1994; Tsuji and Tamai, 1996; Terada and Inui, 2004). Transporters in the liver, kidney and intestine are illustrated in Fig. 1.

Transporters in other tissues are also determinants of the distribution of drugs to the target organs for the pharmacological effects and/or adverse reactions. Since the distribution volume of drugs to the brain is generally low, transporters in the brain do not affect the plasma concentration of drugs. However, they control the drug distribution to the brain, affecting the pharmacological effects or side effects (Tamai and Tsuji, 2000; Kusuhara and Sugiyama, 2004, 2005).

In this manuscript, we shall focus on the transporter functions in the kidney and liver and review the mechanisms of drug elimination. We will also describe a recently developed method of analyzing transporter function by estimating the contribution of each transporter, and the use of transporter double transfectants.

2. Hepatic and renal transporters as a determinant of drug disposition

2.1. *Substrates of hepatobiliary transporters*

Table 1 shows some of therapeutic drugs which are substrates of transporters in the liver. Among them, some drugs are taken up into hepatocytes, followed by metabolism while others are excreted into the bile in intact form (Stieger and Meier, 1998; Keppler and Konig, 2000; van Montfoort et al., 2003; Fujino et al., 2004a). For example, atorvastatin, a 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin), is taken up into liver via transporter(s) including organic anion transporting polypeptide 1B1 (OATP1B1/OATP-C/OATP2/LST-1, gene symbol; SLCO1B1/SLC21A6), subsequently undergoing metabolism by cytochrome P450 3A4 (CYP3A4) (Lennernas, 2003; Kameyama et al., 2005; Lau et al., 2006). In the case of cerivastatin, CYP2C8 and 3A4 are responsible for its metabolism after its hepatic uptake (Muck, 2000; Shitara et al., 2003, 2004a). On the other hand, pravastatin and rosuvastatin are reported to be mainly excreted in intact form (Hatanaka, 2000; White, 2002). Although pitavastatin is metabolized by CYP2C9, the metabolic rate is very low (Fujino et al., 2004b). In fact, it is excreted into bile mainly in the intact form in experimental animals including rats, rabbits and dogs (Kojima et al., 1999). Table 1 also shows the elimination pathway following the hepatic uptake. For some of the drugs listed in Table 1, the hepatic extraction ratio and the fraction excreted into urine as the unchanged form is shown in Table 2. In this table, the hepatic extraction ratio is calculated by the hepatic clearance, which is the total body clearance minus the renal clearance, divided by the hepatic blood flow.

The hepatobiliary transport mechanism of pravastatin has been studied in rats. Its Michaelis constant (K_m) determined in vitro for hepatic uptake is 29–37 μM while that for biliary excretion is 220 μM (Yamazaki et al., 1993, 1997; Ishigami et al., 1995). On the other hand, the K_m value for the hepatic elimination rate in vivo is close to that for the hepatic uptake process in vitro, suggesting that the rate-limiting step for the overall hepatic elimination in rats is the uptake (Yamazaki et al., 1996b).

Table 2 – Hepatic extraction of substrate drugs of hepatic uptake transporter(s)

	CL_{tot} (L/h)	f_e	E_H
Cerivastatin	13	0	0.13
Pravastatin	57	0.47	0.31
Rosuvastatin	49	0.30	0.36
Repaglinide	38	0.080	0.36
Valsartan	2.2	0.29	0.016
Bosentan	13	<0.01	0.14

f_e: fraction excreted in urine as an unchanged form; E_H: hepatic extraction ratio, E_H was calculated based on the following equation: $E_H = CL_H/Q_H$ where, CL_H is hepatic clearance, that is total body clearance minus renal clearance, and Q_H is hepatic blood flow rate (96.6 L/h).

In rats, Oatp1a1 (Oatp1, *Slco1a1/Slc21a1*), Oatp1a4 (Oatp2, *Slco1a4/Slc21a5*) and Oatp1b2 (Oatp4, *Slco1b2/Slc21a10*) are involved in the hepatic uptake of pravastatin (Hsiang et al., 1999; Tokui et al., 1999; Sasaki et al., 2004). Among them, there are conflicting reports of the involvement of Oatp1a4: Hsiang et al. showed that Oatp1a4 does not accept pravastatin as a substrate while Tokui et al. described its saturable transport in Oatp1a4-expressing *Xenopus laevis* oocytes. This discrepancy may be due to the difference in the experimental systems used: i.e. cDNA transfected mammalian cells versus cRNA injected *Xenopus laevis* oocytes. The uptake study in isolated rat hepatocytes showed that most of the hepatic uptake of pravastatin (92–93%) is mediated by a saturable process (Table 3). In humans, OATP1B1 and OATP2B1 (*SLCO2B1/SLC21A9*) accept pravastatin as a substrate among hepatic uptake transporters, and OATP1B1 seems to play a major role (Hsiang et al., 1999; Nakai et al., 2001; Kobayashi et al., 2003; Nozawa et al., 2004). Recent studies using MDCK cells expressing OATP1B1 and efflux transporter (multidrug resistance associated protein 2 (MRP2; *ABCC2*), multidrug resistance 1 (MDR1; *ABCB2*) or breast cancer resistance protein (BCRP; *ABCG2*)) showed that the biliary excretion of pravastatin is mediated by multiple transporters in humans (Sasaki et al., 2002; Matsushima et al., 2005). Not only MRP2 but also BCRP and MDR1 may play roles in its biliary excretion, though the contribution of MRP2 seems to be the highest (Sasaki et al., 2002; Matsushima et al., 2005). Moreover, bile salt export pump (BSEP, *ABCB11*) is also reported to be involved in its biliary excretion (Hirano et al., 2005a). Recent studies indicate that genetic polymorphisms in OATP1B1 alter the pharmacokinetics of pravastatin, suggesting that transporter-mediated hepatic uptake is the main determinant of its plasma clearance (Nishizato et al., 2003; Niemi et al., 2004; Mwinyi et al., 2004). More recently, genetic polymorphism in OATP1B1 was also found to alter the pharmacokinetics of pitavastatin similar to pravastatin (Chung et al., 2005).

Even for drugs, whose clearances are dependent on metabolism, the hepatic uptake can become rate determining in the overall elimination. For cerivastatin, it is clear that the uptake process is the rate-limiting factor in humans and rats in its elimination because cyclosporin A (CsA), which inhibits its transporter-mediated hepatic uptake with mini-

Table 3 – Uptake of drugs into isolated human or rat hepatocytes

	K_m (μM)	V_{max} (pmol/min/mg protein)	V_{max}/K_m (μL/min/mg protein)	P_{dif} (μL/min/mg protein)	References
Human					
Pravastatin	12	10	0.89	0.30	Nakai et al. (2001)
Cerivastatin	180	5200[a]	280[a]	70[a]	Shitara et al. (2003)
	2.6	550[a]	210[a]	65[a]	Shitara et al. (2003)
	3.7	360[a]	97[a]	42[a]	Shitara et al. (2003)
Pitavastatin	3.0	80	27	7.7	Fujino et al. (2004a)
Rat					
Pravastatin	29	550	19	1.6	Yamazaki et al. (1993)
	37	820	22	1.6	Ishigami et al. (1995)
Cerivastatin	5.9	260	44	24	Shitara et al. (2004a)
Pitavastatin	26	3100	120	1.2	Shimada et al. (2003)
Rosuvastatin	9.2	–	–	–	Nezasa et al. (2003)
Glycyrrhizin	11	110[a]	9.9[a]	–	Ishida et al. (1993)
Bumetanide	140	980	6.9	–	Follmann et al. (1990)
Cephalexin	6300	2300	0.36	–	Tamai and Tsuji (1987)
Benzylpenicillin	470	2000	4.3	–	Tsuji et al. (1986)
Glibenclamide	3.1	420	130	–	Petzinger and Fuckel (1992)
Indomethacin	12	1100	93	2.1	Kouzuki et al. (2000)
Grepafloxacin	170	6700	40	28	Sasabe et al. (1997)
Levofloxacin	440	8600	20	14	Sasabe et al. (1997)

[a] Based on per 10^6 cells.

mal effects on metabolism, alters its plasma clearance in vivo (Muck et al., 1999; Shitara et al., 2003, 2004b). Repaglinide, an antidiabetic drug, is also metabolized by CYP2C8 and 3A4 (Bidstrup et al., 2003). Its total clearance is also affected by the genetic polymorphism of OATP1B1, suggesting OATP1B1-mediated uptake is a determinant of its pharmacokinetics (Niemi et al., 2005b) (Fig. 2). More recent studies have shown that CsA alters the pharmacokinetics of repaglinide, supporting that transporter-mediated hepatic uptake is a determinant of its pharmacokinetics (Kajosaari et al., 2005). Bosentan, an endothelin receptor antagonist, is metabolized by CYP2C9 and 3A4 (Dingemanse and van Giersbergen, 2004). Although the mechanism of its hepatic uptake in humans is still to be investigated, its pharmacokinetic behavior in rats is affected by the coadministration of CsA (Treiber et al., 2004) (Fig. 3). Treiber et al. analyzed the mechanism of this pharmacokinetic interaction and concluded that it was mainly due to the inhibition of Oatp-mediated hepatic uptake because the inhibition of Mdr1a/b (*Abcb1a/b*) did not cause a serious alteration in the pharmacokinetics of bosentan and the inhibition of metabolism was insufficient to explain the serious interaction between these two drugs. This pharmacokinetic interaction was also reported in humans in clinical trials, suggesting its pharmacokinetics in humans is also determined by hepatic uptake transporters (Binet et al., 2000) (Fig. 3). Telmisartan, an angiotensin receptor antagonist, is excreted in bile as its glucuronide conjugate, at least partly, via Mrp2 (*Abcc2*) in rats (Nishino et al., 2000). Recently, OATP1B3 (OATP8, *SLCO1B3/SLC21A8*) has been suggested to be involved in the hepatic uptake of telmisartan in humans (Ishiguro et al., 2005). Accordingly, this transporter may be a determinant of the pharmacokinetics of telmisartan. As described here, for some drugs which are taken up into the liver via transporter(s), the uptake can be a limiting process of the elimination rate even when their final elimination pathway is metabolism.

