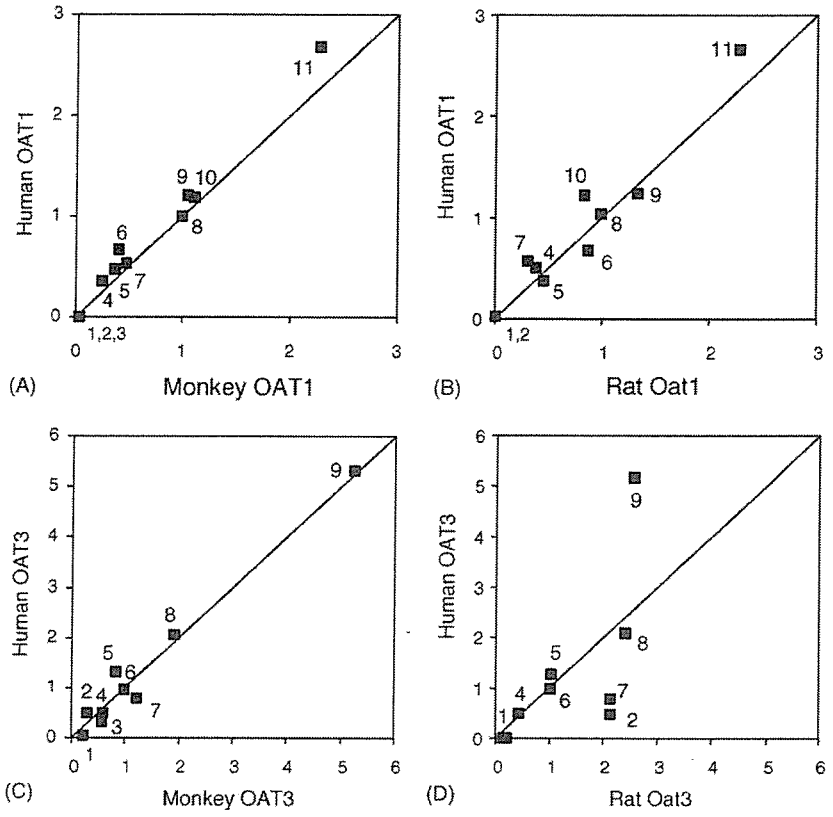

Fig. 11 – Correlation of uptake of various compounds in human, rat and monkey OAT1 and OAT3. The correlation of OAT1-mediated relative uptake activity between humans and monkeys (A) and between humans and rats (B), and that of OAT3-mediated relative uptake activity (C and D) are plotted. No good correlation 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

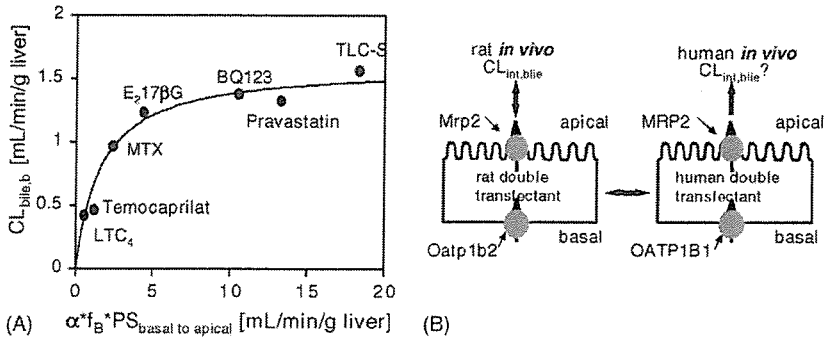

Fig. 12 – Comparison of the in vivo biliary excretion clearances and in vitro transcellular transport examined in rat Oatp1b2/Mrp2 double transfected cells. Epithelial cells expressing double transporters for hepatic uptake and biliary excretion can be used for the estimation of the net biliary excretion. 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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Contents

1. Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) inhibit the synthesis of mevalonate, a rate-limiting step in cholesterol biosynthesis, leading to a reduction in the plasma low density lipoprotein (LDL)-cholesterol level. High plasma LDL-cholesterol is a risk factor of cardiovascular diseases and, therefore, cholesterol-lowering drugs are used to prevent them. Some randomized controlled trials have shown that statins have potent cholesterol-lowering effects and reduce the risk of cardiovascular diseases in everyday medical practice (Scandinavian Simvastatin Survival Study Group, 1994; Shepherd et al., 1995; Sacks et al., 1996; Bertolini et al., 1997; The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group, 1998). On the other hand, some of statins exhibit a number of adverse effects, such as myopathy or rhabdomyolysis (Staffa et al., 2002; Thompson et al., 2003). Concomitant use of other drugs sometimes increases the risk of severe myotoxicity (Pierce et al., 1990; Pogson et al., 1999). Cerivastatin causes a serious myotoxicity, which has resulted in 31 deaths in the USA (Staffa et al., 2002). Among these patients, 12 were concomitantly taking gemfibrozil, suggesting that combination therapy with these drugs might increase the risk of side effects due to a drug–drug interaction (Staffa et al., 2002). Indeed, the plasma concentration of cerivastatin was reported to be increased by

coadministration of gemfibrozil (Backman et al., 2002). Due to this severe side effect, cerivastatin was voluntarily withdrawn from the market in 2001. This review will summarize the mechanism of drug–drug interactions between statins and other drugs, using comparisons of the characteristics of statins, their physicochemical properties, elimination routes, and so on.

Mevastatin, a lead compound of the statins, is a fungal product, initially extracted from *Penicillium citrinum* (Endo et al., 1976). Lovastatin, simvastatin and pravastatin are also derivatives of fungal products (Alberts et al., 1980; Hoffman et al., 1986; Endo, 1992). Among them, lovastatin and simvastatin possess a lactone ring in their structure and are transformed into the active open acid form in the body while pravastatin is administered as the biologically active open acid form (Fig. 1). On the other hand, fluvastatin is a completely synthetic statin with a very different structure from the statins derived from fungal products (Fig. 1). Fluvastatin is a mevalonolactone derivative with a fluorophenyl-substituted indole ring (Fig. 1). Statins, which reached the market after fluvastatin, also have similar structures with fluorophenyl groups. All of the totally synthetic statins have open acid forms. Depending upon their chemical structures, they have different affinities for HMG-CoA reductase, which determines their pharmacological effects, and different pharmacokinetic properties (i.e. tissue distribution, metabolic stability, enzymes and transporters involved in their metabolism, etc.). Thus, the information on the physicochemical properties of statins is useful to understand their pharmacokinetic properties. Drugs which interact with statins depend upon the pharmacokinetic properties of each of statins.

Recently, some reports on genetic polymorphisms in drug metabolizing enzymes and transporters have been published and interindividual differences in the pharmacokinetics of statins associated with them have been reported (Kirchheiner et al., 2003; Nishizato et al., 2003; Kajinami et al., 2004a; Niemi et al., 2004; Wang et al., 2005). This review also summarizes the information on the interindividual differences in pharmacokinetics of statins associated with these genetic factors.

