxin, and a 1.4- and 1.5-fold increase in the expression of duodenal P-gp and CYP3A4, respectively (Table 8) (Durr et al., 2000). Although St. John's wort induces both P-gp and CYP3A4, like rifampin, the reduction in the oral bioavailability of digoxin may be caused by the induction of

TABLE 9
Reduction of fexofenadine bioavailability by fruit juices in humans (Dresser et al., 2002)

	Control	Grapefruit Juice	Orange Juice	Apple Juice
$AUC_{(0-8h)}$ (ng · h/ml)	1330 ± 109	439 ± 44*	373 ± 19*	301 ± 33*
C_{max} (ng/ml)	288 ± 23	110 ± 14*	96 ± 7*	81 ± 13*
$T_{1/2}$ (h)	2.6 ± 0.2	3.1 ± 0.2	3.4 ± 0.3	3.5 ± 0.4
CL_r (ml/min)	78 ± 8	74 ± 7	86 ± 8	92 ± 9

Fexofenadine 120 mg with 1200 ml of water, grapefruit juice, orange juice, or apple juice was administered to 10 healthy volunteers. Data are expressed as mean ± S.E.M. Comparisons are between juice and water treatments.
* $P < 0.001$.

TABLE 10
The effect of rifampin on pharmacokinetics of digoxin in humans (Greiner et al., 1999)

	Digoxin 1 mg p.o.		Digoxin 1 mg i.v.	
	Control	With Rifampin[a]	Control	With Rifampin[a]
$AUC_{(0-144h)}$ (ng/h/ml)	54.8 ± 11.6	38.2 ± 12.4*	87.3 ± 8.3	74.5 ± 10.5*
Bioavailability (%)	63 ± 11	44 ± 14*		
T_{max} (min)	42 ± 12	52 ± 18*		
C_{max} (ng/ml)	5.4 ± 1.9	2.6 ± 0.7**	24.7 ± 5.2	20.9 ± 1.8
Renal clearance (ml/min)	159 ± 30	159 ± 38	151 ± 25	147 ± 18
Nonrenal clearance (ml/min)			17 ± 17	54 ± 29**
$T_{1/2}$ (h)	56 ± 13	54 ± 13	58 ± 12	53 ± 11

[a] 600 mg/day of rifampin were administered to healthy volunteers for 10 days.
* $P < 0.05$; ** $P < 0.01$.

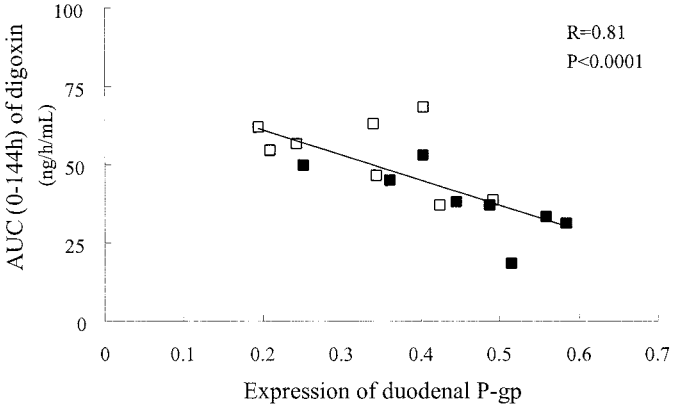


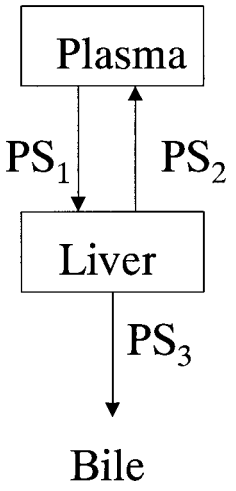
FIG. 4. Correlation between AUC of orally administrated digoxin (1 mg) and expression of duodenal P-gp in humans ($n = 16$). □, without rifampin; ■, with rifampin (600 mg) (Greiner et al., 1999).

intestinal P-gp due to a lack of significant metabolism by CYP3A4.

C. Prediction of in Vivo Drug-Drug Interactions from in Vitro Data

Although remarkable progress has been made in the identification and functional characterization of drug transporters, quantitative evaluation of drug-drug interactions involving the membrane transport process is difficult compared with that of drug-drug interactions involving metabolism. Overall, interactions involving membrane transporters in organs of elimination (e.g., liver and kidney) and absorption (e.g., intestine) alter the blood concentration-time profiles of drugs. In contrast, interactions occurring at the blood-brain barrier and in tumors will not alter the drug exposure in the circulating blood, only the pharmacological and/or toxicological effect of the drugs involved. Although there is not much difference between mdr1a knockout mice and wild-type mice as far as the plasma concentrations of many drugs are concerned, the brain concentrations are markedly increased in mdr1a knockout mice, as shown in Table 4. Evaluating the change in plasma drug concentrations alone is not enough to study drug-drug interactions involving the membrane transport process, and changes in the tissue distribution of drugs should also be taken into consideration.

Ito et al. (1998a) have previously proposed a method for predicting in vivo drug-drug interactions involving hepatic metabolism from in vitro experiments. A similar method can be used to predict in vivo drug-drug interactions involving biliary excretion (Kusuhara and Sugiyama, 2001a). Assuming that the contribution of passive diffusion to the membrane transport process is negligible, the reduction in intrinsic membrane transport clearance (PS_{int}) produced by an inhibitor can be predicted from eq. 1 of Fig. 5 (Ito et al., 1998a): where I_u and K_i represent the unbound concentration of an inhibitor around a transporter and its inhibition constant, respectively. When the substrate concentration is much lower than the K_m value (this assumption holds true for many drugs at their clinical dosages), the degree of inhibition (R) is defined by eq. 1 of Fig. 5, independent of the type of inhibition (Ito et al., 1998a). Compared with drug interactions involving hepatic metabolism, the prediction of biliary excretion is a more complicated procedure because at least three membrane transport processes have to be considered to successfully predict the excretion. The intrinsic clearance for the net biliary excretion from the blood ($CL_{int,bile}$) is a hybrid of the intrinsic clearances for each membrane penetration: hepatic uptake across the sinusoidal membrane (PS_1), efflux across the sinusoidal membrane from the liver (PS_2), and excretion across the canalicular membrane (PS_3), as shown in eq. 4 of Fig. 5. Thus, one can predict a drug interaction involving each type of membrane transport separately based on the Michaelis-Menten equation (eq. 1 of Fig. 5). To demonstrate the validity of the prediction method based on these equations, Ueda et al. attempted to predict the interaction between methotrexate and probenecid involving biliary excretion in rats using in vitro systems (Ueda et al., 2001). This interaction has already been reported in a clinical situation. Coadministration of probenecid reduced the biliary clearance of methotrexate in rats. This inhibition by probenecid was confirmed in vivo for both the uptake and excretion processes of methotrexate across the sinusoidal and canalicular membranes, respectively. Both the hepatic uptake clearance (PS_1), assessed by integration plot analysis, and the steady-state biliary clearance



$$R = \frac{PS_{int}(+inhibitor)}{PS_{int}(control)} = \frac{1}{1 + I_u / K_i} \quad \text{(Eq. 1)}$$

$$R_{uptake} = \frac{PS_1(+inhibitor)}{PS_1(control)} = \frac{1}{1 + I_{u,plasma} / K_{i,1}} \quad \text{(Eq. 2)}$$

$$R_{excretion} = \frac{PS_3(+inhibitor)}{PS_3(control)} = \frac{1}{1 + I_{u,liver} / K_{i,3}} \quad \text{(Eq. 3)}$$

$$CL_{int,bile} = PS_1 \times \frac{PS_3}{PS_2 + PS_3} \quad \text{(Eq. 4)}$$

$$R = \frac{CL_{int,bile}(+inhibitor)}{CL_{int,bile}(control)} \leqq R_{uptake} \times R_{excretion} \quad \text{(Eq. 5)}$$

FIG. 5. Schematic diagram illustrating the model of biliary excretion considering each processes of membrane penetration. PS_{int} represents the intrinsic membrane transport clearance. I_u and K_i represent the unbound concentration of an inhibitor around a transporter and its inhibition constant, respectively. PS_1, PS_2, and PS_3 represent the intrinsic membrane transport clearance for hepatic uptake across the sinusoidal membrane, that for efflux across the sinusoidal membrane from the liver, and that for excretion across the canalicular membrane, respectively. $I_{u,plasma}$ and $I_{u,liver}$ represent the unbound concentration of an inhibitor in plasma and that in liver, respectively. $K_{i,1}$ and $K_{i,3}$ represent the inhibition constant of an inhibitor for a hepatic uptake process across the sinusoidal membrane and that for an excretion process across the canalicular membrane, respectively. $CL_{int,bile}$ represents the intrinsic clearance for the net biliary excretion from the blood.

(PS_3) defined with respect to the hepatic unbound methotrexate, were reduced in the presence of probenecid in vivo. Furthermore, to predict drug interactions associated with membrane transport via sinusoidal and canalicular membranes from in vitro data using eqs. 2 and 3 of Fig. 5, $K_{i,1}$ and $K_{i,3}$ values were obtained in isolated hepatocytes and CMVs, respectively. The unbound concentration of the inhibitor ($I_{u,plasma}$ and $I_{u,liver}$) was directly estimated both in plasma and liver in vivo. As a result, the degree of inhibition of the uptake (PS_1) and excretion (PS_3) processes found in vivo was comparable with the predicted values using the inhibition constant obtained using isolated hepatocytes and CMVs, respectively (Fig. 6). This suggests that the interaction associated with each membrane transport process can be quantitatively estimated from in vitro data.

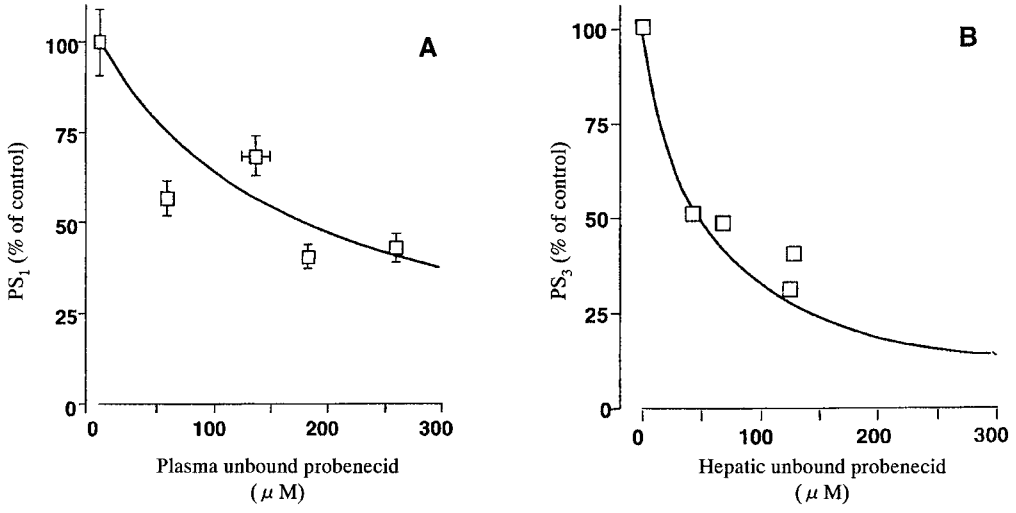


FIG. 6. Extrapolation of the interaction between methotrexate and probenecid across sinusoidal and canalicular membranes in vivo from in vitro data in rats. A, the reduction in the intrinsic clearance for the hepatic uptake of methotrexate across the sinusoidal membrane (PS_1) by probenecid was extrapolated based on eq. 2 of Fig. 5 and is shown as a solid line. The actual PS_1 value (□) obtained from the integration plot analysis was normalized by that in the control, and also plotted. The PS_1 value of the control was 23.9 ± 1.1 ml/min/kg. B, the reduction in the intrinsic clearance for the excretion of methotrexate across the bile canalicular membrane (PS_3) by probenecid was extrapolated based on eq. 3 of Fig. 5 and is shown as a solid line. The actual PS_3 value (□) obtained from the steady-state biliary excretion and hepatic unbound concentration of methotrexate was normalized by that in the control and also plotted. The PS_3 of the control was 60.6 ± 2.4 ml/min/kg (Ueda et al., 2001).

Since it is difficult to measure PS_1 and PS_3 directly in vivo and, in particular, it cannot be estimated in humans in vivo, the degree of inhibition of the net excretion from circulating plasma into bile ($CL_{int,bile}$), expressed by eq. 4 of Fig. 5, has been predicted. It should be noted that there is still only limited information available about the in vitro system that can estimate efflux transport on the sinusoidal membrane (PS_2). In view of this difficulty, a method to avoid false negative predictions, using eq. 5 of Fig. 5, of the interaction involving net biliary excretion has been proposed. If the inhibitor drug also reduces PS_2, the $CL_{int,bile}$ should be increased and, therefore, PS_2 does not need to be considered if we want to avoid any false negative predictions. Thus, in all cases, the reduction in $CL_{int,bile}$ should be, at most, the reduction in PS_1 (sinusoidal uptake) multiplied by that in PS_3 (canalicular efflux) as expressed by eq. 5 of Fig. 5. In actual fact, the predicted values were only slightly lower than the actual values in vivo, suggesting that this method is a suitable way of avoiding any false negative predictions (Ueda et al., 2001). This method can be adapted to the prediction of clinically relevant drug-drug interactions and the calculated R value may be a good criterion for initially investigating the possibility of a drug-drug interaction.

Generally, the substrate specificity of drug transporters is broad and multispecific because they function as detoxification systems. Once the strategy of using drug transporters in the new drug discovery and development processes has been adopted, transporter-mediated drug interactions will always need to be taken into account. However, we believe that pharmaceutical companies should not abandon transporter-based drug discovery, considering it promises greater benefit than risk. We should evaluate which drug candidates are substrates for which transporters, and identify which comedication is likely to cause drug-drug interactions. Furthermore, it is helpful to predict the degree of any changes in pharmacokinetics caused by a drug interaction, using the values of the inhibition constants and clinical concentrations, to provide useful information to clinicians.

IV. Possible Strategies for Drug Discovery Using Drug Transporter Inhibitors

A. P-Glycoprotein Blockade to Overcome Multidrug Resistance

Since over-expression of P-gp or MRP on the surface of tumor cells causes multidrug resistance, the use of a chemomodulator to inhibit efflux transport has been tried in an attempt to overcome this resistance (Cole and Deeley, 1998; Konig et al., 1999a; Kool et al., 1999; Kuwano et al., 1999). Several inhibitors of P-gp, such as PSC-833, LY335979, XR9576, and GF120918, have been discovered and are currently undergoing clinical trials (Ishikawa et al., 2000; Dantzig et al., 2001; Malingre et al., 2001a; Mistry et al., 2001). Since P-gp mediates the biliary and urinary excretion of its substrates (Oude Elferink et al., 1995; Simons et al., 1997) and limits their intestinal absorption (Hunter and Hirst, 1997), inhibition of P-gp will either reduce the hepatic and renal clearance or increase the bioavailability. Thus, some MDR modulators may change not only the concentration of anticancer drugs in tumor cells, but also their plasma concentrations.

Furthermore, it should be noted that P-gp modulators may increase the brain penetration of many drugs, including anticancer drugs, by blocking brain P-gp, leading to CNS side effects (Tsuji and Tamai, 1997; Fromm, 2000; Sadeque et al., 2000; Schinkel, 2001; Troutman et al., 2001). Since the brain is not a clearance organ, it may be that drug interactions involving P-gp will not alter the plasma concentrations, but change only the brain penetration of drugs. In actual fact, the brain distribution of many P-gp substrates is increased significantly in mdr1a knockout mice compared with that in wild-type mice, despite there being only a minor change in the plasma concentrations of these drugs (Table 4). Such cases should be carefully considered because the toxic effect of drugs on the brain cannot be estimated from the change in drug plasma concentrations.

B. P-Glycoprotein Blockade to Improve Efficacy of Human Immunodeficiency Virus Protease Inhibitors

The blockade of P-gp is expected to improve the efficacy of HIV protease-inhibitors (HIV-PIs) (Huisman et al., 2000). Since HIV-PIs are transported by P-gp, their distribution to the target sites is restricted (Lee et al., 1998). P-gp is expressed in several subclasses of lymphocytes, major targets of human immunodeficiency virus type 1 infection (Lucia et al., 1995; Andreana et al., 1996). Cell lines expressing a high level of P-gp significantly reduce the accumulation of HIV-PI, and are less sensitive to HIV-PI antiviral activity than cell lines not expressing P-gp (Turriziani et al., 2000). It is possible that the cells in patients that express P-gp will be relatively resistant to the antiviral effects of HIV-PIs. P-gp is also expressed in the blood-brain and blood-testis barrier and in the materno-fetal barrier formed by placental trophoblasts (Hoetelmans, 1998). Therefore, HIV-PI concentrations in pharmacological sanctuary sites, such as brain, testis, and fetus, are limited by P-gp, and it is assumed that these sites could be a breeding ground for therapy-resistant viruses due to the low drug concentrations (Groothuis and Levy, 1997). Residual viral replication in the CNS is often associated with the acquired immunodeficiency syndrome dementia complex (Achim et al., 1994; Kolson et al., 1998) and that in the testes contributes to the sexual transmission of the infection. Intravenous administration of the novel and potent P-gp inhibitor, LY335979, to mice increased the brain and testes concentrations of nelfinavir up to 37- and 4-fold, respectively (Choo et al., 2000). Oral administration of the P-gp inhibitors valspodar or GG918 completely in-

hibited placental P-gp activity, and the fetal distribution of saquinavir was increased in mice (Smit et al., 1999).

Moreover, P-gp in the small intestine limits the oral bioavailability of HIV-PIs (Kim et al., 1998). Several HIV-PIs, such as saquinavir, have a poor and highly variable oral bioavailability (Perry and Noble, 1998). In addition to rapid metabolism of saquinavir by intestinal and hepatic CYP3A4 (Fitzsimmons and Collins, 1997), intestinal P-gp activity is also likely to be involved in this problem (Kim et al., 1998). It has been reported that coadministration of ritonavir markedly increases the AUC value of orally administrated saquinavir in HIV-infected patients or healthy volunteers (58- and 112-fold, respectively) (Table 11) (Merry et al., 1997; Hsu et al., 1998). Huisman et al. investigated whether the ritonavir effect is primarily mediated by inhibition of CYP3A4 or P-gp (Huisman et al., 2001). In vitro, ritonavir only moderately inhibited P-gp-mediated transport of saquinavir compared with the potent P-gp inhibitor valspodar. When [^{14}C]saquinavir was coadministered orally with the maximum tolerated dose of ritonavir to wild-type and P-gp-deficient mice, saquinavir bioavailability was increased markedly in both strains (Table 11). Furthermore, the brain and fetal penetration of P-gp-deficient mice was markedly higher than that of wild-type mice despite coadministration of a high dose of ritonavir. P-gp still significantly restricts saquinavir penetration into brain and fetus in the presence of ritonavir. These data show that ritonavir is a relatively poor P-gp inhibitor, and the greatly increased bioavailability of saquinavir following ritonavir coadministration most likely results from reduced saquinavir metabolism. Thus, more efficient P-gp inhibitors are needed if one wishes to effectively expose HIV-PIs to pharmacological sanctuary sites.

Effective inhibition of P-gp might improve the oral bioavailability and penetration of HIV-PIs into pharmacological sanctuary sites with the potential to increase the beneficial effects of therapy, but it could also increase unexpected toxicity (Huisman et al., 2003). Careful clinical studies will be needed to establish whether this approach can be applied with a sufficient degree of safety.

