As each transporter accepts multiple drugs and/or xenobiotics as its substrates, it may be competitively inhibited by other coadministered drugs and/or xenobiotics, which may lead to drug-drug interactions involving the transporter (15, 16). In this review, we summarize quantitatively the probability of drug-drug interactions from in vitro and in vivo studies.

THE MECHANISM OF RENAL AND HEPATOBILIARY EXCRETION OF DRUGS

The Mechanism of Renal Excretion—The Role of Transporters

In the kidney, drugs are excreted in the urine as the net result of glomerular filtration, tubular secretion, and reabsorption (4) (Figure 1). The mechanism of glomerular filtration is simply ultrafiltration of drugs and xenobiotics, which do not bind to macromolecules such as plasma proteins, and, therefore, transporters are not involved in this process. Therefore, for drugs eliminated only by filtration, renal excretion is not saturable and cannot be inhibited by other drugs. On the other hand, in the case of tubular secretion, several active secretion mechanisms have been reported in the proximal tubules, which are mainly mediated by transporters.

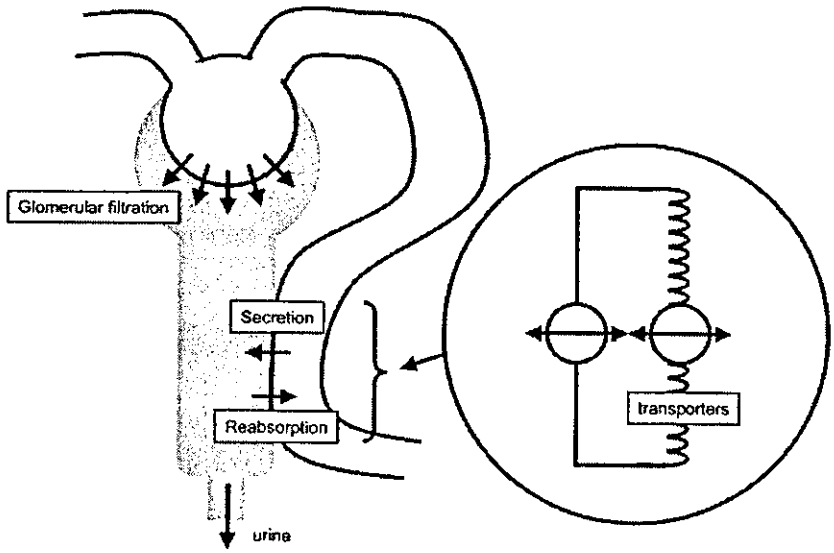

Figure 1 Mechanism of drug elimination in the kidney. Drug elimination in the kidney takes place by glomerular filtration and secretion at the proximal tubules. However, they may return to the systemic circulation via a process of drug reabsorption. Transporters are involved in drug secretion and reabsorption.

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Hence, this process is saturable and may be inhibited by coadministered drugs. As with tubular secretion, the reabsorption process is sometimes mediated by transporters in the proximal tubules. Therefore, the reabsorption process may be, in part, saturable and inhibited by coadministered drugs. Renal clearance can be, in general, described by the following equation:

$$CL_R = (1 - FR) \cdot (f_u \cdot GFR + CL_{scc}) = (1 - FR) \cdot \left(f_u \cdot GFR + \frac{Q_R \cdot f_u \cdot CL_{R,int}}{Q_R + f_u \cdot CL_{R,int}} \right), \quad 1.$$

where f_u, GFR, FR, CL_{scc}, Q_R, and $CL_{R,int}$ represent protein unbound fraction in the blood, glomerular filtration rate [ml min^{-1}], the fraction reabsorbed, renal secretion clearance, renal blood flow rate, and intrinsic clearance of tubular secretion, respectively (4). FR and $CL_{R,int}$ are partly saturable and can be inhibited, suggesting the possibility of drug-drug interactions.

The Mechanism of Hepatobiliary Excretion—The Role of Transporters

In the liver, drugs are first taken up into hepatocytes, followed by metabolism including oxidation (mediated by cytochrome P450, Phase I) and conjugation (mediated by conjugation enzymes, Phase II), and excreted into the bile (Phase III) (3) (Figure 2). Some drugs are excreted as intact drugs without metabolism. In addition, drugs excreted in the intact form may pass into the blood again by enterohepatic circulation. Drugs or their metabolites, once taken up into the liver, may undergo secretion into the blood across the sinusoidal membrane, followed by the hepatobiliary or renal excretion. To date, transporters have been shown to play a role in hepatic uptake, biliary excretion, and the secretion into the blood across the sinusoidal membrane (Figure 2). Hepatic clearance can be described by the following equation (17, 18):

$$CL_H = \frac{Q_H \cdot f_u \cdot CL_{H,int,all}}{Q_H + f_u \cdot CL_{H,int,all}}, \quad 2.$$

where CL_H, Q_H, and $CL_{H,int,all}$ represent the hepatic clearance; hepatic blood flow; and overall intrinsic clearance of biliary excretion, including uptake, metabolism, and biliary excretion, respectively. $CL_{H,int,all}$ can be described by the following equation (3, 19):

$$CL_{H,int,all} = PS_{influx} \times \frac{CL_{H,int}}{PS_{efflux} + CL_{H,int}}, \quad 3.$$

where PS_{influx} and PS_{efflux} are the membrane permeability across the sinusoidal membrane from the outside to the inside and from the inside to the outside of cells, respectively, and $CL_{H,int}$ represents the exact intrinsic clearance for the metabolism and/or biliary excretion of the unbound drugs. When $CL_{H,int}$ is negligibly low

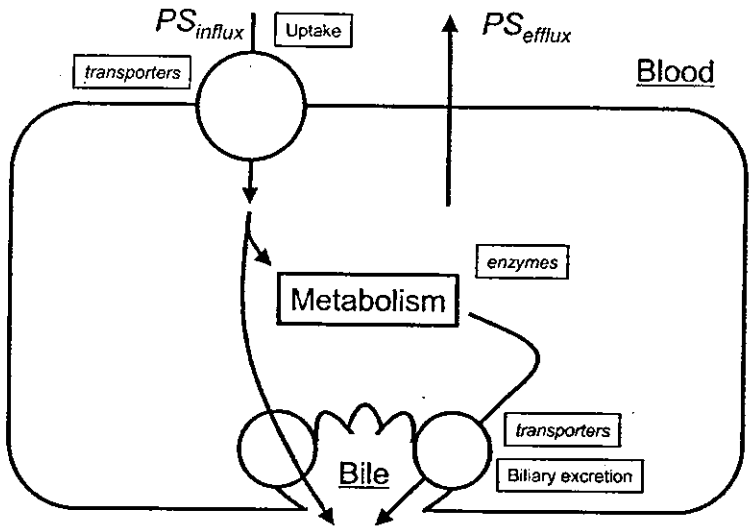

Figure 2 Mechanism of drug elimination in the liver. Drugs are taken up into hepatocytes via transporters and/or passive diffusion, followed by metabolism and/or biliary excretion. Drugs are possibly effluxed into the circulation via sinusoidal membrane.

