JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 419–423
© 2002 The Authors
Received June 14, 2001
Accepted October 19, 2001
ISSN 0022-3573

Simvastatin and lovastatin, but not pravastatin, interact with MDR1

Toshiyuki Sakaeda, Kohji Takara, Mikio Kakumoto, Nobuko Ohmoto, Tsutomu Nakamura, Koichi Iwaki, Yusuke Tanigawara and Katsuhiko Okumura

Abstract

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, pravastatin, was compared with simvastatin and lovastatin from the viewpoint of susceptibility to interaction with or via the multidrug transporter, MDR1 (P-glycoprotein). This was carried out using the MDR1-overexpressing cell line LLC-GA5-COL150, established by transfection of MDR1 cDNA into porcine kidney epithelial LLC-PK_1 cells, and [^{3}H]digoxin, which is a well-documented substrate for MDR1. Pravastatin, at 25–100 μM, had no effect on the transcellular transport of [^{3}H]digoxin whereas simvastatin and lovastatin suppressed the basal-to-apical transport of [^{3}H]digoxin and increased the apical-to-basal transport. It was suggested that recognition by MDR1 was due to the hydrophobicity. In conclusion, simvastatin and lovastatin are susceptible to interaction with or via MDR1, but pravastatin is not. This is important information when selecting the HMG-CoA reductase inhibitors for patients taking drugs that are MDR1 substrates.

Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Toshiyuki Sakaeda, Mikio Kakumoto, Nobuko Ohmoto, Tsutomu Nakamura, Koichi Iwaki, Katsuhiko Okumura

Department of Hospital Pharmacy, Faculty of Pharmaceutical Sciences, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

Kohji Takara

Department of Hospital Pharmacy, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Yusuke Tanigawara

Correspondence: T. Sakaeda, Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. E-mail: sakaedat@med.kobe-u.ac.jp

Acknowledgment: We thank Dr Kazumitsu Ueda, Kyoto University Graduate School, for technical assistance with the LLC-GA5-COL150 cell culture. The authors are grateful to Sankyo Co. Ltd. for kindly supplying pravastatin, simvastatin and lovastatin.

Introduction

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, including pravastatin, simvastatin, lovastatin, fluvastatin, cerivastatin and atorvastatin, are currently used in the treatment of hypercholesterolaemia and mixed dyslipidaemias (Mauro 1993; Quion & Jones 1994). Simvastatin and lovastatin are lactone prodrugs generating the hydroxy acid in-vivo, whereas other HMG-CoA reductase inhibitors are available as active hydroxy acids providing hydrophilicity (Serajuddin et al 1991). Pravastatin differs from the other HMG-CoA reductase inhibitors because it has greater hydrophilicity as a result of the hydroxy group attached to the decalin ring (Quion & Jones 1994). This hydrophilicity accounts for the lower hepatic first-pass effect after oral administration; the extraction ratio is the largest for simvastatin (more than 79%), the least for pravastatin (46%), and lovastatin and fluvastatin show intermediate values (Desager & Horsmans 1996; Lennernäs & Fager 1997). The absolute bioavailability is 18% for pravastatin (Singhvi et al 1990), but less than 5% for simvastatin (Mauro 1993). Consequently, pravastatin has a lower chance of interaction with co-administered drugs via hepatic drug-metabolizing enzymes, and it has been reported that simvastatin is very susceptible to interaction with itraconazole, but pravastatin is not (Neuvonen et al 1998). It has been postulated that there is considerable overlap in substrate

Table 1 Partition coefficients of pravastatin, simvastatin and lovastatin between *n*-octanol and water.

	clogP	mlogP
Pravastatin	2.27	−0.47
Simvastatin	4.70	4.40
Lovastatin	4.30	No data

clog P is the calculated logarithmic value of *n*-octanol–water partition coefficient, and calculated using the computer program $CLOGP_3$ in MedChem Software Release 3.54 programs 1989 (Chemical Information System Inc., USA). mlogP is the measured logarithmic value of *n*-octanol–water (pH 7.4) partition coefficient at 25°C (Ishigami & Yamazoe 1998).