2.2. *Involvement of hepatic uptake transporters in the drug disposition*

2.2.1. *Transporters can be a rate-limiting factor in the elimination of drugs*

Fig. 4(A) shows the scheme of the hepatic elimination of drugs. In this section, the role of transporters in the hepatic uptake and biliary excretion with regard to the overall elimination is presented based on a clearance concept. The overall hepatic intrinsic clearance ($CL_{int,all}$) can be described by the following equation (Pang and Gillette, 1978; Yamazaki et al., 1996a):

$$CL_{int,all} = PS_{u,influx} \times \frac{CL_{int}}{CL_{int} + PS_{u,efflux}} \quad (1)$$

where $PS_{u,influx}$ and $PS_{u,efflux}$ are the membrane permeability–surface area products of unbound drugs across the sinusoidal membrane for the influx and efflux processes, respectively, and CL_{int} is the 'exact' intrinsic clearance for unbound drugs, which includes the metabolism and biliary excretion of unchanged drugs. When the $PS_{u,efflux}$ is negligibly low compared with the CL_{int} ($PS_{u,efflux} \ll CL_{int}$), Eq. (1) can be approximated by the following equation:

$$CL_{int,all} = PS_{u,influx} \quad (2)$$

In this case, the hepatic uptake predominantly determines the net hepatic clearance. On the other hand, when the $PS_{u,efflux}$ is much higher than the CL_{int} ($PS_{u,efflux} \gg CL_{int}$), Eq. (1) can be

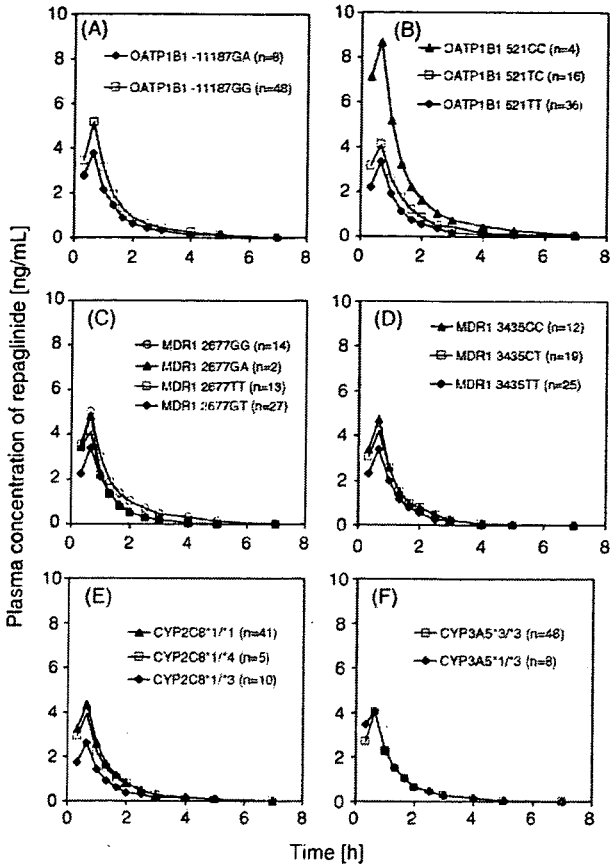

Fig. 2 – Mean plasma concentration of repaglinide in healthy subjects in relation to the single nucleotide polymorphisms in OATP1B1 (A and B) and MDR1 (C and D) and different genotypes in CYP2C8 (E) and CYP3A5 (F). Fifty-six Finnish healthy volunteers were participated in this study and genotyped for the −11187G>A in the promoter region and the 521T>C in exon 5 of the *SLCO1B1* (OATP1B1) gene, the 3435C>T in exon 26 of the *ABCB1* (MDR1) gene, the *CYP2C8*3* and *4 and the *CYP3A4*3* and the mean plasma concentrations of repaglinide after 0.25 mg single oral administration in relation to their genotypes are shown. A statistically significant difference was observed only in the C_{max} and AUC between OATP1B1 (521TT) and (521CC) (Niemi et al., 2005b).

approximated by the following equation:

$$CL_{int,all} = PS_{u,influx} \times \frac{CL_{int}}{PS_{u,efflux}} \quad (3)$$

In this case, all processes shown in Fig. 4(A) affect the net hepatic clearance. When the permeation across the sinusoidal membrane is sufficiently rapid and the transports from the inside of cells to the outside and in the opposite direction are symmetric (i.e. $PS_{u,influx} = PS_{u,efflux}$), Eq. (1) can be approximated by the following equation:

$$CL_{int,all} = CL_{int} \quad (4)$$

In this case, the apparent intrinsic clearance ($CL_{int,all}$) equals the exact intrinsic clearance (CL_{int}). This is applicable to drugs which are lipophilic with rapid membrane permeability. Fig. 4(B) shows the relationship between the apparent $CL_{int,all}$ and 'exact' CL_{int}. When the CL_{int} is low, $CL_{int,all}$ is proportional to CL_{int}. However, when it is high, $CL_{int,all}$ is not affected by CL_{int} and affected only by the uptake clearance, i.e. it is uptake-limited.

Table 4 shows the pharmacokinetic changes in lovastatin, simvastatin and atorvastatin following coadministration of CYP3A4 inhibitors. CYP3A4 inhibitors affect their CL_{int} including the metabolic clearance. Among these statins, lovastatin and simvastatin are lipophilic with lactone ring and are taken up into cells rapidly via passive diffusion while atorvastatin undergoes transporter-mediated uptake (Kameyama et al., 2005; Lau et al., 2006). Following hepatic uptake, they are metabolized in the liver with a minimal excretion in urine as unchanged form. The net hepatic clearance is described by Eq. (1). In the case of lovastatin and simvastatin, which are lipophilic and extensively metabolized by CYP3A4, the net hepatic clearances are close to Eq. (4) (Sirtori, 1993), and their hepatic clearances are directly affected by the change in CL_{int}. On the other hand, the net hepatic clearance of atorvastatin is affected by both metabolism and hepatic uptake (Kameyama et al., 2005; Lau et al., 2006). As atorvastatin is highly taken up into hepatocytes by transporter(s) and well metabolized, its $CL_{int,all}$ is described by Eq. (2) and may be minimally affected by alterations in CL_{int}. In fact, CYP3A4 inhibitors markedly alter the pharmacokinetics of lovastatin and simvastatin, but they have a much weaker effect on that of atorvastatin (Table 4). For example, itraconazole increased the area under the plasma concentration–time curve (AUC) of lovastatin and simvastatin over 10-fold while it increased that of atorvastatin only two- to three-fold (Neuvonen and Jalava, 1996; Neuvonen et al., 1998; Kantola et al., 1998a; Mazzu et al., 2000). Erythromycin increased the AUC of simvastatin acid four-fold although its effect on the AUC of the lactone form of simvastatin is unknown (Kantola et al., 1998b) while it increased that of atorvastatin only 1.3-fold (Siedlik et al., 1999). Nelfinavir, an HIV protease inhibitor, increased the AUC of simvastatin acid six-fold, but that of atorvastatin only 1.7-fold (Hsyu et al., 2001). The combined administration of ritonavir and saquinavir drastically increased the AUC of simvastatin 31-fold while it increased that of atorvastatin 3.5-fold (Fichtenbaum et al., 2002). Grapefruit juice is also known to be a potent inhibitor of CYP3A4 and it increased the AUC of simvastatin and simvastatin acid 3.6- and 3.3-fold, respectively (Lilja et al., 1998). Its effect on the AUC of lovastatin has reported to be marked (2.0- to 15-fold and 1.6- to 5-fold for lovastatin and lovastatin acid, respectively) in two independent reports (Kantola et al., 1998c; Rogers et al., 1999). On the other hand it only produced a minor increase in the AUC of atorvastatin (1.4- to 2.5-fold) (Lilja et al., 1999).

2.2.2. *Transporters determine the tissue distribution of drugs*

Transporters also affect the tissue distribution, and contribute to the selective distribution of drugs to specific tissues. As the pharmacological target of statins is the liver, they should be selectively distributed there. Pravastatin has been reported

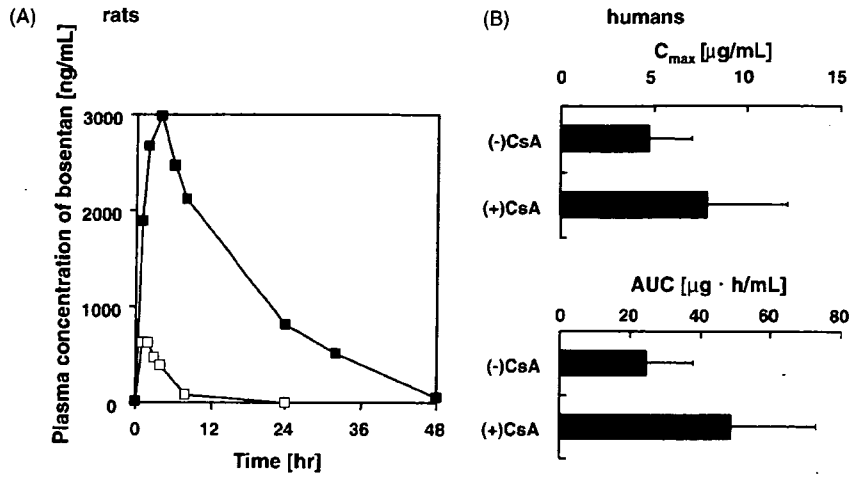

Fig. 3 – Pharmacokinetic alterations of bosentan in rats and humans by coadministration of CsA. (A) Male Wistar rats were orally administered bosentan with (■) or without (□) intraperitoneal administration of CsA. Coadministration of CsA increased the C_{max} and $AUC_{0-\infty}$ of orally administered repaglinide 4.4 and 17 times, respectively. (B) Ten male volunteers were orally given 500 mg bosentan twice daily starting at day 1 in the morning until day 8 and 300 mg CsA (Sandimmun Neoral®) twice daily starting at day 1 in the evening until day 8. The pharmacokinetics of bosentan were evaluated at day 1 ((−)CsA) and day 8 ((+)CsA). The C_{max} and $AUC_{0-\infty}$ of orally administered repaglinide were increased 1.7 and 2.0 times, respectively, by coadministration of CsA ($p = 0.08$ and 0.04 for C_{max} and $AUC_{0-\infty}$, respectively) (Treiber et al., 2004; Binet et al., 2000).