V. Species and Gender Differences in Drug Transporters

There is little published information on species differences in drug transporters, although such information is important for predicting human pharmacokinetics. There is a species difference in transport of organic anions via MRP2 across the bile canalicular membrane. The ATP-dependent transport activity (V_{max}/K_m) of the MRP2 substrate DNP-SG in mouse, rat, guinea pig, rabbit, dog, and human CMVs is 25.5, 64.2, 9.4, 8.4, 7.7, and 3.8 μl/min/mg, respectively (Ishizuka et al., 1999). Another report has shown that the transport activity of glutathione conjugates and unconjugated anions (pravastatin, BQ-123, and methotrexate) in human CMVs was ~3- to 76-fold lower than that in rat CMVs, whereas the transport activity of glucuronides was similar in the two species (Niinuma et al., 1999). If there is a marked species difference in transport activity, it will be necessary to predict the in vivo transport activity in humans from in vitro data using human-based experimental systems. The prediction of in vivo transport activity from in vitro data has been successful in animals. For example, Ishizuka et al. have demonstrated that in vivo excretion clearance across the bile canalicular membrane could be predicted by an uptake experiment with CMVs (Ishizuka et al., 1999).

Several transporters have been shown to exhibit gender differences. Although there were only minor gender differences in the expression of rat Oat1 mRNA, rat Oat2 expression in female kidney was considerably higher than in male kidney (Buist et al., 2002; Kobayashi et al., 2002). Conversely, the Oat3 gender difference occurred in liver, rather than kidney, where male mRNA levels were higher than female levels (Buist et al., 2002; Kobayashi et al., 2002). The expression of the gene product for the Ntcp is higher in the liver of male than female rats (Simon et al., 1999). In addition, the uptake of TEA by isolated kidney slices is higher in male than in female rats (Urakami et al., 1999). This is probably caused by the expression levels of rat Oct2 mRNA and the protein in the kidney of males being much higher than those in females (Urakami et al., 1999).

TABLE 11
Saquinavir bioavailability alone and with ritonavir

	Saquinavir Alone	Saquinavir + Ritonavir	Δ	Reference
HIV-infected patients[a]				Merry et al., 1997
C_{max} (ng/ml)	146 (57–702)	4795 (1420–15810)	33× ↑	
AUC_{0-8h} (ng · h/ml)	470 (293–3446)	27458 (7357–108001)	58× ↑	
Healthy volunteers[b]				Hsu et al., 1998
C_{max} (ng/ml)	0.07 ± 0.03	2.2 ± 0.5	31× ↑	
AUC_{0-40h} (μg · h/ml)	0.24 ± 0.18	26.8 ± 4.9	112× ↑	
Wild-type mice[c]				Huisman et al., 2001
[^{14}C]saquinavir oral availability (%)	9.1 ± 1.1	232 ± 70	25× ↑	
mdr1a/1b knockout mice[c]				Huisman et al., 2001
[^{14}C]saquinavir oral availability (%)	14.1 ± 2.2	865 ± 105	61× ↑	

[a] 600 mg of saquinavir t.i.d. daily alone and with 300 mg ritonavir b.i.d. Data are median (range).
[b] Single 400 mg of saquinavir alone and with single 600 mg of ritonavir.
[c] 50 mg/kg ritonavir was administered 30 min before oral administration of [^{14}C]saquinavir.

However, there are no gender differences in the mRNA expression levels of rat Oct1 and Oct3 (Urakami et al., 1999).

Oatp1 is localized at the sinusoidal membrane of the liver and the apical membrane of the kidney in the S3 segment of the proximal tubules of the outer medulla (Bergwerk et al., 1996). It has been reported that the expression of Oatp1 mRNA in kidney is approximately 6 times higher in male rats compared with females (Lu et al., 1996). In addition, the urinary excretion of the Oatp1 substrate E_2-17βG is more than 250 times lower in male rats than in females (Table 12) (Gotoh et al., 2002). The urinary clearance with respect to the plasma unbound E_2-17βG in male rats is less than 1% of the glomerular filtration rate, indicating extensive reabsorption from the renal tubules of male rats. A marked increase in E_2-17βG urinary excretion was observed in male rats that had undergone orchidectomy (Table 12) (Gotoh et al., 2002). Testosterone injections given to female rats reduced the urinary excretion to a level comparable with control male rats (Table 12) (Gotoh et al., 2002). A concomitant change in the expression of Oatp1 protein has been found in the kidney membrane fractions following such treatments (Gotoh et al., 2002). These results suggest that urinary E_2-17βG excretion is subject to hormonal regulation and the large gender difference can be explained by the difference in Oatp1-mediated reabsorption. The expression of Oatp1 in the liver does not show any clear gender difference, which is compatible with a minimal gender difference in the uptake clearance of E_2-17βG in the liver (Gotoh et al., 2002). Another report has demonstrated a similar phenomenon in the urinary excretion of taurocholate, dibromosulfophthalein, and zenarestat, an aldose reductase inhibitor, used for the treatment of diabetic neuropathy, in rats (Kato et al., 2002). However, there is still little information about human gender differences in drug transporters.

VI. Synergistic Role of Metabolic Enzymes and Transporters

Both CYP3A4 and P-gp are present at high levels in the villus enterocytes of the small intestine, the primary site of absorption of orally administered drugs (Wacher et al., 2001; Zhang and Benet, 2001). Moreover, these proteins are induced by many of the same compounds and exhibit a broad overlap in their substrate and inhibitor specificities (Wacher et al., 1995), suggesting that they may act synergistically in the small intestine as a barrier to drug absorption (Suzuki and Sugiyama, 2000; Wacher et al., 2001; Zhang and Benet, 2001; Cummins et al., 2002). Recent studies demonstrate that the steroid xenobiotic receptor (SXR), a member of the nuclear hormone receptor superfamily, involved in xenobiotic induction of CYP3A (Bertilsson et al., 1998; Blumberg et al., 1998), can also regulate the expression of the MDR1 gene (Geick et al., 2001; Synold et al., 2001). SXR is activated by paclitaxel and is responsible for inducing the expression of not only CYP3A, but also CYP2C9 and MDR1 (Synold et al., 2001). As paclitaxel is metabolized by both CYP3A4 and CYP2C9 and transported by P-gp, induction of all these proteins leads to its enhanced clearance. This indicates a broad role for SXR in the coordinated induction of multiple detoxification pathways. A similar study of pregnane X receptor (PXR), a mouse ortholog of SXR, has been carried out and shows that PXR regulates the expression of Cyp3a11, Cyp7a1 and Oatp2 (Staudinger et al., 2001a; Staudinger et al., 2001b; Guo et al., 2002). PXR is activated by the toxic bile acid lithocholic acid and its metabolite. Activation of PXR results in the repression of Cyp7a1, which blocks bile acid biosynthesis, and induction of Oatp2 and Cyp3a expression, which promotes bile acid uptake and metabolism. PXR coordinately regulates gene expression to reduce the concentrations of the toxic bile acid lithocholic acid in the liver. Since rifampin and the HIV-PI ritonavir are also ligands for human SXR, they activate the transcription of the CYP3A4 and MDR1 (Dussault et al., 2001; Geick et al., 2001). Coordinated induction of intestinal CYP3A and P-gp via SXR will enhance the ability of a barrier as a detoxification system for xenobiotics in the small intestine. However, the induction of these proteins by drug candidates will potentially cause drug-drug interactions, making successful drug development more difficult. Thus, SXR-binding and -activation

TABLE 12

The gender difference in the urinary excretion of E_2-17βG and the effect of androgen hormone on the urinary excretion of E_2-17βG in rats (Gotoh et al., 2002)

	Male		Female		
	Control	Orchidectomy	Control	Sham[a]	+Testosterone
C_{ss} (fmol/ml)	67.9 ± 3.1	59.2 ± 2.5*	63.4 ± 4.9	64.8 ± 4.7	80.1 ± 3.4†
CL_{tot} (ml/min/kg)	54.2 ± 3.8	63.1 ± 5.1	60.4 ± 4.9	58.1 ± 2.8	45.6 ± 2.3**
CL_{urine} (ml/min/kg)	$<8.2 \times 10^{-3}$	1.27 ± 0.36*	1.11 ± 0.27*	0.919 ± 0.051*	$<9.83 \times 10^{-3}$*†
CL_{bile} (ml/min/kg)	43.9 ± 2.0	49.0 ± 2.8	39.3 ± 6.5	42.1 ± 2.1	45.5 ± 4.1
GFR (ml/min/kg)	11.8 ± 0.8	12.1 ± 0.3	12.0 ± 0.8	9.90 ± 0.30	10.6 ± 0.4

Each value represents the mean ± S.E. of four to five independent experiments during constant intravenous infusion of [^{3}H]E_2-17βG (227 pmol/h/kg) to male and female SD rats.

* Significantly different from control male rats ($P < 0.01$).

** Significantly different from control female rats ($P < 0.05$).

† Significantly different from control female rats ($P < 0.01$).

[a] Vehicle only.

assays are useful tool for screening compounds inducible by CYP3A and P-gp.

Some organic anions (carboxylates), formed by carboxyesterases in enterocytes, are sequentially excreted into the lumen via efflux transporters for organic anions. ME3229 is an ester-type prodrug designed to increase the oral bioavailability of the active carboxylate drug ME3277 (a glycoprotein IIb/IIIa receptor antagonist) (Okudaira et al., 2000b). It has been suggested that the low oral bioavailability of ME3277 (~1% in rats) is related to its low lipophilicity. Although esterification of the carboxylate groups in ME3277 results in a much higher lipophilicity, the oral bioavailability of ME3229 remains comparatively low (~10% in rats). It has been found that ME3229 is rapidly taken up by enterocytes, whereas most of its anionic metabolites (ME3277) produced from prodrugs (ME3229) in enterocytes are actively excreted into the lumen (Okudaira et al., 2000b). High efflux activity located on the luminal membrane, along with the metabolic activity of carboxyesterases in enterocytes, hinders the oral absorption of ester-type prodrugs. This active efflux transport of anionic metabolites is similar in normal rats and Eisai hyperbilirubinemic rats, the latter being hereditarily defective in Mrp2 (Okudaira et al., 2000a). These results show that the anionic metabolites of ME3229 are pumped out into the gut lumen by an energy-dependent transport system distinct from MRP2. It has also been shown that P-glycoprotein is not responsible for this efflux (Okudaira et al., 2000a).

A sequential detoxification system operates on other compounds. Platinum drugs are used widely for chemotherapy of lung cancer, but their effectiveness is limited by resistance. They are metabolized to glutathione conjugates in tumor cells. Intracellular glutathione is formed by γ-glutamylcysteine synthetase and the glutathione conjugates of platinum drugs formed in tumor cells are actively excreted via the ATP-dependent glutathione *S*-conjugate export (GS-X) pump, which has been shown to be identical to MRP. The MRP/GS-X pump and γ-glutamylcysteine synthetase are coordinately induced by exposure to platinum drugs or heavy metals in cisplatin-resistant tumor cells (Ishikawa et al., 1996; Gomi et al., 1997; Oguri et al., 1998). Elevated expression of the MRP/GS-X pump and increased glutathione biosynthesis may both be important factors in the cellular metabolism and disposition of cisplatin.

VII. The Regulation Mechanisms of Drug Transporters

A. The Transcriptional Regulation of Transporters

Knowledge of the regulation of transporters is of great help in predicting pharmacokinetics and drug-drug interactions. As mentioned above, the orphan nuclear receptor PXR (SXR) coordinately regulates drug clearance in response to a wide variety of xenobiotic compounds. Although this signaling system protects the body from exposure to toxic compounds, it can also be a serious barrier to drug therapy. In human hepatocytes, the expression of human MRP2 can be up-regulated by human PXR ligands such as rifampicin (Kast et al., 2002; Kauffmann et al., 2002). In PXR knockout mice, no induction of Mrp2 by pregnenolone 16a-carbonitrile, a ligand for the rodent PXR, has been observed (Kast et al., 2002). This suggests that PXR is involved in the induction of Mrp2 (Kast et al., 2002). In addition, it has been suggested that MRP2 expression is regulated by the farnesoid X-activated receptor (FXR) and constitutive androstane receptor (CAR). Treatment of rodent hepatocytes with the FXR ligand chenodeoxycholic acid or the CAR ligand phenobarbital results in a potent induction of Mrp2 mRNA levels (Kast et al., 2002). The presence of potential PXR, CAR, and FXR binding sites has been demonstrated for human MRP2 (Tanaka et al., 1999; Stockel et al., 2000).

The liver-enriched transcription factor hepatocyte nuclear factor 1 alpha (HNF1 alpha) is critical for hepatocyte-specific OATP-C gene expression. Coexpression of HNF1 alpha stimulated OATP-C promoter activity 30-fold in HepG2 cells and 49-fold in HeLa cells, and mutation of the HNF1 site abolished the promoter function (Jung et al., 2001a). The human OATP8 and mouse Oatp4 promoters were also responsive to HNF1 alpha coexpression in HepG2 cells, suggesting a role for HNF1 alpha as a global regulator of liver-specific organic anion transporter genes (Jung et al., 2001a).

BSEP meditates the ATP-dependent transport of bile salts across the canalicular membrane of hepatocytes and the expression of this transporter is sensitive to the flux of bile acids. Luciferase reporter gene assays have shown that the BSEP promoter is positively controlled by FXR, retinoid X receptor alpha, and bile salts (Plass et al., 2002). FXR/ retinoid X receptor alpha heterodimers specifically bind to the inverted repeat (IR)-1 element in the BSEP promoter (Ananthanarayanan et al., 2001). Reducing endogenous FXR levels using RNA interference fully suppresses bile salt-induced BSEP expression (Plass et al., 2002). FXR is required for the bile salt-dependent transcriptional control of the human BSEP gene.

Recently, an in silico approach has been applied to PXR ligands (Ekins et al., 2002c). Using data for the EC50 values of PXR activation derived for 12 human PXR ligands, a pharmacophore has been developed (Ekins and Erickson, 2002). The pharmacophore was able to distinguish between the most potent activators of PXR and poor activators. The model could be useful in drug development, potentially acting as a high-throughput filter for identifying compounds that may bind to PXR before their in vitro determination. This will aid the selection of molecules with a poor ability to be potent PXR ligands, thereby avoiding the induction of drug-metabolizing enzymes and transporters.

B. The Sorting and Polarization of Transporters

The polarization of cells, such as the epithelial cells lining the bile duct or the kidney proximal tubules, is created largely by the differential localization of specific proteins. To create such polarization, proteins destined for specific membranes have been shown to contain molecular targeting signals. The recent identification of a PDZ-interacting domain in the cystic fibrosis transmembrane conductance regulator (CFTR) is the first report of a targeting motif in mammalian ABC transporters (Moyer et al., 1999). CFTR encoded by the cystic fibrosis gene is localized in the apical membrane of epithelial cells, where it functions as a cyclic AMP-regulated chloride channel and as a regulator of other ion channels and transporters (Kleizen et al., 2000). PDZ-interacting domains in CFTR play a key role in the apical polarization of this transporter in epithelial cells (Moyer et al., 1999). In general, PDZ proteins, which are involved in the apical or basal targeting of membrane proteins, bind to the PDZ-interacting domain characterized by the carboxy-terminal amino acid sequence (Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001). A novel PDZ domain-containing protein, PDZ-K1, binds to the carboxy terminus of human MRP2 (Kocher et al., 1999; Harris et al., 2001). In addition, deletion of carboxy-terminal 15 or more amino acids impairs the localization of MRP2 in polarized HepG2 cells (Nies et al., 2002). The amino acid sequence in this region may be involved in the apical targeting, stabilization, and/or in maintaining the conformation of MRP2 to be released from the ER (Nies et al., 2002).

Under cholestatic conditions, it has been suggested that MRP2 is internalized from the cell surface in rats and humans (Rost et al., 1999; Vos et al., 1999; Paulusma et al., 2000; Shoda et al., 2001; Zollner et al., 2001). Although activation of protein kinase C results in the internalization of MRP2 in HepG2 cells (Kubitz et al., 2001), stimulation by cyclic AMP results in the relocalization of MRP2 in isolated rat hepatocyte couplets (Roelofsen et al., 1998). Under cholestatic conditions, tauroursodeoxycholate stimulates the insertion of internalized MRP2 into the bile canalicular membrane in a protein kinase C-dependent manner (Beuers et al., 2001; Fickert et al., 2001).

Recently, it has been demonstrated that radixin (encoded *Rdx*) was involved in the localization of Mrp2 at bile canalicular membranes (Kikuchi et al., 2002). Radixin is a member of the ezrin-radixin-moesin family of proteins, which cross-link actin filaments and integral membrane proteins. *Rdx* knockout mice ($Rdx^{-/-}$ mice) exhibit marked conjugated hyperbilirubinemia. Interestingly, on the bile canalicular membranes of $Rdx^{-/-}$ mice, the level of Mrp2, which secretes conjugated bilirubin into the bile, is reduced compared with that in wild-type mice, while the expression level of Mrp2 in whole liver did not seem to be reduced in $Rdx^{-/-}$ mice. In addition, in vitro binding studies show that radixin associates directly with the carboxy-terminal cytoplasmic domain of human MRP2. These findings indicate that radixin is required for Mrp2 to localize correctly on the apical membranes in the liver.

The Dubin-Johnson syndrome (DJS) is an inherited disorder characterized by conjugated hyperbilirubinemia. The absence of a functional MRP2 transporter from the apical membrane of hepatocytes explains the molecular basis of this disease (Kartenbeck et al., 1996). Several mutations in the MRP2 gene have been identified in patients with DJS (Paulusma et al., 1997; Tsuji et al., 1999). Keitel et al., have investigated the consequences of a mutation in DJS, which leads to the loss of 2 amino acids from the second ATP-binding domain of MRP2 (Keitel et al., 2000). This mutation is associated with the absence of the MRP2 glycoprotein from the apical membrane of hepatocytes. Transfection of mutated MRP2 complementary DNA leads to an MRP2 protein that is only core glycosylated and located in the endoplasmic reticulum (ER) of transfected cells. This indicates that this mutation leads to impaired maturation and trafficking of the protein from the ER to the Golgi complex. This impaired protein sorting may be responsible for the absence of MRP2 protein from the apical membrane.

VIII. Polymorphisms of Drug Transporters

Selecting drugs and dosages according to genetic and specific individual markers has allowed optimized drug therapy to be developed for individual patients (individualized drug therapy). The possibility of defining patient populations genetically may improve drug safety and efficacy by predicting individual responses to drugs. Nevertheless, it is still desirable to develop drugs that are relatively unaffected by polymorphisms and exhibit little interindividual variability with a wide therapeutic spectrum of activity. Genetic polymorphisms in human membrane transporters may also contribute to interindividual differences in the response to drugs. However, our knowledge of the relevant transporters is limited at present.