specificity of CYP3A4 and multidrug transporter MDR1 (P-glycoprotein) (Wacher et al 1995, 1998; Kim et al 1999; Wandel et al 1999). MDR1 is expressed in normal tissues (Thiebaut et al 1987) and can be responsible for drug interactions similarly to hepatic drug-metabolizing enzymes. In this study, hydrophilic pravastatin was compared with hydrophobic simvastatin and lovastatin (Table 1) from the viewpoint of susceptibility to interaction with or via MDR1, using the MDR1-overexpressing cell line LLC-GA5-COL150, which was established by transfection of MDR1 cDNA into porcine kidney epithelial $LLC\text{-}PK_1$ cells (Tanigawara et al 1992; Ueda et al 1992) and has been shown to be useful for assessment of drug interactions via MDR1 (Okamura et al 1993; Takara et al 1999, 2000).

Materials and Methods

Chemicals

Pravastatin, simvastatin and lovastatin were kindly supplied by Sankyo Co. Ltd (Tokyo, Japan). Colchicine was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). [^{3}H]Digoxin (595.7 GBq mmol^{-1}) and [methoxy-^{14}C]inulin (308 MBq mmol^{-1}) were purchased from Du Pont-New England Nuclear (Boston, MA) and Amersham International plc (Buckinghamshire, UK), respectively. Unlabelled digoxin was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were obtained commercially or were of the highest grade requiring no further purification.

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

LLC-GA5-COL150 cells were established by transfection of MDR1 cDNA into porcine kidney epithelial $LLC\text{-}PK_1$ cells (Tanigawara et al 1992; Ueda et al 1992). $LLC\text{-}PK_1$ and LLC-GA5-COL150 cells were maintained in the culture medium consisting of Medium199 (Dainippon Pharmaceutical Co. Ltd, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS; Lot no. AGM7413; HyClone, UT) without antibiotics. Only for LLC-GA5-COL150 cells, 150 ng mL^{-1} of colchicine was added to the culture medium. $LLC\text{-}PK_1$ (1.0×10^6 cells; 1.82×10^4 cells cm^{-2}) and LLC-GA5-COL150 cells (1.5×10^6 cells; 2.73×10^4 cells cm^{-2}) were seeded on plastic culture dishes (100 mm diameter) in 10 mL of the culture medium. These were about 10% of full confluence. Monolayer cultures were grown in a humidified atmosphere of 5% CO_2–95% air at 37°C, and passaged every 4 days and 7 days, respectively, with 0.02% EDTA–0.05% trypsin solution (Gibco BRL, Life Technologies Inc., Grand Island, NY).

Transcellular transport of [^{3}H]digoxin and its inhibition by pravastatin, simvastatin and lovastatin across LLC-GA5-COL150 cell monolayers

Transcellular transport of [^{3}H]digoxin across $LLC\text{-}PK_1$ and LLC-GA5-COL150 cell monolayers was examined as described previously (Tanigawara et al 1992; Takara et al 2000). Cells were seeded on Transwell (Cat. no. 3414; Costar, Cambridge, MA) at a density of 2.0×10^6 cells/well (4.26×10^5 cells cm^{-2}) and 2.4×10^6 cells/well (5.11×10^5 cells cm^{-2}) for $LLC\text{-}PK_1$ and LLC-GA5-COL150 cells, respectively, and cultured under a humidified atmosphere of 5% CO_2–95% air at 37°C for 3 days to form a monolayer. It was confirmed that there was no bilayer area and excess cells were in the culture medium. Three hours before the transport experiments, the culture medium was replaced with fresh culture medium consisting of Medium199 supplemented with 10% FBS without colchicine. The transport study was initiated by replacement of the culture medium on the donor side with 2 mL of fresh culture medium containing [^{3}H]digoxin (100 nM, 18.5 kBq mL^{-1}) together with [methoxy-^{14}C]inulin (6.0 μM, 1.85 kBq mL^{-1}), used as a cell monolayer integrity marker; culture medium on the receiver side was replaced with 2 mL of fresh culture medium alone. The monolayers were incubated at 37°C, and 25-μL samples of the medium were taken from the receiver side at three time points (1, 2 and 3 h). The paracellular leakage estimated by the amount of appearance of [methoxy-^{14}C]inulin in the receiver side was less than 0.4% per h of the initial amount in the donor side. For the inhibition studies, either pravastatin, simvastatin or lovastatin was added to both sides of the