to be a substrate of OATP1B1 and multiple transporters on the bile canalicular membrane, including MRP2 (Sasaki et al., 2002; Matsushima et al., 2005; Hirano et al., 2005b). These transporters assist the liver-specific distribution of pravastatin. Pravastatin is, thus, excreted into the bile, reabsorbed in the intestine to the portal vein and taken up by the liver, and effectively undergoes enterohepatic circulation (Kato et al., 2002). Therefore, the liver concentration should be higher than that in the circulating blood, leading to a high pharmacological effect at a relatively low plasma concentration. Also, in the case of rosuvastatin and pitavastatin, they are effectively and selectively taken up into hepatocytes via OATP1B1 and mainly excreted into bile as the unchanged forms like pravastatin (Fujino et al., 2004b; Simonson et al., 2004). Although atorvastatin is metabolized by CYP3A4, it is also taken up into the liver via OATP1B1, suggesting that it is also effectively dis-

Table 4 – Effect of CYP3A4 inhibitors on the metabolism of simvastatin, lovastatin and atorvastatin

Inhibitor	Substrate	AUC fold increase	C_{max} fold increase	References
Itraconazole	Simvastatin	>10	>10	Neuvonen et al. (1998)
	Simvasatin acid	19	17	Neuvonen et al. (1998)
	Lovastatin	>15–>20	15–>20	Neuvonen and Jalava (1996)
	Lovastatin acid	15	12	Neuvonen and Jalava (1996)
	Atorvastatin	2.5–3	N.S.–1.38	Kantola et al. (1998a), Mazzu et al. (2000)
Erythromycin	Simvastatin acid	3.9	5	Kantola et al. (1998b)
	Atorvastatin	1.3	1.4	Siedlik et al. (1999)
Clarithromycin	Atorvastatin	1.8	1.6	Amsden et al. (2002)
Nelfinavir	Simvastatin acid	6.1	6.2	Hsyu et al. (2001)
	Atorvastatin	1.7	2.2	Hsyu et al. (2001)
Ritonavir + saquinavir	Simvastatin acid	31	31	Fichtenbaum et al. (2002)
	Atorvastatin	3.5	4.3	Fichtenbaum et al. (2002)
Grapefruit juice	Simvastatin	3.6	3.9	Kantola et al. (1998c)
	Simvastatin acid	3.3	4.3	Kantola et al. (1998c)
	Lovastatin	1.9–15	1.7–12	Lilja et al. (1998)
	Lovastatin acid	1.6–5.0	1.7–4	Lilja et al. (1998)
	Atorvastatin	1.4–2.5	1.1	Lilja et al. (1999)

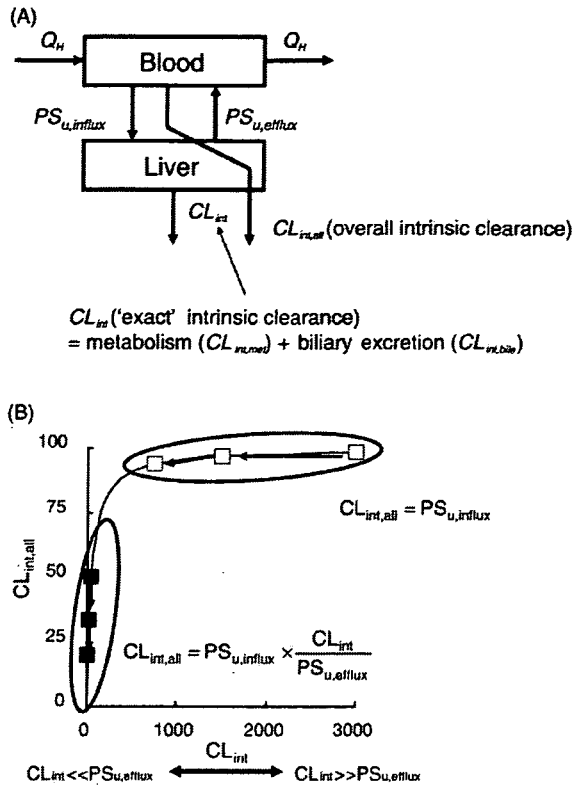

Fig. 4 – The mechanism of the overall hepatic intrinsic clearance of drugs. (A) Drugs that are excreted in the liver are firstly taken up into hepatocytes, followed by metabolism or biliary excretion. At the sinusoidal membrane, transporters can produce back efflux into the blood. Thus, the overall hepatic elimination process includes all these. The overall hepatic intrinsic clearance can be expressed as a hybrid parameter of membrane transport across the sinusoidal membrane from the blood side into hepatocytes (uptake: $PS_{u,influx}$), that from the inside of hepatocytes to the blood side ($PS_{u,efflux}$) and the 'exact' intrinsic clearance (CL_{int}) including drug metabolism and biliary excretion, as described by $CL_{int,all} = PS_{u,influx} \times (CL_{int}/(CL_{int} + PS_{u,efflux}))$ (Eq. (1)). (B) The relation between $CL_{int,all}$ (y-axis) and CL_{int} (x-axis). CL_{int} is higher than the $PS_{u,efflux}$, the overall intrinsic clearance is close to the uptake clearance (uptake-limited). When the intrinsic clearance is quite low, the overall intrinsic clearance can be described by a hybrid parameter of $PS_{u,influx}$, $PS_{u,efflux}$ and CL_{int}. In the case of $CL_{int} \gg PS_{u,influx}$, $CL_{int,all}$ is not affected by the alteration in the CL_{int}. On the other hand, in the case of $CL_{int} \ll PS_{u,influx}$, it depends on the CL_{int}.

tributed to the liver and exert its high pharmacological effect there (Lennernas, 2003; Kameyama et al., 2005; Lau et al., 2006).

Biguanide antidiabetic drugs are taken up into hepatocytes via organic cation transporter 1 (OCT1, *SLC22A1*), but undergoes a minimal metabolism or biliary excretion, mainly resulting in urinary excretion (Wang et al., 2002) (Fig. 5). Wang et al. used Oct1 (*Slc22a1*) knockout mice to show that metformin, a biguanide, is distributed to the liver via Oct1 (Wang et al., 2002). The plasma concentration of metformin was similar in Oct1 (+/+) and (−/−) mice because its elimination is mainly via urinary excretion. However, its liver concentration is affected by Oct1 and it is approximately 30 times lower in Oct1 (−/−) than in Oct1 (+/+). Lactic acidosis is one of the side effects of biguanides and it depends on their liver concentration. In Fig. 6 , the plasma concentration of metformin and lactate in Oct1 (+/+) and (−/−) mice is shown (Wang et al., 2003). The lactate level is markedly different without a difference in the plasma concentration of metformin in Oct1 (+/+) and (−/−). This means that transporters can sometimes affect the pharmacological effects or adverse reactions without any apparent pharmacokinetic alterations.

Rifampicin is well known for its ability to induce drug-metabolizing enzymes and transporters, through activation of the pregnane X receptor (PXR). This activation should be influenced by the concentration of rifampicin in the liver. Thus, hepatic uptake transporters should be a determinant of the induction of enzymes and transporters. Tinora et al. showed that it is a substrate of human OATP1B1, 1B3 and rat Oatp1b2 (Tirona et al., 2003). In humans, OATP1B1 seems to have far greater affinity and capacity for rifampicin transport than OATP1B3. Thus, they suggested that OATP1B1 is a major determinant of rifampin mediated PXR activation. In fact, PXR activation is examined at lower concentration of rifampin in zinc-induced OATP1B1-expressing HeLa cells than in uninduced control cells (Tirona et al., 2003).

2.3. *Substrates of renal transporters*

Transporters are involved in tubular secretion and reabsorption during the renal excretion process (Inui et al., 2000a; Koepsell and Endou, 2004; Shitara et al., 2005). In the kidney, drugs are required to pass through the plasma membranes, the basolateral membrane and the brush border membrane, for transcellular transport. The transporters in the kidney are illustrated in Fig. 1. Table 5 shows a number of drugs which undergo transporter-mediated renal excretion and their urinary excretion ratio as unchanged form. The transporters responsible for their urinary excretion are also shown in Table 5. However, the detailed transport mechanism remains to be investigated for most of drugs because there are few cases where efflux transporters on the brush-border membrane have been characterized.