2. The mechanisms governing the pharmacological effect of statins

2.1. Direct mechanism on 3-hydroxy-3-methylglutaryl coenzyme A reductase

All statins currently on the market possess a HMG-like moiety (Fig. 1): simvastatin and lovastatin have a lactone ring instead of this moiety and are transformed into the

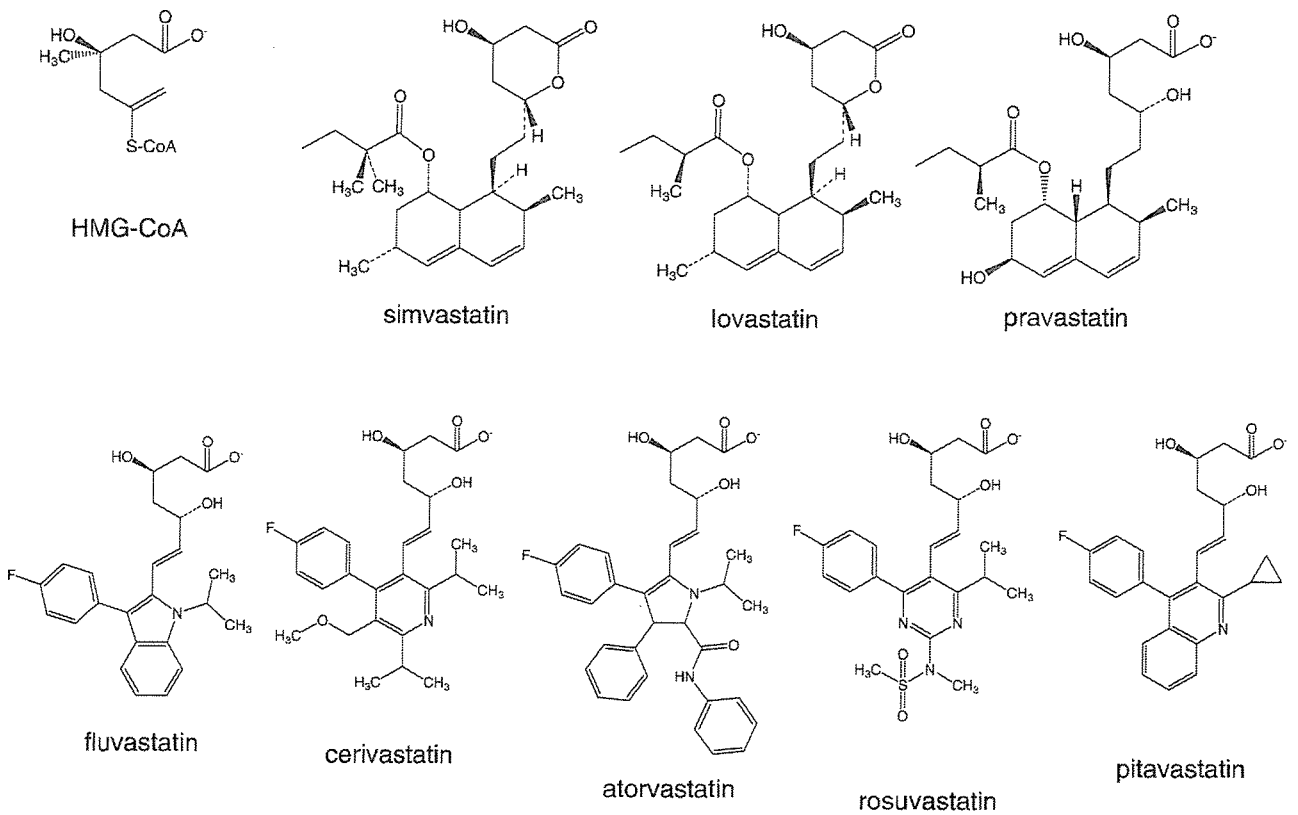

Fig. 1. Chemical structures of HMG-CoA reductase inhibitors (statins). Among statins, simvastatin, lovastatin and pravastatin are derivatives of fungal products while other newly developed statins are completely synthetic. The fungal products, simvastatin, lovastatin and pravastatin, are structurally related and they have a hydronaphthalene ring in common. Simvastatin and lovastatain are orally administered as inactive prodrugs in the lactone forms while pravastatin is given in the active open acid form. Other totally synthetic statins have different structures although they also have an open acid HMG-like moiety between the 4-fluorophenyl- and isopropyl- (or cyclopropyl-) groups. The difference in structure accounts for their different solubility in water.

biologically active form with an open acid in the body while other newer statins are administered as the open acid forms. Newer statins with HMG-moieties have a higher affinity for HMG-CoA reductase and exert more potent inhibitory effects (Istvan & Deisenhofer, 2001; McTaggart et al., 2001; Holdgate et al., 2003). Enzyme activity assay of HMG-CoA reductase substrate catalytic fragments indicates that statins, including simvastatin, pravastatin, fluvastatin, cerivastatin, atorvastatin and rosuvastatin, have a high affinity for HMG-CoA reductase with inhibition constants (K_i) of 5–44 nM while the Michaelis constant (K_m) of HMG-CoA is 4 μM, suggesting that all statins are potent inhibitors of this enzyme (Istvan & Deisenhofer, 2001; McTaggart et al., 2001; Holdgate et al., 2003).

Fig. 2 shows the pathway for the biosynthesis of cholesterol. HMG-CoA reductase-mediated production of mevalonate is a rate-determining step of cholesterol biosynthesis and, thus, inhibition of this enzyme reduces the cholesterol level. A reduced serum cholesterol level leads to an upregulation of LDL-receptors by a transcriptional regulation to maintain the intracellular cholesterol by homeostasis (Brown & Goldstein, 1986; Lennernas & Fager, 1997). However, cytochrome P450 7A1 (CYP7A1, cholesterol 7α-hydroxylase), which is specific to the liver, transforms intracellular cholesterol to bile acids, leading to a reduction of cholesterol in hepatocytes, although it is taken up via upregulated LDL-receptors. Biodegradation of cholesterol in the liver results in a reduction of total cholesterol in the body. In addition, the liver plays an important role in the biosynthesis of lipoprotein and catabolism of LDL (Brown & Goldstein, 1986). About 50% or more of the total cholesterol in the body is endogenous and it is mainly synthesized in the liver (Grundy, 1978; Peters et al., 1993; Gadbut et al., 1995; Transon et al., 1996). Thus, the target organ of statins is the liver.

2.2. The mechanism governing the side effect: the involvement of statin-produced inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase

Patients taking statins sometimes suffer from a number of adverse effects (i.e. myopathy or rhabdomyolysis). Their detailed mechanism is still unknown but some hypotheses have suggested that inhibition of HMG-CoA reductase may directly cause this myotoxicity (Thompson et al., 2003).

Cholesterol is also synthesized in the extrahepatic tissues and this biosynthesis plays an important role in normal cell function and steroid hormone biosynthesis (Corsini et al., 1999). Exposure of statins to extrahepatic tissues suppresses their cell function, leading to adverse effects, with little pharmacological effect (Sirtori, 1993). The reduction in cholesterol biosynthesis in muscle cells by statins leads to a reduction in their cholesterol contents of the plasma membrane. This may cause instability of the plasma membrane and damage to the cells. However, this hypothesis conflicts with the fact that cholesterol reduction by squalene synthase inhibition does not trigger myotoxicity (Flint et al., 1997; see Fig. 2).