monolayers at the indicated concentration 1 h before the start until the end of experiments. The radioactivity associated with the collected media was determined by liquid scintillation counting (LSC-5100, Aloka Co. Ltd, Tokyo). Data are presented as the percentage of the initial amount of total radioactivity added in the donor side. The IC50 values (50% inhibitory concentration) for simvastatin on [^{3}H]digoxin transport were estimated as described previously (Kusunoki et al 1998; Wandel et al 1999).

Statistical analysis

All data presented are the mean±standard error (s.e.). Statistical analysis of the data was performed by one-way analysis of variance followed by Scheffe's test (two-tailed), with $P < 0.05$ considered significant.

Results and Discussion

Transcellular transport of [methoxy-^{14}C]inulin across LLC-PK$_1$ and LLC-GA5-COL150 cell monolayers was time-dependent, showing no alteration of the flux rate for at least 3 h. The apical-to-basal transport and basal-to-apical transport at 3 h were 0.73±0.12 and 0.54±0.05% for LLC-PK$_1$ cells, respectively, and 0.62±0.12 and 0.47±0.12% for LLC-GA5-COL150 cells, respectively (n = 4). There was no effect of [^{3}H]digoxin, pravastatin, simvastatin and lovastatin on the transport of [methoxy-^{14}C]inulin.

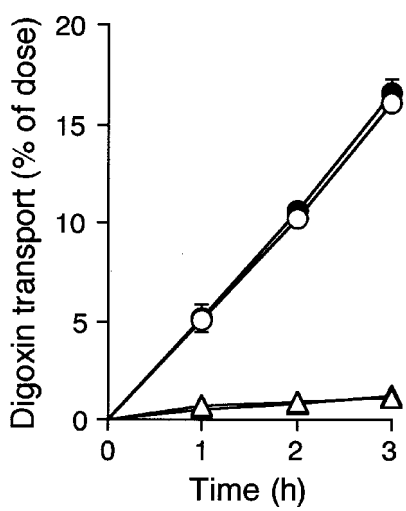

Figure 1 Effects of pravastatin on transcellular transport of [^{3}H]digoxin in LLC-GA5-COL150 cells. Open and closed symbols indicate transport in the absence and presence, respectively, of pravastatin. Circles show the basal-to-apical transport and triangles indicate the apical-to-basal transport. The concentration of pravastatin was 50 μM. Each point represents the mean±s.e. of results from 3–6 independent experiments.

Table 2 Effects of pravastatin, simvastatin and lovastatin on transcellular transport of [^{3}H]digoxin (100 nM) in LLC-PK$_1$ and LLC-GA5-COL150 cells.

	[^{3}H]Digoxin transport at 3 h (% of dose)	
	Basal-to-apical	Apical-to-basal
LLC-PK$_1$ cells	11.8±0.45	5.9±0.40
+Pravastatin 100 μM	12.3±0.69	7.9±0.38**
+Simvastatin 100 μM	10.3±0.11	10.7±0.51**
+Lovastatin 100 μM	9.7±0.29	9.8±0.09**
LLC-GA5-COL150 cells	16.1±0.27	1.2±0.05
+Pravastatin 25 μM	15.3±0.51	1.4±0.10
+Pravastatin 50 μM	16.6±0.68	1.2±0.04
+Pravastatin 100 μM	15.6±0.29	1.5±0.08
+Simvastatin 25 μM	14.9±0.42	2.7±0.10**
+Simvastatin 50 μM	13.3±0.21*	3.5±0.05**
+Simvastatin 100 μM	9.5±0.24**	5.8±0.24**
+Lovastatin 25 μM	16.5±0.30	2.5±0.11**
+Lovastatin 50 μM	11.8±0.40**	3.2±0.08**
+Lovastatin 100 μM	12.0±0.13**	3.9±0.16**

Values are means±s.e. of results from 3–6 independent experiments. *$P < 0.05$, **$P < 0.01$ compared with no addition of pravastatin, simvastatin and lovastatin.