Polymorphisms in drug transporters, such as MDR1, MRP1, MRP2, OATP-A, OATP-C, OATP8, OAT1, OAT2, OAT3, OCT2, and OCTN2 have been demonstrated (Nezu et al., 1999; Mayatepek et al., 2000; Cascorbi et al., 2001; Iida et al., 2001; Kerb et al., 2001; Leabman et al., 2002; Suzuki and Sugiyama, 2002; Tirona and Kim, 2002). A number of single nucleotide polymorphisms (SNPs) have been described in the human MDR1 gene (Kim, 2002; Fromm, 2002). An SNP in exon 26 (C3435T) of MDR1 was found to be associated with reduced intestinal expression of P-gp, along with increased oral bioavailability of digoxin (Hoffmeyer et al., 2000). Since C3435T SNP is a silent or "wobble" polymorphism with no amino acid substitutions of the encoded protein, the

mechanism via which this SNP acts on P-gp function remains unclear. Recently, Kim et al. have found that 95% of subjects who were homozygous for the exon 26 C3435T SNP (C/C) were GG homozygous at base position 2677 (Kim et al., 2001). The C3435T SNP in exon 26 appeared to be linked to a G2677T SNP in exon 21. The G2677T transversion in exon 21 resulted in an alteration in the encoded protein (Ala893Ser). In vitro expression of MDR1 encoding Ala893 (MDR1*1) or a site-directed Ser893 mutation (MDR1*2) led to enhanced efflux of digoxin by cells expressing the Ser893 variants (Kim et al., 2001). Moreover, subjects with the C3435T and G2677T allele (MDR1*2) have over a 40% lower AUC value following oral administration of fexofenadine, a P-gp substrate, compared with subjects with the MDR1*1 variant (Table 13) (Kim et al., 2001). This is consistent with the in vitro data and suggests enhanced in vivo P-gp activity among subjects with the MDR1*2 allele. This finding contradicts previous results about the effect of exon 26 C3435T SNP on digoxin disposition (Hoffmeyer et al., 2000). The reason for this discrepancy and failure to detect exon 21 SNP in a previous study is currently unclear. A polymorphism of MDR1 may change the expression level and/or functional activity of P-gp in the brain as well as that in the small intestine, and contribute to interindividual differences in the brain penetration of P-gp substrates. It is possible that the change in the brain penetration of P-gp substrates caused by P-gp SNPs is more marked than that in the small intestine and may affect interindividual differences in the degree of CNS toxicity exhibited by P-gp substrates. However, assessment of the role of P-gp in BBB function in humans is problematic, since brain concentrations cannot be measured readily. A key area for future investigation of the role of human P-gp in the BBB will be the development of a suitable probe substrate appropriately labeled for continuous monitoring of brain penetration using positron emission tomographic imaging techniques (Hendrikse et al., 2001).

OATP-C is a liver-specific transporter involved in the hepatocellular uptake of a variety of clinically important drugs. The presence of multiple functionally relevant SNPs in OATP-C has been reported (Tirona et al., 2001; Michalski et al., 2002; Nozawa et al., 2002; Tirona and Kim, 2002). In vitro experiments with cultured cells expressing the wild-type and mutated OATP-Cs revealed that several variants exhibited markedly reduced uptake of the OATP-C substrates estrone sulfate and E_2-17βG (Tirona et al., 2001) (Table 14). Specifically, alterations in transport were associated with the SNPs that introduce amino acid changes within the transmembrane-spanning domains and with those that modify extracellular loop 5, suggesting an important role of this region in any substrate-transporter interaction (Table 14). Furthermore, several OATP-C variants were found to have reduced fractional cell membrane expression compared with the wild-type allele (Tirona et al., 2001; Michalski et al., 2002; Nozawa et al., 2002). Interestingly, the variant with A1964G SNP reduced the transport activity of estrone sulfate but not that of E_2-17βG, suggesting possible substrate-dependent polymorphisms. A similar phenomenon has been found in SNPs of polyspecific organic cation transporter OCT1 (Kerb et al., 2002). The Cys88Arg and Gly 401Ser mutants could mediate significant uptake of TEA and serotonin, although they were unable to transport 1-methyl-4-phenylpyridinium (MPP). These mutants exhibit an altered substrate selectivity. In such a case it is possible that the transport activities of some drug candidates can be altered by some SNPs, even if those of typical substrates are unaffected by the SNPs. If so, it will be necessary to evaluate every drug candidate in terms of the transport activity of each variant. Recently, Nishizato et al. have investigated the contribution of the genetic polymorphisms of the OATP-C gene to the pharmacokinetics of pravastatin, a substrate for OATP-C (Nishizato et al., 2003). Among 120 healthy Japanese individuals, five nonsynonymous variants were observed in the OATP-C gene. Subjects with the OATP-C*15 allele (Asp130Ala174) had a reduced total and nonrenal clearance compared with those with the OATP-C*1b allele (Asp130Val174); nonrenal clearance in *1b/*1b (n = 4), *1b/*15 (n = 9), and *15/*15 (n = 1) subjects was 2.01 ± 0.42, 1.11 ± 0.34, and 0.29 (l/h/kg). Mean serum concentration-time curves of pravastatin were different among the three genotypic groups with regard to the *15 allele (Fig. 7). T521C (Val174Ala) SNPs are likely to be associated with altered pharmacokinetics of

TABLE 13
MDR1 alleles and fexofenadine disposition (Kim et al., 2001)

Genotype	Subjects		$AUC_{(0-4)}$ (ng · ml^{-1} · h)	C_{max} (ng · ml^{-1})	T_{max} (h)	$T_{1/2}$ (h)
	No.	%				
Caucasian (n = 37)						
*1/*1	6	16	1316 ± 543	508 ± 205	2.7 ± 0.8	2.8 ± 0.4
*1/*2	4	11	1171 ± 967	400 ± 282	3.3 ± 1.0	3.1 ± 0.7
*2/*2	5	14	837 ± 311	317 ± 185	2.4 ± 1.7	3.5 ± 0.9
African-American (n = 23)						
*1/*1	9	39	1030 ± 435	386 ± 163	1.9 ± 0.9	3.3 ± 0.3
*1/*2	3	13	1099 ± 70	405 ± 29	2.3 ± 1.2	2.7 ± 0.4

180 mg fexofenadine was administered orally. The MDR1*2 allele contain 3 SNPs simultaneously; C1236T in exon 12, G2677T in exon 21, and C3435T in exon 26. The first published MDR1 sequence is shown as the MDR1*1 allele.

pravastatin. As hepatic clearance of pravastatin is rate-limited by uptake (Hsiang et al., 1999), low transport activity of OATP-C may lead to a reduction of hepatocellular uptake of pravastatin, resulting in lower total clearance. OATP-C polymorphisms may also influence the interindividual variability in the pharmacological effects of pravastatin that have the liver as their pharmacological target.

In addition, it has been reported that the genotypic frequencies of MDR1 and OATP-C are dependent on race (Table 14) (Ameyaw et al., 2001; Tirona et al., 2001). Polymorphisms in drug transporters may be involved in not only the interindividual variability but also the ethnic differences in drug disposition, like the polymorphisms of cytochrome P450. In any case, it is important to know whether each polymorphism has any clinical significance. In particular, since a change in the functional properties of transporters frequently does not alter the plasma concentration, unlike metabolizing enzymes, it is difficult to detect a change in these functional properties in vivo. To determine the in vivo function of transporters, positron emission tomography may be a useful tool and a correlation between genotype and phenotype will need to be established in the future. Studies of the polymorphisms in human drug transporters have been recently initiated and, in the future, the information obtained could be used for establishing the most appropriate drug treatment for individual patients.

IX. Methods for Assessing Drug Transporter Activities in Drug Discovery

The pharmaceutical industry is now at a turning point and strategies for drug discovery and development are changing rapidly. A significant number of drug candidates entering clinical development are dropped at some stage due to unacceptable pharmacokinetic properties. Thus, optimizing the pharmacokinetic properties during the early stages of drug development is now widely accepted as being essential (White, 2000; Roberts, 2001). Drug discovery based on the transport mechanisms and substrate specificities of drug transporters will become increasingly important. Identification of compounds that are substrates for transporters can aid the optimization and selection of new drug candidates. High-throughput assays for transporters are needed during the early stages of drug discovery and the expression system of transporters is an efficient tool for screening transport activities.

Recent studies show that in vivo P-gp function can be quantitatively predicted using MDR1-transfectd cell monolayers (Adachi et al., 2001) (Fig. 8). The "$K_{p,brain}$ ratio" ($K_{p,brain\ (mdr1a/1b(-/-))}/K_{p,brain\ (mdr1a/1b(+/+))}$) is the most suitable parameter for describing P-gp function in vivo on the BBB (Fig. 9). By normalizing the brain-to-plasma concentration ratio ($K_{p,brain}$) in mdr1a/1b knock-

TABLE 14
Summary of nonsynonymous polymorphisms in OATP-C (Tirona et al., 2001)

Exon	Region	Nucleic Acid Substitution	Amino Acid Substitution	Allelic Frequency				[^{3}H]Estrone Sulfate Transport Activity by OATP-C Variants		
				European-American (n = 49)	African-American (n = 44)	Japanese[a] (n = 267)	Japanese[b] (n = 120)	K_m (μM)	V_{max} (pmol/min/mg)	V_{max}/K_m (μl/min/mg)
			Wild type					0.54 ± 0.21	19.8 ± 3.4	41 ± 9.7
2	TM	T217C	Phe73Leu	0.02	0.00	NE	0.00	5.9 ± 1.5*	20 ± 2.8	4.3 ± 1.1*
3	TM	T245C	Val82Ala	0.02	0.00	NE	0.00			
4		A388G	Asn130Asp	0.30	0.74††	0.54	0.63	0.33 ± 0.12	18 ± 2.2	90 ± 25
4		A452G	Asn151Ser	0.00	0.00	NE	0.04			
4		C463A	Pro155Thr	0.16	0.02†	NE	0.00	0.72 ± 0.28	17 ± 3.7	30 ± 5.4
4		A467G	Glu156Gly	0.02	0.00	NE	0.00			
5	TM	T521C	Val174Ala	0.14	0.02†	0.11	0.16	0.34 ± 0.089	5.7 ± 0.75**	20 ± 3.2
8	TM	C1007G	Pro336Arg	0.00	0.00	NE	0.01			
8	TM	T1058C	Ile353Thr	0.02	0.00	NE	0.00	2.4 ± 0.9*	20 ± 5.5	9.4 ± 1.4*
9	Ex loop5	A1294G	Asn432Asp	0.01	0.00	NE	0.00	0.37 ± 0.18	12 ± 0.94**	51 ± 16
10	Ex loop5	A1385G	Asp462Gly	0.01	0.00	NE	0.00	0.14 ± 0.073	8.3 ± 1.7*	116 ± 38
10	Ex loop5	G1454T	Cys485Phe	0.00	0.00	NE	0.01			
10	Ex loop5	G1463C	Gly488Ala	0.00	0.09†	NE	0.00	4.3 ± 2.2	13 ± 4.9	5.9 ± 2.1*
14		A1964G	Asp655Gly	0.02	0.00	NE	0.00	2.7 ± 0.56*	19 ± 3.3	8.8 ± 2.2*
14		A2000G	Glu667Gly	0.02	0.34††	NE	0.00	0.63 ± 0.21	16 ± 3.2	33 ± 3.3

NE, not examined.
* $P < 0.05$; ** $P < 0.01$ relative to wild type (GenBank accession numbers AB02657, AJ132573).
† $P < 0.05$; †† $P < 0.01$ relative to European-American using Fisher's exact test.
[a] Adapted from Nozawa et al., 2002.
[b] Adapted from Nishizato et al., 2003.

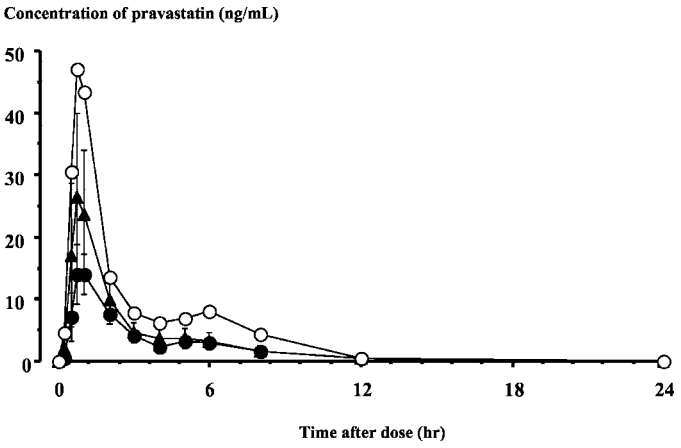


FIG. 7. Mean serum concentration over time after a single oral pravastatin dose of 10 mg in 3 OATP-C genotypic groups. ●, OATP-C*1b/*1b (n = 4); triangles, *1b/*15 subjects (n = 9); ○, *15/*15 subject (n = 1). The OATP-C*1b allele possesses mutations of Asn130Asp, and the OATP*15 allele possesses two SNPs, Asn130Asp and Val174Ala, simultaneously (Nishizato et al., 2003).

out mice with reference to that in normal mice, the P-gp function parameter can be simply estimated as shown in eq. 2 of Fig. 9. Furthermore, in vitro, this parameter corresponds to the "corrected flux ratio" across MDR1-transfected cell monolayers (Fig. 9). The corrected flux ratio represents the normalizing ratio of basal-to-apical (B→A) permeability versus apical-to-basal (A→B) permeability in parent cell monolayers with respect to that in MDR1-transfected cell monolayers (eq. 1 of Fig. 9). Indeed, a clear correlation between both parameters in vitro and in vivo has been obtained experimentally (Fig. 8). Another report also described a similar result (Yamazaki et al., 2001). Although one can calculate the net flux by subtracting the A→B flux from the B→A flux in MDR1-transfected cell monolayers, it is difficult to know to which in vivo parameters these correspond. In the case of CNS-active drugs, the concentration of "free" drug in the brain is very important for predicting pharmacological effects, not the concentration of "total" drug, since the term "total" drug includes the fraction bound nonspecifically to brain macromolecules such as proteins and lipids. We should also note that the value of the $K_{p,brain}$ ratio can be an index of the brain-to-plasma concentration ratio of "free" drug ($K_{p,brain,free}$), the most important parameter as far as the index of pharmacological activity is concerned. Supposing that the activities of passive diffusion and the function of transporters other than P-gp are not altered between mdr1a/1b knockout mice and wild-type mice, the values of PS_1, PS_2, PS_3, and PS_4 of eq. 3 in Fig. 9 are unaffected by P-gp function. Thus, as shown in eq. 3 in Fig. 9, the "$K_{p,brain,free}$" is inversely proportional to the $K_{p,brain}$ ratio. Moreover, this important parameter, which can reflect the pharmacological effect of drugs on the CNS, can be estimated by using a suitable expression system (see the equations in Fig. 9).

Recently, double-transfected cell monolayers have been established that express uptake transporters (OATP-C or OATP8) and MRP2 on the basolateral and apical membranes, respectively (Cui et al., 2001a; Sasaki et al., 2002). Most substrates of MRP2 are negatively charged under physiological conditions and thus cannot penetrate the plasma membrane without an uptake transporter. Therefore, it remains difficult to study MRP2 function in whole cells and MRP2 has been mostly studied using inside-out membrane vesicles prepared from MRP2-expressing cells. The use of double-transfected cell monolayers makes it possible to assess MRP2 activity more easily with intact cells. The basal-to-apical transport of pravastatin, which is a substrate of OATP-C and MRP2, was 2.5 times higher than that in the opposite direction in double-transfected cells (Fig. 10D), whereas a symmetrical flux of pravastatin was observed across the MRP2-expressing cell monolayer (Fig. 10 C) (Sasaki et al., 2002). Because of the easier handling of double-transfected cells grown on Transwell membrane inserts compared with the preparation and handling of membrane vesicles, it may be possible to develop throughput screening systems using double-transfected cells. These in vitro models, reproducing the polarity of transporters and the direction of transport, may be useful for predicting the in vivo hepatic vectorial transport of drugs from blood to bile. Moreover, the combination of an uptake and efflux transporter may be modified for certain purposes. For example, a combina-

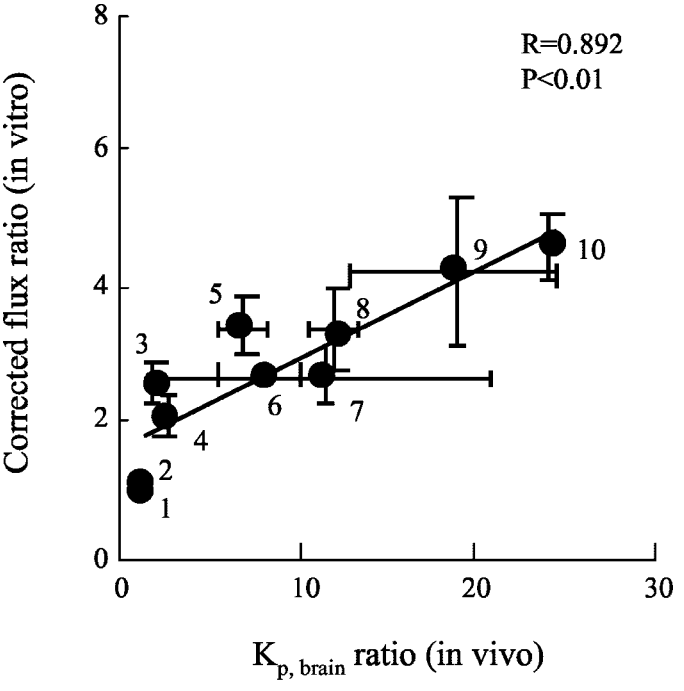


FIG. 8. Correlation of P-gp function determined in in vitro transcellular transport studies using MDR1 transfected cells/control cells and in vivo brain penetration studies using mdr1a/1b knockout mice/wild-type mice. 1, diazepam; 2, progesterone; 3, daunomycin; 4, dexamethasone; 5, loperamide; 6, verapamil; 7, vinblastine; 8, cyclosporin A; 9, digoxin; 10, quinidine (Adachi et al., 2001).

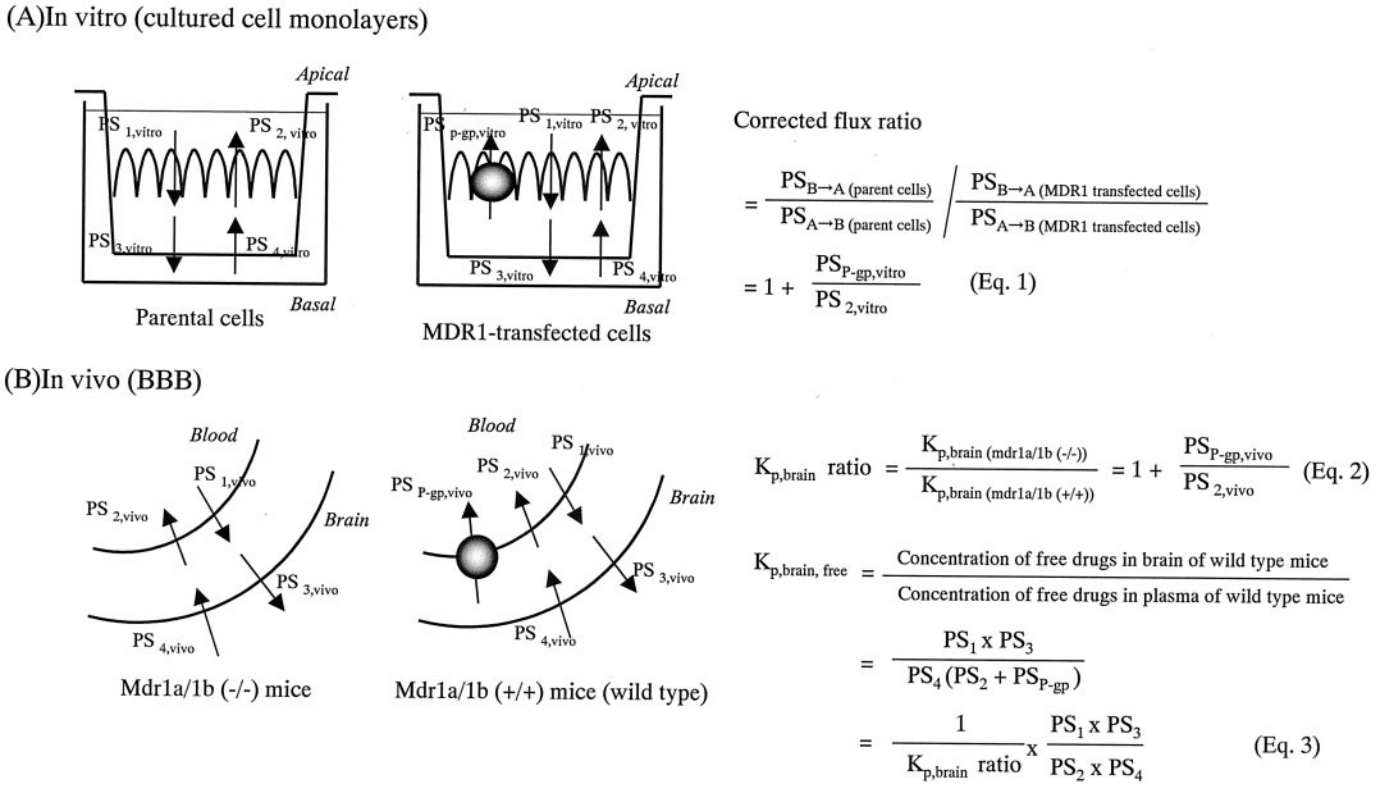


FIG. 9. Schematic diagram illustrating the permeability-surface area products (PS) for the penetration of ligands across the plasma membrane. A and B represent the PS products across the cultured cell monolayers and those across the cerebral endothelial cells. $PS_{1,vitro}$ and $PS_{2,vitro}$ represent the PS products for the influx and non-P-gp-mediated efflux across the apical membrane of the cultured cell monolayers, respectively. $PS_{3,vitro}$ and $PS_{4,vitro}$ represent the PS products for the efflux and influx across the basal membrane, respectively. $PS_{P\text{-}gp,vitro}$ represents the PS products for P-gp-mediated efflux across the apical membrane. $PS_{1,vivo}$ and $PS_{2,vivo}$ represent the PS products for the influx and non-P-gp-mediated efflux across the luminal membrane of cerebral endothelial cells, respectively. $PS_{3,vivo}$ and $PS_{4,vivo}$ represent the PS products for the efflux and influx across the abluminal membrane of cerebral endothelial cells, respectively. $PS_{P\text{-}gp,vivo}$ represents the PS products for P-gp-mediated efflux across the luminal membrane. $PS_{A\text{-}B}$ and $PS_{B\text{-}A}$ represent the PS products across the monolayer in the apical-to-basal direction and the basal-to-apical, respectively (Adachi et al., 2001).

tion of OCT1 with P-gp may serve to study the biliary excretion of organic cations. Although the identification of many transporters localized on the apical membrane in the kidney is awaited, a combination of transporters in human kidney tubule cells may be a suitable system for studying the urinary excretion of drugs. Sample analysis is a major limitation of the throughput in a monolayer transport assay. Liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), due to its superior sensitivity, selectivity, and rapidity along with significantly reduced method development time, is an ideal analytical tool for high-throughput analysis of transport samples. In addition, the exceptional capability of LC/MS/MS for the simultaneous determination of multiple drug mixtures has allowed sample pooling (i.e., multiple samples to be pooled before analysis), which forms the throughput of the monolayer transport assay (Bu et al., 2000).