2.4. *The balance of hepatic and renal clearances determine the elimination pathway*

Drugs are predominantly metabolized or excreted in the urine and bile. Therefore, the total body clearance (CL_{tot}) is a summation of CL_H and renal clearance (CL_R) as described by the following equation:

$$CL_{tot} = CL_R + CL_H \quad (5)$$

This equation shows that the balance of CL_R and CL_H determines the elimination pathway. In addition, CL_R and CL_H are regulated by the affinity and capacity of drugs for transporters in the kidney and liver, respectively. As described in

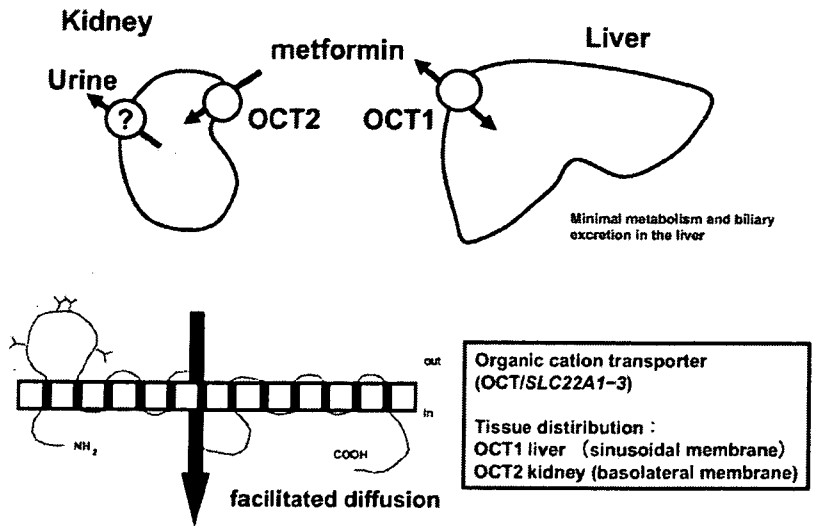

Fig. 5 – The mechanism of tissue distribution of metformin. OCT1 is expressed in the liver while OCT2 is localized on the basolateral membrane in the renal epithelial cells. They transport substrate drugs by a facilitated diffusion. Although metformin is taken up into liver via OCT1, it undergoes a minimal metabolism and biliary excretion. Thus, it mainly undergoes urinary excretion, after renal uptake via OCT2.

Section 2.2, pravastatin is distributed to the liver by hepatic uptake mediated by transporters. On the other hand, its urinary excretion as unchanged form in humans is relatively high (41–47% after intravenous administration) (Hatanaka, 2000). This is attributed to the fact that pravastatin is a substrate of renal transporter(s). Yamazaki et al. studied the renal transport mechanism of pravastatin in rats and showed that its renal uptake is inhibited by *p*-aminohippurate, suggesting the existence of a saturable transport system for it (Yamazaki et al., 1996c). Hasegawa et al. analyzed the molecular mechanism of renal uptake of pravastatin in rats (Hasegawa et al., 2002). They showed that rat organic anion transporter 3 (Oat3; *Slc22a8*) accepts pravastatin as a substrate while Oat1 (*Slc22a6*) does not. The K_m value for its uptake in rat kidney slices was similar with that in rat Oat3-expressing LLC-PK_1 cells. In addition, the inhibition studies of the uptake of pravastatin by *p*-aminohippurate (a relatively selective inhibitor of rat Oat1), benzylpenicillin (a relatively selective inhibitor of rat Oat3) and dibromosulfophthalein (a non-specific inhibitor of rat Oat1 and Oat3) revealed their inhibition constants were similar in rat kidney slices and Oat3-expressing cells. These results suggested that its renal uptake is mediated by Oat3 (Hasegawa et al., 2002). Takeda et al. showed that it is a substrate of human OAT3 (*SLC22A8*) (Takeda et al., 2004). As OAT3 is expressed on the basolateral membrane of kidney proximal tubules, it may help the renal uptake of pravastatin in humans.

The pharmacokinetics of temocaprilat, the active metabolite of temocapril, an angiotensin converting enzyme (ACE) inhibitor, is relatively unaffected by renal failure (Oguchi et al., 1993). This is thought to be due to the fact that temo-

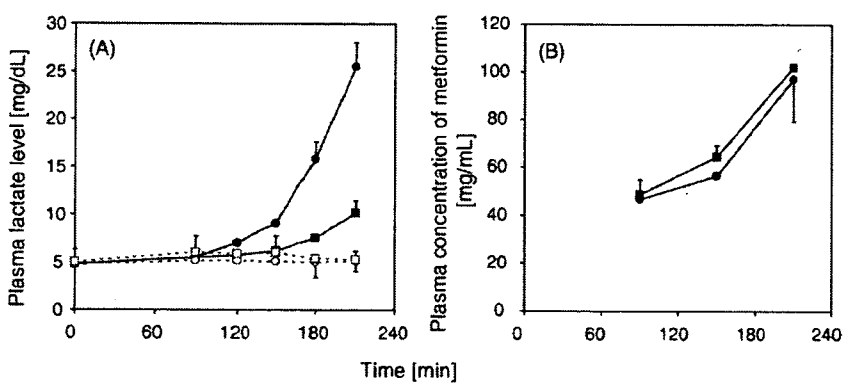

Fig. 6 – Plasma lactate level after metformin administration in Oct1(+/+) and (−/−) mice. The plasma lactate level in Oct1(+/+) (■, □) or (−/−) (●, ○) mice with (■, ●) or without (□, ○) intravenous infusion of metformin at the rate of 150 mg/h/kg (A). Although the plasma concentration of metformin is similar in Oct1(+/+) (■) and (−/−) mice (●) (B), its effect on the plasma lactate level was different. This different lactate level in the plasma is due to the difference in the hepatic uptake of metformin as the target organ for lactic acidosis is the liver. This result suggests the involvement of OCT1 in the lactic acidosis caused by biganides including metformin (Wang et al., 2003).

Table 5 – Urinary excretion ratio of substrate drugs of kidney transporters

	f_e	Involved transporters	References
Acyclovir	0.75	Human OAT1[a]	Takeda et al. (2002a)
		Human OCT1[a]	Takeda et al. (2002a)
		Rat Oat1[a]	Wada et al. (2000)
		Rat Oat3[b]	Ohtsuki et al. (2002)
Adefovir	0.70–0.80	Human OAT1[a]	Cihlar et al. (1999), Ho et al. (2000)
		Rat Oat1[a]	Cihlar et al. (1999)
Cidofovir	0.70	Human OAT1[a]	Cihlar et al. (1999), Ho et al. (2000)
		Rat Oat1[a]	Cihlar et al. (1999)
Amoxicillin	0.86	Human PEPT1[b]	Wenzel et al. (1996)
		Rat Oat1[b]	Jariyawat et al. (1999)
		Rat Pept1[b]	Wenzel et al. (1996), Jariyawat et al. (1999), Terada et al. (1997)
		Rat Pept2[b]	Wenzel et al. (1996), Jariyawat et al. (1999), Terada et al. (1997)
Enalapril	0.88	Human PEPT1[b]	Han et al. (1998), Han et al. (1999)
		Rat Oatp1a1[a]	Pang et al. (1998), Abu-Zahra et al. (2000)
		Rat Pept1[b]	Temple and Boyd (1998)
Temocaprilat	0.090–0.32[c]	Rat Mrp2[a]	Ishizuka et al. (1997)
		Rat Oatp1a1[a]	Ishizuka et al. (1997)
Cefadroxil	0.84	Human OAT1[b]	Takeda et al. (2002b)
		Human OAT3[b]	Takeda et al. (2002b)
		Rat Oat1[b]	Jung et al. (2002)
		Rat Oat3[b]	Jung et al. (2002)
		Human PEPT1[b]	Wenzel et al. (1996), Ganapathy et al. (1995)
		Human PEPT2[b]	Ganapathy et al. (1995)
		Rat Pept1[a]	Terada et al. (1997), Ganapathy et al. (1995)
		Rat Pept2[a]	Terada et al. (1997)
Cefamandole	0.71	Human OAT1[b]	Takeda et al. (2002b)
		Human OAT3[b]	Takeda et al. (2002b)
		Human OAT4[b]	Takeda et al. (2002b)
		Rat Oat1[b]	Jariyawat et al. (1999), Jung et al. (2002)
		Rat Oat3[b]	Jung et al. (2002)
Cefazolin	0.80	Human OAT1[b]	Takeda et al. (2002b)
		Human OAT3[b]	Takeda et al. (2002b)
		Human OAT4[b]	Takeda et al. (2002b)
		Rat Oat1[b]	Jariyawat et al. (1999)
		Rat Oat3[b]	Jung et al. (2002)
Cimetidine	0.62	Human OAT1[a]	Burckhardt et al. (2003)
		Human OAT3[a]	Cha et al. (2001)
		Rat Oat3[a]	Kusuhara et al. (1999)
		Rat Oct1[a]	Grundemann et al. (1999)
Pravastatin	0.47	Human OAT3[a]	Takeda et al. (2004)
		Rat Oat3[a]	Hasegawa et al. (2002)

f_e: Fraction excreted in urine as an unchanged form.
[a] Reported as a substrate.
[b] Reported as an inhibitor.
[c] Excreted as temocaprilat, a prodrug of temocapril.

caprilat undergoes both renal and hepatic elimination. This is a special feature among classic ACE inhibitors, and this is helped by the presence of MRP2/Mrp2 in the bile canalicular membrane of hepatocytes (Ishizuka et al., 1997). Although other classic ACE inhibitors are also taken up into hepatocytes via transporter(s), they are not excreted into the bile so much and are mainly excreted in the urine. On the other hand, temocaprilat is a substrate of MRP2/Mrp2, and excreted in the bile. Therefore, the total clearance of temocaprilat is the sum of the hepatic and renal clearances while that of other ACE inhibitors is mainly due to their renal clearance. Due to this dual elimination pathway, changes in renal function do not affect the plasma concentration of temocaprilat so much. However, other ACE inhibitors are markedly affected by renal failure because they have no other elimination pathways than in the urine. It is, therefore, very necessary to be able to predict the substrate specificity of MRP2 in silico. The authors were recently involved in this in silico prediction (Hirono et al., 2005). In this study, key functional groups of multiple rat Mrp2 ligand molecules were identified, their relative locations were determined and substrate specificity of rat Mrp2 was examined using the three-dimensional quantitative

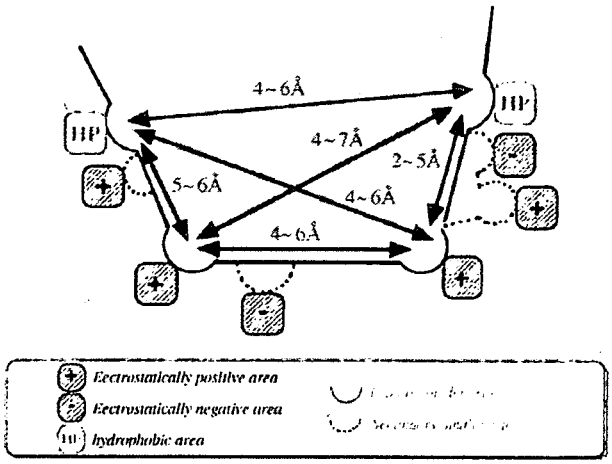

Fig. 7 – Ligand binding region of rat Mrp2 estimated by in silico study. Ligand binding region of rat Mrp2 was estimated by using the three-dimensional (3D) pharmacophore (key functional groups of ligand molecules) of multiple substrates of this transporter and the method of quantitative structure–activity relationship (3D-QSAR) comparative molecular-field analysis (CoMFA). This method revealed a primary binding region, including two hydrophobic and two electrostatically positive sites, and a secondary binding region, including two electrically positive and two electrostatically negative sites, which is attributable to a broad substrate specificity of rat Mrp2 (Hirono et al., 2005).

structure–activity relationship analysis. From this analysis, they estimated the ligand binding region of rat Mrp2 (Fig. 7) including two hydrophobic and two electrostatically positive sites (primary binding sites). In addition, they estimated secondary binding sites including two electrostatically positive and two electrostatically negative sites, which attribute to a broad substrate specificity of rat Mrp2.

