



タイトル Title	Effects of Acid and Lactone Forms of Eight HMG-CoA Reductase Inhibitors on CYP-Mediated Metabolism and MDR1-Mediated Transport
著者 Author(s)	Sakaeda, Toshiyuki / Fujino, Hideki / Komoto, Chiho / Kakumoto, Mikio / Jin, Jiang-shu / Iwaki, Koichi / Nishiguchi, Kohshi / Nakamura, Tsutomu / Okamura, Noboru / Okumura, Katsuhiko
掲載誌・巻号・ページ Citation	Pharmaceutical Research,23(3):506-512
刊行日 Issue date	2006-03
資源タイプ Resource Type	Journal Article / 学術雑誌論文
版区分 Resource Version	author
権利 Rights	
DOI	10.1007/s11095-005-9371-5
JaLDOI	
URL	http://www.lib.kobe-u.ac.jp/handle_kernel/90000351

**Effects of acid and lactone forms of eight HMG-CoA reductase inhibitors on
CYP-mediated metabolism and MDR1-mediated transport**

Toshiyuki Sakaeda,^{1,*} Hideki Fujino,² Chiho Komoto,¹ Mikio Kakumoto,¹ Jiang-shu Jin,¹
Koichi Iwaki,¹ Kohshi Nishiguchi,¹ Tsutomu Nakamura,¹ Noboru Okamura,³ Katsuhiko
Okumura^{1,3}

1) Department of Hospital Pharmacy, School of Medicine, Kobe University, Kobe
650-0017, Japan

2) Tokyo New Drug Research Laboratories I, Kowa Company, Ltd., Higashimurayama
189-0022, Japan

3) Department of Clinical Evaluation of Pharmacotherapy, Kobe University Graduate
School of Medicine, Kobe 650-0047, Japan

* To whom correspondence should be addressed.

Running Title: Inhibition of Statins on CYP and MDR1

Corresponding Author:

Toshiyuki Sakaeda, Ph. D.

Address: 7-5-2, Kusunoki-Cho, Chuo-Ku, Kobe 650-0017, Japan

Tel: +81-78-382-6641 Fax: +81-78-382-6676

E-mail: sakaedat@med.kobe-u.ac.jp

No. of pages 33 pages

No. of tables 4

No. of figures 2

No. of references 40

Abstract 248 words

Introduction 481 words

Methods 888 words

Results 436 words

Discussion 753 words

Abstract (up to 250 words)

The inhibitory effects of acid and lactone forms of eight 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), including withdrawn cerivastatin, and recently developed pitavastatin and rosuvastatin, on CYP2C8, 2C9/10, 2C19 and 3A4/5 metabolic activities and MDR1 transporting activity were investigated using human liver microsomes and MDR1-overexpressing LLC-GA5-COL150 cell line, respectively. Acid forms had minimal inhibitory effects with $IC_{50} > 25 \mu\text{M}$ on all tested CYP activities, except for fluvastatin on CYP2C9/10-mediated tolbutamide 4-hydroxylation ($1.7 \mu\text{M}$) and simvastatin on CYP3A4/5-mediated paclitaxel 3-hydroxylation ($12.0 \mu\text{M}$). Lactone forms also showed no or minimal inhibitory effects on CYP2C8, 2C9/10 and 2C19 activities, except for rosuvastatin on the CYP2C9/10 activity ($20.5 \mu\text{M}$), whereas they showed stronger inhibitory effects on the CYP3A4/5 activity with rank order of atorvastatin ($5.6 \mu\text{M}$), cerivastatin ($8.1 \mu\text{M}$), fluvastatin ($14.9 \mu\text{M}$), simvastatin ($15.2 \mu\text{M}$), rosuvastatin ($20.7 \mu\text{M}$) and lovastatin ($24.1 \mu\text{M}$). Pitavastatin and pravastatin hardly inhibited it, and similar order was found also for testosterone 6β -hydroxylation. MDR1-mediated transport of [^3H]digoxin was inhibited only by lactone forms, and its rank order was correlated with those of inhibitory effects on both CYP3A4/5 activities. Inhibitory effects on MDR1

activity, but on both CYP3A4/5 activities, could be explained by the lipophilicity, however, a strong significant correlation was found between the lipophilicity and inhibitory effects on CYP2C8-mediated paclitaxel 6 α -hydroxylation. Finally, the analysis using the clinical data in the literature suggested that an increase of AUC by grapefruit juice was explained by CYP3A4/5-mediated inhibition rather than those via other CYPs and MDR1.

Introduction (up to 750 words)

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (statins) are widely used in the treatment of hypercholesterolemia and mixed dyslipidemias to reduce the risk of coronary heart disease and stroke through lowering low density lipoprotein and triglyceride. Additionally, recent reports have suggested that statins show an anti-inflammatory effect and induce the production of nitric oxide, so called the pleiotropic effects, resulting in the reduction of the risk of coronary heart disease. Based on the accumulation of in vitro and clinical evidences, statins are now being tried to be used for other diseases, including Alzheimer's disease, cancer and osteoporosis (Miida et al., 2004). As statins would be used more frequently for complicated diseases, we should use them more carefully with paying attention to drug-drug interaction, since it raises the risk for adverse events (Graham et al., 2004). In 2001, cerivastatin was withdrawn from the market because of rhabdomyolysis found especially in the patients co-prescribed with gemfibrosil. To date, little information is available concerning the mechanisms of this interaction, and therefore the safety of other statins in the market.