In addition, higher throughput assays to detect indirectly compounds interacting with P-gp have also been described. Such methods are based on inhibition of the efflux of radiolabeled or fluorescent P-gp substrates (Doppenschmitt et al., 1998; Wang et al., 2000; Eneroth et al., 2001). When radiolabeled ligands are used, the scintillation proximity assay is a useful tool for the sequential detection of radioactivity in the 96-well plate format (Fernandes, 1998). Also shown are assays measuring drug-stimulated ATPase activity in human P-gp-expressing cells (Polli et al., 2001), whereas the monolayer transport assay is regarded as the standard for identifying P-gp substrates because it measures efflux in the most direct manner. However, monolayer transport assays are relatively labor-intensive due to the cell culture and analytical requirements, which limit assay throughput. Indirect assays offer higher throughput, a generic readout (release of inorganic phosphate or increase in fluorescence or radioactivity), and are readily automated. However, these assays are not designed to distinguish P-gp substrates from inhibitors and do not directly measure transport. Polli et al. have compared assays used to determine whether compounds are P-gp substrates (Polli et al., 2001). Sixty-six compounds were tested in a transcellular transport assay using an MDR1-transfected cell monolayer and an inhibition assay for calcein-AM uptake. Although more than half of the compounds exhibited concordance across the assays, there were compounds that exhibited interassay differences that related to their apparent permeability (P_{app}).

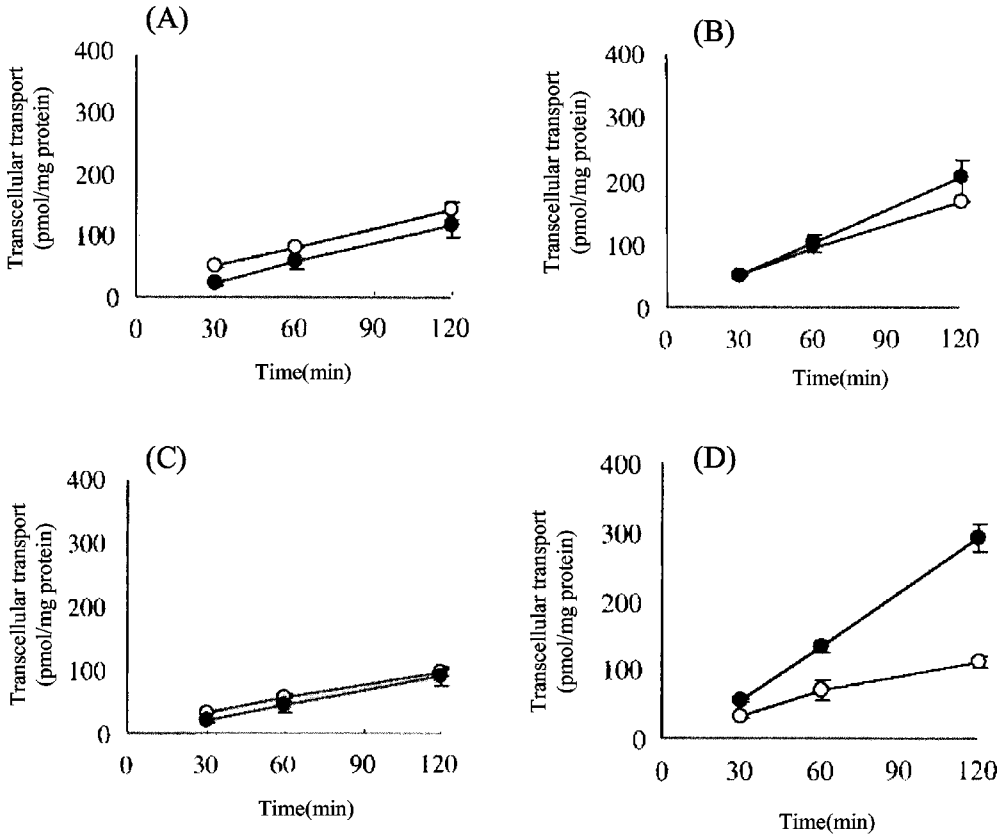


FIG. 10. Time profiles for the transcellular transport of [^{3}H]pravastatin across MDCK II monolayers. Transcellular transport of [^{3}H]pravastatin (1 μM) across MDCK II monolayers expressing OATP-C (B), MRP2 (C), and both OATP-C and MRP2 (double transfectant, D) was compared with that across the control MDCK II monolayer (A). Open and closed circles represent the transcellular transport in the apical-to-basal and basal-to-apical directions, respectively (Sasaki et al., 2002).

All assays detected substrates across a broad range of P_{app} values but the monolayer efflux assay was more prone to fail with high-P_{app} compounds, whereas the calcein-AM assay was more prone to fail with low-to-moderate P_{app} compounds. In the calcein-AM assay, tested compounds cannot inhibit calcein transport via P-gp unless they enter the cells and, thus, it may be difficult to observe P-gp activity with low-P_{app} compounds. As shown in eq. 1 in Fig. 8, P-gp activity is estimated as the ratio of P-gp clearance to passive permeability clearance in the monolayer efflux assay. Therefore, highly permeable compounds may be difficult to detect due to the masking of transport via P-gp. We need to choose suitable assays, depending on the properties of the drug candidates or the purpose of the evaluation. The monolayer efflux assay is more reliable at low-to-moderate P_{app} values and is the method of choice for evaluating drug candidates, despite the relatively low throughput and reliance on LC/MS/MS. In addition, computational (in silico) studies of transporter activity are being studied intensively. An attempt has been made to predict the transport activity of P-gp, MRP2, PEPT1, or ASBT from the structure or physicochemical parameters of compounds (Ekins et al., 2000; Seelig and Landwojtowicz, 2000; Han et al., 2001; Ekins et al., 2002a,b; Stouch and Gudmundsson, 2002). The in silico approach allows the design and optimization of the structures of drug candidates before their synthesis, resulting in an extremely efficient drug discovery process.

The prediction of pharmacokinetics in humans from an understanding of transport mechanisms should allow therapeutic agents to be used more safely. When there are species differences in transporters, the prediction of in vivo transport activity from in vitro data are important. Thus, methods allowing the rational prediction and extrapolation of in vivo drug disposition from in vitro data are also essential (Kusuhara and Sugiyama, 2001a). Since there are drugs that are recognized by several transporters localized on the same membrane, multiple transporters are expected to be involved in membrane transport of one drug. Therefore, the contribution of each transporter to the net membrane transport has to be taken into consideration when observations made in gene expression systems are extrapolated to in vivo situations. For example, Sugiyama et al. have estimated the contribution of each transporter to the efflux of E_2-17βG via the BBB using cDNA-transfected cells and specific inhibitors of each transporter (Table 15) (Sugiyama et al., 2001). Using the Brain Efflux Index method, the inhibitory effects of probenecid, taurocholate (TCA), PAH, and digoxin on the total efflux of

TABLE 15
Efflux transport of estradiol-17β-glucuronide from the brain across the blood-brain barrier in rats (Sugiyama et al., 2001)

	Effects of Each Inhibitor (K_i) on the uptake of [^{3}H]$E_2$17βG into Gene-Transfected LLC-PK1 Cells				Maximum Inhibitory Effects of Each Inhibitor on the Efflux of [^{3}H]$E_2$17βG from the Brain in Rats[a]
	Oatp1	Oatp2	Oat1	Oat3	
[^{3}H]$E_2$17βG	K_m = 2.58	K_m = 17.0	Not Transported	K_m = 8.43	
Probenecid	74.4	72.9	31.0[b]	20.0	100%
TCA	10.8	39.4	2770[b]	790	100%
PAH	>5000	>5000	K_m = 85.1[b]	301	20%
Digoxin	>300	0.037	>330[b]	>330	40%

[a] Effects at the maximum inhibitor concentration on the efflux of [^{3}H]E_2-17βG from the brain after microinjection into the rat cerebrum.
[b] Effects on the uptake of [^{3}H]PAH into Oat1-transfected LLC-PK1 cells.

E_2-17βG from the brain were investigated. Probenecid and TCA inhibited the elimination of E_2-17βG via the BBB completely, whereas PAH and digoxin reduced the total efflux to about 80% and 60% of the control value, respectively. The selectivity of these inhibitors was confirmed by examining their inhibitory effects on the transport via each type of organic anion transporter gene-transfected cell. Digoxin specifically inhibited the transport via Oatp2, TCA inhibited Oatp1 and Oatp2, PAH inhibited Oat1 and Oat3, and probenecid inhibited all these transporters. Taking the selectivity of these inhibitors into consideration, the maximum contribution made by the Oatp2 and Oat family to the total efflux of E_2-17βG from the brain appears to be about 40 and 20%, respectively. A similar analysis has been applied to the renal uptake mechanism of PAH and pravastatin (Hasegawa et al., 2002). Furthermore, the contribution of rat Oat1 and Oat3 to the total renal uptake of anionic compounds and nucleoside derivatives has been examined (Hasegawa et al., 2003). The uptake of test compounds was investigated using kidney slices from male rats and rOat1- and rOat3-expressed LLC-PK1 cells. The uptake clearance of test compounds by kidney slices was compared with the value predicted from the transport activity by cDNA transfectants using PAH and pravastatin as reference compounds. The renal uptake of PAH and pravastatin was predominantly accounted for by rat Oat1 and Oat3, respectively (Hasegawa et al., 2002), and these drugs can be used as reference compounds for rat Oat1 and Oat3. The Oatp family is responsible for the hepatic uptake of pravastatin. Thus, pravastatin is taken up by the liver and kidney via different transporters. Furthermore, it is suggested that, using specific inhibitors, rat Oat3 is mainly responsible for the uptake of benzylpenicillin and PAH by the choroid plexus and the efficient removal of its substrates from the cerebrospinal fluid (Nagata et al., 2002).

Recently, drug-drug interactions involving drug transporters and genetic polymorphisms of drug transporters have been described. The changes in pharmacokinetics due to genetic polymorphisms and drug-drug interactions can often directly affect the therapeutic safety and efficacy of many important drugs. Due to the rapid progress in the analysis of SNPs, it is likely that functionally relevant SNPs will be found for many transporters in the near future. Furthermore, the SNP mutations in the promoter/enhancer region of transporters and/or those in the DNA binding proteins (such as PXR) may be taken into consideration in accounting for interindividual differences in the expression level of transporters (Forman, 2001; Hustert et al., 2001; Zhang et al., 2001). Since the substrate specificity of drug transporters is generally broad and multispecific, if the strategy of targeting specific transporters is adopted then one should develop drug candidates that take into consideration the possibility of drug-drug interactions involving transporters. If the genetic polymorphisms and drug-drug interactions involving transporters mentioned above are likely, it will be crucial to predict quantitatively the degree of any changes in pharmacokinetics caused by these factors. Pharmaceutical companies need to identify which drug candidates are substrates for which transporters, and should investigate the contribution of each transporter to total transport by integrating the data from transporter gene-transfected cells. Providing this information to clinicians should lead to the safer use of drugs.

Current information regarding the molecular and cellular aspects of drug transporters has grown steadily and encouraged studies of the mechanisms of drug disposition. Clarification of the role of each transporter in drug disposition in vivo is of potential importance. The information on substrate selectivity and tissue distribution of the drug transporters will aid in the prediction of the in vivo kinetic profile of drugs from in vitro data. Research on drug transporters will lead to the more efficient development of new safer and more effective drugs.

References

Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, Nomura H, Unno M, Suzuki M, Naitoh T, et al. (1999) Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J Biol Chem* **274:**17159–17163.

Abu-Zahra TN, Wolkoff AW, Kim RB, and Pang KS (2000) Uptake of enalapril and expression of organic anion transporting polypeptide 1 in zonal, isolated rat hepatocytes: role of organic anion transporting polypeptide. *Drug Metab Dispos* **28:**801–806.

Achim CL, Wang R, and Miners DK (1994) Brain viral burden in HIV infection. *J Neuropathol Exp Neurol* **53:**284–294.

Adachi Y, Suzuki H, and Sugiyama Y (2001) Comparative studies on in vitro methods for evaluating in vivo function of MDR1 P-glycoprotein. *Pharm Res (NY)* **18:**1660–1668.

Akita H, Suzuki H, Ito K, Kinoshita S, Sato N, Takikawa H, and Sugiyama Y (2001) Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. *Biochim Biophys Acta* **1511:**7–16.