compared with PS_{efflux} ($CL_{H,int} \ll PS_{efflux}$), Equation 3 gives

$$CL_{H,int,all} = PS_{influx} \times \frac{CL_{H,int}}{PS_{efflux}}. \quad 4.$$

If PS_{efflux} is much lower than $CL_{H,int}$ ($CL_{H,int} \gg PS_{efflux}$), Equation 3 gives

$$CL_{H,int,all} = PS_{influx}. \quad 5.$$

It should be noted that the uptake of drugs via the sinusoidal membrane (PS_{influx}), which is partly mediated by transporters, is a determinant of the net hepatic clearance regardless of the other processes, i.e., $CL_{H,int}$ and PS_{efflux}. Therefore, hepatic clearance may be affected when the uptake clearance of drugs is altered, even if the drug finally undergoes metabolism. On the other hand, the excretion of drugs via the bile canalicular membrane, which is partly mediated by transporters, is a determinant of the net hepatic clearance, unless PS_{efflux} is negligibly low compared with $CL_{H,int}$. Therefore, except in this case, the change in the biliary excretion may affect the net hepatic clearance. If PS_{efflux} is much lower than $CL_{H,int}$, only a drastic reduction in the biliary excretion will affect the net hepatic clearance, possibly leading to a transporter-mediated drug-drug interaction.

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TRANSPORTERS IN THE KIDNEY AND LIVER

Recently, many types of transporters have been isolated in the kidney and liver from animals and humans. Their substrate specificity has been characterized using cRNA-injected oocytes and/or cDNA transfected cells. Generally, amphipathic organic anions with relatively high molecular weights are eliminated from the liver by metabolism and/or biliary excretion, whereas small and hydrophilic organic anions are excreted into urine (7). In this section, the characteristics of transporters expressed in the kidney and liver and their functions are summarized.

Transporters in the Kidney

Figure 3 shows transporters expressed in the kidney of rats and humans. Some transporters are located on the basolateral membrane (blood side), whereas others are located on the brush border membrane (luminal side), and these transporters contribute to membrane transport, resulting in tubular secretion and/or reabsorption. In this section, the molecular aspects of renal transporters are summarized.

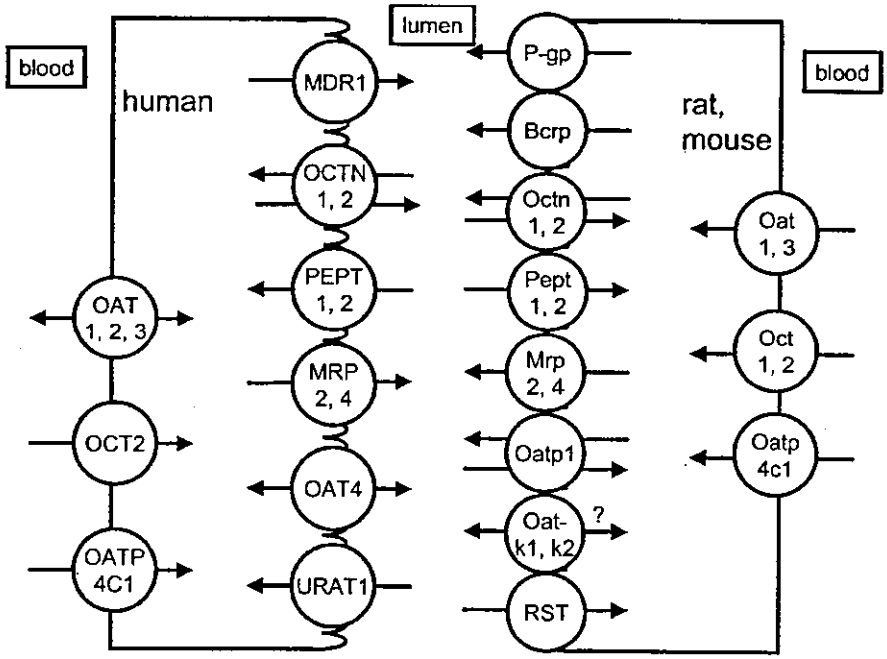

Figure 3 Transporters in the kidney. Transporters expressed in human and rodent kidney are summarized in this figure. Some of the transporters in rodents are expressed only in either rats or mice.

ORGANIC ANION TRANSPORTERS The transport of organic anions is mainly mediated by organic anion transporters (OATs). Rat Oat1 has been isolated as a renal transporter that is involved in the renal uptake of organic anions like p-aminohippuric acid (PAH) in an exchange of dicarboxylates (20). OAT1–5 have been identified as human OAT family transporters (21–24). Among them, OAT1–4 are expressed in the human kidney and OAT2 and 5 are expressed in the liver (21–24). In the kidney, OAT1–3 are localized on the basolateral membrane, whereas OAT4 is localized on the brush border membrane (24). Each of these transporters in the OAT family has a similar substrate specificity. These transporters accept organic anions with a relatively small molecular weight with some exceptions. They accept PAH, methotrexate (MTX), nonsteroidal antiinflammatory drugs, and antiviral nucleoside analogues as substrates (25–27). They also accept more lipophilic organic anions, such as estrone 3-sulfate and ochratoxin A, and even an organic cation, cimetidine (24, 25, 28).

P-GLYCOPROTEIN P-glycoprotein (P-gp) consists of two subclasses: MDR1 (MDR1 in humans, Mdr1a and 1b in rats and mice) and MDR2 [MDR2 (or 3) in humans and Mdr2 in rats and mice] (8). The former is the well-known multidrug resistance transporter, whereas the latter is a translocator for phospholipids (8). In the kidney, P-gp is localized on the brush border membrane and acts as an efflux transporter into the urine (8). P-gp is also expressed in the liver (8). In the liver, it is localized on the bile canalicular membrane (8). P-gp was originally found as an overexpressing transporter in tumor tissues, and it acts as a multidrug resistance protein, although it has also been identified in normal tissues such as kidney, liver, blood-brain barrier, and intestine (8). P-gp substrates include anticancer drugs (such as vincristine, vinblastine, doxorubicin, daunorubicin, etoposide, and paclitaxel), immunosuppresants (such as cyclosporin A), verapamil, digoxin, and steroids (such as aldosterone and cortisole) (29–36).

PEPTIDE TRANSPORTERS In the kidney, two isoforms of peptide transporters have been identified: PEPT1 and PEPT2 (37). PEPT1 and 2 are localized on the brush border membrane of the proximal tubule (38, 39). PEPT1 is expressed in the early part of the proximal tubule (pars convoluta), whereas PEPT2 is expressed further along the proximal tubule (pars recta) (38, 39). PEPT1 and 2 accept not only di- or tri-peptides but also several therapeutic drugs. PEPT1 accepts therapeutic drugs such as β-lactam antibiotics (such as cephalexin, ceftibuten, cephradine), ACE inhibitors (enalapril and temocapril), and valacyclovir (37, 40, 41). Although there are few therapeutic drugs that have been reported to be substrates of PEPT2 (cephalexin), there are many drugs that interact with PEPT2 as inhibitors (37).