Inhibition of HMG-CoA reductase results in the reduced biosynthesis of farnesyl pyrophosphate, an intermediate metabolite of ubiquinone/coenzyme Q_{10} (CoQ_{10}; Fig. 2). CoQ_{10} is a steroid isoprenoid which plays an important role in the cellular energy transduction in the mitochondrial electron transport system. In addition, it is a vital electron and proton carrier and supports ATP synthesis in the mitochondrial inner membrane, and stabilizes cell membranes, preserving cellular integrity and function. Thus, a reduced CoQ_{10} level may be one of reasons causing myotoxicity. Indeed, a reduction of serum CoQ_{10} level was reported in patients of myofiber atrophy and muscular dystrophy although it was not observed in patients of myofiber predominance and lower motor unit disease (Miles et

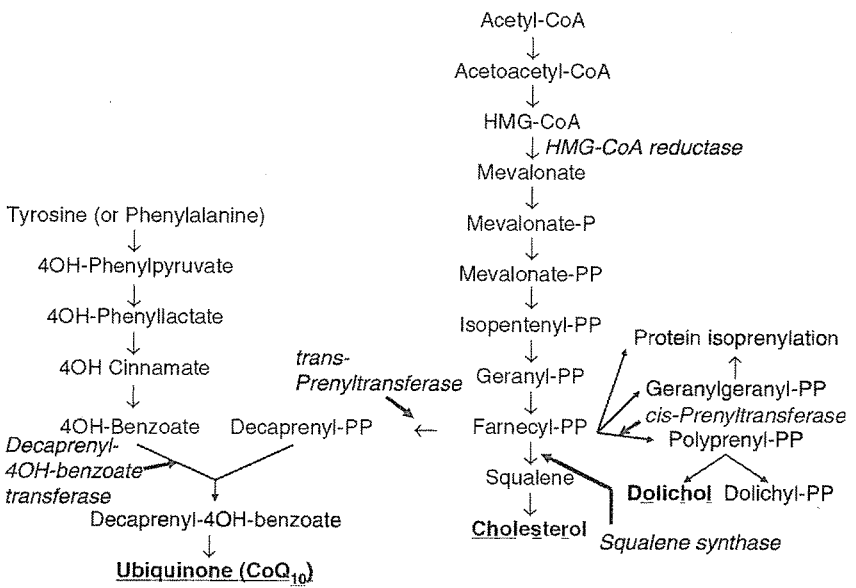

Fig. 2. Biosynthesis of cholesterol. Cholesterol is synthesized from acetyl-CoA. The synthesis of mevalonate, mediated by HMG-CoA reductase, is the rate-limiting step, which regulates the cholesterol synthesis. Farnesyl pyrophosphate is a branch point for the biosynthesis of other polyisoprenoids, dolichol and ubiquinone.

al., 2005). In addition, myopathy was reported to be associated with muscle CoQ_{10} deficiency (Lalani et al., 2005). 50% of total CoQ_{10} in the body is endogenously synthesized and, in patients taking statins, reduced serum CoQ_{10} levels have been reported (Folkers et al., 1990; Ghirlanda et al., 1993). Laakson et al. reported that CoQ_{10} in the skeletal muscle is not reduced by administration of statins while Paiva et al. showed that it is reduced in patients taking a high dose of simvastatin (Laaksonen et al., 1995, 1996; Paiva et al., 2005). Thus, some reports do not support the relationship between statin-induced myotoxicity and CoQ_{10}. However, the hypothesis that statin-induced myotoxicity is associated with reduced CoQ_{10} cannot be refuted because there are no data about the CoQ_{10} level in the skeletal muscles in patients routinely taking statins. There are other data supporting this hypothesis. In patients taking statins, the ratio of lactate to pyruvate increases, suggesting mitochondrial dysfunction (De Pinieux et al., 1996). In addition, biopsy samples from patients with statin-induced myopathy suggest mitochondrial dysfunction (i.e. increased lipid storage and ragged red muscle fibers), although the intracellular CoQ_{10} level has not been measured (Phillips et al., 2002).

On the other hand, there is a report that reduced synthesis of farnesyl pyrophosphate and geranylgeranyl pyrophosphate led to a reduction in the prenylation of small GTP-binding proteins such as Ras, Rac and Rho, which is thought to result in apoptosis of muscle cells (see Fig. 2). Pravastatin and lovastatin reduce the synthesis of these proteins in neonatal rat myocytes (Flint et al., 1997). This reduction can be reversed by the addition of farnesol and geranyl geraniol. On the other hand, myotoxocity was not observed when cholesterol was reduced by the inhibition of squalene synthase. These facts suggest that statin-associated myotoxicity is caused by farnesol and geranyl geraniol, intermediate metabolites in cholesterol synthesis, but not by cholesterol itself. Johnson et al. showed apoptosis of differentiated L-6 myoblast cells was not associated with the intracellular CoQ_9 and CoQ_{10} levels (Johnson et al., 2004). On the other hand, they showed this apoptosis was suppressed by the addition of mevalonate and geranylgeraniol, but not by the addition of farnesol (Johnson et al., 2004). Their results support the possibility of the involvement of the reduction in the protein geranylgeranylation in the myotoxicity associated with statins.

2.3. Other effects involving antiatherosclerosis

The direct mechanism of statins in preventing atherosclerosis and cardiovascular diseases by inhibition of cholesterol biosynthesis associated with the inhibition of HMG-CoA reductase is described above. However, statins also prevent cardiovascular diseases by other mechanisms (Corsini et al., 1999). As described above, mevalonic acid, the synthesis of which is inhibited by statins, is a precursor of not only cholesterol but other metabolites including isopentyl adenosine contained in transfer RNA, dolichols for the synthesis of glycoproteins and CoQ_{10} (Fig. 2). In addition, metabolites of mevalonic acid including farnesyl pyrophosphate and geranylgeranylpyrophosphate mediate the prenylation of some specific proteins (Fig. 2). Prenylated proteins are involved in a number of processes including cell signal transduction, differentiation, proliferation, myelination and cytoskeleton dynamics. Thus, statins may protect from the atherosclerosis by modulating such cell function triggered by the inhibition of protein prenylation.

The effects of statins, in addition to the inhibition of cholesterol biosynthesis, are summarized in Table 1. Statins affect arterial myocytes, macrophages and metaloproteases by these mechanisms and prevent atheroscelosis.

2.4. Association between the pharmacological effect of statins and genetic polymorphisms which affect the cholesterol level

Until now, there have been a large number of pharmacogenetic studies to examine the interindividual difference in the lipid level in the circulating blood and outcome response by statin therapy. By these studies, more than 30 genes have been investigated looking at statin responsiveness. Among them, some genes are directly related to the cholesterol biosynthesis or hypercholesterolemia while others are studied in relation to the pharmacokinetics of statins (Kajinami et al., 2004b, 2005). Table 2 summarized the candidate genes which have been investigated in pharmacogenetic studies to explore the determinants of statin therapy (Kajinami et al., 2004b, 2005). The relationship between the pharmacological and/or toxic effects of statins and genetic polymorphisms of factors which affect the cholesterol biosynthesis, degradation and elimination, or which are associated with hypercholesterolemia, is well summarized in the review article by Kajinami et al. (2004b). According to their review, a statistically significant

Table 1
Mechanisms for direct vascular actions and side effects of statins

Lipid effects
Inhibition of cholesterol biosynthesis Increased uptake and degradation of LDL
Inhibition of LDL oxidation
Inhibition of lipoprotein secretion
Inhibition of modified LDL endocytosis
Antiatherosclerotic effects
Inhibition of migration and proliferation of arterial myocytes
Inhibition of macrophage growth
Inhibition of cholesterol accumulation in macrophages
Inhibition of cell adhesion
Inhibition of tissue factor expression and activity
Inhibition of superoxide generation
Inhibition of endothelin-1 synthesis and expression
Increased expression and activity of eNOS
Increased fibrinolytic activity
Induction of myocytes apoptosis in proliferative lesions
Adverse effects on muscle injury
Reduced cholesterol contents in skeletal muscle cell membranes
Reduced levels of isoprenoids, such as ubiquinone, or regulatory proteins by the inhibition of HMG-CoA reductase
Reduced production of farnesyl pyrophosphate that is required for the activation of small GTP-binding regulatory protein

References: Corsini et al. (1999) and Thompson et al. (2003).