3. The mechanism of transporter-mediated drug–drug interactions

We analyzed the mechanism of the drug–drug interaction between cerivastatin and CsA and showed that CsA inhibited the transporter (including OATP1B1)-mediated uptake with only a minimal effect on the microsomal metabolism, suggesting that this drug–drug interaction is due to the transporter-mediated uptake process (Shitara et al., 2003). The pharmacokinetics of cerivastatin is also affected by the coadministration of gemfibrozil (Backman et al., 2002). This is due to inhibition of the microsomal metabolism by gemfibrozil glucuronide (Shitara et al., 2004b). It should be noted that CsA, a transporter inhibitor, alters the AUC with only a minimal effect on the elimination half-life ($t_{1/2}$) while gemfibrozil, a metabolic inhibitor, increased both of the AUC and $t_{1/2}$ (Muck et al., 1999; Backman et al., 2002; Shitara et al., 2005). This can be explained as follows. The $t_{1/2}$ can be described by the following equation using CL_{tot} and distribution volume (V_d).

$$t_{1/2} = \frac{\ln 2 \cdot V_d}{CL_{tot}} \quad (6)$$

Cerivastatin is highly distributed to the liver, due to the efficient transporter-mediated hepatic uptake and extensive protein binding in the liver, and the V_d mainly depends on the distribution to the liver. For this drug, inhibition of the transporter-mediated hepatic uptake leads to a reduction in V_d. Transporter-mediated hepatic uptake is also a determinant of its hepatic clearance. Thus, CsA simultaneously reduces V_d and CL_{tot} to similar levels, resulting in no alteration in $t_{1/2}$. On the other hand, gemfibrozil inhibits the metabolism with only a minor effect on the transporter-mediated hepatic uptake of cerivastatin. Thus, it reduces the CL_{tot} but does not change the V_d so much, leading to a prolongation of $t_{1/2}$. The pharmacokinetics of bosentan was reported to be changed by coadministration of CsA in rats and humans (Binet et al., 2000; Treiber et al., 2004). It is also due to inhibition of the transporter-mediated hepatic uptake as described above (Fig. 3(A)). The plasma concentration of repaglinide is also altered by coadministration of CsA in humans (Kajosaari et al., 2005). This interaction is, at least partly, caused by the inhibition of OATP1B1-mediated hepatic uptake. The extent of the alteration caused by this interaction was significantly lower in patients with a genetic polymorphism in OATP1B1 (T521C), suggesting the involvement of OATP1B1-mediated hepatic uptake in this interaction (Kajosaari et al., 2005).

Transporter-mediated drug–drug interactions can occur in the biliary excretion process as well as the uptake process. Ueda et al. described a method to quantitatively predict the pharmacokinetic alteration caused by a drug–drug interaction, considering the inhibition of biliary excretion as well as uptake (Ueda et al., 2001). They examined the effect of probenecid on the uptake of methotrexate in isolated rat hepatocytes and rat bile canalicular membrane vesicles to determine the inhibition constants (K_i) of probenecid for the purpose of a quantitative prediction of the extent of drug–drug interaction between these two drugs in vivo from the in vitro studies (Fig. 8(A)). They also examined the inhibitory effect of probenecid on the in vivo biliary excretion of methotrexate, which is excreted in the bile in rats, to validate their prediction (Fig. 8(A)). The uptake of methotrexate by isolated rat hepatocytes and bile canalicular membrane vesicles were inhibited by probenecid with K_i of 180 and 52 μM, respectively. Probenecid also reduced the biliary excretion clearance in rats in vivo in a concentration-dependent manner. Although the degree of the reduction in the hepatic uptake and biliary excretion are well predicted by the reduction in the uptake in isolated hepatocytes and bile canalicular membrane vesicles, the degree of the reduction in the net clearance is found to be overestimated by a simple calculation of the product of the reduction in the hepatic uptake and biliary excretion (Fig. 8(B)–(D)). This suggests that the actual degree of the drug–drug interaction should be estimated considering that the hepatic clearance ranges between Eqs. (2) and (3) described in Section 2.2.

Matsushita et al. examined the effect of benzylpenicillin on the pharmacokinetics of cefodizime in rats (Matsushita et

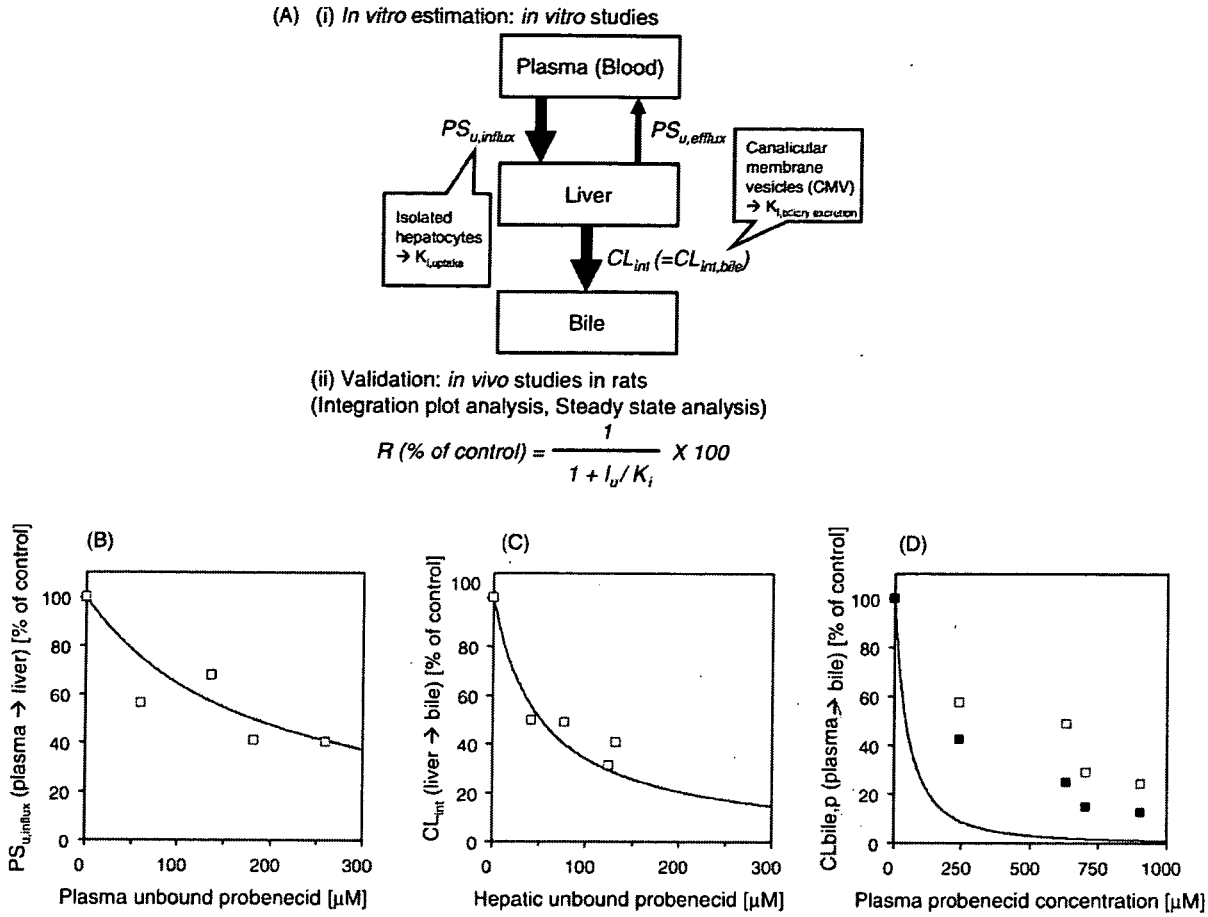

Fig. 8 – Extrapolation of the drug interaction between methotrexate and probenecid in the process of transport across the sinusoidal and canalicular membrane, and the net biliary excretion from in vitro data. For the in vitro to in vivo extrapolation of transporter-mediated drug–drug interaction between methotrexate (MTX) and probenecid (PBN) in the process of biliary excretion, the inhibitory effect of PBN on the uptake of MTX into isolated rat hepatocytes and bile canalicular membrane vesicles was examined by in vitro studies. The results obtained in these studies were extrapolated to in vivo to predict the in vivo drug–drug interaction. This extrapolation was validated by the comparison with the pharmacokinetic alterations observed in vivo. The reduction in the hepatic uptake of unbound MTX across the sinusoidal membrane ($PS_{u,influx}$) by PBN was extrapolated from in vitro data (—) based on the inhibition constant (K_i) for the uptake of MTX into isolated rat hepatocytes and inhibitor unbound concentration in vivo (I_u) by ($1/(1 + I_u/K_i)$) × 100 [% of control] (B). This prediction was well matched with the observed value in vivo (□). The reduction of the biliary excretion was also predicted using in vitro data based on the K_i for the uptake of MTX into rat bile canalicular membrane vesicles and I_u and shown in (C). The reduction in the net biliary excretion of MTX was also predicted by the simple calculation of the product of the reduction in the hepatic uptake and biliary excretion (D). The predicted values based on inhibitor unbound concentration in the plasma (—) and in the liver (■) are shown. This simple prediction resulted in an overestimation of the reduction of the net biliary excretion compared with the observed value (□) (Ueda et al., 2001).