ORGANIC CATION TRANSPORTERS OCT1 and OCT2 are expressed in the kidney, whereas only OCT1 is expressed in the liver (14, 42). OCT2 is highly expressed in the kidney (14, 42). In human kidney, these transporters are localized in the basolateral membrane and are important organic cation transporters for renal

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tubular secretion (14, 42). These are pH-independent, electrogenic, and polyspecific transporters (14, 42). These transporters accept organic cations with relatively low molecular weight (type I cations), such as tetraethylammonium (TEA), as substrates (42, 43). Cimetidine, choline, dopamine, acyclovir, and zidovudine are also reported to be substrates (44–46).

OCTN OCTN1 is strongly expressed in the kidney but not in the adult liver (47). In OCTN1-expressing HEK293 cells, pH-sensitive uptake of TEA has been observed (47). An inward proton concentration gradient stimulated the efflux of TEA in OCTN1-expressing *Xenopus leavis* oocytes, indicating that OCTN1-mediated transport couples with proton antiport (48). OCTN1 is considered to be localized on the brush border membrane of the kidney. Substrates include quinidine and adriamycin as well as TEA (47, 48). OCTN2, an isoform of OCTN1, was isolated from human placenta and it was also found to be expressed in the kidney (49, 50). Although OCTN2 accepts TEA as its substrate, the transporter activity is not as high as that of OCTN1. OCTN2 can also accept carnitine, a zwitterion that is a cofactor essential for β-oxidation of fatty acids, and several mutations in mRNA encoding OCTN2 result in systemic carnitine deficiency owing to the poor renal reabsorption of carnitine (51). This fact suggests that OCTN2 plays a role in the renal reabsorption of carnitine. This transporter also accepts cephaloridine and other cationic compounds, such as verapamil, quinidine, and phyrilamine, in addition to TEA and carnitine, although it is not yet known whether this transporter takes part in the renal reabsorption and/or excretion of these compounds together with OCTN1 (52, 53).

Transporters in the Liver

Figure 4 shows transporters in the liver. In the liver, uptake transporters are located on the sinusoidal membrane (blood side) and efflux transporters are found on the bile canalicular membrane, although some efflux transporters are on the sinusoidal membrane and take part in the secretion into the blood.

ORGANIC ANION TRANSPORTING POLYPEPTIDES In the liver, the uptake of many organic anions is mediated by organic anion transporting polypeptides (OATPs), although OAT2 and 5 are also reported to be expressed in the liver. In humans, OATP-A, B, C, D, E, F, and 8 have been identified, and OATP-B, C and 8 are expressed in the liver (54–58). In rats, the OATP family is also conserved and Oatp1, 2, and 4 are expressed in the liver (59–61). These transporters are localized on the sinusoidal membrane of the liver. OATPs mainly accept bulky and amphipathic organic anions as substrates, although they also accept neutral compounds such as digoxin. The substrates of OATP family transporters include therapeutic drugs such as HMG-CoA reductase inhibitors, ACE inhibitors [enalapril and temocaprilat (an active form of temocapril)], and digoxin (55, 56, 58, 62–65). Many other therapeutic drugs also interact with OATP family transporters as inhibitors,

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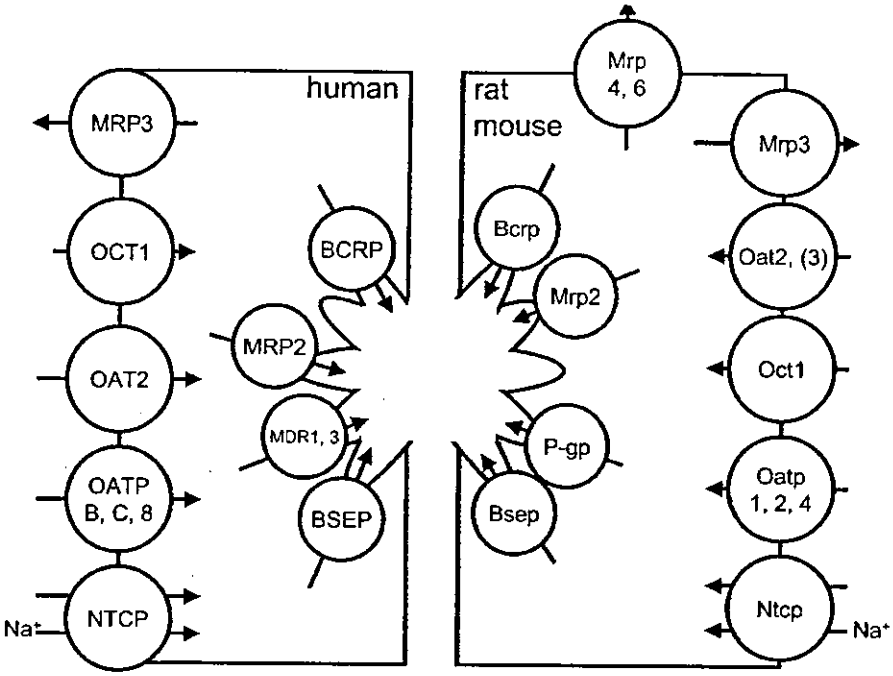

Figure 4 Transporters in the liver. Transporters expressed in human and rodent liver are summarized in this figure. Some of the transporters in rodents are expressed only in either rats or mice.

suggesting there may be other drugs that are taken up into the liver via OATP family transporters.

MULTIDRUG RESISTANCE ASSOCIATED PROTEINS In TR$^-$ and Eisai hyperbilirubinemic rats (EHBRs), which exhibit hyperbilirubinemia owing to a deficiency in the biliary excretion of bilirubin glucuronide, mutations in the transporter, multidrug resistance associated protein 2 (Mrp2), have been found (66, 67). This finding and the comparison of the biliary excretion of several compounds between normal rats and Mrp2-deficient rats suggested that it played an important role in the biliary excretion of multispecific organic anions, including glucuronide conjugates [bilirubin glucuronide, E3040 glucuronide, estradiol 17β-D-glucuronide ($E_2$17βG), grepafloxacin glucuronide, SN-38 glucuronide, glycyrrhizin, etc.], glutathione conjugate [glutathione bimane (GSB), dinitrophenyl glutathione (DNP-SG), leucotrienes (LTC_4, D_4 and E_4), etc.], grepafloxacine, MTX, pravastatin, SN-38, and temocaprilat (8, 68–72). This transporter is localized in the bile canalicular membrane of the liver (73–75). Its human counterpart (MRP2) has been isolated from the cisplatin-resistant tumor cells, KCP4 (76). This transporter is

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also localized on the bile canalicular membrane of the liver and accepts multiple organic anions, including glucuronides (bilirubin monoglucuronide, bilirubin bisglucuronide, E3040 glucuronide, $E_2 17\beta G$, grepafloxacin glucuronide, LTC_4, etc.), glutathione conjugates (DNP-SG, GSB, glutathione-methylfluorescein, etc.), pravastatin, MTX, vinblastine, vincristine, etoposide, etc. (33, 77–88).

Human MRP3 is also a MRP family transporter, which is expressed in the liver. However, this transporter is localized on the sinusoidal membrane and considered to be involved in secretion into the blood. It also accepts many glucuronides, glutathione conjugates, MTX, etc. (89, 90).