Drug-drug interaction has been well investigated in terms of drug metabolizing enzymes, especially of cytochrome P450 (CYP) enzymes, and of them, CYP3A4 is

understood to be the most important, since it contributes to a major role for metabolism of many drugs (Bjornsson et al., 2003). Recently, drug transporters have been recognized to be another class of key molecules to define drug disposition (Lin and Yamazaki, 2003; Sakaeda et al., 2004; Okamura et al., 2004). A number of drug transporters has been cloned and characterized, and the best characterized is MDR1 (P-glycoprotein). MDR1 was originally cloned in multidrug resistant cancer cells (Roninson et al., 1986), and it has been revealed that MDR1 is expressed in the normal tissue (Thiebaut et al., 1987) and involved in drug-drug interaction (Tanigawara et al., 1992; Ueda et al., 1992; Okamura et al., 1993; Hori et al., 1993; Sakaeda et al., 2002). It has been serendipitously noted that CYP3A4 and MDR1 show significant overlap in substrate or inhibitor specificity and proposed that MDR1 would regulate the access of drugs to CYP3A4 in the intestine (Benet et al.; 2004).

In this study, we compared the inhibitory effects of eight statins, including withdrawn cerivastatin, and recently developed pitavastatin and rosuvastatin, on CYP2C8, 2C9/10, 2C19 and 3A4/5 activities and MDR1 activity using human liver microsomes and MDR1-overexpressing LLC-GA5-COL150 cell line, respectively. Lovastatin and simvastatin in the medicine are of the lactone form, whereas others are of the acid form, but

both forms are found in human plasma after their administration (Lilja et al., 1998; 1999; Kivistö et al., 1998; Kantola et al., 1998; Backman et al., 2002; Tomlinson, 2003; Schneck et al., 2004), and the effects of both of acid and lactone forms of statins were examined independently herein to understand and estimate the possibility of the drug interaction of statins found in clinical.

Methods

Materials

All statin acid and lactone forms were synthesized or extracted from products and purified by Kowa Co. Ltd (Tokyo, Japan). Testosterone, tolbutamide and colchicine were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Paclitaxel and digoxin were obtained Sigma-Aldrich Co. (St. Louis, MO). 4-Hydroxyl tolbutamide, S-mephenytoin, 4'-hydroxyl S-mephenytoin, 6 α -hydroxyl paclitaxel, 3-hydroxyl paclitaxel and 6 β -hydroxyl-testosterone were purchased from Ultrafine Ltd. (Manchester, UK). [¹⁴C]Testosterone (2.00 GBq/mmol), [¹⁴C]tolbutamide (2.28 GBq/mmol), [¹⁴C]-S-mephenytoin (2.05 GBq/mmol) and [methoxy-¹⁴C]inulin (303 MBq/mmol) were purchased from Amersham Biosciences (Little Chalfont, UK). [¹⁴C]Paclitaxel (1.96 GBq/mmol) and [³H]digoxin (866 GBq/mmol) were purchased from Sigma-Aldrich Co. and New England Nuclear (Boston, MA), respectively. All other chemicals were obtained commercially or were of the highest grade requiring no further purification.

Inhibitory Effects of statins on activities of CYP enzymes in human liver microsomes

Paclitaxel 6 α -hydroxylation, tolbutamide 4-hydroxylation and S-mephenytoin

4'-hydroxylation were used probe reactions for CYP2C8, 2C9/10 and 2C19, respectively in pooled human liver microsomes (BD-GENESTTM, Becton, Dickinson and Co., Franklin Lakes, NJ). In addition, paclitaxel 3-hydroxylation and testosterone 6 β -hydroxylation were assessed for CYP3A4 activity. The activities of CYP enzymes were evaluated by the method previously reported (Fujino et al., 2003; 2004). Briefly, the incubation mixture was final volume of 250 μ L containing 0.5 – 1.0 mg microsomal protein/mL, 1.3 mM β -NADP⁺, 3.3 mM G-6-P and 0.4 U/mL G6P-DH in 0.1 M phosphate buffer (pH7.4). The reaction was started by the adding microsomal protein solution following 5 min of pre-warming. The reaction was stopped by the adding acetonitrile at the designated time. The substrate concentrations of tolbutamide, paclitaxel, S-mephenytoin and testosterone were 40, 4, 100 and 50 μ M, respectively. The inhibitory effects of statins were assessed by the presence of inhibitors at concentrations of 1, 3, 10, 30 and 100 μ M throughout experiments. Formed metabolites were determined by the method of TLC-RLG as previously reported (Shimada et al., 1985; Ludwig et al., 1998; Fujino et al., 2001; 2002). The formation rates of metabolites were evaluated as the activities of CYP enzymes.