al., 1992). Cefodizime undergoes biliary excretion in the liver and glomerular filtration in the kidney. Therefore, the CL_{tot} of cefodizime can be described by the following equation.

$$CL_{tot} = CL_H + CL_R = CL_H + f_B\,GFR \quad (7)$$

where f_B and GFR are the blood protein unbound fraction and the glomerular filtration rate, respectively. Coadministration of benzylpenicillin apparently had no effect on the pharmacokinetics of cefodizime. However, benzylpenicillin reduced the hepatobiliary transport of cefodizime, resulting in a reduction in hepatobiliary clearance. In addition, benzylpenicillin reduced its plasma protein binding, leading to an increased renal clearance (Fig. 9). The reduced hepatobiliary excretion and increased urinary excretion resulted in no alteration in the plasma concentration of cefodizime. It is, thus, possible that drug–drug interaction might not be revealed by measuring only the plasma concentration profile.

Although renal excretion of famotidine is affected by coadministration of probenecid in humans, it is not altered in rats, suggesting there is an inter-species difference in the renal clearance mechanism of famotidine (Lin et al., 1988; Inotsume

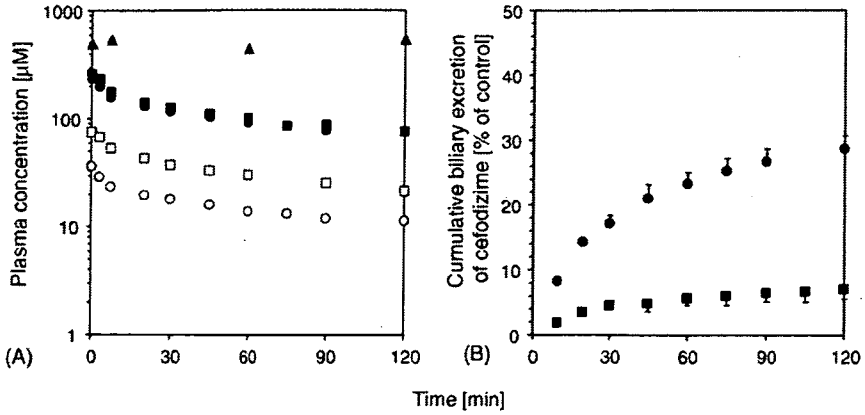

Fig. 9 – Effect of benzylpenicillin on the disposition of cefodizime. Data are taken from the result by Matsushita et al. (A) (▲) plasma concentration of benzylpenicillin; (■, ●) plasma concentration of cefodizime with or without coadministration of benzylpenicillin, respectively; (□, ○) plasma unbound concentration of cefodizime with or without coadministration of benzylpenicillin, respectively. (B) Coadministration of benzylpenicillin does not affect the plasma concentration of cefodizime (A). However, benzylpenicillin reduces the biliary excretion. On the other hand, it increases the plasma protein unbound fraction, resulting in the enhanced urinary excretion. Reduced hepatic clearance and increased renal clearance resulted in no apparent alteration in the disposition of cefodizime (■ and ●) (Matsushita et al., 1992).

et al., 1990) (Fig. 10(A)). Tahara et al. examined the effect of probenecid on the uptake of famotidine by renal transporters in order to clarify the mechanism of this inter-species difference (Tahara et al., 2005a). H_2 receptor antagonists are reported to be substrates of solute carrier 22 (SLC22) family transporters including OCTs and OATs. Tahara et al. examined the uptake of H_2 receptor antagonists in human and rat OAT and OCT family transporters and the inhibitory effects produced by other drugs (Tahara et al., 2005a). They analyzed the inter-species differences and found that the transport activity of famotidine by rat Oat3 was not as high as that by human OAT3. In humans, the inhibition of the OAT3-mediated transport of famotidine by probenecid caused a drug–drug interaction. However, in rats, this inhibition does not cause a marked interaction, possibly due to the smaller contribution made by Oat3 to the renal clearance in rats. In addition, Oct1 as well as Oct2 (*Slc22a2*) accepts famotidine as a substrate and possibly contributes to the renal clearance of famotidine in rats while, in humans, OCT1 (*SLC22A1*) is not expressed in the kidney. Probenecid does not interact with OCTs. Thus, the inter-species difference, exhibited by the fact that probenecid alters the pharmacokinetics of famotidine in humans but not in rats, is caused by an inter-species difference in the activities of the OAT3/Oat3-mediated transport of famotidine and the existence of Oct1 in rat kidney (Fig. 10(B)). Tahara et al. also examined inter-species differences in the transport activities of OAT1/Oat1 and OAT3/Oat3 in humans, rats and monkeys, using different substrates (Tahara et al., 2005b). A good correlation was observed in the case of humans versus rats and humans versus monkeys as far as OAT1/Oat1-mediated transport was concerned (Fig. 11). However, in the case of OAT3/Oat3-mediated transport, a good correlation was observed in the case of humans versus monkeys, while a relatively poor correlation was observed in the case of humans versus rats (Fig. 11). This suggests that an extrapolation from rat to human data is not necessarily appropriate for OAT3 substrates.

More recently, Tahara et al. examined the effect of probenecid on the pharmacokinetics of famotidine in monkeys in vivo (Tahara et al., in press). In monkeys, probenecid caused a 65% reduction in the renal clearance and a 90% reduction in the tubular secretion, which is very similar to the corresponding interaction observed in humans. In addition, they showed the absence of Oct1 in monkey kidney. This result also supports the belief that the monkey is more appropriate animal model for predicting OAT3-mediated drug–drug interactions involving renal excretion in humans.

4. A method for estimating the contribution of each transporter to the total hepatic uptake

4.1. *The importance of the contribution of transporters*

Currently, great progress is being made in the molecular cloning of transporters. These studies help to characterize the molecular mechanisms of drug transport by using transporter-expressing systems. However, the contribution of each transporter to drug transport in vivo has not yet been evaluated. Using these contributions, the uptake clearance in the transporter-expressing systems can be extrapolated to that in the tissue, and it is possible to quantitatively predict the transporter-mediated drug–drug interactions and pharmacokinetic alterations in humans with single nucleotide polymorphisms (SNPs) in their transporter(s), in specific pathophysiological disorders and so on. In this section, the estimation of the transporter contributions is described.

4.2. *Estimation of the contributions of specific transporters to the total hepatic uptake*

Studies to characterize the contributions of transporters to the total hepatic uptake have been performed. For example, Hagenbuch et al. used the method to estimate the contri-

(A) **Rat**

	CL_{renal} [mL/min/kg]
Famotidine alone	42.3 ± 8.9
with probenecid	45.8 ± 10.3

Human

	CL_{renal} [mL/min]	$CL_{secretion}$ [mL/min]
Famotidine alone	297 ± 19	196 ± 21
with probenecid	107 ± 5	22.0 ± 4.2

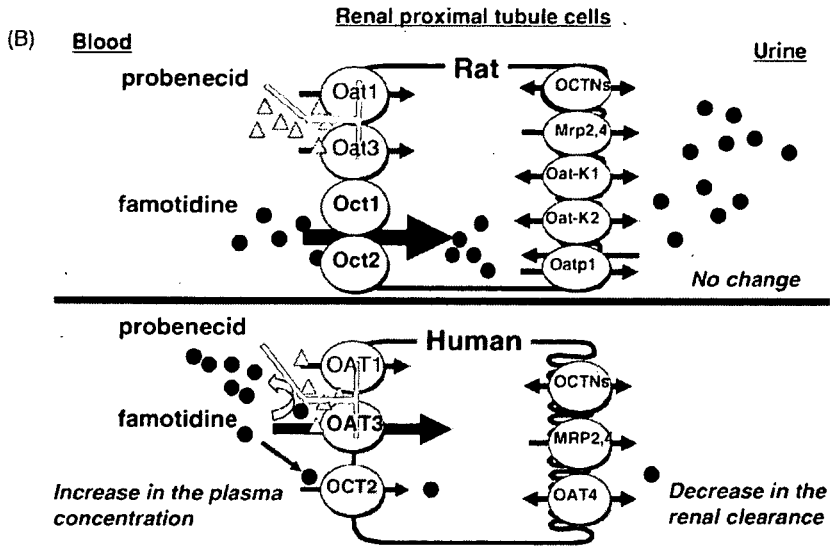

Fig. 10 – Inter-species difference between rats and humans in the drug–drug interaction between famotidine and probenecid. (A) Probenecid does not alter the renal clearance of famotidine in rats while it decreases that in humans (Inotsume et al., 1990; Lin et al., 1988). (B) This inter-species difference can be explained by the different mechanism of renal excretion of famotidine in rats and humans. The contribution made by Oat3 to the uptake of famotidine across the basolateral membrane in renal epithelial cells in rats is smaller than that in humans. In rats, Oct1 exists in the kidney and it contributes to the renal uptake of famotidine. In humans, OAT3-mediated transport of famotidine is inhibited by probenecid while, in rats, Oct1-mediated transport is not inhibited and Oat3-mediated transport is minor. Thus, the renal secretion of famotidine is affected by the coadministration of probenecid in humans but not in rats.