BREAST CANCER RESISTANT PROTEIN Breast cancer resistant protein (BCRP) has been cloned from human MCF-7 breast cancer cells as a multidrug resistance transporter (91–93). This transporter also belongs to the ABC transporter family (91–93). However, its structure differs from that of other ABC transporters, such as MDR1 and MRP, which contain two tandem repeats of transmembrane and ABC domains. BCRP consists of only one ABC and one transmembrane domain, and, therefore, it is referred to as a half-sized ABC transporter (91–93). This transporter is also expressed in normal tissues including the liver (94). In the liver, it is located on the bile canalicular membrane (94). Many sulfated conjugates, such as estrone 3-sulfate (E_1S), dehydroepiandrosterone sulfate, 4-methylumbelliferone sulfate, etc., are transported by BCRP (95). MTX, estradiol 17β-D-glucuronide, and 2,4-dinitrophenyl-S-glutathione are also transported but to a lesser extent compared with E_1S (95). BCRP preferentially transports sulfate conjugates (95).

METHODS FOR EVALUATING TRANSPORTER-MEDIATED DRUG-DRUG INTERACTIONS IN THE KIDNEY AND THE LIVER

In Vitro Transport Systems Using Tissues, Cells, Membrane Vesicles, and Transporter-Expressing Systems

In vitro studies using tissues, cells, and membrane vesicles prepared from animals have made it easy to characterize the mechanism of drug transport and estimate the elimination rates of drugs via liver or kidney. Recently, these systems prepared from human sources have also become available, and they are likely to be of great help in the drug discovery and other related research areas. Transporter cDNA-transfected cells or cRNA-injected oocytes are also available for drug transport studies. Because of the scarcity of human tissue sources, transporter-expressing systems will be useful for predicting transporter-mediated drug-drug interactions.

KIDNEY SLICES Kidney slices were used for the study to evaluate the renal uptake of compounds/drugs from the basolateral side (96–99). In rat kidney slices, the

uptake of compounds via Oct2, Oat1, Oat3, and a novel peptide transporter has been observed (96–99). The uptake of compounds into kidney slices is much lower than the renal intrinsic uptake clearance in vivo, which may be partly due to diffusion from the surface of the slices. Although extrapolation of the renal clearance using kidney slices has not been reported, it may be used as a tool for the prediction of transporter-mediated drug-drug interactions in the kidney. At the time of writing, there have been no reports using human kidney slices; however, the use of this tool will be useful for the prediction of transporter-mediated drug-drug interactions in the kidney.

ISOLATED AND CULTURED HEPATOCYTES Isolated and cultured hepatocytes have been used as an in vitro model of the liver (100, 101). The hepatic uptake of peptidic endothelin antagonists using isolated rat hepatocytes showed that their in vitro uptake clearance could be extrapolated to give their in vivo uptake clearance, assuming a well-stirred model (100). Thus, isolated hepatocytes are a good tool for the evaluation of drug uptake in the liver and transporter-mediated drug-drug interactions in the liver. Recently, because of the progress in the techniques of cryopreservation, it seems possible to preserve frozen human hepatocytes in such a way that most of their enzymatic activity is retained (102). They have been used to examine drug metabolism interactions, including induction of metabolic enzymes (103–105). Recently, we have examined the uptake of taurocholate (TC) and estradiol-17β-D-glucuronide in freshly-isolated and cryopreserved human hepatocytes (106). This study suggested that their active transports were retained even in cryopreserved human hepatocytes, although the activity was decreased after cryopreservation in some lots of hepatocytes (106). Therefore, cryopreserved human hepatocytes, at least, retain transporter function and they can be used as a useful experimental system for examining the mechanism of the hepatic uptake of drugs and interactions with other drugs (106).

LIVER SLICES Liver slices are also used for the study of drug uptake in the liver (107, 108). Olinga et al. examined the uptake of digoxin, a substrate of human OATP8 [OATP1B3] and rat Oatp2 [Oatp1a4], and temperature-dependent uptake was observed (107). Liver slices are supplemented with nonparenchymal cells, and, therefore, the interaction between hepatocytes and other cells and the effect of other cells on the function of hepatocytes can also be examined.

MEMBRANE VESICLES Today, membrane vesicles prepared from the brush border and basolateral membrane in the kidney and from the sinusoidal and bile canalicular membrane in the liver are readily available for the study of renal and hepatobiliary transport (109–114). The advantages of using this system for transport studies are (*a*) drug transport across the basolateral (sinusoidal) and apical (brush border or bile canalicular) membrane can be measured separately, (*b*) intracellular binding and/or metabolism can be ignored, and (*c*) buffers inside and outside vesicles can be changed easily. On the other hand, using this system has limitations because it

requires a driving force for transport, so it is impossible to use this system without prior characterization.

STUDIES USING GENE EXPRESSION SYSTEMS Using transporter expression systems, the kinetic parameters for the target transporter can be obtained. Once the responsible transporter for the drugs in question has been identified, the possibility of drug-drug interactions can be examined using the gene expression system, i.e., without hepatocytes, membrane vesicles, and tissue slices. As human tissue samples are scarcely distributed, transporter-expressing systems greatly help drug transport studies. With the information of contributions of specific transporter(s) to the total uptake of drugs in human liver or kidney, quantitative prediction of drug uptake in human tissues is possible. The method to estimate the contributions of specific transporters is described below. cDNA-transfected cells and cRNA-injected oocytes can be used as gene expression systems. More recently, cultured cells stably transfected with both uptake and efflux transporters have become available (85, 115). OATP-C/OATP2 [OATP1B1] and MRP2 transfected cells and OATP8 [OATP1B3] and MRP2 transfected cells have been reported (Figure 5) (85, 115). Using them, hepatobiliary transport can be measured as vectorial transcellular transport when these cells are cultured on a porous membrane. This will make it easy to predict transporter-mediated drug-drug interactions in the liver.

ESTIMATION OF THE CONTRIBUTION OF A SPECIFIC TRANSPORTER The estimation of the contribution of specific transporter(s) is important for the quantitative prediction of the uptake in human tissues, including liver and kidney from the in vitro data using transporter-expressing systems, and even for the quantitative

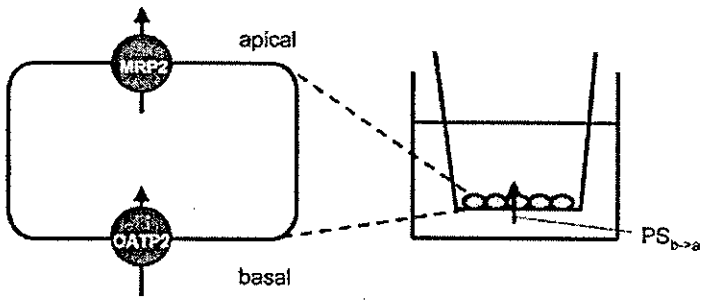

Figure 5 Experimental system for the estimation of transcellular transport of drugs mediated by OATP-C/OATP2 [OATP1B1] and MRP2. OATP-C/OATP2 [OATP1B1] and MRP2 double-transfected MDCK cells are seeded in a membrane insert. The basal-to-apical flux of drugs across the MDCK cell monolayer was examined to estimate the transcellular transport mediated by OATP-C/OATP2 [OATP1B1] and MRP2.

prediction of transporter-mediated drug-drug interaction. Here, we show the method to estimate it in in vitro assays.