Table 2
Genes previously investigated in pharmacogenetic studies to explore the determinants of statin therapy

Category	Locus	Outcome
Pharmacokinetics	*CYP2C8*	AE
	CYP2C9	AE, LR, PK
	CYP2D6	AE, LR, PK
	CYP3A4	LR, PK
	CYP3A5	LR, PK
	ABCB1 (MDR1)	LR, PK
	ABCC2 (MRP2)	PK
	SLCO1B1 (OATP1B1)	PK
Pharmacodynamics	*ABCA1*	CVDER, LR
	ABCG5/G8	LR
	APOA1	LR
	APOA4	LR
	APOB	LR
	APOE	CVDER, LR
	CYP7A1	LR
	HMGCR	LR
	LDLR	LR
	LIPC	CVDER
	MTP	CVDER, LR
	SCAP	CVDER
Hypothesis-oriented (disease-related)	*ACE*	CVDER
	CETP	CVDER, LR
	ESR1	LR
	FDFT1	LR
	FGB	CVDER
	GP3A	CVDER
	IL6	CVDER
	LEPR	CVDER, LR
	MMP3	CVDER
	PON1	CVDER, LR
	PPARs	LR
	SREBPF1	CVDER, LR
	TLR4	CVDER, LR
	TNFα	CVDER, LR

AE: adverse events; CVDER: cardiovascular event response; LR: lipid response; PK: pharmacokinetics.
Reference: Kajinami et al. (2005).

difference in lipid response was observed associated with the genetic polymorphism in ABCA1, ABCG5/G8, apolipoprotein A1 (APOA1), APOB, APOE, CYP7A1, HMG-CoA reductase and LDL receptor although they sometimes failed to find a statistically significant difference in other studies. Thus, the genetic variation in these factors may influence the pharmacological effect of statins, leading to an interindividual difference in the pharmacological and/or toxic side effects.

3. The mechanism governing the pharmacokinetics of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors

3.1. The characteristics of statins

Statins have different pharmacokinetic profiles that are associated with their physicochemical properties. Table 3 shows the log*D* values reflecting the lipophilicity of statins. Simvastatin and lovastatin, which are administered as prodrugs with a lactone ring, have high log*D* values while other statins with open acid structures are less lipophilic. Among them, the log*D* of pravastatin is the lowest. Generally, compounds with high log*D* values can easily cross lipid bilayer membranes by passive diffusion and, thus, distribute to tissues nonspecifically (via passive diffusion). Table 4 shows the inhibitor concentration to produce a 50% inhibition (IC_{50}) of different statins on HMG-CoA reductase. Using purified catalytic fragment of human HMG-CoA reductase and cell-free enzyme system from rat liver, statins directly inhibit this enzyme and, thus, the IC_{50} values obtained in these experimental systems reflect the absolute potency as statins. However, using cell systems to inhibit this enzyme, statins are required to pass through the cell membrane, enter the cells and reach the HMG-CoA reductase in the cells. Thus, the IC_{50} values obtained in these systems reflect the hybrid parameter including membrane permeability of statins and their potency as statins (Table 4). Lipophilic statins such as simvastatin and lovastatin have low IC_{50} values even in the cell systems because they can easily cross the cell membrane via passive diffusion. However, hydrophilic statins (i.e. pravastatin and rosuvastatin) have much higher IC_{50} values than simvastatin and lovastatin in the cell systems except for rat and human hepatocytes although all of these statins have relatively close IC_{50} values in the cell-free systems. This observation supports that hydrophilic statins cannot easily cross the cell membrane. However, they inhibit HMG-CoA reductase potently in rat and human hepatocytes, since these statins are taken up by hepatocytes via active transport systems. The hepatic uptake of statins is described in the following section in detail.

3.2. The mechanism governing the hepatic uptake and biliary excretion of statins

Table 5 shows the pharmacokinetic properties of statins. As all statins available at present are cleared mainly by the liver as shown in Table 5, the processes of the hepatic uptake and biliary excretion as well as the metabolism play important roles in the mechanism governing their total body clearances (CL_{tot}). According to the clearance concept (Rowland et al., 1973; Winkler et al., 1973; Wilkinson & Shand, 1975; Pang & Rowland, 1977; Roberts & Rowland, 1986), hepatic clearance can be described by the following equations in terms of hepatic

Table 3
The log*D* values of statins

	log*D* (pH 7.0)[a]	log*D* (pH 7.4)[b]
Simvastatin (simvastatin acid)	4.4 (1.88)	
Lovastatin (lovastatin acid)	3.91 (1.51)	
Pravastatin	−0.47	−0.75–−1.00
Fluvastatin	1.75	1.00–1.25
Atorvastatin	1.53	1.00–1.25
Cerivastatin	2.32	1.50–1.75
Pitavastatin	1.5	
Rosuvastatin		−0.25–−0.50

[a] Ishigami et al. (2001) and Hirano et al. (2005b).
[b] Holdgate et al. (2003).

Table 4
Comparison of IC_{50} values of statins for HMG-CoA reductase activity measured in different experimental systems

Experimental system	IC_{50} values [nM]							Reference
	Simvastatin	Lovastatin	Pravastatin	Fluvastatin	Cerivastatin	Atorvastatin	Rosuvastatin	
Cell free systems								
HMG-CoA reductase activity in purified human catalytic fragment of the enzyme (peptide 419–888)	11		44	28	10	8.2	3.5–5.4	1, 2
Cell-free enzyme system from rat liver	1.2	1.2	1.9					3
	2.7		6.9	3.8	3.5	1.2	0.16	1
Hepatocytes								
Primary cultured rat hepatocytes	5.2		5.0	4.8	2.5	0.82	0.30	4
Rat hepatocytes	3.3	4.7	5.2					3
Primary cultured human hepatocytes	8.0	4.1	2.0					5
Non-hepatic cells								
HUVEC (human umblical vein endothelial)	1.0		1900	0.56	3.1	5.5	41	4
	5.5	2.4	1200					5
HCF (human cornea fibroblast)	4.6	15	1300					5
Human skin fibroblasts	2.9	4.0	470					3
NRK-49F (rat fibroblast)	7.9		14000	3.4	1.2	340	310	4
HRPEC (human retinal pigment epithelial cells)	8.0	18	4100					5
HGC (human granulosa cells)	16	27	1500					5
Rat spleen cells	5.3	3.5	170					3
Mouse L-cells	3.8	2.0	1400					3
Rat lenses	23	40	450					3

References: (1) McTaggart et al. (2001), (2) Holdgate et al. (2003), (3) Koga et al. (1990), (4) Buckett et al. (2000), (5) van Vliet et al. (1995).

blood flow (Q_H), blood unbound fraction (f_b) and overall intrinsic clearance ($CL_{int,all}$) of drugs.