Alexandridis G, Pappas GA, and Elisaf MS (2000) Rhabdomyolysis due to combination therapy with cerivastatin and gemfibrozil. *Am J Med* **109:**261–262.
Alsenz J, Steffen H, and Alex R (1998) Active apical secretory efflux of the HIV protease inhibitors saquinavir and ritonavir in Caco-2 cell monolayers. *Pharm Res (NY)* **15:**423–428.
Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, Thornton N, Folayan GO, Githangs J, Indalo A, et al. (2001) MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics* **11:**217–221.
Amsden GW, Nafziger AN, Foulds G, and Cabelus LJ (2000) A study of the pharmacokinetics of azithromycin and nelfinavir when coadministrated in healthy volunteers. *J Clin Pharmacol* **40:**1522–1527.
Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorg DJ, and Suchy FJ (2001) Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem* **276:**28857–28865.
Andreana A, Aggarwal S, Gollapudi S, Wien D, Tsuruo T, and Gupta S (1996) Abnormal expression of a 170-kilodalton P-glycoprotein encoded by MDR1 gene, a metabolically active efflux pump, in CD4+ and CD8+ T cells from patients with human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses* **11:**893–901.
Apiwattanakul N, Sekine T, Chairoungdua A, Kanai Y, Nakajima N, Sophasan S, and Endou H (1999) Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in Xenopus laevis oocytes. *Mol Pharmacol* **55:**847–854.
Araki E, Ishikawa M, Iigo M, Koide T, Itabashi M, and Hoshi A (1993) Relationship between development of diarrhea and the concentration of SN-38, an active metabolite of CPT-11, in the intestine and the blood plasma of athymic mice following intraperitoneal administration of CPT-11. *Jpn J Cancer Res* **84:**697–702.
Asaba H, Hosoya K, Takanaga H, Ohtsuki S, Tamura E, Takizawa T, and Terasaki T (2000) Blood-brain barrier is involved in the efflux transport of a neuroactive steroid, dehydroepiandrosterone sulfate, via organic anion transporting polypeptide 2. *J Neurochem* **75:**1907–1916.
Ayrton A and Morgan P (2001) Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica* **31:**469–497.
Balimane PV, Tamai I, Guo A, Nakanishi T, Kitada H, Leibach FH, Tsuji A, and Shinko PJ (1998) Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. *Biochem Biophys Res Commun* **250:**246–251.
Barone GW, Gurley BJ, Ketel BL, Lightfoot ML, and Abul-Ezz SR (2000) Drug interaction between St. John's wort and cyclosporine. *Ann Pharmacother* **34:**1013–1016.
Bergwerk AJ, Shi X, Ford AC, Kanai N, Jacquemin E, Burk RD, Bai S, Novikoff PM, Stieger B, Meier PJ, et al. (1996) Immunologic distribution of an organic anion transport protein in rat liver and kidney. *Am J Physiol Gastrointest Liver Physiol* **271:**G231–G238.
Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P, and Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci USA* **95:**12208–12213.
Beuers U, Bilzer M, Chittattu A, Kullak-Ublick GA, Keppler D, Paumgaetner G, and Dombrowski F (2001) Tauroursodeoxycholic acid inserts the apical conjugate export pump, Mrp2, into canalicular membranes and stimulates organic anion secretion by protein kinase C-dependent mechanisms in cholestatic rat liver. *Hepatology* **35:**1206–1216.
Bezprozvanny I and Maximov A (2001) PDZ domains: more than just a glue. *Proc Natl Acid Sci USA* **98:**787–789.
Blumberg B, Sabbagh W Jr, Jugulion H, Bolado J Jr, van Meter CM, Ong ES, and Evans RM (1998) SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev* **12:**3195–3205.
Borst P, Evers R, Kool M, and Wijnholds J (1999) The multidrug resistance protein family. *Biochim Biophys Acta* **1461:**347–357.
Boyd RA, Stern RH, Stewart BH, Wu X, Reyner EL, Zegarac EA, Randinitis EJ, and Whitfield L (2000) Atrovastatin coadministration may increase digoxin concentrations by inhibition of intestinal P-glycoprotein-mediated secretion. *J Clin Pharmacol* **40:**91–98.
Breidert T, Spitzenberger F, Grundemann D, and Schomig E (1998) Catecholamine transport by the organic cation transporter type 1 (OCT1). *Br J Pharmacol* **125:**218–224.
Britten CD, Baker SD, Denis LJ, Johnson T, Drengler R, Siu LL, Duchin K, Kuhn J, and Rowinsky EK (2000) Oral paclitaxel and concurrent cyclosporin A: targeting clinically relevant systemic exposure to paclitaxel. *Clin Cancer Res* **6:**3459–3468.
Briz O, Serrano MA, Rebollo N, Hagenbuch B, Meier PJ, Koepsell H, and Marin JJG (2002) Carriers involved in targeting the cytostatic bile acid-cisplatin derivatives *cis*-diammine-chloro-cholylglycinate-platinum(II) and *cis*-diammine-bisursodeoxycholate-platinum(II) toward liver cells. *Mol Pharmacol* **61:**853–860.
Bu H, Poglod M, Micetich RG, and Khan JK (2000) High-throughput Caco-2 cell permeability screening by cassette dosing and sample pooling approaches using direct injection/on-line guard cartridge extraction/tandem mass spectrometry. *Rapid Commun Mass Spectrom* **14:**523–528.
Buist SCN, Cherrington NJ, Choudhuri S, Hartley DP, and Klaassen CD (2002) Gender-specific and developmental influence on the expression of rat organic anion transporters. *J Pharmacol Exp Ther* **301:**145–151.
Burckhardt G and Wolff NA (2000) Structure of renal organic anion and cation transporters. *Am J Physiol Renal Physiol* **278:**F853–F866.
Calvo MV, Martin-Suarez A, Martin Luengo C, Avila C, Cascon M, and Dominguez-Gil Hurle A (1989) Interaction between digoxin and propafenone. *Ther Drug Monit* **11:**10–15.
Cascorbi I, Gerlogg T, Johne A, Meisel C, Hoffmeyer S, Schwab M, Schaeffeler E, Eichelbaum M, Brinkmann U, and Roots I (2001) Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* **69:**169–174.
Cha SH, Sekine T, Fukushima JI, Kanai Y, Kobayashi Y, Goya T, and Endou H (2001) Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol Pharmacol* **59:**1277–1286.
Chang T, Benet LZ, and Hebert MF (1996) The effect of water-soluble vitamin E on cyclosporine pharmacokinetics in healthy volunteers. *Clin Pharmacol Ther* **59:**297–303.
Charatan F (2001) Bayer decides to withdraw cholesterol lowering drug. *BMJ* **323:**359.
Choo EF, Leake B, Wandel C, Imamura H, Wood AJ, Wilkinson GR, and Kim RB (2000) Pharmacological inhibition of p-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug Metab Dispos* **28:**655–660.
Chu XY, Kato Y, Niinuma K, Sudo KI, Hakusui H, and Sugiyama Y (1997b) Multispecific organic anion transporter (cMOAT) is responsible for the biliary excretion of the camptothecin derivative irinotecan, CPT-11 and its metabolites in rats. *J Pharmacol Exp Ther* **281:**304–314.
Chu XY, Kato Y, and Sugiyama Y (1997a) Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan, CPT-11 and its metabolites in rats. *Cancer Res* **57:**1934–1938.
Chu X, Kato Y, Ueda K, Suzuki H, Niinuma K, Tyson CA, Weizer V, Dabbs JE, Froehlich R, Green CE, et al. (1998) Biliary excretion mechanism of CPT-11 and its metabolites in humans: involvement of primary active transporters. *Cancer Res* **58:**5137–5143.
Cihlar T and Ho ES (2000) Fluorescence-based assay for the interaction of small molecules with the human renal organic anion transporter 1. *Anal Biochem* **283:**49–55.
Cihlar T, Ho ES, Lin DC, and Mulato AS (2001) Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. *Nucleosides Nucleotides* **20:**641–648.
Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, and Sweet DH (1999) The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol Pharmacol* **56:**570–580.
Cobbe SM (1997) Using the right drug. A treatment algorithm for atrial fibrillation. *Eur Heart J* **18(Suppl C):**33–39.
Cole SP and Deeley RG (1998) Multidrug resistance mediated by the ATP-binding cassette transporter protein MRP. *Bioessays* **20:**931–940.
Cui Y, Konig J, and Keppler D (2001a) Vectorial transport by double-transfected cells expressing the human uptake transporter SLC21A8 and the apical export pump ABCC2. *Mol Pharmacol* **60:**934–943.
Cui Y, Konig J, Leier I, Buchholz U, and Keppler D (2001b) Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. *J Biol Chem* **276:**9626–9630.
Cummins CL, Jacobsen W, and Benet LZ (2002) Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. *J Pharmacol Exp Ther* **300:**1036–1045.
Cvetkovic M, Leake B, Fromm MF, Wilkinson GR, and Kim RB (1999) OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos* **27:**866–871.
Dantzig AH, Law KL, Cao J, and Starling IJ (2001) Reversal of multidrug resistance by the P-glycoprotein modulator, LY335989, from the bench to the clinic. *Curr Med Chem* **8:**39–50.
Davit B, Reynolds K, Yuan R, Ajayi F, Conner D, Fadiran E, Gillespir B, Sahajwalla C, Huang S, and Lesko LJ (1999) FDA evaluations using in vitro metabolism to predict and interpret in vivo metabolic drug-drug interactions: Impact on labeling. *J Clin Pharmacol* **39:**899–910.
de Bruin M, Miyake K, Litman T, Robey R, and Bates SE (1999) Reversal of resistance by GF120918 in cell lines expressing the ABC half-transporter, MXR. *Cancer Lett* **146:**117–126.
de Lange ECM, de Bock G, Schinkel AH, de Boer AG, and Breimer DD (1998) BBB transport and P-glycoprotein functionality using MDR1a (−/−) and wild-type mice. Total brain versus microdialysis concentration profiles of Rhodamine-123. *Pharm Res (NY)* **15:**1657–1665.
Desrayaud S, de Range ECM, Lemaire M, Bruelisauer A, de Boer AG, and Breimer DD (1998) Effect of the mdr1a P-glycoprotein gene disruption on the tissue distribution of SDZ PSC 833, a multidrug resistance-reversing agent, in mice. *J Pharmacol Exp Ther* **285:**438–443.
Doan KMM, Humphreys JE, Webster LO, Wring SA, Shampine LJ, Serabjit-Singh CJ, Adkison KK, and Polli JW (2002) Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J Pharmacol Exp Ther* **303:**1029–1037.
Dominguez MF, Macias RIR, Izco-Basurko I, Fuente ADL, Pascual MJ, Craido JM, Monte MJ, Yajeya J and Marin JJG (2001) Low in vivo toxicity of a novel cisplatin-ursodeoxycholic derivative (Bamet-UD2) with enhanced cytostatic activity versus liver tumors. *J Pharmacol Exp Ther* **297:**1106–1112.
Donner MG and Keppler D (2001) Up-regulation of basolateral multidrug resistance protein 3 (Mrp3) in cholestatic rat liver. *Hepatology* **34:**351–359.
Doppenschmitt S, Spahn-Langguth H, Regardh CG, and Langguth P (1998) Radioligand-binding assay employing P-glycoprotein-overexpressing cells: testing drug affinities to the secretory intestinal multidrug transporter. *Pharm Res (NY)* **15:**1001–1006.
Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, and Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* **95:**15665–15670.
Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, and Kim RB (2002) Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* **71:**11–20.
Dresser MJ, Leabman MK, and Giacomini KM (2001) Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters. *J Pharm Sci* **90:**397–421.
Durr D, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, and

Fattinger K (2000) St. John's wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin Pharmacol Ther* **68:**598–604.
Dussault I, Lin M, Hollister K, Wang EH, Synold TW, and Forman BM (2001) Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. *J Biol Chem* **276:**33309–33312.
Ekins S and Erickson JA (2002) A pharmacophore for human pregnane X receptor ligands. *Drug Metab Dispos* **30:**96–99.
Ekins S, Kim RB, Leake BF, Dantzig AH, Schuetz EG, Lan L, Yasuda K, Shepard RL, Winter MA, Schuetz JD, et al. (2002a) Three-dimensional quantitative structure-activity relationships of inhibitors of P-glycoprotein. *Mol Pharmacol* **61:**964–973.
Ekins S, Kim RB, Leake BF, Dantzig AH, Schuetz EG, Lan L, Yasuda K, Shepard RL, Winter MA, Schuetz JD, et al. (2002b) Application of three-dimensional quantitative structure-activity relationships of P-glycoprotein inhibitors and substrates. *Mol Pharmacol* **61:**974–981.
Ekins S, Mirny L, and Schuetz EG (2002c) A ligand-based approach to understanding selectivity of nuclear hormone receptors PXR, CAR, FXR, LXRα and LXRβ. *Pharm Res (NY)* **19:**1788–1800.
Ekins S, Waller CL, Swaan PW, Cruciani G, Wrignton SA, and Wikel JH (2000) Progress in predicting human ADME parameters in silico. *J Pharmacol Toxicol Methods* **44:**251–272.
Eneroth A, Astrom E, Hoogstaate J, Schrenk D, Conrad S, Kauffmann HM, and Gjellan K (2001) Evaluation of a vincristine resistant Caco-2 cell line for use in a calcein AM extrusion screening assay for P-glycoprotein interaction. *Eur J Pharm Sci* **12:**205–214.
Faber KN, Muller M, and Jansen PLM (2003) Drug transport proteins in the liver. *Adv Drug Deliv Rev* **55:**107–124.
Fernandes PB (1998) Technological advances in high-throughput screening. *Curr Opin Chem Biol* **2:**597–603.
Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Pojer C, Zenz R, Lammert F, Stieger B, Meier PJ, Zatloukal K, et al. (2001) Effects of ursodeoxycholic and cholic acid feeding on hepatocellular transporter expression in mouse liver. *Gastroenterology* **121:**170–183.
Fitzsimmons ME and Collins JM (1997) Selective biotransformation of the human immunodeficiency virus protease inhibitor saquinavir by human small-intestinal cytochrome P4503A4: potential contribution to high first-pass metabolism. *Drug Metab Dispos* **25:**256–266.
Floren LC, Bekersky I, Benet LZ, Mekki Q, Dressler D, Lee JW, Roberts JP, and Hebert MF (1997) Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. *Clin Pharmacol Ther* **62:**41–49.
Forman BM (2001) Polymorphisms in promiscuous PXR: an explanation for inter-individual differences in drug clearance? *Pharmacogenetics* **11:**551–552.
Freeman DJ, Martell R, Carruthers SG, Heinrichs D, Keown PA, and Stiller CR (1987) Cyclosporin-erythromycin interaction in normal subjects. *Br J Clin Pharmacol* **23:**776–778.
Fromm MF (2000) P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. *Int J Clin Pharmacol Ther* **38:**69–74.
Fromm MF (2002) The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. *Adv Drug Deliv Rev* **54:**1295–1310.
Fromm MF, Kauffmann HM, Fritz P, Burk O, Kroemer HK, Warzok RW, Eichelbaum M, Siegmund W, and Schrenk D (2000) The effect of rifampin treatment on intestinal expression of human MRP transporters. *Am J Pathol* **157:**1575–1580.
Funk C, Pantze M, Jehle L, Ponelle C, Scheuermann G, Lazendic M, and Gasser R (2001a) Troglitazone-induced intrahepatic cholestasis by an interference with the hepatobiliary export of bile acids in male and female rats. Correlation with the fender difference in troglitazone sulfate formation and the inhibition of the canalicular bile salt export pump (Bsep) by troglitazone and troglitazone sulfate. *Toxicology* **167:**83–98.
Funk C, Ponelle C, Scheuermann G, and Pantze M (2001b) Cholestatic potential of troglitazone as a possible factor contributing to troglitazone-induced hepatotoxicity: in vivo and in vitro interaction at the canalicular bile salt export pump (Bsep) in the rat. *Mol Pharmacol* **59:**627–635.
Gao B, Hagenbuch B, Kullak-Ublick GA, Benke D, Aguzzi A, and Meier PJ (2000) Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J Pharmacol Exp Ther* **294:**73–79.
Gao B and Meier PJ (2001) Organic anion transport across the choroid plexus. *Microsc Res Tech* **52:**60–64.
Gao J, Murase O, Schowen RL, Aube J, and Borchardt RT (2001) A functional assay for quantitation of the apparent affinities of ligands of P-glycoprotein in Caco-2 cells. *Pharm Res (NY)* **18:**171–176.
Gao B, Stieger B, Noe B, Fritschy JM, and Meier PJ (1999) Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. *J Histochem Cytochem* **47:**1255–1264.
Geick A, Eichelbaum M, and Burk O (2001) Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* **276:**14581–14587.
Gerloff T, Stieger B, Hagenbuch B, Madon J, Landmann L, Roth J, Hofmann AF, and Meier PJ (1998) The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. *J Biol Chem* **273:**10046–10050.
Gomi A, Masuzawa T, Ishikawa T, and Kuo MT (1997) Posttranscriptional regulation of MRP/GS-X pump and gamma-glutamylcysteine synthetase expression by 1-(4-amino-2-methyl-5-pyrimidinyl) methyl-3-(2-chloroethyl)-3-nitrosourea and by cycloheximide in human glioma cells. *Biochem Biophys Res Commun* **239:**51–56.
Gorboulev V, Ulzheimer JC, Akhoundova A, Ulzheimer-Teuber I, Karbach U, Quester S, Baumann C, Lang F, Busch AE, and Koepsell H (1997) Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol* **16:**871–881.
Gotoh Y, Kato Y, Stieger B, Meier PJ, and Sugiyama Y (2002) Gender difference in the Oatp1-mediated tubular reabsorption of estradiol 17β-D-glucuronide in rats. *Am J Physiol Endocrinol Metab* **282:**E1245–E1254.
Gotoh Y, Suzuki H, Kinoshita S, Hirohashi T, Kato Y, and Sugiyama Y (2000) Involvement of an organic anion transporter (canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2) in gastrointestinal secretion of glutathione conjugates in rats. *J Pharmacol Exp Ther* **292:**433–439.
Greiner B, Eichelbaum M, Fritz P, Kreichigauer HP, von Richter O, Zundler J, and Kroemer HK (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Investig* **104:**147–153.
Groothuis DR and Levy RM (1997) The entry of antiviral and antiretroviral drugs into the central nervous system. *J Neuroviral* **3:**387–400.
Grub S, Bryson H, Goggin T, Ludin E, and Jorga K (2001) The interaction of saquinavir (soft gelatin capsule) with ketoconazole, erythromycin and rifampicin: comparison of the effect in healthy volunteers and in HIV-infected patients. *Eur J Clin Pharmacol* **57:**115–121.
Grundemann D, Gorboulev V, Gambaryan M, Veyhl M, and Koepsell H (1994) Drug excretion mediated by a new prototype of polyspecific transporter. *Nature (Lond)* **372:**549–552.
Guo GL, Staudinger J, Ogura K, and Klaassen CD (2002) Induction of rat organic anion transporting polypeptide 2 by pregnenolone-16alpha-carbonitrile is via interaction with pregnane X receptor. *Mol Pharmacol* **61:**832–839.
Gupta SK, Bakran A, Johnson RWG, and Rowland M (1988) Erythromycin enhances the absorption of cyclosporin. *Br J Clin Pharmacol* **25:**401–402.
Gupta SK, Bakran A, Johnson RWG, and Rowland M (1989) Cyclosporin-erythromycin interaction in renal transplant patients. *Br J Clin Pharmacol* **27:**475–481.
Hagenbuch B, Gao B, and Meier PJ (2002) Transport of xenobiotics across the blood-brain barrier. *News Physiol Sci* **17:**231–234.
Hagenbuch B and Meier PJ (2003) The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* **1609:**1–18.
Hagenbuch B, Stieger B, Foguet M, Lubbert H, and Meier PJ (1991) Functional expression cloning and characterization of the hepatocyte Na+/bile acid cotransport system. *Proc Natl Acad Sci USA* **88:**10629–10633.
Hamman MA, Bruce MA, Haehner-Daniels BD, and Hall SD (2001) The effect of rifampin administration on the disposition of fexofenadine. *Clin Pharmacol Ther* **69:**114–121.
Han Y, Kato Y, Harashima M, Ohta M, Matsuoka H, and Sugiyama Y (2001) Physicochemical parameters responsible for the affinity of methotrexate analogs for rat canalicular multispecific organic anion transporter (cMOAT/MRP2). *Pharm Res (NY)* **18:**579–586.
Harris MJ, Kuwano M, Webb M, and Board PG (2001) Identification of the apical membrane targeting signal of the multidrug resistance-associated protein 2 (MRP2/MOAT). *J Biol Chem* **276:**20876–20881.
Hasegawa M, Kusuhara H, Endou H, and Sugiyama Y (2003) Contribution of organic anion transporters to the renal uptake of anionic compounds and nucleoside derivatives. *J Pharmacol Exp Ther* **305:**1087–1097.
Hasegawa M, Kusuhara H, Sugiyama D, Ito K, Ueda S, Endou H, and Sugiyama Y (2002) Functional involvement of rat organic anion transporter 3 (rOAT3) in the renal uptake of organic anions. *J Pharmacol Exp Ther* **300:**746–753.
Hashida T, Masuda S, Umemoto S, Saito H, Tanaka K, and Inui K (2001) Pharmacokinetic and prognostic significance of intestinal MDR1 expression in recipients of living-donor liver transplantation. *Clin Pharmacol Ther* **69:**308–316.
Hebert MF, Fisher RM, Marsh CL, Dressler D, and Bekersky I (1999) Effects of rifampin on tacrolimus pharmacokinetics in healthy volunteers. *J Clin Pharmacol* **39:**91–96.
Hebert MF and Lam AY (1999) Diltiazem increases tacrolimus concentrations. *Ann Pharmacother* **33:**680–682.
Hedman A, Angelin B, Arvidsson A, Beck O, Dahlqvist R, Nilsson B, Olsson M, and Schenck-Gustafsson K (1991) Digoxin-verapamil interaction: reduction of biliary but not renal digoxin clearance in humans. *Clin Pharmacol Ther* **49:**256–262.
Hedman A, Angelin B, Arvidsson A, Dahlqvist R, and Nilsson B (1990) Interactions in the renal and biliary elimination of digoxin: stereoselective difference between quinine and quinidine. *Clin Pharmacol Ther* **47:**20–26.
Hendrikse NH, Bart J, de Vries EG, Groen HJ, van der Graaf WT, and Vaalburg W (2001) P-glycoprotein at the blood-brain barrier and analysis of drug transport with positron-emission tomography. *J Clin Pharmacol* **(Suppl):**48S–54S.
Hendrikse NH, Schinkel AH, De Vries EGE, Fluks E, Van der Graaf WTA, Willemsen ATM, Vaalburg W, and Franssen EJF (1998) Complete in vivo reversal of P-glycoprotein pump function in the blood-brain barrier visualized with positron emission tomography. *Br J Pharmacol* **124:**1413–1418.
Henney JE (2000) Risk of drug interactions with St. John's wort. *J Am Med Assoc* **283:**1679.
Hirohashi T, Suzuki H, Chu XY, Tamai I, Tsuji A, and Sugiyama Y (2000a) Functional and expression of multidrug resistance-associated protein family in human colon adenocarcinoma cells (Caco-2). *J Pharmacol Exp Ther* **292:**265–270.
Hirohashi T, Suzuki H, Ito K, Ogawa K, Kume K, Shimizu T, and Sugiyama Y (1998) Hepatic expression of multidrug resistance-associated protein-like proteins maintained in Eisai hyperbilirubinemic rats. *Mol Pharmacol* **53:**1068–1075.
Hirohashi T, Suzuki H, and Sugiyama Y (1999) Characterization of the transport properties of cloned rat multidrug resistance-associated protein 3 (Mrp3). *J Biol Chem* **274:**15181–15185.
Hirohashi T, Suzuki H, Takikawa H, and Sugiyama Y (2000b) ATP-dependent transport of bile salts by rat multidrug resistance-associated protein 3 (Mrp3). *J Biol Chem* **275:**2905–2910.
Hoetelmans RMW (1998) Sanctuary site in HIV-1 infection. *Antivir Ther* **3 (Suppl 4):**13–17.
Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, et al. (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* **97:**3473–3478.
Hooiveld GJ, van Montfoort JE, Meijer DK, and Muller M (2001) Function and regulation of ATP-binding cassette transport proteins involved in hepatobiliary transport. *Eur J Pharm Sci* **12:**525–543.
Hori R, Tomita Y, Katsura T, Yasuhara M, Inui K, and Takano M (1993) Transport