First, injection of cRNA coding a transporter results in its expression on the plasma membrane of *Xenopus laevis* oocytes that have been used for expression cloning, functional analysis, or transport assays (117, 118). However, hybridization of mRNA with antisense oligonucleotide coding a specific sequence for the target transporter specifically reduces the expression of the transporter (117, 118). Comparison of the transporter activity in cRNA-injected oocytes in the presence and absence of antisense nucleotides gives the contribution of each transporter to the net transport (117, 118).

Kouzuki et al. have proposed a method using reference compounds (119, 120). They measured the uptake of reference and test compounds at the same time in transporter cDNA-transfected COS7 cells and rat hepatocytes and calculated the contribution using the following equation (119, 120):

$$\text{Contribution} = \frac{CL_{hep,ref}/CL_{COS,ref}}{CL_{hep,test}/CL_{COS,fest}}, \quad (6)$$

where CL_{hep} and CL_{COS} represent the uptake clearance of compounds into hepatocytes and transporter cDNA transfected cells, respectively. $CL_{\#,ref}$ and $CL_{\#,test}$ represent the uptake clearance of the reference and test compounds, respectively. The reference compounds should be specific substrates, otherwise the contribution will be overestimated (119, 120). More recently, Hirano et al. proposed a method to estimate the contributions of human transporters (OATP-C/OATP2 [OATP1B1] and OATP8 [OATP1B3]) to the total hepatic uptake using estrone 3-sulfate and cholecystokinine octapeptide (CCK8) as specific substrates, respectively, and actually estimated their contributions to the hepatic uptake of pitavastatin (121). They also estimated their contributions by uptake in human hepatocytes and transporter expression systems normalized by their transporter expression levels measured by Western blot analysis (121). The contributions of OATP-C/OATP2 [OATP1B1] and OATP8 [OATP1B3] estimated by these two different methods were comparable, suggesting the validity of this method (121).

A specific inhibitor of a transporter also helps to estimate its contribution to the total uptake. To identify a specific inhibitor, we examined the comparative inhibitory effects of many compounds on rat Oatp1 [Oatp1a1] and Oatp2 [Oatp1a4] (122). Among them, we found that digoxin specifically inhibited Oatp2 [Oatp1a4] with no effect on Oatp1 [Oatp1a1] (122). We also found several compounds which preferentially inhibited one of these transporters (122). These inhibitors may be used to estimate the contributions of Oatp1 [Oatp1a1] and Oatp2 [Oatp1a4] at appropriate concentrations (122). However, the selectivity of most of the preferential inhibitors in this report was not very high, and inhibitors that act as selective inhibitors over a wider range of concentrations are needed (122).

EVALUATION OF TRANSPORTER-MEDIATED DRUG-DRUG INTERACTIONS

In this section, the inhibitory effects of therapeutic drugs and the possibility of clinically relevant drug-drug interactions based on transporter-mediated processes are described.

How to Evaluate the Extent of Transporter-Mediated Drug-Drug Interactions

Previously, our group has suggested how to predict the extent of drug-drug interactions based on drug metabolism using in vitro studies (123, 124). This method can also be applied to transporter-mediated drug-drug interactions (125). As transporter-mediated influx or efflux follows the Michaelis-Menten equation, the clearance can be described as follows:

$$CL = \frac{V_{max}}{K_m + S_u} + P_{dif}, \quad (7)$$

where CL is the influx or efflux clearance; V_{max}, K_m, and P_{dif} are the maximum transport rate, Michaelis constant, and nonsaturable transport clearance, respectively; and S_u is the protein-unbound substrate concentration. In the presence of competitive inhibitors, it can be described as follows:

$$CL\,(+\text{inhibitor}) = \frac{V_{max}}{K_m \cdot (1 + I_u/K_i) + S_u + P_{dif}}, \quad (8)$$

where I_u is the protein-unbound inhibitor concentration and K_i is the inhibition constant. It should be noted that the I_u value is the protein-unbound inhibitor concentration outside the cells for influx transporters, whereas it is that inside the cells for efflux transporters. On the other hand, in the case of noncompetitive inhibition, it can be described as follows:

$$CL\,(+\text{inhibitor}) = \frac{V_{max}/(1 + I_u/K_i)}{K_m + S_u} + P_{dif}. \quad (9)$$

When the protein unbound substrate concentration is negligibly low compared with the K_m value, the influx or efflux clearance via transporters can be described by the following equation, both for competitive and noncompetitive inhibition:

$$CL\,(+\text{inhibitor}) = \frac{V_{max}}{K_m \cdot (1 + I_u/K_i)} + P_{dif}. \quad (10)$$

Therefore, transporter-mediated influx or efflux clearance (i.e., net influx or efflux clearance subtracted by the nonsaturable clearance) is decreased by the following equation:

$$\frac{CL_{transporter}\,(+inhibitor)}{CL_{transporter}\,(control)} = \frac{1}{1 + I_u/K_i} = R, \tag{11}$$

where $CL_{transporter}$ represents transporter-mediated influx or efflux clearance.

When a transporter, which is a key determinant of the disposition of a drug, is inhibited by a concomitantly administered drug, the area under the blood/plasma concentration (AUC) after an oral administration will increase by at most $\frac{1}{R}$-fold when the drug is predominantly excreted in the liver. In such cases, hepatic or renal intrinsic clearances decrease by R-fold and, therefore, this R value is one of the indicators of the severity of a drug-drug interaction. It should be particularly useful for the evaluation of in vivo drug-drug interactions to avoid false negative predictions.

For the liver transporters, the estimation of I_u should account for the inhibitors in the portal vein as well as the hepatic artery when the inhibitor drug is orally administered. In this case, I_u is not equal to the inhibitor concentration in the circulating blood. Ito et al. have suggested a method to estimate the inhibitor concentration at the inlet to the liver using the following equation (Figure 6) (123, 124):

$$I_u = f_u \cdot (I_{sys} + I_{pv}) = f_u \cdot \left(I_{sys} + \frac{v_{abs}}{Q_H}\right), \tag{12}$$

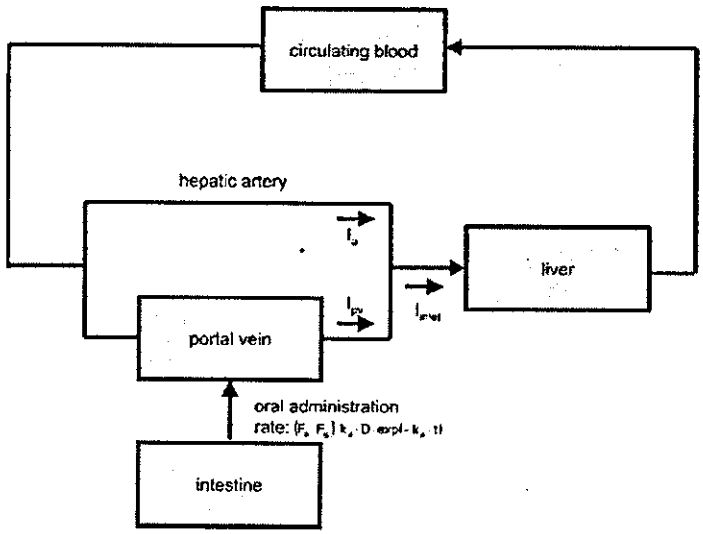

Figure 6 A model for estimating the inhibitor concentration at the inlet to the liver after oral administration. I_{inlet} is the inhibitor concentration at the inlet to the liver. It can be estimated from the inhibitor concentration in the hepatic artery (I_a) plus that in the portal vein (I_{pv}).