(1) Well-stirred model

$$CL_H = \frac{Q_H \cdot f_b \cdot CL_{int,all}}{Q_H + f_b \cdot CL_{int,all}} \quad (1)$$

(2) Parallel-tube model

$$CL_H = Q_H \cdot \left(1 - e^{-\frac{f_b \cdot CL_{int,all}}{Q_H}}\right) \quad (2)$$

(3) Dispersion model

$$CL_H = Q_H \cdot \left\{1 - \frac{4a}{(1+a)^2 \cdot e^{\frac{a-1}{2D_N}} - (1-a)^2 \cdot e^{\frac{a+1}{2D_N}}}\right\} \quad (3)$$

where,

$$a = \sqrt{1 + 4R_N \cdot D_N} \quad (4)$$

$$R_N = \frac{f_b \cdot CL_{int,all}}{Q_H} \quad (5)$$

D_N is a dispersion number and this is taken as 0.17 (Iwatsubo et al., 1996, 1997). $CL_{int,all}$ includes not only metabolism and/or biliary excretion but also the membrane permeability as described by the following equation:

$$CL_{int,all} = PS_{u,influx} \cdot \frac{CL_{int}}{PS_{u,efflux} + CL_{int}} \quad (6)$$

where $PS_{u,influx}$ and $PS_{u,efflux}$ represent the membrane permeability clearance of unbound drugs for the influx and efflux processes, respectively, and CL_{int} represents the 'exact' intrinsic clearance for metabolism and/or biliary excretion of the unbound drugs (Pang & Gillette, 1978; Miyauchi et al., 1993; Yamazaki et al., 1996a; Mizuno et al., 2003). As shown in Eq. (6), the rate-limiting step in $CL_{int,all}$ depends on the relative values of $PS_{u,efflux}$ and CL_{int}. If $PS_{u,efflux}$ is much smaller than CL_{int} ($PS_{u,efflux} \ll CL_{int}$), Eq. (6) gives

$$CL_{int,all} = PS_{u,influx} \quad (7)$$

This equation shows that only the influx process influences $CL_{int,all}$. If $PS_{u,efflux}$ is much larger than CL_{int}, Eq. (6) gives

$$CL_{int,all} = CL_{int} \times \frac{PS_{u,influx}}{PS_{u,efflux}} \quad (8)$$

This equation indicates $CL_{int,all}$ reflects the asymmetry of the membrane permeability (influx/efflux) and the intrinsic metabolic and/or biliary excretion ability. If there is no asymmetry associated with the membrane transport process ($PS_{u,influx} = PS_{u,efflux}$), $CL_{int,all}$ becomes

$$CL_{int,all} = CL_{int} \quad (9)$$

If drugs can rapidly penetrate the cell membrane by passive diffusion, there is no asymmetry associated with the membrane transport and Eq. (9) can be applied. Except for this case, hepatic uptake can be one of the determinants of hepatic clearance.

The specific transporting systems for the hepatic uptake of many drugs including statins have been characterized. These drug transporters can also be caused of drug–drug interactions except for the case where drugs mainly undergo rapid penetration across the cell membrane via passive diffusion (see Eq. (9)). Especially in the case of statins, a transporter-mediated drug–drug interaction in the process of hepatic uptake

Table 5
Pharmacokinetic properties of statins

		Simvastatin		Lovastatin	Pravastatin	Fluvastatin		Atorvastatin	Cerivastatin	Pitavastatin	Rosuvastatin		
	Dose (mg)	40	60	40	40	20	40	40	0.3	2	20	40	80
	Dose form	Lactone	Lactone	Lactone	Open acid	Open acid	Open acid	Open acid	Open acid	Open acid	Open acid	Open acid	Open acid
Acid:	t_{max} (h)	1	4	3	1	0.43–2.1	0.5–1.5	1–2.5	3	1.2	5	5	4–5
	C_{max} (ng/mL)	6.9	3.1	2.7	45–66	53–370	200–440	13–67	3.2	41	6.1	19	39–50
	$t_{1/2}$ (h)	3.5	2.8		1.8–2.0		0.8–2.4	7.8–21	3.2	13		20	17
	AUC (ng h/mL)	25	22	34	110–140	110–440	320–570	58–620	21	120	63	180	310–410
Lactone	t_{max} (h)	4	1	4	2			3	3	1.6	–	5.1	4.5
	C_{max} (ng/mL)	3.2	16	2.8	1.6			4.2	0.30	22			7.1
	$t_{1/2}$ (h)		3.4					8.3	4.8	12			21
	AUC (ng h/mL)	20	47	28	3.3			47	1.9	170			110
	Absorption (%)	60–80		30	34	98		30					
	Bioavailability (%)	<5		5	18	19–29		12	60	80	20		
	Fraction excreted in urine as unchanged form (% of dose)	Negligible		10	47	Negligible		<2	Negligible		<10		

References: Tse et al. (1992), Smith et al. (1993), Kivisto et al. (1998), Muck et al. (1999), Backman et al. (2000), Kyrklund et al. (2001), Backman et al. (2002), Cooper et al. (2003a, 2003b), Klotz (2003), Martin et al. (2003), Schneck et al. (2004), Schachter (2005).

may affect their pharmacological effects because the target organ of statins is the liver, as shown in Section 2.1.

Our research group has studied the mechanism governing the hepatic uptake of statins, especially pravastatin (Yamazaki et al., 1993, 1996b, 1996c; Ishigami et al., 1995). Yamazaki et al. analyzed the mechanism of hepatic uptake and biliary excretion of pravastatin as well as the mechanism of its overall elimination in vivo (Yamazaki et al., 1993, 1996b, 1996c). They clarified that the K_m value for the overall elimination was similar with that for its uptake in isolated hepatocytes but quite different from that for the biliary excretion, estimated from the study using isolated bile canalicular membrane vesilces, suggesting the rate-limiting step for the overall elimination is the hepatic uptake process (Yamazaki et al., 1993, 1996b, 1997). This phenomenon can be explained by the Eq. (7). Yamazaki et al. have shown that pravastatin is selectively distributed to the liver and kidney, with most of it being distributed to the liver (Yamazaki et al., 1996c). Interestingly, liver selective uptake is inhibited by dibromosulphophthaleine but not by *p*-aminohippurate while kidney selective uptake is inhibited both of them. It suggests the different mechanism exists for the uptake of pravastatin in the liver and kidney. Later studies demonstrated that pravastatin uptakes by rat liver and kidney are accounted for by organic anion transporting polypeptides (Oatps) and organic anion transporter 3 (Oat3: gene symbol, *Slc22a8*), respectively (Hsiang et al., 1999; Tokui et al., 1999; Hasegawa et al., 2002).

Table 6
Uptake of statins in isolated or cultured hepatocytes

	K_m [μM]	V_{max} [pmol/min/mg protein]	P_{dif} [μL/min/mg protein]	V_{max}/K_m [μL/min/mg protein]	Ref
Pravastatin	11.5	10.2	0.3	0.887	(1)
Cerivastatin	180	5200*	280*	70*	(2)
	2.6	550*	210*	65*	
	3.7	360*	97*	42*	
Pitavastatin	2.99	79.7	7.73	26.7	(3)
Pravastatin	29.1	546	1.6	18.8	(4)
	36.5	816	1.6	21.5	(5)
Cerivastatin	5.9	260	24	44.4	(6)
Pitavastatin	26.0	3124	1.2	120	(7)

References: (1) Nakai et al. (2001), (2) Shitara et al. (2003), (3) Fujino et al. (2004a), (4) Ishigami et al. (1995), (5) Yamazaki et al. (1993), (6) Hirayama et al. (2000), (7) Shimada et al. (2003).
* per 10 cells.