of bestatin in rat renal brush-border membrane vesicles. *Biochem Pharmacol* **45:**1763–1768.
Horikawa M, Kato Y, and Sugiyama Y (2002a) Reduced gastrointestinal toxicity in rats following inhibition of the biliary excretion of irinotecan and its metabolites by probenecid. *Pharm Res (NY)* **19:**1345–1353.
Horikawa M, Kato Y, Tyson CA, and Sugiyama Y (2002b) The potential for an interaction between MRP2 (ABCC2) and various therapeutic agents: Probenecid as a candidate inhibitor of the biliary excretion of irinotecan metabolites. *Drug Metabol Pharmacokin* **17:**23–33.
Horikawa M, Kato Y, Tyson CA, and Sugiyama Y (2003) Potential cholestatic activity of various therapeutic agents assessed by bile canalicular membrane vesicles isolated from rats and humans. *Drug Metab Pharmacokinet* **18:**16–22.
Hosoyamada M, Sekine T, Kanai Y, and Endou H (1999) Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am J Physiol Renal Physiol* **276:**F122–F128.
Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, and Kirchgessner TG (1999) A novel human hepatic organic anion transporting polypeptide (OATP2). *J Biol Chem* **274:**37161–37168.
Hsu A, Granneman R, Cao G, Carothers L, El-Shourbagy T, Baroldi P, Erdman K, Brown F, Sun E, and Leonard JM (1998) Pharmacokinetic interactions between two human immunodeficiency virus protease inhibitors, ritonavir and saquinavir. *Clin Pharmacol Ther* **63:**453–464.
Huisman MT, Smit JW, and Schinkel AH (2000) Significance of P-glycoprotein for the pharmacology and clinical use of HIV protease inhibitors. *AIDS* **14:**237–242.
Huisman MT, Smit JW, Wiltshire HR, Beijnen JH, and Schinkel AH (2003) Assessing safety and efficacy of directed P-glycoprotein inhibition to improve the pharmacokinetic properties of saquinavir coadministered with ritonavir. *J Pharmacol Exp Ther* **304:**596–602.
Huisman MT, Smit JW, Wiltshire HR, Hoetelmans RMW, Beijnen JH, and Schinkel AH (2001) P-glycoprotein limits oral availability, brain and fetal penetration of saquinavir even with high doses of ritonavir. *Mol Pharmacol* **59:**806–813.
Hunter J and Hirst BH (1997) Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption. *Adv Drug Deliv Rev* **25:**129–157.
Hustert E, Zibat A, Presecan-Siedel E, Eiselt R, Mueller R, Fuss C, Brehm I, Brinkmann U, Eichelbaum M, Wojnowski L, et al. (2001) Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. *Drug Metab Dispos* **29:**1454–1459.
Iida A, Saito S, Sekine S, Mishima C, Kondo K, Kitamura Y, Harigae S, Osawa S, and Nakamura Y (2001) Catalog of 258 single-nucleotide polymorphisms (SNPs) in genes encoding three organic anion transporters, three organic anion-transporting polypeptides and three NADH:ubiquinone oxidoreductase flavoproteins. *J Hum Genet* **46:**668–683.
International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature (Lond)* **409:**860–921.
Inui K, Masuda S, and Saito H (2000a) Cellular and molecular aspects of drug transport in the kidney. *Kidney Int* **58:**944–958.
Inui K, Terada T, Masuda S, and Saito H (2000b) Physiological and pharmacological implications of peptide transporters, PEPT1 and PEPT2. *Nephrol Dial Transplant* **15:**11–13.
Ishikawa T (1992) The ATP-dependent glutathione S-conjugate export pump. *Trends Biochem Sci* **17:**463–468.
Ishikawa T, Bao JJ, Yamane Y, Akimaru K, Frindrich K, Wright CD, and Kuo MT (1996) Coordinate induction of MRP/GS-X pump and gamma-glutamylcysteine synthetase by heavy metals in human leukemia cells. *J Biol Chem* **271:**14981–14988.
Ishikawa T, Kuo MT, Furuta K, and Suzuki M (2000) The human multidrug resistance-associated protein (MRP) gene family: from biological function to drug molecular design. *Clin Chem Lab Med* **38:**893–897.
Ishizuka H, Konno K, Naganuma H, Nishimura K, Kouzuki H, Suzuki H, Stieger B, Meier PJ, and Sugiyama Y (1998) Transport of temocaprilat into rat hepatocytes: role of organic anion transporting polypeptide. *J Pharmacol Exp Ther* **287:**37–42.
Ishizuka H, Konno K, Naganuma H, Sasahara K, Kawahara Y, Niinuma K, Suzuki H, and Sugiyama Y (1997) Temocaprilat, a novel angiotensin converting enzyme inhibitor, is excreted into bile via an ATP-dependent active transporter (cMOAT) that is deficient in Eisai hyper-bilirubinemic mutant rats (EHBR). *J Pharmacol Exp Ther* **280:**1304–1311.
Ishizuka H, Konno K, Shiina T, Naganuma H, Nishimura K, Ito K, Suzuki H, and Sugiyama Y (1999) Species differences in the transport activity for organic anions across the bile canalicular membrane. *J Pharmacol Exp Ther* **290:**1324–1330.
Ismair MG, Stieger B, Cattori V, Hagenbuch B, Fried M, Meier PJ, and Kullak-Ublick GA (2001) Hepatic uptake of cholecystokinin octapeptide by organic anion-transporting polypeptides OATP4 and OATP8 of rat and human liver. *Gastroenterology* **121:**1185–1190.
Ito K, Iwatsubo T, Kanamitsu S, Ueda H, Suzuki H, and Sugiyama Y (1998a) Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* **50:**387–411.
Ito K, Suzuki H, Hirohashi T, Kume K, Shimizu T, and Sugiyama Y (1998b) Functional analysis of a canalicular multispecific organic anion transporter cloned from rat liver. *J Biol Chem* **273:**1684–1688.
Ito K, Suzuki H, Hirohashi T, Kume K, Shimizu T, and Sugiyama Y (1997) Molecular cloning of canalicular multispecific organic anion transporter defective in EHBR. *Am J Physiol Gastrointest Liver Physiol* **272:**G16–G22.
Jaehde U, Sorgel F, Reiter A, Sigl G, Naber KG, and Schunack W (1995) Effect of probenecid on the distribution and elimination of ciprofloxacin in humans. *Clin Pharmacol Ther* **58:**532–541.
Jalava KM, Partanen J, and Neuvonen PJ (1997) Itraconazole decreases renal clearance of digoxin. *Ther Drug Monit* **19:**609–613.
Jariyawat S, Sekine T, Takeda M, Apiwattanakul N, Kanai Y, Sophasan S, and Endou H (1999) The interaction and transport of beta-lactam antibiotics with the cloned rat renal organic anion transporter 1. *J Pharmacol Exp Ther* **290:**672–677.
Johne A, Brockmoller J, Bauer S, Maurer A, Langheinrich M, and Roots I (1999) Pharmacokinetic interaction of digoxin with an herbal extract from St. John's wort. *Clin Pharmacol Ther* **66:**338–345.
Jonker JW, Smit JW, Brinkhuis RF, Maliepaaed M, Beijnen JH, Schellens JHM, and Schinkel AH (2000) Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J Natl Cancer Inst* **92:**1651–1656.
Jonker JW, Wagenaar E, Mol CA, Buitelaar M, Koepsell H, Smit JW, and Schinkel AH (2001) Reduced hepatic uptake and intestinal excretion of organic cations in mice with a targeted disruption of the organic cation transporter 1 (Oct1 [Slc22a1]) gene. *Mol Cell Biol* **21:**5471–5477.
Jonker JW, Wagenaar E, van Deemter L, Gottschlich R, Bender HM, Dasenbrock J, and Schinkel AH (1999) Role of blood-brain barrier P-glycoprotein in limiting brain accumulation and sedative side-effects of asimadoline, a peripherally acting analgesic drug. *Br J Pharmacol* **127:**43–50.
Jung D, Hagenbush B, Gresh L, Pontoglio M, Meier PJ, and Kullak-Ublick GA (2001a) Characterization of the human OATP-C (SLC21A6) gene promoter and regulation of liver-specific OATP genes by hepatocyte nuclear factor 1 alpha. *J Biol Chem* **276:**37206–37214.
Jung KY, Takeda M, Kim DK, Tojo A, Narikawa S, Yoo BS, Hosoyamada M, Cha SH, Sekine T, and Endou H (2001b) Characterization of ochratoxin A transport by human organic anion transporters. *Life Sci* **69:**2123–2135.
Kakyo M, Unno M, Tokui T, Nakagomi R, Nishio T, Iwasashi H, Nakai D, Seki M, Suzuki M, Naitoh T, et al. (1999) Molecular characterization and functional regulation of a novel rat liver-specific organic anion transporter rlst-1. *Gastroenterology* **117:**770–775.
Kaneda N and Yokokura T (1990) Nonlinear pharmacokinetics of CPT-11 in rats. *Cancer Res* **50:**1721–1725.
Kaplan B, Meier-Kriesche HU, Napoli KL, and Kahan BD (1998) The effects of relative timing of sirolimus and cyclosporine microemulsion formulation coadministration on the pharmacokinetics of each agent. *Clin Pharmacol Ther* **63:**48–53.
Kartenbeck J, Leuschner U, Mayer R, and Keppler D (1996) Absence of the canalicular isoform of the MRP gene-encoded conjugate export pump from the hepatocytes in Dubin-Johnson syndrome. *Hepatology* **23:**1061–1066.
Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, and Edwards PA (2002) Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor and constitutive androstane receptor. *J Biol Chem* **277:**2908–2915.
Kato Y, Kuge K, Kusuhara H, Meier PJ, and Sugiyama Y (2002) Gender difference in the urinary excretion of organic anions in rats. *J Pharmacol Exp Ther* **302:**483–489.
Kauffmann HM, Pfannschmidt S, Zoller H, Benz A, Vorderstemann B, Webster JI, and Schrenk D (2002) Influence of redox-active compounds and PXR-activators on human MRP1 and MRP2 gene expression. *Toxicology* **171:**137–146.
Kaukonen KM, Olkkola KT, and Neuvonen PJ (1997) Itraconazole increases plasma concentrations of quinidine. *Clin Pharmacol Ther* **62:**510–517.
Kawahara I, Kato Y, Suzuki H, Achira M, Ito K, Crespi CL, and Sugiyama Y (2000) Selective inhibition of human cytochrome P450 3A4 by *N*-[2(*R*)-hydroxy-1(*S*)-indanyl]-5-[2(*S*)-(1,1-dimethylethylaminocarbonyl)-4-[(furo[2, 3-b]pyridin-5-yl)methyl]piperazin-1-yl]-4(*S*)-hydroxy-2(*R*)-phenylmethylpentanamide and P-glycoprotein by valspodar in gene transfectant systems. *Drug Metab Dispos* **28:**1238–1243.
Keitel V, Kartenbeck J, Nies AT, Spring H, Brom M, and Keppler D (2000) Impaired protein maturation of the conjugate export pump multidrug resistance protein 2 as a consequence of a deletion mutation in Dubin-Johnson syndrome. *Hepatology* **32:**1317–1328.
Kekuda R, Prasad PD, Wu X, Wand H, Fei YJ, Leibach FH, and Ganapathy V (1998) Cloning and functional characterization of a potential-sensitive polyspecific organic cation transporter (OCT3) most abundantly expressed in placenta. *J Biol Chem* **273:**15971–15979.
Keppler D and Konig J (2000) Hepatic secretion of conjugated drugs and endogenous substance. *Semin Liver Dis* **20:**265–272.
Keppler D, Konig J, and Buchler M (1997) The canalicular multidrug resistance protein, cMRP/MRP2, a novel conjugate export pump expressed in the apical membrane of hepatocyte. *Adv Enzyme Regul* **37:**321–333.
Kerb R, Brinkmann U, Chatskaia N, Gorbunov D, Gorboulev V, Mornhinweg E, Keil A, Eichelbaum M, and Koepsell H (2002) Identification of genetic variants of the human organic cation transporter hOCT1 and their functional consequences. *Pharmacogenetics* **12:**591–595.
Kerb R, Hoffmeyer S, and Brinkmann U (2001) ABC drug transporters: hereditary polymorphisms and pharmacological impact in MDR1, MRP1 and MRP2. *Pharmacogenetics* **2:**51–64.
Kikuchi S, Hata M, Fukumoto K, Yamane Y, Matsui T, Tamura A, Yonemura S, Yamagishi H, Keppler D, Tsukita S, et al. (2002) Radixin deficiency causes conjugated hyperbilirubinemia with loss of Mrp2 from bile canalicular membranes. *Nat Genet* **31:**320–325.
Kim RB (2000) Transporters in drug disposition. *Curr Opin Drug Discov Develop* **3:**94–101.
Kim RB (2002) MDR1 single nucleotide polymorphisms: multiplicity of haplotypes and functional consequences. *Pharmacogenetics* **12:**425–427.
Kim RB, Fromm MF, Wandel C, Leake B, Wood AJJ, Roden DM, and Wilkinson GR (1998) The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Investig* **101:**289–294.
Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, et al. (2001) Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* **70:**189–199.
Kim RB, Wandel C, Leake B, Cvetkovic M, Fromm MF, Dempsey PJ, Roden MM, Belas F, Chaudhary AK, Roden DM, et al. (1999) Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm Res (NY)* **16:**408–414.

Kleizen B, Braakman I, and de Jonge HR (2000) Regulated trafficking of the CFTR chloride channel. *Eur J Cell Biol* **79:**544–556.
Kobayashi Y, Horikawa N, Ohshiro N, Sekine T, Sasaki T, Tokuyama S, Endou H, and Yamamoto T (2002) Differential gene expression of organic anion transporters in male and female rats. *Biochem Biophys Res Commun* **290:**482–487.
Kocher O, Comella N, Gilchrist A, Pal R, Tognazzi K, Brown LF, and Knoll JH (1999) PDZK1, a novel PDZ domain-containing protein up-regulated in carcinomas and mapped to chromosome 1q21, interacts with cMOAT (MRP2), the multidrug resistance-associated protein. *Lab Investig* **79:**1161–1170.
Koepsell H (1998) Organic cation transporters in intestine, kidney, liver and brain. *Annu Rev Physiol* **60:**243–266.
Kojima R, Sekine T, Kawachi M, Cha SH, Suzuki Y, and Endou H (2002) Immunolocalization of multispecific organic anion transporters OAT1, OAT2 and OAT3, in rat kidney. *J Am Soc Nephrol* **13:**848–857.
Kolson DL, Lavi E, and Gonzalez-Scarano F (1998) The effects of human immunodeficiency virus in the central nervous system. *Adv Virus Res* **50:**1–47.
Konig J, Cui Y, Nies AT, and Keppler D (2000a) A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *Am J Physiol Gastrointest Liver Physiol* **278:**G156–G164.
Konig J, Cui Y, Nies AT, and Keppler D (2000b) Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J Biol Chem* **275:**23161–23168.
Konig J, Nies AT, Cui Y, Leier I, and Keppler D (1999a) Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity and MRP2-mediated drug resistance. *Biochim Biophys Acta* **1461:**377–394.
Konig J, Rost D, Cui Y, and Keppler D (1999b) Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. *Hepatology* **29:**1156–1163.
Kool M, van der Linden M, de Haas M, Scheer GL, de Vree JML, Smith AJ, Jansen G, Peters GJ, Ponne N, Scheper RJ, et al. (1999) MRP3 and organic anion transporter able to transport anticancer drugs. *Proc Natl Acad Sci USA* **96:**6914–6919.
Kovarik JM, Purba HS, Pongowski M, Gerbeau C, Humbert H, and Mueller EA (1998) Pharmacokinetics of dexamethasone and valspodar, a P-glycoprotein (mdr1) modulator: implications for coadministration. *Pharmacotherapy* **18:**1230–1236.
Kovarik JM, Rigaudy L, Guerret M, Gerbeau C, and Rost KL (1999) Longitudinal assessment of a P-glycoprotein-mediated drug interaction of valspodar on digoxin. *Clin Pharmacol Ther* **66:**391–400.
Kremer JM and Hamilton RA (1995) The effects of nonsteroidal anti-inflammatory drugs on methotrexate (MTX) pharmacokinetics: impairment of renal clearance of MTX at weekly maintenance doses but not at 7.5 mg. *J Rheumatol* **22:**2072–2077.
Kubitz R, Huth C, Schmitt M, Horbach A, Kullak-Ublick G, and Haussinger D (2001) Protein kinase C-dependent distribution of the multidrug resistance protein 2 from the canalicular to the basolateral membrane in human HepG2 cells. *Hepatology* **34:**340–350.
Kullak-Ublick GA, Ismair MG, Stieger B, Landmann L, Huber R, Pizzagalli F, Fattinger K, Meier PJ, and Hagenbuch B (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120:**525–533.
Kullak-Ublick GA, Stieger B, Hagenbuch B, and Meier PJ (2000) Hepatic transport of bile salts. *Semin Liver Dis* **20:**273–292.
Kusuhara H, Sekine T, Utsunomiya-Tate N, Tsuda M, Kojima R, Cha SH, Sugiyama Y, Kanai Y, and Endou H (1999) Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. *J Biol Chem* **274:**13675–13680.
Kusuhara H and Sugiyama Y (2001a) Drug-drug interactions involving the membrane transport process, in *Drug-Drug Interactions* (Rodrigues AD ed) pp. 123–188, Marcel Dekker, New York.
Kusuhara H and Sugiyama Y (2001b) Efflux transport system for drugs at the blood-brain barrier and blood-cerebrospinal fluid barrier (Parts 1, 2). *Drug Discov Today* **6:**150–156 (1), 206–212 (2).
Kusuhara H and Sugiyama Y (2002) Role of transporters in the tissue-selective distribution and elimination of drugs: transporters in the liver, small intestine, brain and kidney. *J Controlled Release* **78:**43–54.
Kusuhara H, Suzuki H, and Sugiyama Y (1998) The role of P-glycoprotein and canalicular multispecific organic anion transporter (cMOAT) in the hepatobiliary excretion of drugs. *J Pharm Sci* **87:**1025–1040.
Kusuhara H, Suzuki H, Terasaki T, Kakee A, Lemaire M, and Sugiyama Y (1997) P-glycoprotein mediates the efflux of quinidine across the blood-brain barrier. *J Pharmacol Exp Ther* **283:**574–580.
Kuwano M, Toh S, Uchiumi T, Takano H, Kohno K, and Wada M (1999) Multidrug resistance-associated protein subfamily transporters and drug resistance. *Anticancer Drug Des* **14:**123–131.
Lautier D, Canitrot Y, Deeley RG, and Cole SP (1996) Multidrug resistance mediated by the multidrug resistance protein (MRP) gene. *Biochem Pharmacol* **52:**967–977.
Leabman MK, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, DeYoung J, Taylor T, Clark AG, Herskowitz I, et al. (2002) Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *J Hum Genet* **46:**668–683.
Lee G, Dallas S, Hong M, and Bendayan R (2001a) Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. *Pharmacol Rev* **53:**569–596.
Lee CG, Gottesman MM, Cardarelli CO, Ramachandra M, Jeang KT, Ambudkar SV, Pastan I, and Dey S (1998) HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry* **37:**3594–3601.
Lee VH, Sporty JL, and Fandy TE (2001b) Pharmacogenomics of drug transporters: the next drug delivery challenge. *Adv Drug Deliv Rev* **50:**S33–S40.
Lentz K, Polli JW, Wring SA, Humphreys JE, and Polli JE (2000) Influence of passive permeability on apparent P-glycoprotein kinetics. *Pharm Res (NY)* **17:**1456–1460.
Lin JH (2003) Drug-drug interaction mediated by inhibition and induction of P-glycoprotein. *Adv Drug Deliv Rev* **55:**53–81.
Lo YL, Hsu CY, and Huang JD (1998) Comparison of effects of surfactants with other MDR reversing agents on intracellular uptake of epirubicin in Caco-2 cell line. *Anticancer Res* **18:**3005–3009.
Lu R, Kanai N, Bao Y, Wolkoff AW, and Schuster VL (1996) Regulation of renal oatp mRNA expression by testosterone. *Am J Physiol Renal Physiol* **270:**F332–F337.
Lucia MB, Cauda R, Landay AL, Malorni W, Donelli G, and Ortona L (1995) Transmembrane P-glycoprotein (P-gp/P-170) in HIV infection: analysis of lymphocyte surface expression and drug-unrelated function. *AIDS Res Hum Retroviruses* **11:**893–901.
Lum BL, Kaubisch S, Yahanda AM, Adler KM, Jew L, Ehsan MN, Brophy NA, Halsey J, Gosland MP, and Sikic BI (1992) Alteration of etoposide pharmacokinetics and pharmacodynamics by cyclosporine in a phase 1 trial to modulate multidrug resistance. *J Clin Oncol* **10:**1635–1642.
Mackstaller LL and Alpert JS (1997) Atrial fibrillation: a review of mechanism, etiology and therapy. *Clin Cardiol* **20:**640–650.
Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg ACLM, Schinkel AH, van de Vijver MJ, Scheper RJ, and Schellens JHM (2001) Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res* **61:**3458–3464.
Malingre MM, Beijnen JH, Rosing H, Koopman FJ, Jewell RC, Paul EM, Ten Bokkel Huinink WW, and Schellens JH (2001a) Co-administration of GF120918 significantly increases the systemic exposure to oral paclitaxel in cancer patients. *Br J Cancer* **84:**42–47.
Malingre MM, Richel DJ, Beijnen JH, Rosing H, Koopman FJ, Huinink WWTB, Schot ME, and Schellens JHM (2001b) Coadministration of cyclosporine strongly enhances the oral bioavailability of docetaxel. *J Clin Oncol* **19:**1160–1166.
Martel F, Vetter T, Russ H, Grundemann D, Azevedo I, Koepsell H, and Schomig E (1996) Transport of small organic cations in the rat liver. The role of the organic cation transporter OCT1. *Naunyn-Schmiedeberg's Arch Pharmacol* **354:**320–326.
Masereeuw R and Russel FGM (2001) Mechanisms and clinical implications of renal drug excretion. *Drug Metab Rev* **33:**299–351.
Masuda M, Iizuka Y, Yamazaki M, Nishigaki R, Kato Y, Niinuma N, Suzuki H, and Sugiyama Y (1997) Methotrexate is excreted into the bile by canalicular multispecific organic anion transporter in rats. *Cancer Res* **57:**3506–3510.
Masuda S, Uemoto S, Hashida T, Inomata Y, Tanaka K, and Inui K (2000) Effect of intestinal P-glycoprotein on daily tacrolimus trough level in a living-donor small bowel recipient. *Clin Pharmacol Ther* **68:**93–103.
Maxwell DL, Gilmour-White SK, and Hall MR (1989) Digoxin toxicity due to interaction of digoxin with erythromycin. *Br Med J* **298:**572.
Mayatepek E, Nezu J, Tamai I, Oku A, Katsura M, Shimane M, and Tsuji A (2000) Two novel missense mutations of the OCTN2 gene (W283R and V446F) in a patient with primary systemic carnitine deficiency. *Hum Mutat* **15:**118.
Mayer U, Wagenaar E, Dorobek B, Beijnen JH, Borst P, and Schinkel AH (1997) Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier P-glycoprotein by oral treatment of mice with PSC833. *J Clin Investig* **100:**2430–2436.
Meerum Terwogt JM, Malingre MM, Beijnen JH, ten Bokkel Huinink WW, Rosing H, Koopman FJ, van Tellingen O, Swart M, and Schellens JH (1999) Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. *Clin Cancer Res* **5:**3379–3384.
Meier PJ (1995) Molecular mechanisms of hepatic bile salt transport from sinusoidal blood into bile. *Am J Physiol Gastrointest Liver Physiol* **269:**G801–GG812.
Meier PJ, Eckhardt U, Schroeder A, Hagenbuch B, and Stieger B (1997) Substrate specificity of sinusoidal bile acid and organic anion uptake systems in rat and human liver. *Hepatology* **26:**1667–1677.
Meijer DK, Hooiveld GJ, Schinkel AH, van Montfoort JE, Haas M, de Zeeuw D, Moolenaar F, Smit JW, and Meier PJ (1999) Transport mechanisms for cationic drugs and proteins in kidney, liver and intestine: implication for drug interactions and cell-specific drug delivery. *Nephrol Dial Transplant* **14:**1–3.
Merry C, Barry MG, Mulcahy F, Ryan M, Heavey J, Tjia JF, Gibbons SE, Breckenridge AM, and Back DJ (1997) Saquinavir pharmacokinetics alone and in combination with ritonavir in HIV-infected patients. *AIDS* **11:**F29–F33.
Meyer-Wentrup F, Karback U, Gorvoulev V, Arndt P, and Koepsell H (1998) Membrane localization of the electrogenic cation transporter roct1 in rat liver. *Biochem Biophys Res Commun* **248:**673–678.
Michalski C, Cui Y, Nies AT, Nuessler AK, Neuhaus P, Zanger UM, Klein K, Eichelbaum M, Keppler D, and Konig J (2002) A naturally occurring mutation in the SLC21A6 gene causing impaired membrane localization of the hepatocyte uptake transporter. *J Biol Chem* **277:**43058–43063.
Mistry P, Stewart AJ, Dangerfield W, Okiji S, Liddle C, Bootle D, Plumb JA, Templeton D, and Charlton P (2001) In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res* **61:**749–758.
Mizuno N and Sugiyama Y (2002) Drug transporters: their role and importance in the selection and development of new drugs. *Drug Metabol Pharmacokin* **17:**93–108.
Morita N, Kusuhara H, Sekine T, Endou H, and Sugiyama Y (2001) Functional characterization of rat organic anion transporter 2 in LLC-PK1 cells. *J Pharmacol Exp Ther* **298:**1179–1184.
Moyer BD, Denton J, Karlson KH, Reynolds D, Wang S, Mickle JE, Milewski M, Cutting GR, Guggino WB, Li M, et al. (1999) A PDZ-interacting domain in CFTR is an apical membrane polarization signal. *J Clin Investig* **104:**1353–1361.
Muck W, Mai I, Fritsche L, Ochmann K, Rohde G, Unger S, Johne A, Bauer S, Budde K, Roots I, et al. (1999) Increase in cerivastatin systemic exposure after single and multiple dosing in cyclosporine-treated kidney transplant recipients. *Clin Pharmacol Ther* **65:**251–261.
Muck W, Ritter W, Ochmann K, Unger S, Ahr G, Wingender W, and Kuhlmann J (1997) Absolute and relative bioavailability of the HMG-CoA reductase inhibitor cerivastatin. *Int J Clin Phamacol Ther* **35:**255–260.
Mulato AS, Ho ES, and Cihlar T (2000) Nonsteroidal anti-inflammatory drugs

efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. *J Pharmacol Exp Ther* **295:**10–15.
Muller M and Jansen PL (1997) Molecular aspects of hepatobiliary transport. *Am J Physiol Gastrointest Liver Physiol* **272:**G2185–G1303.
Murata M, Tamai I, Kato H, Nagata O, Kato H, and Tsuji A (1999) Efflux transport of a new quinolone antibacterial agent, HSR-903, across the blood-brain barrier. *J Pharmacol Exp Ther* **290:**51–57.
Nagata Y, Kusuhara H, Endou H, and Sugiyama Y (2002) Expression and functional characterization of rat organic anion transporter 3 (Slc22a8) in the choroid plexus. *Mol Pharmacol* **61:**982–988.
Nakai D, Nakagomi R, Furuta Y, Tokui T, Abe T, Ikeda T, and Nishimura K (2001) Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. *J Pharmacol Exp Ther* **297:**861–867.
Nakanishi T, Tamai I, Sai Y, Sasaki T, and Tsuji A (1997) Carrier-mediated transport of oligopeptides in the human fibrosarcoma cell line HT1080. *Cancer Res* **58:**4118–4122.
Nakanishi T, Tamai I, Takagi A, and Tsuji A (2000) Cancer cell-targeted drug delivery utilizing oligopeptide transport activity. *Int J Cancer* **88:**274–280.
Nezu J, Tamai I, Oku A, Ohashi R, Yabuuchi H, Hashimoto N, Nikaido H, Sai Y, Koizumi A, Shoji Y, et al. (1999) Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. *Nat Genet* **21:**91–94.
Niemi M, Backman JT, Neuvonen M, Neuvonen PJ, and Kivisto KT (2001) Effects of rifampin on the pharmacokinetics and pharmacodynamics of glyburide and glipizide. *Clin Pharmacol Ther* **69:**400–406.
Nies AT, Konig J, Cui Y, Brom M, Spring H, and Keppler D (2002) Structural requirements for the apical sorting of human multidrug resistance protein 2 (ABCC2). *Eur J Biol Chem* **269:**1866–1876.
Niinuma K, Kato Y, Suzuki H, Tyson CA, Weizer V, Dabbs JE, Froehlich R, Green CE, and Sugiyama Y (1999) Primary active transport of organic anions on bile canalicular membrane in humans. *Am J Physiol Gastrointest Liver Physiol* **276:**G1153–G1164.
Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, Urasaki Y, et al. (2003) Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* **73:**554–565.
Nozawa T, Nakajima M, Tamai I, Noda K, Nezu J, Sai Y, Tsuji A, and Yokoi T (2002) Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. *J Pharmacol Exp Ther* **302:**804–813.
Oguchi H, Miyasaka M, Koiwai T, Tokunaga S, Hara K, Sato K, Yoshie T, Shioya H, and Furuta S (1993) Pharmacokinetics of temocapril and enalapril in patients with various degree of renal insufficiency. *Clin Pharmacokinet* **24:**421–427.
Oguri T, Fujiwara Y, Isobe T, Katoh O, and Yamakido M (1998) Expression of γ-glutamylcysteine synthetase (γ-GCS) and multidrug resistance-associated protein (MRP), but not human canalicular multispecific organic anion transporter (cMOAT), genes correlates with exposure of human lung cancers to platinum drugs. *Br J Cancer* **77:**1089–1096.
Oh DM, Han HK, and Amidon GL (1999) Drug transport and targeting. Intestinal transport. *Pharm Biotechnol* **12:**59–88.
Ohtsuki S, Asaba H, Takanaga H, Deguchi T, Hosoya K, Otagiri M, and Terasaki T (2002) Role of blood-brain barrier organic anion transporter 3 (OAT3) in the efflux of indoxyl sulfate, a uremic toxin: its involvement in neurotransmitter metabolite clearance from the brain. *J Neurochem* **83:**57–66.
Okudaira N, Komiya I, and Sugiyama Y (2000a) Polarized efflux of mono- and diacid metabolites of ME3229, an ester-type prodrug of a glycoprotein IIb/IIIa receptor antagonist, in rat small intestine. *J Pharmacol Exp Ther* **295:**717–723.
Okudaira N, Tatebayashi T, Speirs GC, Komiya I, and Sugiyama Y (2000b) A study of the intestinal absorption of and ester type prodrug, ME3229 in rats: active efflux transport as a cause of poor bioavailability of the active drug. *J Pharmacol Exp Ther* **294:**580–587.
Oude Elferink RPJ, Meijer DKF, Kuipers F, Jansen PLM, Groen AK, and Groothuis GMM (1995) Hepatobiliary secretion of organic compounds: molecular mechanisms of membrane transport. *Biochim Biophys Acta* **1241:**215–268.
Overbosch D, Van Gulpen C, Hermans J, and Mattie H (1988) The effect of probenecid on the renal tubular excretion of benzylpenicillin. *Br J Clin Pharmacol* **25:**51–58.
Ozvegy C, Litman T, Szakacs G, Nagy Z, Bates S, Varadi A, and Sarkadi B (2001) Functional characterization of the human multidrug transporter, abcg2, expressed in insect cells. *Biochem Biophys Res Commun* **285:**111–117.
Paulusma CC, Bosma PJ, Zaman GJR, Bakker CTM, Otter M, Scheffer GL, Scheper RJ, Borst P, and Oude Elferink RPJ (1996) Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science (Wash DC)* **271:**1126–1128.
Paulusma CC, Kool M, Bosma PJ, Scheffer GL, ter Borg F, Scheper RJ, Tytgat GN, Borst P, Baas F, and Oude Elferink RP (1997) A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin-Johnson syndrome. *Hepatology* **25:**1539–1542.
Paulusma CC, Kothe MJ, Bakker CT, Bosma PJ, van Bokhoven I, van Marle J, Bolder U, Tytgat GN, and Oude Elferink RP (2000) Zonal down-regulation and redistribution of the multidrug resistance protein 2 during bile duct ligation in rat liver. *Hepatology* **31:**684–693.
Perry CM and Noble S (1998) Saquinavir soft-gel capsule formulation. A review of its use in patients with HIV infection. *Drugs* **55:**461–486.
Piscitelli SC, Burstein AH, Chaitt D, Alfaro RM, and Falloon J (2000) Indinavir concentrations and St John's Wort. *Lancet* **355:**547–548.
Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, and Meier PJ (2002) Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol Endocrinol* **16:**2283–2296.
Plass JR, Mol O, Heegsma J, Geuken M, Faber KN, Jansen PL, and Muller M (2002) Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump. *Hepatology* **35:**589–596.
Polli JW, Wring SA, Humphreys JE, Huang L, Morgan JB, Webster LO, and Serabjit-Singh CS (2001) Rational use of in vitro P-glycoprotein assays in drug discovery. *J Pharmacol Exp Ther* **299:**620–628.
Rabindran SK, He H, Singh M, Brown E, Collins KI, Annable T, and Greenberger LM (1998) Reversal of a novel multidrug resistance mechanism in human colon carcinoma cells by fumitremorgin C. *Cancer Res* **58:**5850–5858.
Reichel C, Gao B, Van Montfoort J, Cattori V, Rahner C, Hagenbuch B, Stieger B, Kamisako T, and Meier PJ (1999) Localization and function of the organic anion-transporting polypeptide Oatp2 in rat liver. *Gastroenterology* **117:**688–695.
Roberts SA (2001) High-throughput screening approaches for investigating drug metabolism and pharmacokinetics. *Xenobiotica* **31:**557–589.
Rocchi E, Khodjakov A, Volk EL, Yang CH, Litman T, Bates SE, and Schneider E (2000) The product of the ABC half-transporter gene ABCG2 (BCRP/MXR/ABCP) is expressed in the plasma membrane. *Biochem Biophys Res Commun* **271:**42–46.
Roelofsen H, Soroka CJ, Keppler D, and Boyer JL (1998) Cyclic AMP stimulates sorting of the canalicular organic anion transporter (Mrp2/cMoat) to the apical domain in hepatocyte couplets. *J Cell Sci* **111:**1137–1145.
Rost D, Kartenbeck J, and Keppler D (1999) Changes in the localization of the rat canalicular conjugate export pump Mrp2 in phalloidin-induced cholestasis. *Hepatology* **29:**814–821.
Rowinsky EK, Grochow LB, Ettinger DS, Sartorius SE, Lubejko BG, Chen TL, Hendricks C, Rock MK, and Donehower RC (1994) Phase I and pharmacological study of novel topoisomerase I inhibitor 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin (CPT-11) administered as a ninety-minute infusion every 3 weeks. *Cancer Res* **54:**427–436.
Rushing DA, Raber SR, Rodvold KA, Piscitelli SC, Plank GS, and Tewksbury DA (1994) The effects of cyclosporine on the pharmacokinetics of doxorubicin in patients with small cell lung cancer. *Cancer* **74:**834–841.
Russel FG, Masereeuw R, and van Aubel RA (2002) Molecular aspects of renal anionic drug transport. *Annu Rev Physiol* **64:**563–594.
Sadeque AJM, Wandel C, He H, Shah S, and Wood AJJ (2000) Increased drug delivery to the brain by P-glycoprotein inhibition. *Clin Pharmacol Ther* **68:**231–237.
Saito H and Inui K (1995) Dipeptide transporters in apical and basolateral membranes of the human intestinal cell line Caco-2. *Am J Physiol Gastrointest Liver Physiol* **265:**G289–G294.
Sasabe H, Tsuji A, and Sugiyama Y (1998) Carrier-mediated mechanism for the biliary excretion of the quinolone antibiotic grepafloxacin and its glucuronide in rats. *J Pharmacol Exp Ther* **284:**1033–1039.
Sasaki M, Suzuki H, Ito K, Abe T, and Sugiyama Y (2002) Transcellular transport of organic anions across double-transfected MDCK II cell monolayer expressing both human organic anion transporting polypeptide (OATP2/SLC21A6) and multidrug resistance associated protein 2 (MRP2/ABCC2). *J Biol Chem* **277:**6497–6503.
Sathirakul K, Suzuki H, Yasuda K, Hanano M, Tagaya O, Hori T, and Sugiyama Y (1993) Kinetic analysis of hepatobiliary transport of organic anions in Eisai hyperbilirubinemic mutant rats. *J Pharmacol Exp Ther* **265:**1301–1312.
Sawada K, Terada T, Saito H, Hashimoto Y, and Inui K (1999) Recognition of L-amino acid ester compounds by rat peptide transporters PEPT1 and PEPT2. *J Pharmacol Exp Ther* **291:**705–709.
Schellens JH, Creemers GJ, Beijnen JH, Rosing H, de Boer-Dennert M, and McDonald M (1996) Bioavailability and pharmacokinetics of oral topotecan: a new topoisomerase I inhibitor. *Br J Cancer* **73:**1268–1271.
Schinkel AH (2001) The role of P-glycoprotein and MRP1 in the blood-brain and blood-cerebrospinal fluid barriers. *Adv Exp Med Biol* **500:**365–372.
Schinkel AH and Jonker JW (2003) Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv Drug Deliv Rev* **55:**3–29.
Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JM, van der Valk MA, Voordouw AC, Spits H, van Tellingen O, et al. (1997) Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci USA* **94:**4028–4033.
Schinkel AH, Mol CA, Wagenaar E, van Deemter L, Smit JJM, and Borst P (1995a) Multidrug resistance and the role of P-glycoprotein knockout mice. *Eur J Cancer* **31A:**1295–1298.
Schinkel AH, Smit JJM, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HPJ, et al. (1994) Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77:**491–502.
Schinkel AH, Wagenaar E, Mol CA, and van Deemter L (1996) P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Investig* **97:**2517–2524.
Schinkel AH, Wagenaar E, van Deemter L, Mol CA, and Borst P (1995b) Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin and cyclosporin A. *J Clin Investig* **96:**1698–1705.
Schwarz UI, Gramatte T, Krappweis J, Oertel R, and Kirch W (2000) P-glycoprotein inhibitor erythromycin increases oral bioavailability of talinolol in humans. *Int J Clin Pharmacol Ther* **38:**161–167.
Seelig A and Landwojtowicz E (2000) Structure-activity relationship of P-glycoprotein substrates and modifiers. *Eur J Pharm Sci* **12:**31–40.
Seitz S, Kretz-Rommel A, Oude Elferink RP, and Boelsterli UA (1998) Selective protein adduct formulation of diclofenac glucuronide is critically dependent on the rat canalicular conjugate export pump (Mrp2). *Chem Res Toxicol* **11:**513–519.
Sekine T, Cha SH, and Endou H (2000) The multispecific organic anion transporter (OAT) family. *Pflueg Arch* **440:**337–350.
Sekine T, Cha SH, Tsuda M, Apiwattanakul N, Nakajima N, Kanai Y, and Endou H (1998) Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett* **429:**179–182.
Sekine T, Watanabe N, Hosoyamada M, Kanai Y, and Endou H (1997) Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* **272:**18526–18529.