Culture of LLC-PK₁ and LLC-GA5-COL150 cells

LLC-GA5-COL150 cells were established by transfection of MDR1 cDNA into porcine kidney epithelial LLC-PK₁ cells (Tanigawara et al., 1992; Ueda et al., 1992). Both lines were maintained in the culture medium consisting of Medium 199 (Dainippon Pharmaceutical Co., Ltd, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS; Lot no. AKH12368 or AMJ17247, HyClone, Logan, UT, USA) without antibiotics. For LLC-GA5-COL150 cells, 150 ng/mL of colchicine was added for stable expression of MDR1. LLC-PK₁ (1.0×10^6 cells; 1.82×10^4 cells/cm²) and LLC-GA5-COL150 (1.5×10^6 cells; 2.73×10^4 cells/cm²) cells were seeded on the plastic culture dishes (100 mm diameter) in 10 mL culture medium. They were grown in a humidified atmosphere of 5% CO₂/95% air at 37°C, and subcultured every 4 and 7 days, respectively, with 0.02% EDTA-0.05% trypsin solution (Invitrogen Corp., Carlsbad, CA, USA).

Transepithelial transport of [³H]digoxin with or without statins across LLC-PK₁ and LLC-GA5-COL150 cell monolayers

The transepithelial transport of [³H]digoxin across LLC-PK₁ and LLC-GA5-COL150 cell monolayers was examined as described previously (Tanigawara et al., 1992; Sakaeda et al., 2002). Basal-to-apical transport and apical-to-basal transport were assayed

independently. Both of cells were seeded onto Transwell® (Cat. No. 3414, Corning Costar Corp., Cambridge, MA, USA) at a cell density of 2.0×10^6 cells/well (4.26×10^5 cells/cm²) and 2.4×10^6 cells/well (5.11×10^5 cells/cm²) for LLC-PK₁ and LLC-GA5-COL150 cells, respectively. They were cultured under a humidified atmosphere of 5% CO₂/95% air at 37°C for 3 days. At 3 hr before the start of transport experiments, the culture medium was replaced with the fresh culture medium. The transport experiment was initiated by replacement of the culture medium on the donor side with 2 mL of the fresh culture medium containing [³H]digoxin (100 nM, 18.5 kBq/mL) together with [methoxy-¹⁴C]inulin (6.0 μM, 1.85 kBq/mL) and that on the receiver side with 2 mL of the fresh culture medium. The monolayers were incubated at 37°C, and 25 μL aliquot were taken from the receiver side at 1, 2 and 3 hr. The paracellular leakage estimated by the transport of inulin was less than 0.4% per hour in all experiments. The inhibitory effects of acid and lactone forms of statins were evaluated by adding inhibitors to both sides from 1 hr before and through 3 hours after the initiating the experiment. The radioactivity of samples was determined by liquid scintillation counting (LSC-5100, Aloca Co. Ltd, Tokyo, Japan). In the assessment, the net basal-to-apical transport was calculated by subtracting the apical-to-basal transport from the basal-to-apical transport of

[³H]digoxin, and the ratio of net basal-to-apical transport with a statin to that without was calculated.

Calculation of logarithm of octanol/water partition coefficient

To assess impact of the lipophilicity on inhibitory potency, we investigated the relationship between calculated logarithm of octanol/water partition coefficient (cLog P) of statins and IC₅₀ values for CYP and MDR1 activities. cLog P values were calculated by Crippen's fragmentation method (Ghose and Crippen, 1987) using CS ChemDraw Ultra ver 5.0 (Cambridge Soft Corp., Cambridge, MA), except rosuvastatin, which cannot be calculated by the Crippen's method.

Statistical analysis

Spearman's correlation test was applied for the correlation among the IC₅₀ values for CYP activities and MDR1 activity and the values of clog P of the acid and lactone forms of eight statins, giving a correlation coefficient, ρ , and associated probability, P .

Results

Table 1 lists the values of IC_{50} of acid and lactone forms of eight statins for CYP2C8, 2C9/10, 2C19 and 3A4/5 metabolic activities investigated using human liver microsomes. Acid forms had minimal inhibitory effects with IC_{50} being more than 25 μ M on all tested CYP activities, except for fluvastatin on CYP2C9/10-mediated tolbutamide 4-hydroxylation (1.7 μ M) and simvastatin on CYP3A4/5-mediated paclitaxel 3-hydroxylation (12.0 μ M). Lactone forms also showed no or minimal inhibitory effects on CYP2C8, 2C9/10 and 2C19 activities, except for rosuvastatin on CYP2C9/10-mediated tolbutamide 4-hydroxylation (20.5 μ M), whereas they showed stronger inhibitory effects on CYP3A4/5-mediated paclitaxel 3-hydroxylation with rank order of atorvastatin (5.6 μ M), cerivastatin (8.1 μ M), fluvastatin (14.9 μ M), simvastatin (15.2 μ M), rosuvastatin (20.7 μ M) and lovastatin (24.1 μ M). Pitavastatin and pravastatin hardly inhibited it with IC_{50} being more than 25 μ M. CYP3A4/5-mediated testosterone 6 β -hydroxylation was inhibited by atorvastatin with IC_{50} of 9.7 μ M. The lactone forms of other statins showed IC_{50} being more than 25 μ M, but the rank order was similar to that obtained by CYP3A4/5-mediated paclitaxel 3-hydroxylation.

In Table 1, the values of IC_{50} for MDR1 transporting activity are also listed, which

assessed using MDR1-overexpressing LLC-GA5-COL150 cell line and a typical MDR1 substrate, [³H]digoxin. MDR1-mediated transport of [³H]digoxin was inhibited only by lactone forms with the rank order of atorvastatin (15.1 μM), cerivastatin (28.2 μM), pitavastatin (34.9 μM), lovastatin (44.5 μM) and simvastatin (59.6 μM). Fluvastatin, pravastatin and rosuvastatin showed no inhibition even at 100μM.