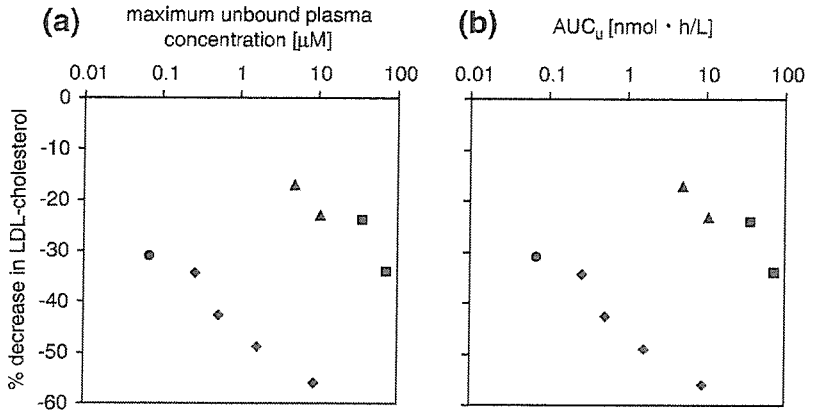

Fig. 3. Relationship between cholesterol-lowering effects of statins and the maximum plasma unbound concentration (**a**) or the area under the plasma unbound concentration–time curve (AUC_u, **b**). Cholesterol lowering effects are expressed as a % reduction in serum LDL-cholesterol. All data are modified from the reported values and the references are shown in Table 7. ◆, ■, ▲ and ● represent plots for atorvastatin, pravastatin, fluvastatin and cerivastatin, respectively.

Table 6 summarizes the reports of the uptake of statins into human or rat hepatocytes. Among the statins shown in Table 6, cerivastatin is taken up avidly by human hepatocytes. Nonspecific uptake, mainly by passive diffusion, is also high for cerivastatin as well as transporter-mediated uptake, possibly due to its lipophilicity. The specific carrier mediated hepatic uptake also contributes to the liver selective distribution (Sirtori, 1993). Table 4 shows that the IC_{50} value of pravastatin for the HMG-CoA reductase inhibition in extrahepatic cells is much higher than that of simvastatin while the values measured in hepatic cells are comparable. It is attributed to the specific uptake of pravastatin into hepatocytes as described above. Atorvastatin and rosuvastatin behave similar to pravastatin, suggesting that they are also taken up into the liver via specific transporters (Table 6). Although cerivastatin is taken up into hepatocytes via transporter-mediated mechanism, it has a marked inhibitory effect on HMG-CoA reductase even in extrahepatic cells. This is attributed to uptake by passive diffusion as cerivastatin is taken up into hepatocytes via passive diffusion as well as a specific transporter-mediated system because it is relatively lipophilic (Table 6). The high uptake of statins specifically by the liver, a target organ, should correlates

Table 7
Comparison of the pharmacological activities of statins in relation to their pharmacokinetics

	f_u	Dose (mg/day)	ΔLDL cholesterol (%)	C_{max} (ng/mL)	$C_{max,u}$ (ng/mL)	AUC (ng h/mL)	AUC_u (ng h/mL)
Atorvastatin	<0.02	10	−38, −30.5	7.41	0.741–1.48	77.6	15.5
		20	−46, −39.2	14.9	1.49–2.98	164	32.8
		40	51, 46.7	27–66.8	2.7–13.2	618	124
		80	54, 57.8	252	25.2–50.4	1293	259
Pravastatin	0.45–0.57	10	19				
		20	24	27	12–16	66	30–38
		40	34	45–66	20–38	140	63–80
Simvastatin	0.02–0.06	10	28				
		20	−35				
		40	−41	10–34			
				6.9 (as a lactone form)		25 (as a lactone form)	
				3.2 (as an open acid form)		20 (as an open acid form)	
		80	−46				
Fluvastatin	<0.01	20	−17	53–370	<3.7	110–440	<4.4
		40	−23	440–450	<4.5	570	<5.7
		80	−36				
Lovastatin	<0.05	20	−29				
		40	−32	10–20			
				2.7 (as a lactone form)		34 (as a lactone form)	
				2.8 (as an open acid form)		28 (as an open acid form)	
		80	48				
Cerivastatin	<0.01	0.2		1.6–2.0	<0.020	9.5	<0.095
		0.3	31	3.2	0.032	21	<0.21
		0.4	36				
		0.8	45				

Reference: Chong et al. (2001). Data in Table 5 were also referred.

with their pharmacological effects in vivo. Fig. 3 shows the correlation between the unbound plasma concentrations and serum LDL-cholesterol reduction produced by statins (also refer to Table 7). This shows that atorvastatin and cerivastatin exert a higher pharmacological effect at a lower plasma unbound concentration than pravastatin and fluvastatin. It cannot be quantitatively explained by the difference in their affinities for HMG-CoA reductase, which ranges within 6-fold (Table 4) and is, partly, due to their higher hepatic uptake.

Currently, the human transporters involved in the hepatic uptake of statins have been characterized and it has shown that many statins are specifically distributed to the liver via a transporter-mediated system. To date, it has been reported that pravastatin, cerivastatin, pitavastatin, rosuvastatin and atorvastatin are substrates of human OATP1B1 (also refered to as OATP-C/OATP2/LST-1, *SLCO1B1/SLC21A6*) (Hsiang et al., 1999; Nakai et al., 2001; Shitara et al., 2003; Fujino et al., 2004a; Schneck et al., 2004; Kameyama et al., 2005; Lau et al., 2006). Pitavastatin is reported to be also a substrate of OATP1B3 (OATP8/LST-2, *SLCO1B3*, *SLC21A8*) (Hirano et al., 2004). Hirano et al. estimated the contributions of OATP1B1 and 1B3 to the hepatic uptake of pitavastatin by so-called RAF (relative activity factor) method applied to drug metabolism as shown in Fig. 4 (Crespi & Penman, 1997; Hirano et al., 2004). This result suggests that pitavastatin is predominantly taken up into human hepatocytes via OATP1B1 with a minor contribution of OATP1B3. Simvastatin and lovastatin inhibit OATP1B1-mediated transport, suggesting that they may also be substrates of OATP1B1 (Hsiang et al., 1999). However, the contribution of this transporter to the hepatic uptake of simvastatin and lovastatin seems to be low because these lactone statins are lipophilic and are taken up by hepatocytes, and even by other tissues, by passive diffusion (Sirtori, 1993).

Our research group also examined the biliary excretion mechanism of statins, especially for pravastatin (Yamazaki et al., 1996d, 1997; Niinuma et al., 1999). Our investigations suggest that pravastatin is efficiently excreted into the bile via transporters, and then subsequently eliminated. Comparative studies involving normal rats and Eisai hyperbilirubinemic rats (EHBR), whose multidrug resistance associated protein 2 (Mrp2, *Abcc2*) is hereditarily deficient, showed that pravastatin is a substrate of Mrp2 (Yamazaki et al., 1996d, 1997). Thus, pravastatin is efficiently transported via transporters associated with hepatic uptake and biliary excretion and, both transporters for uptake and excretion help its specific distribution to the liver (Fig. 5). In humans, Sasaki et al. showed that pravastatin is a substrate of MRP2 (*ABCC2*), a counterpart of rat Mrp2, using a double transporter (OATP1B1 and MRP2) expressing system