Sheng M and Sala C (2001) PDZ domains and the organization of supramolecular complex. *Annu Rev Neurosci* **24:**1–29.
Shitara Y, Itoh T, Sato H, Li AP, and Sugiyama Y (2003) Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. *J Pharmacol Exp Ther* **304:**610–616.
Shoda J, Kano M, Oda K, Kamiya J, Nimura Y, Suzuki H, Sugiyama Y, Miyazaki H, Todoroki T, Stengelin S, et al. (2001) The expression levels of plasma membrane transporters in the cholestatic liver of patients undergoing biliary drainage and their association with the impairment of biliary secretory function. *Am J Gastroenterol* **96:**3368–3378.
Siedlik PH, Olson SC, Yang BB, and Stern RH (1999) Erythromycin coadministration increases plasma atrovastatin concentrations. *J Clin Pharmacol* **39:**501–504.
Sikic BI, Advani R, Fisher GA, Halsey J, Cohen P, and Lum BL (2000) Enhanced bioavailability of oral paclitaxel by valspodar (PSC833), an inhibitor of small bowel P-glycoprotein and cytochrome P450. *Clin Cancer Res* **6:**4580S.
Simon FR, Fortune M, Iwahashi S, Bowman A, Wolkoff A, and Sutherland E (1999) Characterization of the mechanisms involved in the gender differences in hepatic taurocholate uptake. *Am J Physiol Gastrointest Liver Physiol* **276:**G556–G565.
Simons NL, Humter J, and Jepson MA (1997) Renal secretion of xenobiotics mediated by P-glycoprotein: importance to renal function in health and exploitation for targeted drug delivery to epithelial cysts in polycystic kidney disease. *Adv Drug Deliv Rev* **25:**243–256.
Simonson GD, Vincent AC, Roberg KJ, Huang Y, and Iwanij V (1994) Molecular cloning and characterization of a novel liver-specific transport protein. *J Cell Sci* **107:**1065–1072.
Smit JW, Huisman MT, van Tellingen O, Wiltshire HR, and Schinkel AH (1999) Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. *J Clin Investig* **104:**1441–1447.
Sokol RJ, Johnson KE, Karrer FM, Narkewicz MR, Smith D, and Kam I (1991) Improvement of cyclosporin absorption in children after liver transplantation by means of water-soluble vitamin E. *Lancet* **338:**212–214.
SoRelle R (2001) Baycol withdrawn from market. *Circulation* **104:**E9015–E9016.
Soroka CJ, Lee JM, Azzaroli F, and Boyer JL (2001) Cellular localization and up-regulation of multidrug resistance-associated protein 3 in hepatocytes and cholangiocytes during obstructive cholestasis in rat liver. *Hepatology* **33:**783–791.
Spahn-Langguth H and Langguth P (2001) Grapefruit juice enhances intestinal absorption of the P-glycoprotein substrate talinolol. *Eur J Pharm Sci* **12:**361–367.
Sparreboom A, Planting AS, Jewell RC, van der Burg ME, van der Gaast A, de Bruijn P, Loos WJ, Nooter K, Chandler LH, Paul EM, et al. (1999) Clinical pharmacokinetics of doxorubicin in combination with GF120918, a potent inhibitor of MDR1 P-glycoprotein. *Anticancer Drugs* **10:**719–728.
Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, et al. (2001b) The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci USA* **98:**3369–3374.
Staudinger J, Liu Y, Madan A, Habeebu S, and Klaassen CD (2001a) Coordinate regulation of xenobiotic and bile acid homeostasis by pregnane X receptor. *Drug Metab Dispos* **29:**1467–1472.
Stieger B, Fattinger K, Madon J, Kullak-Ublick GA, and Meier PJ (2000) Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. *Gastroenterology* **118:**422–430.
Stockel B, Konig J, Nies AT, Cui Y, Brom M, and Keppler D (2000) Characterization of the 5′-flanking region of the human multidrug resistance protein 2 (MRP2) gene and its regulation in comparison with the multidrug resistance protein 3 (MRP3) gene. *Eur J Biochem* **267:**1347–1358.
Stouch TR and Gudmundsson O (2002) Progress in understanding the structure-activity relationships of P-glycoprotein. *Adv Drug Deliv Rev* **54:**315–328.
St-Pierre MV, Hagenbuch B, Ugele B, Meier PJ, and Stallmach T (2002) Characterization of an organic anion-transporting polypeptide (OATP-B) in human placenta. *J Clin Endocrinol Metab* **87:**1856–1863.
Sugiyama D, Kusuhara H, Shitara Y, Abe T, Meier PJ, Sekine T, Endou H, Suzuki H, and Sugiyama Y (2001) Characterization of the efflux transport of 17β-estradiol-D-17β-glucuronide from the brain across the blood-brain barrier. *J Pharmacol Exp Ther* **298:**316–322.
Sun H, Dai H, Shaik N, and Elmquist WF (2003) Drug efflux transporters in the CNS. *Adv Drug Deliv Rev* **55:**83–105.
Suzuki H and Sugiyama Y (1999) Transporters for bile acids and organic anions, in *Membrane Transporters as Drug Targets* (Sadee W and Amidon G eds) pp. 387–439, Kluwer Academic/Plenum Publishing Co, New York.
Suzuki H and Sugiyama Y (2000) Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. *Eur J Pharm Sci* **12:**3–12.
Suzuki H and Sugiyama Y (2002) Single nucleotide polymorphisms in multidrug resistance associated protein 2 (MRP2/ABCC2): its impact on drug disposition. *Adv Drug Deliv Rev* **54:**1311–1331.
Suzuki H, Terasaki T, and Sugiyama Y (1997) Role of efflux transport across the blood-brain barrier and blood-cerebrospinal fluid barrier on the disposition of xenobiotics in the central nervous system. *Adv Drug Deliv Rev* **25:**257–285.
Swaan PW, Stehouwer MC, and Tukker JJ (1995) Molecular mechanism for the relative binding affinity to the intestinal peptide carrier: comparison of three ACE-inhibitors, enalapril, enalaprilat and lisinopril. *Biochim Biophys Acta* **1236:** 31–38.
Sweet DH, Miller DS, Pritchard JB, Fujiwara Y, Beier DR, and Nigam SK (2002) Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 [Slc22a8]) knockout mice. *J Biol Chem* **277:**26934–26943.
Synold TW, Dussault I, and Forman BM (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* **7:**584–590.
Taipalensuu J, Tornblom H, Lindberg G, Einarsson C, Sjoqvist F, Melhus H, Garberg P, Sjostrom B, Lundgren B, and Artursson P (2001) Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. *J Pharmacol Exp Ther* **299:**164–170.
Takano M, Hasegawa R, Fukuda T, Yamoto R, Nagai J, and Murakami T (1998) Interaction with P-glycoprotein and transport of erythromycin, midazolam and ketoconazole in Caco-2 cells. *Eur J Pharmacol* **358:**289–294.
Takeda M, Babu E, Narikawa S, and Endou H (2002a) Interaction of human organic anion transporters with various cephalosporin antibiotics. *Eur J Pharmacol* **438:** 137–142.
Takeda M, Khamdang S, Narikawa S, Kimura H, Hosoyamada M, Cha SH, Sekine T, and Endou H (2002b) Characterization of methotrexate transport and its drug interactions with human organic anion transporters. *J Pharmacol Exp Ther* **302:** 666–671.
Takeda M, Tojo A, Sekine T, Hosoyamada M, Kanai Y, and Endou H (1999) Role of organic anion transporter 1 (OAT1) in cephaloridine (CER)-induced nephrotoxicity. *Kidney Int* **56:**2128–2136.
Tamai I, Nezu J, Uchino H, Sai Y, Oku A, Shimane M, and Tsuji A (2000a) Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem Biophys Res Commun* **273:**251–260.
Tamai I, Nozawa T, Koshida M, Nezu J, Sai Y, and Tsuji A (2001) Functional characterization of human organic anion transporting polypeptide B (OATP-B) in comparison with liver-specific OATP-C. *Pharm Res (NY)* **18:**1262–1269.
Tamai I, Takanaga H, Maeda H, Ogihara T, Yoneda M, and Tsuji A (1995) Proton-cotransport of pravastatin across intestinal brush-border membrane. *Pharm Res (NY)* **12:**1727–1732.
Tamai I and Tsuji A (2000) Transporter-mediated permeation of drugs across the blood-brain barrier. *J Pharm Sci* **89:**1371–1388.
Tamai I, Yamashita J, Kido Y, Ohnari A, Sai Y, Shima Y, Naruhashi K, Koizumi S, and Tsuji A (2000b) Limited distribution of new quinolone antibacterial agents into brain caused by multiple efflux transporters at the blood-brain barrier. *J Pharmacol Exp Ther* **295:**146–152.
Tanaka T, Uchiumi T, Hinoshita E, Inokuchi A, Toh S, Wada M, Takano H, Kohno K, and Kuwano M (1999) The human multidrug resistance protein 2 gene: functional characterization of the 5′-flanking region and expression in hepatic cells. *Hepatology* **30:**1507–1712.
Terada T, Saito H, Murai M, and Inui K (1997) Recognition of beta-lactam antibiotics by rat peptide transporters, PEPT1 and PEPT2, in LLC-PK1 cells. *Am J Physiol Renal Physiol* **273:**F706–F711.
Thyss A, Milano G, Kubar J, and Namer M (1986) Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. *Lancet* **1:**256–258.
Tiberghien F and Loor F (1996) Ranking of P-glycoprotein substrates and inhibitors by a calcein-AM fluorometry screening assay. *Anticancer Drugs* **7:**568–578.
Tirona RG and Kim RB (2002) Pharmacogenomics of organic anion-transporting polypeptides (OATP). *Adv Drug Deliv Rev* **54:**1343–1352.
Tirona RG, Leake BF, Merino G, and Kim RB (2001) Polymorphisms in oatp-c. Identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* **276:**35669–35675.
Tracy TS, Krohn K, Jones DR, Bradley JD, Hall SD, and Brater DC (1992) The effects of a salicylate, ibuprofen and naproxen on the disposition of methotrexate in patients with rheumatoid arthritis. *Eur J Clin Pharmacol* **42:**121–125.
Troutman MD, Luo G, Gan LS, and Thakker DR (2001) The role of P-glycoprotein in drug disposition: significance to drug development, in *Drug-Drug Interactions* (Rodrigues AD ed) pp. 295–358, Marcel Dekker, New York.
Tsuda M, Sekine T, Takeda M, Cha SH, Kanai Y, Kimura M, and Endou H (1999) Transport of ochratoxin A by renal multispecific organic anion transporter 1. *J Pharmacol Exp Ther* **289:**1301–1305.
Tsuji H, Konig J, Rost D, Stockel B, Leuschner U, and Keppler D (1999) Exon-intron organization of the human multidrug resistance protein 2 (MRP2) gene mutated in Dubin-Johnson syndrome. *Gastroenterology* **117:**653–660.
Tsuji A and Tamai I (1996) Carrier-mediated intestinal transport of drugs. *Pharm Res (NY)* **13:**963–977.
Tsuji A and Tamai I (1997) Blood-brain barrier function of P-gp. *Adv Drug Deliv Rev* **25:**285–298.
Tsuji A, Terasaki T, Tamai I, and Takeda K (1990) In vivo evidence for carrier-mediated uptake of beta-lactam antibiotics through organic anion transport systems in rat kidney and liver. *J Pharmacol Exp Ther* **253:**315–320.
Turriziani O, Marco PD, and Antonelli G (2000) May the drug transporter P-glycoprotein affect the antiviral activity of human immunodeficiency virus type 1 protease inhibitors? *Antimicrob Agents Chemother* **44:**473–474.
Ueda K, Kato Y, Komatsu K, and Sugiyama Y (2001) Inhibition of the biliary excretion of methotrexate by probenecid in rats: quantitative prediction of the interaction from in vitro data. *J Pharmacol Exp Ther* **297:**1036–1043.
Urakami Y, Nakamura N, Takahashi K, Okuda H, Saito Y, Hashimoto Y, and Inui K (1999) Gender differences in expression of organic cation transporter OCT2 in rat kidney. *FEBS Lett* **461:**339–342.
Urakami Y, Okuda M, Masuda S, Saito H, and Inui K (1998) Functional characteristics and membrane localization of rat multispecific organic cation transporters, oct1 and oct2, mediating tubular secretion of cationic drugs. *J Pharmacol Exp Ther* **287:**800–805.
Uwai Y, Okuda M, Takami K, Hashimoto Y, and Inui K (1998) Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. *FEBS Lett* **438:**321–324.
Uwai Y, Saito H, Hashimoto Y, and Inui K (2000) Interaction and transport of thiazide diuretics, loop diuretics and acetazolamide via rat renal organic anion transporter rOAT1. *J Pharmacol Exp Ther* **295:**261–265.
van Aubel RAMH, Masereeuw R, and Russel FGM (2000) Molecular pharmacology of renal organic anion transporters. *Am J Physiol Renal Physiol* **279:**F216–F232.
van Zuylen L, Verweij J, and Sparreboom A (2001) Role of formulation vehicles in taxane pharmacology. *Investig New Drugs* **19:**125–141.
Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, et al. (2001) The sequence of the human genome. *Science (Wash DC)* **291:**1304–1351.
Vos TA, Ros JE, Havinga R, Moshage H, Kuipers F, Jansen PL, and Muller M (1999)

Regulation of hepatic transport systems involved in bile secretion during liver regeneration in rats. *Hepatology* **29:**1833–1839.
Wacher VJ, Salphati L, and Benet LZ (2001) Active secretion and enterocytic drug metabolism barriers to drug absorption. *Adv Drug Deliv Rev* **46:**89–102.
Wacher VJ, Wu CY, and Benet LZ (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog* **13:**129–134.
Wada S, Tsuda M, Sekine T, Cha SH, Kimura M, Kanai Y, and Endou H (2000) Rat multispecific organic anion transporter 1 (rOAT1) transports zidovudine, acyclovir and other antiviral nucleoside analogs. *J Pharmacol Exp Ther* **294:**844–849.
Wakasugi H, Yano I, Ito T, Hashida T, Futami T, Nohara R, Sasayama S, and Inui K (1998) Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. *Clin Pharmacol Ther* **64:**123–128.
Walters HG, Craddock AL, Fusegawa H, Willingham MC, and Dawson PA (2000) Expression, transport properties and chromosomal location of organic anion transporter subtype 3. *Am J Physiol Gastrointest Liver Physiol* **279:**G1188–G1200.
Wandel C, Kim RB, Kajiji S, Guengerich FP, Wilkinson GR, and Wood AJJ (1999) P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. *Cancer Res* **59:**3944–3948.
Wang EJ, Casciano CN, Clement RP, and Johnson WW (2000) In vitro flow cytometry method to quantitatively assess inhibitors of P-glycoprotein. *Drug Metab Dispos* **28:**316–322.
Wang D, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, and Sugiyama Y (2002) Involvement of organic cation transporter 1 in the hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther* **302:**510–515.
Wang D, Kusuhara H, Kato Y, Jonker JW, Schinkel AH, and Sugiyama Y (2003) Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. *Mol Pharmacol* **63:**1–5.
Westphal K, Weinbrenner A, Giessmann T, Stuhr M, Franke G, Zschiesche M, Oertel R, Terhaag B, Kroemer HK, and Siegmund W (2000a) Oral bioavailability of digoxin is enhanced by talinolol: evidence for involvement of intestinal P-glycoprotein. *Clin Pharmacol Ther* **68:**6–12.
Westphal K, Weinbrenner A, Zschiesche M, Franke G, Knoke M, Oertel R, Fritz P, von Richter O, Warzok R, Hachenberg T, et al. (2000b) Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug-drug interaction. *Clin Pharmacol Ther* **68:**345–355.
White RE (2000) High-throughput screening in drug metabolism and pharmacokinetic support of drug discovery. *Annu Rev Pharmacol Toxicol* **40:**133–157.
Wijnholds J, de Lange ECM, Scheffer GL, van den Berg DJ, Mol CA, van der Valk M, Schinkel AH, Scheper RJ, Breimer DD, and Borst P (2000) Multidrug resistance protein 1 protects the choroid plexus epithelium and contributes to the blood-cerebrospinal fluid barrier. *J Clin Investig* **105:**279–285.
Wijnholds J, Evers R, van Leusden MR, Mol CA, Zaman GJ, Mayer U, Beijnen JH, van der Valk M, Krimpenfort P, and Borst P (1997) Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. *Nat Med* **3:**1275–1279.
Wu X, Huang W, Prasad PD, Seth P, Pajan DP, Leibach FH, Chen J, Conway SJ, and Ganapathy V (1999) Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2) and organic cation /carnitine transporter. *J Pharmacol Exp Ther* **290:**1482–1492.
Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, Conway SJ, and Ganapathy V (1998) Identity of organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake$_2$) and evidence for the expression of the transporter in the brain. *J Biol Chem* **273:**32776–32786.
Wu X, Whitfield LR, and Stewart BH (2000) Atrovastatin transport in the Caco-2 cell model: Contributions of P-glycoprotein and the proton-monocarboxylic acid co-transporter. *Pharm Res (NY)* **17:**209–215.
Yamaguchi H, Yano I, Hashimoto Y, and Inui K (2000) Secretory mechanisms of grepafloxacin and levofloxacin in the human intestinal cell line Caco-2. *J Pharmacol Exp Ther* **295:**360–366.
Yamazaki M, Neway WE, Ohe T, Chen IW, Rowe JF, Hochman JH, Chiba M, and Lin JH (2001) In vitro substrate identification studies for P-glycoprotein-mediated transport: species difference and predictability of in vivo results. *J Pharmacol Exp Ther* **296:**723–735.
Yamazaki M, Suzuki H, and Sugiyama Y (1996) Recent advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. *Pharm Res (NY)* **13:**497–513.
Yokogawa K, Takahashi M, Tamai I, Konishi H, Nomura M, Moritani S, Miyamoto K, and Tsuji A (1999) P-glycoprotein-dependent disposition kinetics of tacrolimus: studies in *mdr*1a knockout mice. *Pharm Res (NY)* **16:**1213–1218.
Yu DK (1999) The contribution of P-glycoprotein to pharmacokinetic drug-drug interactions. *J Clin Pharmacol* **39:**1203–1211.
Yu L, Bridgers A, Polli J, Vickers A, Long S, Roy A, Winnike R, and Coggin M (1999) Vitamin E-TPGS increases absorption flux of an HIV protease inhibitor by enhancing its solubility and permeability. *Pharm Res (NY)* **16:**1812–1817.
Yumoto R, Murakami T, Nakamoto Y, Hasegawa R, Nagai J, and Takano M (1999) Transport of Rhodamine 123, a P-glycoprotein substrate, across rat intestine and Caco-2 cell monolayers in the presence of cytochrome P-450 3A-related compounds. *J Pharmacol Exp Ther* **289:**149–155.
Zhang Y and Benet LZ (1998) Characterization of P-glycoprotein mediated transport of K02, a novel vinylsulfone peptidomimetic cysteine protease inhibitor, across MDR1-MDCK and Caco-2 cell monolayers. *Pharm Res (NY)* **15:**1520–1524.
Zhang Y and Benet LZ (2001) The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein. *Clin Pharmacokinet* **40:**159–168.
Zhang L, Brett CM, and Giacomini KM (1998) Role of organic cation transporters in drug absorption and elimination. *Ann Rev Pharmacol Toxicol* **38:**431–460.
Zhang L, Dresser MJ, Gray AT, Yost SC, Terashita S, and Giacomini KM (1997) Cloning and functional expression of a human liver organic cation transporter. *Mol Pharmacol* **51:**913–921.
Zhang J, Kuehl P, Green ED, Touchman JW, Watkins PB, Daly A, Hall SD, Maurel P, Relling M, Brimer K, et al. (2001) The human pregnane X receptor: genomics structure and identification and fictional characterization of natural allelic variants. *Pharmacogenetics* **11:**555–572.
Zollner G, Fickert P, Zenz R, Fuchsbichler A, Stumptner C, Kenner K, Ferenci P, Stauber RE, Krejs GJ, Denk H, et al. (2001) Hepatobiliary transporter expression in percutaneous liver biopsies of patients with cholestatic liver diseases. *Hepatology* **33:**633–646.