Table 2 lists the Spearman's correlation coefficients (ρ) and associated probability (P) for the correlations among the values of the IC₅₀ for and cLog P of acid and lactone forms. The values of cLog P of seven statins (acid form / lactone form) are 5.05/5.58, 4.72/5.26, 3.79/4.32, 3.15/3.68, 4.09/4.63, 1.69/2.23 and 3.85/4.39 for atorvastatin, cerivastatin, fluvastatin, lovastatin, pitavastatin, pravastatin and simvastatin, respectively. Those for rosuvastatin cannot be calculated by the Crippen's method. All of lactone forms are more lipophilic than corresponding acid forms. The rank order of the inhibitory effects on MDR1 activity was correlated with those of the inhibitory effects on both CYP3A4/5 activities obtained by paclitaxel 3-hydroxylation ($\rho = 0.621$, $P = 0.010$) and testosterone 6 β -hydroxylation ($\rho = 0.560$, $P = 0.024$). Inhibitory effects on MDR1 activity were explained by the lipophilicity (Figure 1; $\rho = -0.546$, $P = 0.044$). However, there was no correlation between those on both CYP3A4/5 activities and the lipophilicity ($\rho = -0.497$, P

= 0.071 and $\rho = -0.302$, $P = 0.294$, respectively). It is noted that a strong significant correlation was found between the lipophilicity and that on CYP2C8-mediated paclitaxel 6α -hydroxylation (Figure 2; $\rho = -0.943$, $P < 0.001$).

Discussion (up to 1500 words)

With growing of clinical usage of statins, the number of reports concerning serious drug-drug interaction has been increased as following clinical reports. Fluvastatin and rosuvastatin enhanced the anticoagulant effect of warfarin (Barry 2004; Andrus 2004). Atorvastatin has no effects on warfarin efficacy (Stern et al., 1997), but reduces the antiplatelet effect of clopidogrel (Lau et al., 2004). Atorvastatin and rosuvastatin elevate the blood concentration of immunosuppressant, cyclosporine and ethynyl-estradiol, respectively (Renders et al., 2001; Simonson et al., 2004). Warfarin is mainly metabolized by CYP2C9, and CYP3A4 is understood to be responsible for transformation of a prodrug of clopidogrel to active metabolite, and metabolism of cyclosporine or ethynyl-estradiol. Atorvastatin, fluvastatin and rosuvastatin in the medicine are of the acid form, and atorvastatin and rosuvastatin has no or minimal effects on CYP2C9/10 or CYP3A4/5 activities (Table 1). However, recently conducted clinical investigations has suggested the conversion from the acid forms to lactone forms, and the opposite conversion after the administration (Lilja et al., 1998; 1999; Kivistö et al., 1998; Kantola et al., 1998; Backman et al., 2002; Tomlinson, 2003; Schneck et al., 2004). As summarized in Table 3, the AUC ratio of acid form to lactone form varies from 0.03 of pravastatin to 2.15 of

simvastatin. Herein, we elucidates that the lactone forms of atorvastatin and rosuvastatin inhibit CYP2C9/10 or CYP3A4/5 activities (Table 1). Taken together, the recently reported drug interaction of atorvastatin or rosuvastatin in clinical can be explained by action of lactone form, which can be transformed in the body, not by that of acid form, which is that in the medicine.

In this study, we clearly showed the difference between the acid and lactone forms of statins in terms of interaction with CYPs and MDR1. The results could be explained by the difference of chemical structures, but as shown in Table 2 and Figures 1 and 2, the lipophilicity is, at least, a key factor for the affinity for them. Generally, it is well-accepted that CYPs convert lipophilic substances to hydrophilic ones, and in turn, it is not surprising that the affinity for CYPs defined by the lipophilicity. For MDR1, Tanaka *et al.* have reported that the MDR1-mediated interaction between daunorubicin and cyclosporine analogs is defined by the lipophilicity (Tanaka et al., 1996). To our knowledge, this is the first time to show the relationship between the lipophilicity and the effects on CYP2C8 activity (Table 1, Figure 2). In the last 5 years, it has been recognized that CYP2C8 is an important CYP enzymes, because substrates of CYP2C8 are distinct from those of other CYP2C families, genetic polymorphisms affect the disposition

of CYP2C8 substrates and induction is mediated pregnane X receptor (Totah and Rettiew, 2005). The result is useful to avoid development new chemical entities, which is likely to cause drug-drug interaction mediated inhibition of CYP2C8.

In Table 3, the alteration of systemic exposure of statins by intake with grapefruit juice is also summarized (Lilja et al., 1998; 1999; Kivistö et al., 1998; Kantola et al., 1998; Backman et al., 2002; Tomlinson, 2003; Schneck et al., 2004). We evaluated the correlation of AUC fold increase of statins with grapefruit juice compared those without grapefruit juice, and the IC₅₀ values for the activities of CYPs and MDR1, and found a significant negative correlation for CYP3A4/5-mediated testosterone 6β-hydroxylation (Table 4). In addition, based on the assumption that the grapefruit juice would act in the intestinal wall before entering the systemic circulation of the statins, we evaluated the relationship between the IC₅₀ values of the forms of statins in the medicine and the fold increase of total AUC, that is, the sum of AUC of acid form and lactone form, and found the strong negative correlation still for CYP3A4/5-mediated paclitaxel 3-hydroxylation (Table 4). Although further studies are needed, this suggested that grapefruit juice-statin interaction can be explained CYP3A4/5-mediated process, being consistent with that grapefruit juice has been reportedly to raise plasma concentration of CYP3A4

substrates through the inactivation of CYP3A4 (Guo and Yamazoe, 2004).