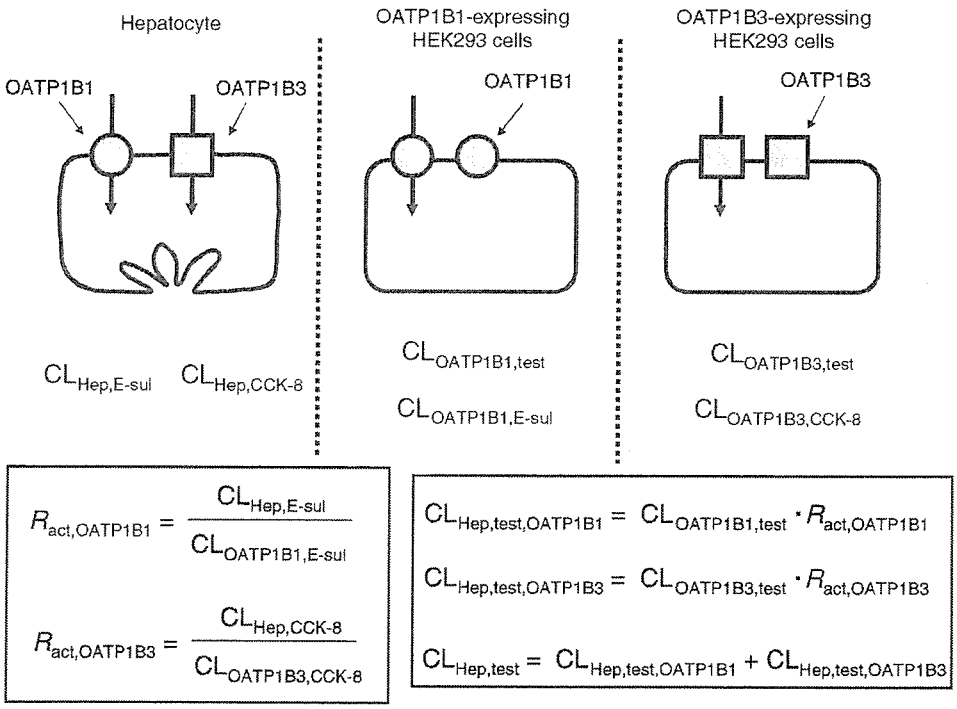

Fig. 4. Estimation of the contributions of OATP1B1 and OATP1B3 to the hepatic uptake of test compounds using selective substrate for each of transporters. The method for estimating the contribution of each transporter (OATP1B1 and 1B3) to the total hepatic uptake using reference compounds which are selectively taken up by a single transporter, OATP1B1 or 1B3, is shown. The uptake clearances of test compounds by OATP1B1 and 1B3 in human hepatocytes ($CL_{Hep,test,OATP1B1}$ and $CL_{Hep,test,OATP1B3}$) can be estimated by comparison with the uptake of reference compounds. Estrone 3-sulfate (E-sul) and cholecystokinine octapeptide (CCK-8) are used as selective substrates for OATP1B1 and 1B3, respectively. $CL_{Hep,E\text{-}sul}$ and $CL_{Hep,CCK\text{-}8}$ represent the uptake clearances of E-sul and CCK-8 in human hepatocytes, respectively, and $CL_{OATP1B1,E\text{-}sul}$ and $CL_{OATP1B3,CCK\text{-}8}$ represent the uptake clearances of E-sul and CCK-8 in OATP1B1- and 1B3-expressing HEK293 cells, respectively. $R_{act,OATP1B1}$ and $R_{act,OATP1B3}$ are ratio of the uptake clearances of reference compounds in human hepatocytes to those in transporter-expressing cells. The uptake clearance of test compounds mediated by OATP1B1 or 1B3 can be estimated by multiplying the uptake clearance of test compounds in transporter expressing cells by the R_{act} value for OATP1B1 or 1B3, respectively, as shown in the figure.

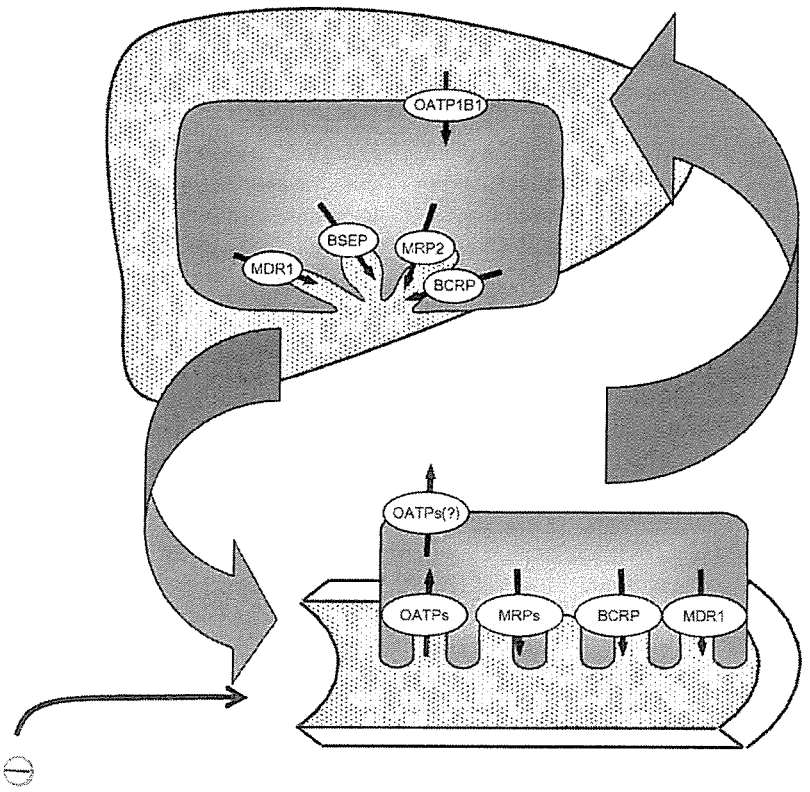

Fig. 5. Liver-selective distribution of pravastatin by transporters in the liver and intestine. Pravastatin is taken up into the liver via transporters including OATP1B1 and excreted into the bile via multiple transporters including MRP2, BCRP, MDR1 and BSEP in the unchanged form although the contribution of MRP2 seems to be the highest among the transporters on the bile canalicular membrane. The excreted pravastatin passes into the intestine and its absorption into the blood is mediated by transporters. OATP2B1 has recently been reported to be one of the transporters responsible for the intestinal absorption although its contribution to the intestinal absorption is unknown. Other members of the OATP family may also be involved in its absorption. Most of the other open-acid statins are also substrates of these transporters. These transporters may also be involved in the selective disposition of other statins to the liver.

(Sasaki et al., 2002). More recently, Matsushima et al. clarified multidrug resistance 1 (MDR1, *ABCB1*) and breast cancer resistance protein (BCRP, *ABCG2*) as well as MRP2 are involved in the biliary excretion of pravastatin and cerivastatin although their contributions are not estimated (Matsushima et al., 2005; Fig. 6). For pravastatin, MRP2 has the most potent transport activity among these efflux transporters while, for cerivastatin, MDR1 and MRP2 have relatively higher transport activity in their experimental systems (Matsushima et al., 2005). Hirano et al. reported that pitavastatin is also a substrate of human BCRP, MDR1 and MRP2 (Hirano et al., 2005a). On the other hand, in the case of experimental animals, its biliary excretion was not changed either in EHBR or Mdr1a/1b (*Abcb1a/1b*) knock-out mice compared with the corresponding wild type animals (Fujino et al., 2002). On the other hand, the biliary excretion of pitavastatin was extensively decreased in Bcrp (*Abcg2*) knock-out mice (Hirano et al., 2005a). In addition, Hirano et al. showed that human and rat bile salt exporting pump (BSEP/Bsep, *ABCB11/Abcb11*) also accept pravastatin as their substrates while they do not accept cerivastatin, fluvastatin and pitavastatin (Hirano et al., 2005b). These reports suggest that biliary excretion of statins is mediated by multiple transporters. Table 8 summarizes the human and rat transporters involved in the transport of statins.