Transcellular transport of [^{3}H]digoxin across LLC-GA5-COL150 cell monolayers was also time-dependent with no alteration of the flux rate for at least 3 h, and the basal-to-apical transport of [^{3}H]digoxin was markedly higher than the apical-to-basal transport (Figures 1 and 2, Table 2). MDR1 is highly expressed on the apical membrane of the cells (Tanigawara et al 1992; Ueda et al 1992), and the basal-to-apical transport was increased and the apical-to-basal transport was decreased in the LLC-GA5-COL150 cells as compared with the parent LLC-PK$_1$ cells.

Pravastatin, at 25–100 μM, had no effect on the transcellular transport of [^{3}H]digoxin across LLC-GA5-COL150 cell monolayers (Figure 1, Table 2). In contrast, simvastatin and lovastatin suppressed the basal-to-apical transport of [^{3}H]digoxin and increased the apical-to-basal transport in a concentration-dependent manner (Figure 2, Table 2). The estimated IC50 value for simvastatin on [^{3}H]digoxin transport in LLC-GA5-COL150 cells was 48.2 μM, which was higher than those of the Ca^{2+} blockers, nicardipine (4.54 μM), manidipine (4.65 μM) and benidipine (4.96 μM), and comparable with that of nisoldipine (44.1 μM) (unpublished results). The interaction of nisoldipine with digoxin was observed in clinical use, but the degree of the interaction was not

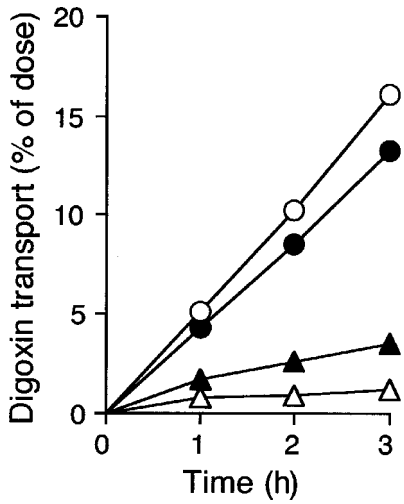

Figure 2 Effects of simvastatin on transcellular transport of [^{3}H] digoxin in LLC-GA5-COL150 cells. Open and closed symbols indicated the transport in the absence and presence, respectively, of simvastatin. Circles show the basal-to-apical transport and triangles indicate the apical-to-basal transport. The concentration of simvastatin was 50 μM. Each point represents the mean$\pm$s.e. of results from 3–6 independent experiments.

significant (Kirch et al 1986). The plasma concentration of simvastatin (40 mg/day) was around 50 μg equiv. L^{-1} (0.12 μM) at steady state (Cheng et al 1992), which was markedly lower than the estimated IC50 value. Thus, the interaction of simvastatin with digoxin might be insignificant. However, the clinical relevance of these results remains unclear.

Pravastatin, simvastatin and lovastatin, at 100 μM, had no effect on the transcellular transport of [^{3}H] digoxin across LLC-PK$_1$ cell monolayers (Figure 1, Table 2). It was suggested that simvastatin and lovastatin, but not pravastatin, interacted with MDR1. Patients are usually treated with many drugs. In this regard, simvastatin and lovastatin are more dangerous than pravastatin, as they are substrates for MDR1 and many drugs have been shown to be transported via MDR1, such as amiodarone, quinidine, itraconazole and cyclosporin A (Hunter & Hirst 1997).