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where I_{sys} and I_{pv} are the inhibitor concentration in the circulating blood and portal vein, respectively; f_u is the blood protein unbound fraction; v_{abs} is the absorption rate from the intestine to the portal vein; and Q_H is the hepatic blood flow. When the intestinal absorption is described by a first-order rate constant, this equation becomes (123, 124)

$$I_u = f_u \cdot \left(I_{sys} + \frac{F \cdot D \cdot k_a \cdot e^{-k_a \cdot t}}{Q_H} \right) \leq f_u \cdot \left(I_{sys} + \frac{F \cdot D \cdot k_a}{Q_H} \right), \qquad (13)$$

where F is the fraction absorbed from the gastrointestinal tract, D is the dose, and k_a is the absorption rate constant. To avoid a false negative prediction, the unbound inhibitor concentration should be estimated by $f_u \cdot (I_{sys} + \frac{F \cdot D \cdot k_a}{Q_H})$ for a drug-drug interaction based on a hepatic transporter-mediated process.

To date, there are many published inhibition studies of renal and hepatic uptake transporters: OATs and OATPs. In this section, the inhibitory effects of therapeutic drugs on these transporters are evaluated using K_i values, comparing them with the therapeutic concentrations.

OAT-Mediated Drug-Drug Interactions

In the kidney, the OAT family transporters are involved in the uptake of organic anions with relatively low molecular weights into the renal tubules, although OAT2 and 5 are localized in the liver and OAT4 is expressed in the brush border membrane of the kidney and may be involved in efflux from the renal tubules into the urine (21–24). These OAT family transporters are inhibited by several compounds, including therapeutic drugs (Supplemental Table 1, Follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org). Supplemental Table 1 gives a partial list of therapeutic drugs that interact with OAT family transporters, together with their maximum plasma concentration and maximum plasma unbound concentration in a clinical situation and R value.

The calculated R values suggest that many inhibitor drugs of OAT family transporters do not cause a serious drug-drug interaction because of the relatively low plasma concentrations compared with their K_i values (Supplemental Table 1). However, some cephalosporin antibiotics and probenecid exhibited low R values and, therefore, may lead to clinically relevant drug-drug interactions (Supplemental Table 1). These results suggest that the concomitant use of these drugs with OAT substrate drugs, which are mainly excreted in the urine, should be very carefully monitored. Such use may cause at least a partial reduction in the intrinsic clearance for renal secretion, possibly leading to an increase in plasma concentration.

OATP-Mediated Drug-Drug Interactions

Among OATP family transporters, OATP-B [OATP2B1], OATP-C/OATP2 [OATP1B1], and OATP8 [OATP1B3] are expressed in the human liver and are involved in the hepatic uptake of several compounds, including therapeutic drugs (54–58). Although, in rats, some Oatp family transporters, such as Oatp1 [Oatp1a1],

Oat-k1 [Oatp1a3], and k2, are reported to be expressed in the kidney (126–130), their human counterparts have not been characterized. As shown in Supplemental Table 2 (Follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org), several therapeutic drugs are reported to inhibit OATP family transporters. Because they are hepatic uptake transporters, R values were calculated based on not only the maximum inhibitor unbound therapeutic concentration in the circulating blood but also that in the inlet to the liver, calculated by Equation 13 (123, 124). Values calculated based on the unbound concentration in the inlet to the liver are given as R'. Inhibitors of OATP family transporters consist of bulky compounds, including anions, neutral compounds, and even cations (Supplemental Table 2). In Supplemental Table 2, only cyclosporin A and rifampicin exhibited relatively low R and R' values and may lead to clinically relevant drug-drug interactions. On the other hand, pravastatin, an HMG-CoA reductase inhibitor, is not a cause of a severe drug-drug interaction based on OATP-mediated hepatic uptake because of its low plasma unbound concentration. As pravastatin is a potent HMG-CoA reductase inhibitor and is highly distributed to the liver, its target organ, a low plasma concentration is sufficient for its pharmacological effect, leading to a low risk of inhibition of transporter function (132). A small number of inhibitors with relatively low R values may be due to a lack of inhibition studies involving human OATP family transporters, and further studies may provide other inhibitors that cause clinically relevant drug-drug interactions. More inhibition studies on human OATP transporters are needed to allow the quantitative prediction of transporter-mediated drug-drug interactions.

MDR-Mediated Drug-Drug Interactions

MDR1 is expressed in the liver and kidney (7, 8, 15). Therefore, MDR1-mediated drug-drug interactions result in a reduction in renal and hepatobiliary excretion. It is also expressed in the intestine and the blood-brain barrier and, therefore, MDR1-mediated transport affects intestinal absorption and even distribution to the brain (7). MDR1-mediated drug-drug interactions cause complex effects. MDR1 has a broad substrate specificity and is inhibited by a large number of compounds. Quinidine is one MDR1 inhibitor (35). As the K_m value of quinidine for ATP-dependent efflux via MDR1 is approximately 5 μM (32), its K_i value for MDR1 can be assumed to be 5 μM. The therapeutic steady-state concentration of quinidine is approximately 4.5 μM and its unbound concentration is 0.59 μM. As MDR1 is an efflux transporter, the R value should be calculated using the unbound concentration of inhibitor in the cell. However, it is practically impossible to measure the intracellular unbound concentration of inhibitors in humans. Assuming the cell-to-medium concentration ratio to be 10 as a safety margin, the R value can be calculated to be $\frac{1}{1+10\times0.59/5} = 0.46$, suggesting that renal efflux will be reduced to at most 46% of the control. For hepatobiliary efflux, the blood concentration at the inlet to the liver should be used. The plasma concentration of quinidine at the inlet to the liver is calculated to be 4.6 μM using $Q_H = 1.6$ liters min^{-1}, $F_a{}^*F_g =$

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0.8, $k_a = 0.1\ min^{-1}$, and $f_u = 0.13$. Using this and assuming a cell-to-medium concentration ratio of 10, the calculated R value is $\frac{1}{1+10\times4.6/5} = 0.098$, suggesting that hepatobiliary excretion will be reduced to at most 9.8% of the control. Actually, both the hepatobiliary and renal clearances of digoxin have been reported to be reduced when concomitantly administered with quinidine (133).