bution of specific transporters by coinjecting antisense DNA to this transporter with total mRNA isolated from rat liver into *Xenopus laevis* oocytes (Hagenbuch et al., 1996). They estimated the contributions of Na^+-taurocholate cotransporting polypeptide (Ntcp; Slc10a1) and Oatp1a1 to the uptake of taurocholate (TC) and bromosulfophthalein (BSP) by this method. In their report, they revealed that the Na^+-dependent and Na^+-independent uptakes of TC are mostly mediated by Ntcp and Oatp1a1, respectively. They also revealed that only half of the uptake of BSP attributes to Oatp1a1, suggesting an existence of other transporter(s) responsible for its uptake. In fact, as of now, other transporting systems than Oatp1a1, which include Oatp1b2, has been reported (Cattori et al., 2000). Nakai et al. applied this method to examine the contribution of human OATP1B1 to the hepatic uptake of pravastatin and estradiol 17β-D-glucuronide ($E_2$17βG) (Nakai et al., 2001). They have shown that microinjection of cRNA for human OATP1B1 and human liver polyadenylated RNA into *Xenopus laevis* oocytes enhanced the uptake of pravastatin and $E_2$17βG and coinjection of antisense DNA for human OATP1B1 with human liver polyadenylated RNA resulted in a decrease of the uptake of these compounds to the similar level with that in water-injected oocytes. They concluded that OATP1B1 mainly contributes to the hepatic uptake of them. As of now, as many

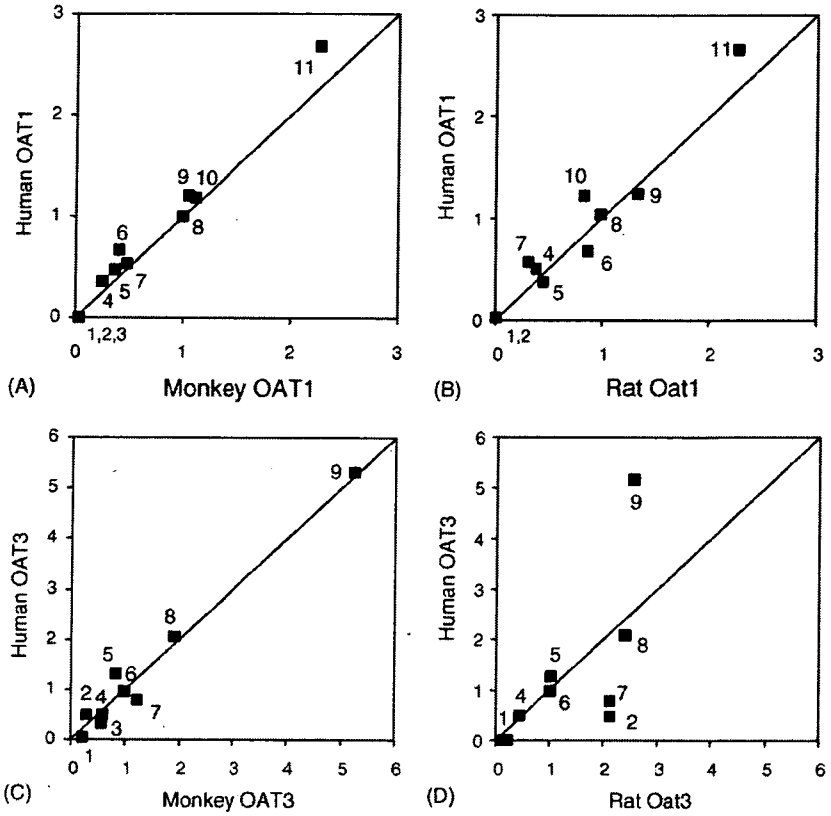

Fig. 11 – Correlation of uptake of various compounds in human, rat and monkey OAT1 and OAT3. The correlation of OAT1-mediated relative uptake activity between humans and monkeys (A) and between humans and rats (B), and that of OAT3-mediated relative uptake activity (C and D) are plotted. No good correlation was observed between human and rat Oat3. OAT1/Oat1 substrates (in (A) and (B)): (1) benzylpenicillin; (2) acyclovir; (3) cimetidine; (4) 3-carboxy-4-methyl-5-propyl-2-furanpropionate; (5) indoleacetate; (6) indoxyl sulfate; (7) 3′-azido-3′-deoxythymidine; (8) p-aminohippurate; (9) ochratoxin A; (10) hippurate; (11) 2,4-dichloro-phenoxyacetate. OAT3/Oat3 substrates (in (C) and (D)): (1) acyclovir; (2) indoxyl sulfate; (3) 2,4-dichloro-phenoxyacetate; (4) p-aminohippurate; (5) cimetidine; (6) benzylpenicillin; (7) 3-carboxy-4-methyl-5-propyl-2-furanpropionate; (8) ochratoxin A; (9) estrone 3-sulfate (Tahara et al., 2005b).

homologues of these transporters have been reported and their sequences are similar, the specificity of antisense DNA to the target DNA should be considered cautiously. More recently, Takagi et al. introduced a method using 2′-O,4′-C-ethylene-bridged nucleic acids residues incorporated into antisense oligonucleotides and selectively inhibited the Oatp subtypes (Takagi et al., 2004).

Kouzuki et al. have described an estimation method using a reference compound (Kouzuki et al., 1998, 1999). They used a compound the hepatic uptake of which can be completely explained by a single transporter as a reference compound. They measured the uptake of sample drugs and 'reference compounds' in transporter-expressing cells and primary cultured hepatocytes, which were cultured for a short term allowing a minimal reduction in transporters. By comparison of the uptake clearances (CL_{uptake}) in these experimental systems, they estimated the contributions from the following equation:

$$\text{contribution}\,(\%) = \frac{R_{exp}}{R_{hep}} \times 100 \tag{8}$$

where R_{exp} and R_{hep} are defined as follows:

$$R_{exp} = \frac{CL_{uptake,exp}\,(\text{sample})}{CL_{uptake,exp}\,(\text{reference})} \tag{9}$$

$$R_{hep} = \frac{CL_{uptake,hep}\,(\text{sample})}{CL_{uptake,hep}\,(\text{reference})} \tag{10}$$

$CL_{uptake,exp}$ (sample) and $CL_{uptake,exp}$ (reference) are the uptake clearances of sample drugs and reference compounds in the transporter expressing systems, respectively, and $CL_{uptake,hep}$ (sample) and $CL_{uptake,hep}$ (reference) are the uptake clearances of the corresponding compounds in hepatocytes. They estimated the contributions of Ntcp and Oatp1a1 using TC and $E_2$17βG as reference compounds, respectively. However, as of this writing, their assumption that $E_2$17βG is taken up into hepatocytes predominantly via Oatp1a1 is incorrect (Cattori et al., 2000) and careful interpretation should be made. Their results using the Ntcp expression system suggest this transporter is responsible for the hepatic uptake of bile salts but not for other organic anions and the contribution of Ntcp to their uptakes of bile salts is varied. They suggest the

Table 6 – Estimation of OATP1B1- and 1B3-mediated uptake clearances of pitavastatin in human hepatocytes

Hepatocyte lot	Estimated uptake clearances	
	OATP1B1 (μL/min/10^6 cells)	OATP1B3 (μL/min/10^6 cells)
(1) Method using transporter-selective substrates		
OCF	63.8 (87.7%)	8.92 (12.3%)
094	77.8 (95.1%)	4.01 (4.91%)
ETR	33.5 (93.5%)	2.32 (6.47%)
(2) Method using relative transporter expression levels		
OCF	222 (85.7%)	37.0 (14.3%)
094	121 (80.9%)	28.4 (19.1%)
ETR	68.1 (75.1%)	22.6 (24.9%)

Data are taken from the report by Hirano et al. (2004). The parenthetical values represent the percentage of OATP1B1- or 1B3-mediated uptake clearance relative to the sum of the estimated clearances mediated by OATP1B1 and 1B3.

existence of other transporters than Ntcp responsible for the Na^+-dependent uptake of bile salts including glycocholate and cholate.

Hirano et al. applied this method to human hepatocytes and estimated the contributions of OATP1B1 and 1B3 to the total uptake of pitavastatin and $E_2$17βG using cryopreserved human hepatocytes (Hirano et al., 2004). They used estrone 3-sulfate (E_1S) and cholecystokinin octapeptide, as the reference compounds for OATP1B1 and 1B3, respectively. In addition, they also measured the expression levels of OATP1B1 and 1B3 in hepatocytes and transporter-expressing cells by Western blot analyses and estimated their contributions by normalization with these data. The results obtained by these two different methods suggested that OATP1B1 is the major transporter responsible for the uptake of pitavastatin and $E_2$17βG (Table 6).

Shimizu et al. examined the uptake of fexofenadine in OATP1B1, 1B3 and 2B1 expressing cells (Shimizu et al., 2005). OATP1B3-mediated transport was observed but OATP1B1- and 2B1-mediated transport was negligible. They also calculated the contribution of each of transporters to the hepatic uptake of fexofenadine on the basis of the method by Hirano et al. using the uptake of reference compounds and concluded that the contribution of OATP1B3 is over 50%, suggesting that this transporter plays an important role in the hepatic uptake of fexofenadine.

Using a specific or selective inhibitor for a single transporter, its contribution can be estimated. We have compared the inhibitory effects of different compounds on Oatp1a1- and 1a4-mediated transport (Shitara et al., 2002). This study showed that some inhibitors preferentially inhibit either of them, but, in general, the inhibitor specificities are very similar. Ishiguro et al. examined the uptake of telmisartan and $E_2$17βG in cryopreserved human hepatocytes in the presence of E_1S, which preferentially inhibits OATP1B1 rather than OATP1B3 (Ishiguro et al., 2005). The uptake of telmisartan was not inhibited by E_1S while that of $E_2$17βG was mostly inhibited, suggesting OATP1B1 does not contribute to the uptake of telmisartan.