Recently, it has been elucidated that UDP-glucuronosyl transferase (UGT) 1A1, 1A3 and 2B7 are thought to be responsible for conversion of statins from acid to lactone forms (Prueksaritanont et al., 2002; Fujino et al., 2002; Yamada et al., 2003). Since genetic polymorphisms are reported, at least, for UGT1A1 and 2B7 (Miners et al., 2002), there will be ethnic difference of the interaction between statins and other drugs, and the magnitude of the interaction will depend on their genotypes. Here, we showed the difference between the acid and lactone forms in terms of drug interaction. In the case of statins, it is important to examine the effects of both forms to understand the events found in clinical, including the pleiotropic effects.

References (Up to 40 refs)

- Andrus MR (2004) Oral anticoagulant drug interactions with statins: case report of fluvastatin and review of the literature. *Pharmacotherapy* **24**:285-290.
- Barry M (2004) Rosuvastatin-warfarin drug interaction. *Lancet* **363**:328.
- Backman JT, Kyrklund C, Neuvonen M and Neuvonen PJ (2002) Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther* **72**:685-691.
- Benet LZ, Cummins CL and Wu CY (2003) Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharm* **277**:3-9.
- Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, Kao J, King SP, Miwa G, Ni L, Kumar G, McLeod J, Obach SR, Roberts S, Roe A, Shah A, Snikeris F, Sullivan JT, Tweedie D, Vega JM, Walsh J, Wrighton SA and Pharmaceutical Research and Manufacturers of America Drug Metabolism/Clinical Pharmacology Technical Working Groups (2003) The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *J Clin Pharmacol* **43**:443-469.
- Fujino H, Yamada I, Shimada S, Yoneda M. (2001) Simultaneous determination of taxol and its metabolites in microsomal samples by a simple thin-layer chromatography

radioactivity assay--inhibitory effect of NK-104, a new inhibitor of HMG-CoA reductase. *J Chromatogr B Biomed Sci Appl* **757**:143-150.

Fujino H, Yamada I, Shimada S, Nagao T, Yoneda M. (2002) Metabolic fate of pitavastatin (NK-104), a new inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase. Effects on drug-metabolizing systems in rats and humans. *Arzneimittelforschung* **52**:745-753.

Fujino H, Yamada I, Shimada S, Yoneda M and Kojima J (2003) Metabolic fate of pitavastatin, a new inhibitor of HMG-CoA reductase: human UDP-glucuronosyltransferase enzymes involved in lactonization. *Xenobiotica* **33**:27-41.

Fujino H., Saito T, Tsunenari Y, Kojima J, Sakaeda T (2004) Metabolic properties of the acid and lactone form of HMG-CoA reductase inhibitors. *Xenobiotica* **34**:961-971.

Ghose AK and Crippen GM (1987) Atomic physicochemical parameters for three-dimensional-structure-directed quantitative structure-activity relationships. 2. Modeling dispersive and hydrophobic interactions. *J Chem Inf Comput Sci* **27**:21-35.

Graham DJ, Staffa JA, Shatin D, Andrade SE, Schech SD, La Grenade L, Gurwitz JH, Chan KA, Goodman MJ and Platt R (2004) Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs. *JAMA* **292**:2585-2590.

Guo LQ and Yamazoe Y (2004) Inhibition of cytochrome P450 by furanocoumarins in grapefruit juice and herbal medicines. *Acta Pharmacol Sin* **25**:129-136.

Hori R, Okamura N, Aiba T and Tanigawara Y (1993) Role of P-glycoprotein in renal tubular secretion of digoxin in the isolated perfused rat kidney. *J Pharmacol Exp Ther* **266**:1620-1625.

Kantola T, Kivistö KT and Neuvonen PJ (1998) Effect of itraconazole on the pharmacokinetics of atorvastatin. *Clin Pharmacol Ther* **64**: 58-65.

Kivistö KT, Kantola T and Neuvonen PJ (1998) Different effects of itraconazole on the pharmacokinetics of fluvastatin and lovastatin. *Br J Clin Pharmacol* **46**: 49-53.

Lau WC, Carville DG and Bates ER (2004) Clinical significance of the atorvastatin-clopidogrel drug-drug interaction. *Circulation* **110**:e66-e67.

Lilja JJ, Kivistö KT and Neuvonen PJ (1998) Grapefruit juice-simvastatin interaction: Effects on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther* **64**: 477-483.

Lilja JJ, Kivistö KT and Neuvonen PJ (1999) Grapefruit juice increases serum concentrations of atorvastatin and has no effect on pravastatin. *Clin Pharmacol Ther* **66**:118-127.