MRP2-Mediated Drug-Drug Interactions

MRP2 also has a broad substrate specificity and is inhibited by a large number of therapeutic drugs, including cyclosporin A, daunomycin, etoposide, probenecid, and pravastatin (33, 134, 135). MRP2 functions as an efflux transporter for CPT-11 and its metabolites, SN-38 and SN-38 glucuronide (SN38-glu) (136). CPT-11 is excreted into the bile mainly via MDR1 and, to a minor extent, via MRP2, whereas SN-38 and SN38-glu are excreted via MRP2 (136). The biliary excretion of its metabolites causes severe diarrhea as a side effect (137, 138). To prevent this side effect, inhibition of MRP2-mediated transport by coadministration of its inhibitor may be effective. Horikawa et al. have investigated the inhibitory effects of several compounds on rat Mrp2 function (139). Among them, probenecid, sulfobromophthalein, and the glutathione-conjugate of sulfobromophthalein had potent inhibitory effects (139). The inhibitory effects of probenecid were also confirmed for the in vitro human biliary excretion of SN-38 with a K_i value of 42 μM (139). The same authors also confirmed these inhibitors of rat Mrp2 significantly reduced the biliary excretion of CPT-11, SN-38, and SN38-glu (140). They suggested the possibility of using MRP2 inhibitors such as probenecid to prevent the clinically observed toxicity of diarrhea by CPT-11.

EXAMPLES OF CLINICALLY RELEVANT DRUG-DRUG INTERACTIONS BASED ON RENAL AND HEPATOBILIARY TRANSPORT

In this section, examples of clinically relevant drug-drug interactions based on membrane transport in the kidney and the liver are described.

HMG-CoA Reductase Inhibitors Versus Cyclosporin A

As cerivastatin, a potent HMG-CoA reductase inhibitor (statin), is metabolized by two different enzymes, cycochrome P450 2C8 (CYP2C8) and 3A4, the likelihood of a severe drug-drug interaction was believed to be low (141). However, the plasma concentration of cerivastatin was reported to be increased when coadministered with cyclosporin A (142).

The plasma AUC and maximum plasma concentration of cerivastatin increased by four- and fivefold, respectively, when concomitantly administered with cyclosporin A (142). Our group investigated the mechanism underlying this drug-drug interaction (62). We have shown that the transporter-mediated uptake of

cerivastatin is inhibited by cyclosporin A at a low concentration (K_i was 0.3 ~ 0.7 μM), whereas the in vitro metabolism of cerivastatin is inhibited with an IC_{50} value of more than 30 μM, suggesting that this clinically relevant drug-drug interaction was caused by a transporter-mediated process rather than a metabolic one (62). The unbound concentration of cyclosporin A in the circulating blood and at the inlet to the liver, calculated by Equation 13, are, at most, 0.1 μM and 0.6 μM, respectively, which may explain the clinically relevant drug-drug interaction, although there may be other mechanisms involved (62). We also showed that the OATP-C/OATP2 [OATP1B1]-mediated transport of cerivastatin was inhibited by cyclosporin A with a K_i value of less than 0.2 μM (Figure 7) (62).

In addition to cerivastatin, the plasma concentrations of pravastatin, pitavastatin, and HMG-CoA reductase inhibitory activity of atorvastatin are reported to be affected by concomitantly administered cyclosporin A (143–145). Among them, pravastatin and pitavastatin undergo only minimal metabolism, and the likelihood of a drug-drug interaction owing to this is quite low. As these statins are substrates of OATP-C/OATP2 [OATP1B1], interactions with cyclosporin A may also be caused by a transporter-based mechanism (55, 56, 121). Interaction between atorvastatin and cyclosporin A may be occurred by a transporter-mediated

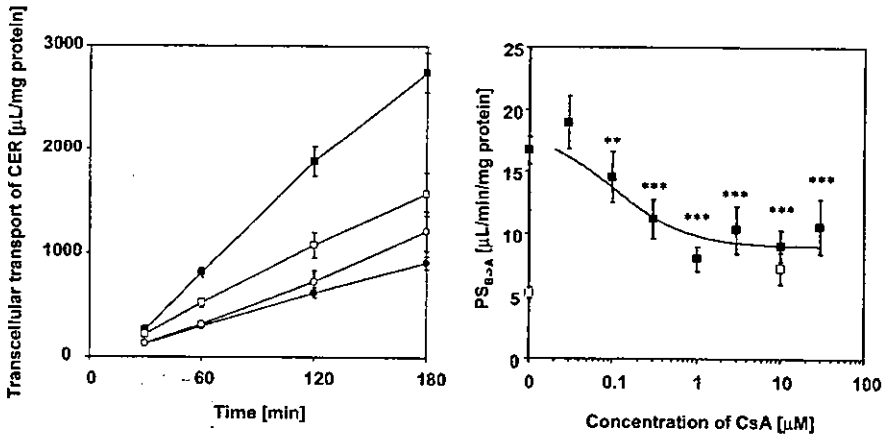

Figure 7 Transcellular transport of cerivastatin (CER) mediated by OATP-C/OATP2 [OATP1B1] and MRP2 and the inhibitory effect of cyclosporin A. (*a*) Transcellular transport of [^{14}C]CER in OATP-C/OATP2 [OATP1B1] and MRP2 double-transfected MDCK cells (*closed squares*) and in vector-transfected cells (*closed circles*) was examined. Addition of cyclosporin A (10 μM) inhibited OATP-C/OATP2 [OATP1B1]- and MRP2-mediated transport of CER (*open squares*), whereas it did not change the transcellular transport in vector transfected cells (*open circles*). (*b*) Cyclosporin A inhibited the transcellular transport ($PS_{B->A}$) in a concentration-dependent manner. The IC_{50} value obtained in this experimental system was 0.084 ± 0.015 μM. $^{**}p < 0.01$, $^{***}p < 0.001$.

TABLE 1 Kinetic parameters of HMG-CoA reductase inhibitors coadministered with cyclosporin A

HMG-CoAreductase inhibitors	Cyclosporin A (+/−) Cmax [ng/mL]	Ratio	AUC [ng · hr/mL]	Ratio	Major clearance mechanism	Reference
Simvastatin	18.9/2.5**	7.56	78.1/9.8**	7.97	CYP3A4	193
	20.6/9.9*	2.08	101/39.6*	2.55		194
Pravastatin	223/28.0	7.95	1300/ 57.1***		OATP-C	143
Fluvastatin	155/119	1.30	373/192	1.94	CYP2C9	195
Cerivastatin	7.82/1.56	5.01	36.2/9.53	3.80	CYP2C8/ 3A4OATP-C	142
Atorvastatin	58.0/8.8#*	6.59	595/79.9#*	7.45	CYP3A4-OATP-C	145
Pitavastatin	179/27.6***	6.49	347/76.9***	4.51	OATP-C	144

#ng eq./mL or ng eq. · hr/mL
*$p<0.05$, **$p<0.01$, ***$p<0.001$

and metabolism-based mechanism as atorvastatin is metabolized by CYP3A4 and cyclosporin A inhibits CYP3A4-mediated metabolism (146). In Table 1, we summarize pharmacokinetic interactions between HMG-CoA reductase inhibitors and cyclosporin A.