5. Transport studies using double transfected cells

For transporter-mediated transcellular transport, substrates need to be taken up into cells and excreted to the opposite side via two different transporters. To evaluate the transcellular transport, transporter double transfected cells have been developed and used. In this section, analyses using these cells are described.

Double transfected cells were introduced by Cui et al. in 2001 and by Sasaki et al. in 2002 (Cui et al., 2001; Sasaki et al., 2002). They constructed OATP1B3-MRP2 and OATP1B1-MRP2 double expressing MDCKII cells, respectively. Cui et al. showed that transfection of two different transporters enhanced the transcellular transport of BSP, leukotriene C_4, $E_2$17βG, dehydroepiandrosterone sulfate, fluo-3 and rifampicin (Cui et al., 2001). Sasaki et al. found that transfection of both OATP1B1 and MRP2 enhanced the transcellular transport of $E_2$17βG, pravastatin and leukotriene C_4 (Sasaki et al., 2002). These results indicate that these compounds are substrates of the two transfected transporters. On the other hand, the transcellular transport of E_1S was similar in OATP1B1 single expressing cells and OATP1B1-MRP2 double transfected cells. However, more recent study by Spears et al. revealed an enhancement of the transcellular transport of E_1S in LLC-PK_1 cells expressing OATP1B1 and MRP2 comparing with OATP1B1 single expressing LLC-PK_1 cells (Spears et al., 2005). This discrepancy may be caused by the difference in the host cells. Spears et al. suggested that the expression of endogenous MRP transporter in MDCKII cells is too high to detect the transport mediated by transfected human MRP2. Thus, E_1S also may be a substrate of OATP1B1 and MRP2.

Sasaki et al. analyzed the transcellular transport of many compounds in rat Oatp1b2-Mrp2 expressing cells for the purpose of extrapolation of the net biliary excretion in vivo from the in vitro data (Sasaki et al., 2004). The biliary excretion clearances in vivo in rats can be extrapolated by using the following equation (Fig. 12(A)):

$$CL_{bile,b} = \alpha \times \frac{Q_H f_B CL_{int,in\ vitro}}{Q_H + f_B CL_{int,in\ vitro}} \quad (11)$$

In this equation, $CL_{bile,b}$ is the biliary excretion clearance defined with respect to the blood concentration of the drug, f_B is the protein unbound fraction of the drug in blood and $CL_{int,in\ vitro}$ is in vitro intrinsic clearance calculated from the result of the transcellular transport in double transfected cells, by considering that 1 g liver contains 160 mg protein. Also, α is the scaling factor between the predicted biliary excretion

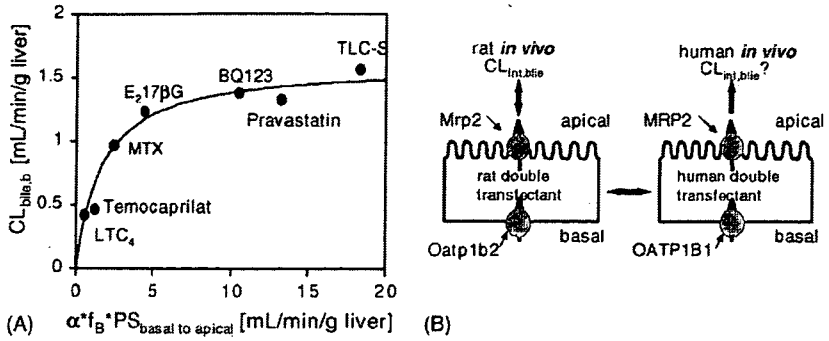

Fig. 12 – Comparison of the in vivo biliary excretion clearances and in vitro transcellular transport examined in rat Oatp1b2/Mrp2 double transfected cells. Epithelial cells expressing double transporters for hepatic uptake and biliary excretion can be used for the estimation of the net biliary excretion. Sasaki et al. examined the transcellular transport of compounds in rat Oatp1b2/Mrp2 double transfected system and their net biliary excretion in rats in vivo (Sasaki et al., 2004). (A) An extrapolation of the net biliary excretion of compounds in vivo in rats from the transcellular transport data obtained in rat Oatp1b2/Mrp2 double expressing cells is shown (Sasaki et al., 2004). The x-axis represents the basal-to-apical transport clearance in rat Oatp1b2/Mrp2 double transfected cells multiplied by the blood protein unbound fraction (f_B) and the scaling factor ($\alpha = 17.9$). The solid line represents the fitted line based on the well-stirred model. As shown here, the net biliary excretion clearance in vivo can be quantitatively predicted from the results of in vitro transcellular transport in transporter double expressing cells. (B) If a good in vitro–in vivo correlation was obtained for rats, a similar method can be also applied for the prediction of the net biliary excretion clearance of drugs in humans. TLC-S: taurolithocholate sulfate, $E_2$17βG: estradiol 17β-D-glucuronide, BQ123: cyclo-[D-Asp-Pro-D-Val-Leu-D-Trp], LTC_4: leukotriene C_4, MTX: methotrexate (Sasaki et al., 2004).

clearance from the in vitro transcellular transport in double transfected cells and the observed value in vivo. The α value was assumed constant irrespective of the kinds of ligands. As shown in Fig. 12(B), it was found that extrapolation of the result of the transcellular transport in double transfected cells can be extrapolated to the in vivo hepatic clearance. This may be also applied to humans using double transfectants expressing human transporters (Fig. 12(B)).

More recently, our group has used other double transfected cells, such as human OATP1B1-MDR1, OATP1B1-BCRP and rat Ntcp-Bsep (*Abcb11*) expressing cells, and characterized their mechanisms for the hepatobiliary transport of drugs or endogenous substrates (Matsushima et al., 2005; Hirano et al., 2005a; Mita et al., 2005). Transport studies using double transfected cells allow a longer time to evaluate the transport because the steady-state transport rate continues for a longer time while uptake studies using single transfected cells or transporter-expressing vesicles should be conducted within a limited time in which the initial uptake rate is maintained and, accordingly, the system using double transfected cells enables the evaluation of transcellular transport to be carried out more easily.

More recently, Kopplow et al. constructed quadruple transfected cells with OATP1B1, 1B3, 2B1 and MRP2 (Kopplow et al., 2005). As this system resembles hepatocytes more closely, it will help in the prediction of the hepatobiliary transport of drugs with unknown transport mechanisms.

6. Conclusion

This review has examined the involvement of transporters in the hepatobiliary and renal transport of drugs. In addition, we have introduced a recently developed method to evaluate the transporter-mediated transport of drugs. Until now, the number of reports of pharmacokinetic alterations caused by transporter-mediated drug–drug interactions or genetic polymorphisms in transporters is less than those involving in metabolism. However, there may be increasing numbers of reports of such pharmacokinetic alterations triggered by the altered activity of transporters because this is also a determinant of the pharmacokinetics of a number of drugs.

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Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: Drug–drug interactions and interindividual differences in transporter and metabolic enzyme functions

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Abstract

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are widely used for the treatment of hypercholesterolemia. Their efficacy in preventing cardiovascular events has been shown by a large number of clinical trials. However, myotoxic side effects, sometimes severe, including myopathy or rhabdomyolysis, are associated with the use of statins. In some cases, such toxicity is associated with pharmacokinetic alterations. In this review, the pharmacokinetic aspects and physicochemical properties of statins are reviewed in order to understand the mechanism governing their pharmacokinetic alterations. Among the statins, simvastatin, lovastatin and atorvastatin are metabolized by cytochrome P450 3A4 (CYP3A4) while fluvastatin is metabolized by CYP2C9. Cerivastatin is subjected to 2 metabolic pathways mediated by CYP2C8 and 3A4. Pravastatin, rosuvastatin and pitavastatin undergo little metabolism. Their plasma clearances are governed by the transporters involved in the hepatic uptake and biliary excretion. Also for other statins, which are orally administered as open acid forms (i.e. fluvastatin, cerivastatin and atorvastatin), hepatic uptake transporter(s) play important roles in their clearances. Based on such information, pharmacokinetic alterations of statins can be predicted following coadministration of other drugs or in patients with lowered activities in drug metabolism and/or transport. We also present a quantitative analysis of the effect of some factors on the pharmacokinetics of statins based on a physiologically based pharmacokinetic model. To avoid a pharmacokinetic alteration, we need to have information about the metabolizing enzyme(s) and transporter(s) involved in the pharmacokinetics of statins and, along with such information, model-based prediction is also useful.

Keywords: HMG-CoA reductase inhibitor; Transporter; Metabolism; Drug–drug interaction

Abbreviations: AUC, area under the plasma concentration–time profile; BCRP/Bcrp, breast cancer resistance protein; BSEP/Bsep, bile salt exporting pump; CL_{int}, intrinsic clearance; $CL_{int,all}$, overall intrinsic clearance; CL_{tot}, total body clearance; C_{max}, maximum plasma concentration; CNS, central nervous system; CoQ_{10}, ubiquinone/coenzyme Q_{10}; CsA, cyclosporin A; CYP, isoforms of cytochrome P450; EHBR, Eisai hyperbilirubinemic rat; f_b, blood unbound fraction; HIV, human immunodeficiency virus; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IC_{50}, inhibitor concentration to produce a 50% reduction; K_i, inhibition constant; K_m, Michaelis constant; LDL, low density lipoprotein; MDR/Mdr, multidrug resistance; MRP/Mrp, multidrug resistance associated protein; OAT/Oat, organic anion transporter; OATP/Oatp, organic anion transporting polypeptide; P450, cytochrome P450; P-gp, P-glycoprotein; $PS_{u,efflux}$, membrane permeability clearance of unbound drugs for the efflux process; $PS_{u,influx}$, membrane permeability clearance of unbound drugs for the influx process; Q_H, hepatic blood flow; statins, HMG-CoA reductase inhibitors; $t_{1/2}$, elimination half life; UGT, UDP glucuronosyl transferase; V_d, distribution volume.

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