- Lin JH and Yamazaki M (2003) Role of P-glycoprotein in pharmacokinetics. *Clin Pharmacokinet* **42**:59-98.
- Ludwig E, Wolfinger H and Ebner T (1998) Assessment of microsomal tolbutamide hydroxylation by a simple thin-layer chromatography radioactivity assay. *J Chromatogr B Biomed Sci Appl* **707**:347-350.
- Miida T, Hirayama S and Nakamura Y (2004) Cholesterol-independent effects of statins and new therapeutic targets: Ischemic stroke and dementia. *J Atheroscler Thromb* **11**:253-264.
- Miners JO, McKinnon RA and Mackenzie PI (2002) Genetic polymorphisms of UDP-glucuronosyltransferases and their functional significance. *Toxicology* **181-182**:453-456.
- Okamura N, Hirai M, Tanigawara Y, Tanaka K, Yasuhara M, Ueda K, Komano T and Hori R (1993) Digoxin-cyclosporin A interaction: modulation of the multidrug transporter P-glycoprotein in the kidney. *J Pharmacol Exp Ther* **266**:1614-1619.
- Okamura N, Sakaeda T and Okumura K (2004) Pharmacogenomics of MDR and MRP subfamilies. *Personalized Med* **1**:85-104.
- Prueksaritanont T, Subramanian R, Fang X, Ma B, Qiu Y, Lin JH, Pearson PG and Baillie

- TA (2002) Glucuronidation of statins in animals and humans: a novel mechanism of statin lactonization. *Drug Metab Dispos* **30**:505-512.
- Renders L, Mayer-Kadner I, Koch C, Scharffe S, Burkhardt K, Veelken R, Schmieder RE and Hauser IA (2001) Efficacy and drug interactions of the new HMG-CoA reductase inhibitors cerivastatin and atorvastatin in CsA-treated renal transplant recipients. *Nephrol Dial Transplant*. **16**:141-146.
- Roninson IB, Chin JE, Choi KG, Gros P, Housman DE, Fojo A, Shen DW, Gottesman MM and Pastan I (1986) Isolation of human mdr DNA sequences amplified in multidrug-resistant KB carcinoma cells. *Proc Natl Acad Sci USA* **83**:4538-4542.
- Sakaeda T, Takara K, Kakumoto M, Ohmoto N, Nakamura T, Iwaki K, Tanigawara Y and Okumura K (2002) Simvastatin and lovastatin, but not pravastatin, interact with MDR1. *J Pharm Pharmacol* **54**:419-423.
- Sakaeda T, Nakamura T and Okumura K (2004) Pharmacogenetics of drug transporters and its impact on the pharmacotherapy. *Curr Top Med Chem* **4**:1385-1398.
- Schneck DW, Birmingham BK, Zalikowski JA, Mitchell PD, Wang Y, Martin PD, Lasseter KC, Brown CD, Windass AS and Raza A (2004) The effect of gemfibrozil on the pharmacokinetics of rosuvastatin. *Clin Pharmacol Ther* **75**:455-463.

- Shimada T, Shea JP and Guengerich FP (1985) A convenient assay for mephenytoin 4-hydroxylase activity of human liver microsomal cytochrome P-450. *Anal Biochem* **147**:174-179.
- Simonson SG, Martin PD, Warwick MJ, Mitchell PD, Schneck DW (2004) The effect of rosuvastatin on oestrogen & progestin pharmacokinetics in healthy women taking an oral contraceptive. *Br J Clin Pharmacol* **57**:279-286.
- Stern R, Abel R, Gibson GL, Besserer J (1997) Atorvastatin does not alter the anticoagulant activity of warfarin. *J Clin Pharmacol* **37**:1062-1064.
- Tanaka K, Hirai M, Tanigawara Y, Yasuhara M, Hori R, Ueda K and Inui K (1996) Effect of cyclosporin analogues and FK506 on transcellular transport of daunorubicin and vinblastine via P-glycoprotein. *Pharm Res* **13**:1073-1077.
- Tanigawara Y, Okamura N, Hirai M, Yasuhara M, Ueda K, Kioka N, Komano T and Hori R (1992) Transport of digoxin by human P-glycoprotein expressed in a porcine kidney epithelial cell line (LLC-PK₁). *J Pharmacol Exp Ther* **263**:840-845.
- Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I and Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* **84**:7735-7738.

Tomlinson B (2003) Pharmacokinetics of pitavastatin with grapefruit juice. *Medical View*

Point **24**:11

Total RA and Rettie AE (2005) Cytochrome P450 2C8: Substrates, inhibitors,

pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* **77**:341-352.

Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T and Hori R

(1992) Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem* **267**:24248-24252.

Yamada I, Fujino H, Shimada S and Kojima J (2003) Metabolic fate of pitavastatin, a new

inhibitor of HMG-CoA reductase: similarities and difference in the metabolism of pitavastatin in monkeys and humans. *Xenobiotica* **33**:789-803.