HMG-CoA Reductase Inhibitors Versus Gemfibrozil

Gemfibrozil also interacts with a wide range of statins (Table 2). In particular, interactions with cerivastatin have been reported to cause the severe side effect of myotoxicity, including lethal rhabdomyolysis (147). In addition, pharmacokinetic interaction between cerivastatin and gemfibrozil was reported (148, 149). Although our group examined the inhibitory effects of gemfibrozil and its major metabolites on the OATP-C/OATP2 [OATP1B1]-mediated uptake of cerivastatin, we found gemfibrozil and its glucuronide inhibited it with IC_{50} values of 72 and 24 μM, respectively, which were higher than their therapeutic unbound concentrations, suggesting a low possibility of a transporter-mediated drug-drug interaction (150). On the other hand, an interaction with rosuvastatin was reported to be caused by the inhibition of OATP-C/OATP2 [OATP1B1]-mediated uptake by Schneck et al. (151). In their report, gemfibrozil inhibited the OATP-C/OATP2 [OATP1B1]-mediated transport of cerivastatin with a low IC_{50} value of 4 μM (151). Although it is still higher than the therapeutic unbound concentration of cerivastatin, this value is lower than that we have obtained (150). This gap may be partly due to the difference in the experimental system, i.e., we used transporter-expressing MDCK cells, whereas Schneck et al. used cRNA-injected

TABLE 2 Kinetic parameters of HMG-CoA reductase inhibitors coadministered with gemfibrozil

HMG-CoA reductase inhibitors	Gemfibrozil (+/−)				Major clearance mechanism	Reference
	Cmax [ng/mL]	Ratio	AUC [ng · hr/mL]	Ratio		
Lovastatin	2.38/2.69	0.885	33.1/34.4	0.962	CYP3A4	196
Simvastatin	6.15/6.87	0.895	36.2/25.2**	1.44	CYP3A4	197
Pravastatin	120/66.3*	1.81	281/139*	2.02	OATP-C	198
Fluvastatin	54.3/48.4	1.12	213/227	0.938	CYP2C9	199
Cerivastatin	8.0/3.2**	2.5	91.1/20.9***	4.36	CYP2C8/3A4 OATP-C	148
	2.93/1.61	1.82	41.9/9.92	4.22		149
Pitavastatin	no data	1.30	no data	1.45	OATP-C	200
Rosuvastatin	109/49.5	2.20	771/410	1.88	CYP2C9 OATP-C	151

*p<0.05, **p<0.01, ***p<0.001

Xenopus laevis oocytes (150, 151). We also analyzed the inhibitory effects of gemfibrfozil and its metabolites on the P450-mediated metabolism of cerivastatin and found that gemfibrozil and its glucuronide inhibited the CYP2C8-mediated metabolism with IC_{50} values of 28 and 4 μM, respectively (150). They are still higher than the therapeutic unbound concentrations in the circulating blood. However, there are reports that, in rat perfusion studies, gemfibrozil-1-O-glucuronide is actively taken up into the liver and accumulates there (152–154). If this also took place in human liver, the concentrated gemfibrozil-1-O-glucuronide might act as an inhibitor of CYP2C8-mediated metabolism, leading to a drug-drug interaction. In this case, a transporter plays an important role, i.e., an inhibitor of the metabolism leading to accumulation in the liver via transporter-mediated uptake. Our hypothesis that interaction with gemfibrozil is not a transporter-mediated one, but a metabolism-mediated one is supported by the fact that gemfibrozil does not cause a severe interaction with pravastatin and pitavastatin, which are mainly cleared by the OATP-C/OATP2 [OATP1B1]-mediated hepatic uptake (Table 2). Therefore, we should also be more cautious about drug-drug interactions when inhibitors of the metabolism are substrates of hepatic uptake transporters (Figure 8).

Digoxin Versus Quinidine and Quinine

Digoxin undergoes biliary and renal excretion. Drug-drug interactions between digoxin and quinidine and between digoxin and quinine (a stereoisomer of quinidine) have been reported by Hedmann et al. (133). Quinidine reduced the renal and biliary excretion of digoxin, whereas quinine reduced only the biliary excretion of digoxin (133).

Because quinidine is a well-known P-gp inhibitor, its effect on biliary and urinary excretion may be related to P-gp (MDR1)- mediated transport (35). As de-

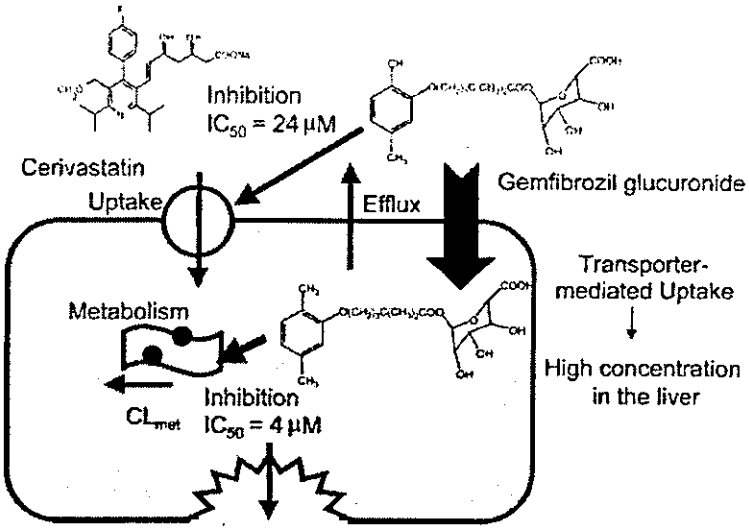

Figure 8 Possible mechanism of drug-drug interaction between cerivastatin and gemfibrozil. Gemfibrozil-1-O-glucuronide is actively taken up via transporter(s) and accumulates in the liver. In the liver, its concentration is hypothesized to be high enough to inhibit the P450-mediated metabolism of cerivastatin.

scribed in MDR-Mediated Drug-Drug Interactions (above), the K_i value of quinidine for the MDR1-mediated efflux can be assumed to be 5 μM. On the other hand, the steady-state plasma concentration of quinidine in this study was 4.5 μM, with a protein unbound fraction of 0.13. Therefore, the protein unbound concentration in the circulating blood is estimated to be 0.59 μM. The unbound concentration of quinidine at the inlet to the liver estimated by Equation 13 is 4.6 μM using Q_H = 1.6 liters min^{-1}, $F_a{}^*F_g$ = 0.8, and k_a = 0.1 min^{-1}. With a safety margin of 1 ~ 10 as a cell-to-medium concentration ratio, the estimated reduction in the renal excretion of digoxin is 46% to 89% of the control, and the estimated reduction in the hepatobiliary excretion of digoxin is 9.8% to 52% of the control. In clinical situations, the hepatobiliary excretion was reduced to 42% of the control, whereas the renal excretion was reduced to 60% of the control, which was within the predicted range (133).

In rat hepatocytes, the inhibitory effect on the uptake of digoxin was more potent for quinine than for quinidine, and the same tendency was observed using the rat Oatp2 [Oatp1a4] expression system (122, 155). Therefore, the mechanism of the drug-drug interaction between digoxin and quinine may be caused by the inhibition of the transporter-mediated uptake. However, there is a study that shows that both quinine and quinine had no inhibitory effects on the uptake of digoxin into isolated human hepatocytes, although both of them inhibited the uptake of digoxin into rat hepatocytes (156).