3.3. Cytochrome P450-mediated metabolism

Statins, which are metabolized by members of the cytochrome P450 (P450), are susceptible to metabolism-mediated drug–drug interactions. Different drugs affect the pharmacokinetics of statins, depending on the isoforms of P450 that are involved in their metabolism. Simvastatin, lovastatin and atorvastatin are predominantly metabolized by cytochrome P450 3A4 (CYP3A4) (Wang et al., 1991; Prueksaritanont et al., 1997; Lennernas, 2003). On the other hand, fluvastatin is metabolized by CYP2C9, which is unique among statins (Transon et al., 1995, 1996). Pravastatin and rosuvastatin undergo very little metabolism by P450 and, therefore, they are not susceptible to the drug–drug interactions involving metabolism (Hatanaka, 2000; McCormick et al., 2000; White, 2002). Pitavastatin undergoes a minor degree of metabolism by CYP2C9, but the rate of metabolism is very slow and P450-mediated metabolism does not play an important role in its elimination (Fujino et al., 2004b). Thus, also for pitavastatin, P450-mediated metabolism is not susceptible to the mechanism governing drug–drug interactions. Cerivastatin is metabolized by 2 different P450 isoforms: CYP2C8 and 3A4 (Muck, 2000). Because of this dual metabolic pathway, it was believed to be less likely to be affected by a drug–drug interaction. In fact, its

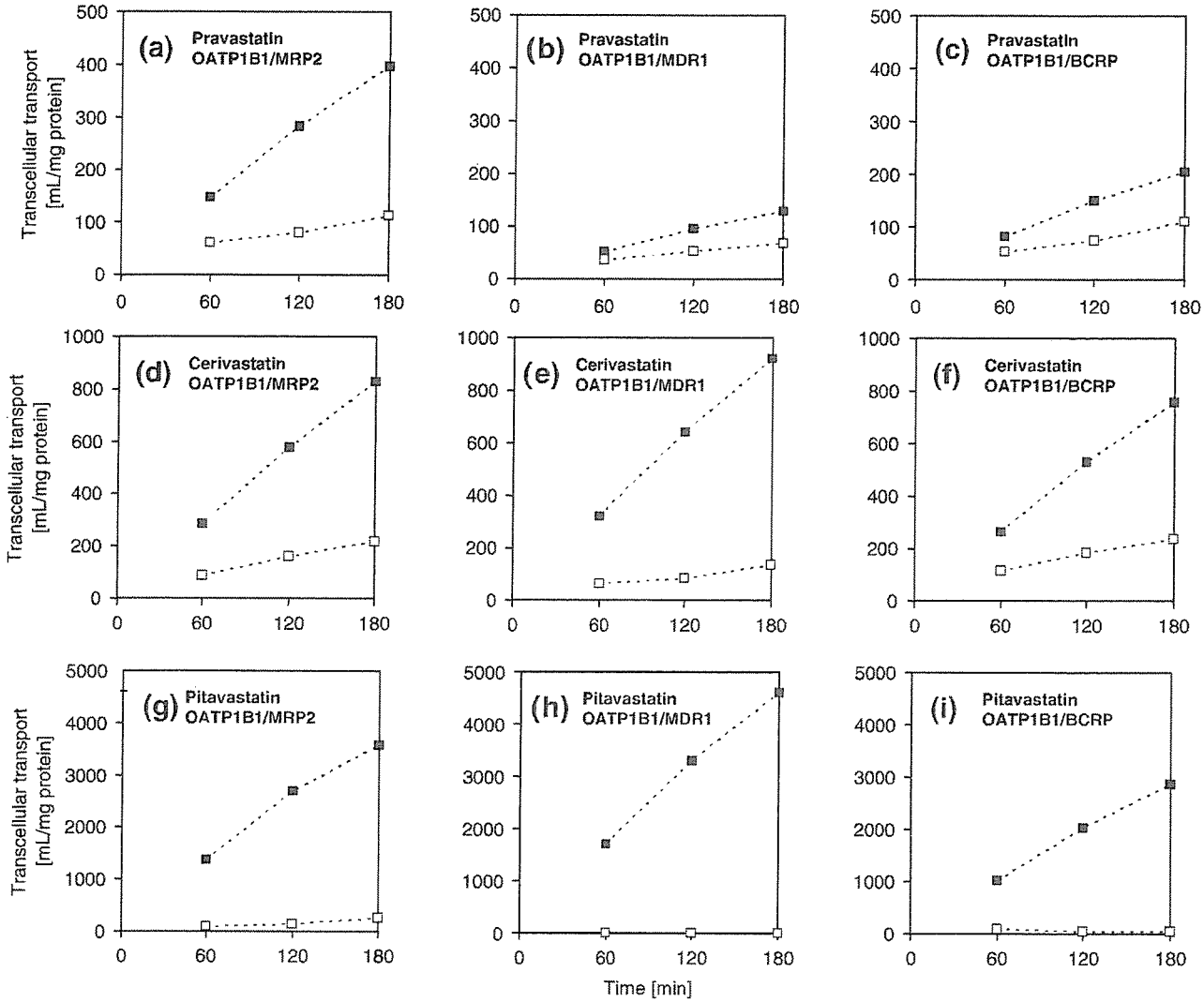

Fig. 6. Transcellular transport of pravastatin, cerivastatin and pitavastatin in OATP1B1/BCRP, OATP1B1/MDR1 and OATP1B1/MRP2 double expressing MDCK II cells. Transcellular transport of pravastatin (**a**, **b**, **c**), cerivastatin (**d**, **e**, **f**) and pitavastatin (**g**, **h**, **i**) in OATP1B1/BCRP (**a**, **d**, **g**), OATP1B1/MDR1 (**b**, **e**, **h**) and OATP1B1/MRP2 (**c**, **f**, **i**) is shown. All transport was measured bidirectionally and □ and ■ represent the apical-to-basal and basal-to-apical transports, respectively. Basal-to-apical transport of statins was enhanced by the expression of BCRP, MDR1 and MRP2 compared with the transport in OATP1B1 single expressing cells, suggesting they are substrates of these hepatic efflux transporters. Reprint from "Identification of the hepatic efflux transporters of organic anions using double-transfected Madin-Darby canine kidney II cells expressing human organic anion-transporting polypeptide 1B1 (OATP1B1)/multidrug resistance-associated protein 2, OATP1B1/multidrug resistance 1, and OATP1B1/breast cancer resistance protein" by Matsushima et al., 2005, and "Involvement of BCRP (ABCG2) in the biliary excretion of pitavastatin" by Hirano et al., 2005, with the permissions from the American Society for Pharmacology and Experimental Therapeutics.

plasma concentration is not affected by coadministration of itraconazole and erythromycin, potent inhibitors for CYP3A4 (Muck et al., 1998; Kantola et al., 1999; Mazzu et al., 2000). However, this phenomenon appears to be only due to the smaller contribution of CYP3A4 compared with that of CYP2C8 and, thus, this statin is also susceptible to the drug–drug interactions during the process of CYP2C8-mediated metabolism (Shitara et al., 2004). Table 9 shows the metabolic rates of statins (open acid and lactone forms) in human liver microsomes reported by Fujino et al. (2004c). It also shows P450 isoforms involved in their metabolism (Fujino et al., 2004c).

3.4. UDP glucuronosyl transferase-mediated lactonization of statins

Recently, a possible metabolic pathway leading to the lactone form of statins via UDP glucuronosyl transferase (UGT)-mediated glucuronidation was reported (Prueksaritanont et al., 2002a; Fujino et al., 2003). HMG-like moieties of open acid statins are glucuronosylated by UGT (UGT1A1 and 1A3), subsequently followed by lactonization of the acyl glucuronide HMG-like moieties (Fig. 7). This mechanism of lactonization, via acyl glucuronide, is common metabolic pathway for all statins with the open acid form. Due to this metabolic pathway, statins