Footnotes

¹ Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2,

Kusunoki-Cho, Chuo-ku, Kobe 650-0017, Japan

² Tokyo New Drug Research Laboratories I, Kowa Company, Ltd., Higashimurayama

189-0022, Japan

³ Department of Clinical Evaluation of Pharmacotherapy, Kobe University Graduate School

of Medicine, 1-5-6, Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan

Table 1 IC₅₀ values for CYP-mediated metabolic activities and MDR1-mediated transporting activity for eight statins

Model substrates		IC ₅₀ values (μM)					
		Paclitaxel 6α-hydroxylation CYP2C8	Tolbutamide 4-hydroxylation CYP2C9/10	s-Mephenytoin 4-hydroxylation CYP2C19	Testosterone 6β-hydroxylation CYP3A4/5	Paclitaxel 3-hydroxylation CYP3A4/5	Digoxin Net B to A transport MDR1
Statins							
Atorvastatin	acid form ^a	38.4	>100	>100	>100	74.6	>100
	lactone form	28.8	61.0	>100	9.7	5.6	15.1
Cerivastatin	acid form ^a	29.8 ^b	>100	>100	>100	>100 ^b	>100
	lactone form	44.3 ^b	42.9	>100	80.7	8.1 ^b	28.2
Fluvastatin	acid form ^a	70.2	1.7 ^b	>100	>100	>100	>100
	lactone form	55.4	81.8 ^b	>100	48.1	14.9	>100
Lovastatin	acid form	74.6	>100	>100	>100	>100	>100
	lactone form ^a	79.9	>100	>100	26.9	24.1	44.5 +
Pitavastatin	acid form ^a	57.0	>100 ^b	>100	>100	>100	>100
	lactone form	50.5	>100 ^b	>100	>100	67.2	34.9
Pravastatin	acid form ^a	>100	>100	>100	>100	>100	>100 +
	lactone form	99.3	>100	>100	>100	73.7	>100
Rosuvastatin	acid form ^a	>100 ^b	>100 ^b	>100	>100	>100 ^b	>100
	lactone form	32.5 ^b	20.5 ^b	>100	82.7	20.7 ^b	>100
Simvastatin	acid form	51.5	>100	>100	79.0	12.0	>100
	lactone form ^a	44.1	>100	>100	76.8	15.2	59.6 +

^a the form contained in oral medicine.

^b already reported in 2004 (Fujino et al., 2004).

^c already reported in 2002 (Sakaeda et al., 2002).

Table 2 Spearman's correlation coefficients (ρ) and associated probabilities (P) for the correlation among the values of clog P and IC₅₀ for CYP-mediated metabolic activities and MDR1-mediated transporting activity for eight statins

		Paclitaxel 6α-hydroxylation CYP2C8	Tolbutamide 4-hydroxylation CYP2C9/10	Testosterone 6β-hydroxylation CYP3A4/5	Paclitaxel 3-hydroxylation CYP3A4/5	Digoxin Net B to A transport MDR1
cLog P	ρ	-0.943	-0.358	-0.302	-0.497	-0.546
	P	<0.001	0.209	0.294	0.071	0.044
	n	n = 14	n = 14	n = 14	n = 14	n = 14
Paclitaxel 6α-hydroxylation CYP2C8	ρ		0.336	0.347	0.496	0.354
	P		0.203	0.188	0.051	0.178
	n		n = 16	n = 16	n = 16	n = 16
Tolbutamide 4-hydroxylation CYP2C9/10	ρ			0.331	0.382	0.183
	P			0.210	0.144	0.498
	n			n = 16	n = 16	n = 16
Testosterone 6β-hydroxylation CYP3A4/5	ρ				0.852	0.560
	P				<0.001	0.024
	n				n = 16	n = 16
Paclitaxel 3-hydroxylation CYP3A4/5	ρ					0.621
	P					0.010
	n					n = 16

Table 3 Effects of grapefruit juice on pharmacokinetic parameters of eight statins

Statin		cLogP	without Grapefruit Juice				with Grapefruit Juice			
			Cmax ng/mL	AUC ng h/mL	AUC ratio	T _{1/2} h	Cmax ng/mL	AUC ng h/mL	AUC fold increase	T _{1/2} h
Atorvastatin^a	acid form	5.05	12.7	61.4	1	7.8	13.4	150.9	2.46	13.3
	lactone form	5.58	4.2	53.0	0.86	8.3	10.8	173.8	3.28	12.6
Cerivastatin^b	acid form	4.72	3.2	20.9	1	3.2	N. A.	N. A.	N. A.	N. A.
	lactone form	5.26	0.27	1.9	0.09	4.8	N. A.	N. A.	N. A.	N. A.
Fluvastatin^c	acid form	3.79	197	324	1	2.4	N. A.	N. A.	N. A.	N. A.
	lactone form	4.32	N. A.	N. A.	N. A.	N. A.	N. A.	N. A.	N. A.	N. A.
Lovastatin^d	acid form	3.15	17.6	76.9	1	2.5	69.6	384	5.0	2.5
	lactone form	3.68	7	28.1	0.37	2.5	82.4	429	15.3	3.0
Pitavastatin^e	acid form	4.09	38.5	87.2	1	9.10	33.2	101	1.16	9.19
	lactone form	4.63	24.7	177	2.03	12.4	21.2	203	1.15	15.2
Pravastatin^a	acid form	1.69	45.3	112.3	1	2	42.6	103.0	0.92	1.7
	lactone form	2.23	1.6	3.3	0.03	N. A.	1.6	3.7	1.12	N. A.
Rosuvastatin^f	acid form	N. C.	49.5	410	1	17.1	N. A.	N. A.	N. A.	N. A.
	lactone form	N. C.	7.1	110	0.27	20.5	N. A.	N. A.	N. A.	N. A.
Simvastatin^g	acid form	3.85	3.1	21.7	1	2.8	20.3	147	6.8	2.5
	lactone form	4.39	15.6	46.6	2.15	3.4	147	752	16.1	2.9

N. A.; Not available. N. C.; Not calculated.

^a PK parameters are referred from the report by Lilja et al., 1999.

^b PK parameters are referred from the report by Backman et al., 2002.

^c PK parameters are referred from the report by Kivistö et al., 1998.

^d PK parameters are referred from the report by Kantola et al., 1998.

^e PK parameters are referred from the report by Tomlinson, 2003.

^f PK parameters are referred from the report by Schneck et al., 2004.

^g PK parameters are referred from the report by Lilja et al., 1998.

Table 4 Spearman's correlation coefficients (ρ) and associated probabilities (P) for the correlation between grapefruit juice effect and the values of cLog P and IC₅₀ for CYP-mediated metabolic activities and MDR1-mediated transporting activity for five statins, whose AUC values are available with and without grapefruit juice

		Paclitaxel	Tolbutamide	Testosterone	Paclitaxel	Digoxin
		6α-hydroxylation	4-hydroxylation	6β-hydroxylation	3-hydroxylation	Net B to A
		cLog P	CYP2C8	CYP2C9/10	CYP3A4/5	MDR1
		CYP2C8	CYP2C9/10	CYP3A4/5	CYP3A4/5	MDR1
Fold increase of acid and lactone AUC	ρ	0.273	-0.382	-0.058	-0.703	-0.546
	p	0.446	0.276	0.873	0.023	0.102
	n	n = 10	n = 10	n = 10	n = 10	n = 10
Fold increase of total AUC	ρ	0.500	-0.500	N. C.	-0.783	-0.975
	p	0.391	0.391	N. C.	0.118	0.005
	n	n = 5	n = 5	N. C.	n = 5	n = 5

N. C.; not calculated.

Legends for Figures

Figure 1. Relationship between the values of $\text{clog } P$ and IC_{50} for MDR1 of 8 statins. Closed and open circles represent lactone and acid forms, respectively. Significant correlation was found with $\rho = -0.546$ and $P = 0.044$.

Figure 2. Relationship between the values of $\text{clog } P$ and IC_{50} for CYP2C8 of 8 statins. Closed and open circles represent lactone and acid forms, respectively. Significant correlation was found with $\rho = -0.943$ and $P < 0.001$.

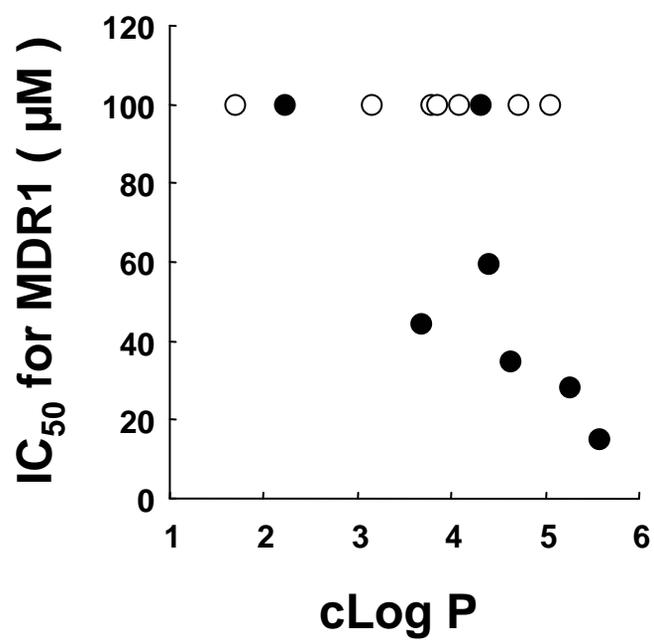


Fig. 1. Sakaeda et al.

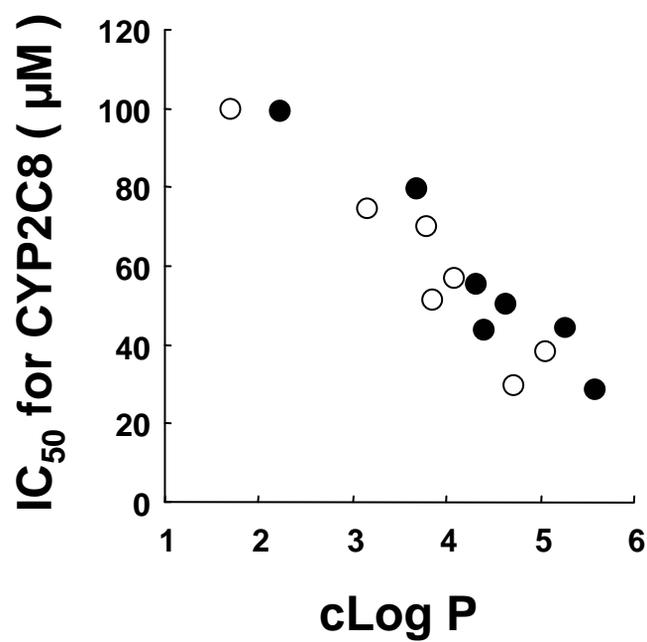


Fig. 2. Sakaeda et al.