Simvastatin and lovastatin are more hydrophobic than pravastatin (Table 1). Very recently, it has been demonstrated that MDR1- or MDR1a/b-mediated transport of rhodamine-123, a well-documented substrate, was inhibited by hydrophobic atorvastatin (clogP = 4.90), like simvastatin and lovastatin, but not by simvastatin acid and lovastatin acid (Bogman et al 2001; Wang et al 2001). Thus, it was suggested that recognition by MDR1 was due to hydrophobicity. The importance of hydrophobicity in the MDR1 recognition has been proposed by Ueda et al (1997), who used steroids to demonstrate this. It is still unclear whether these statins are substrates for MDR1 or simple inhibitors of MDR1-mediated transport. Recently, atorvastatin was shown to be a substrate for MDR1 (Wu et al 2000). As for steroids, hydroxylation was the key factor in the chemical structure for becoming a substrate. For example, progesterone interacts strongly with MDR1, but is not transported by MDR1 (Ueda et al 1992; Gruol & Bourgeois 1994). Future work should consider characterization of the statins themselves as to whether they are transported.

Conclusion

Simvastatin and lovastatin are susceptible to interaction with or via MDR1, but pravastatin is not. This is important when selecting an HMG-CoA reductase inhibitor for treatment of patients who are also taking drugs that are MDR1 substrates.

References

Bogman, K., Peyer A.-K., Torok, M., Kusters, E., Drewe, J. (2001) HMG-CoA reductase inhibitors and P-glycoprotein modulation. *Br. J. Pharmacol.* **132**: 1183–1192

Cheng, H., Rogers, J. D., Sweany, A. E., Dobrinska, M. R., Stein, E. A., Tate, A. C., Amin, R. D., Quan, H. (1992) Influence of age and gender on the plasma profiles of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitory activity following multiple doses of lovastatin and simvastatin. *Pharm. Res.* **9**: 1629–1633

Desager, J. P., Horsmans, Y. (1996) Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. *Clin. Pharmacokinet.* **31**: 348–371

Gruol, D. J., Bourgeois, S. (1994) Expression of the mdr1 P-glycoprotein gene: a mechanism of escape from glucocorticoid-induced apoptosis. *Biochem. Cell Biol.* **72**: 561–571

Hunter, J., Hirst, B. H. (1997) Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption. *Adv. Drug Delivery Rev.* **25**: 129–157

Kim, R. K., Wandel, C., Leake, B., Cvetkovic, M., Fromm, M. F., Dempsey, P. J., Roden, M. M., Belas, F., Chaudhary, A. K., Roden, D. M., Wood, A. J. J., Wilkinson, G. R. (1999) Interrelationship between substrates and inhibitors of human CYP3A4 and P-glycoprotein. *Pharm. Res.* **16**: 408–414

Kirch, W., Stenzel Dylewicz, P., Hutt, H. J., Santos, S. R., Ohnhaus, E. E. (1986) Influence of nisoldipine on haemodynamic effects and plasma levels of digoxin. *Br. J. Clin. Pharmacol.* **22**: 155–159

Kusunoki, N., Takara, K., Tanigawara, Y., Yamauchi, A., Ueda, K., Komada, F., Ku, Y., Kuroda, Y., Saitoh, Y., Okumura, K. (1998) Inhibitory effects of a cyclosporin derivative, SDZ PSC 833, on transport of doxorubicin and vinblastine via human P-glycoprotein. *Jpn. J. Cancer Res.* **89**: 1220–1228

Lennernäs, H., Fager, G. (1997) Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. *Clin. Pharmacokinet.* **32**: 403–425

Mauro, V. F. (1993) Clinical pharmacokinetics and practical applications of simvastatin. *Clin. Pharmacokinet.* **24**: 195–202

Neuvonen, P. J., Kantola, T., Kivisto, K. T. (1998) Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. *Clin. Pharmacol. Ther.* **63**: 332–341

Okamura, N., Hirai, M., Tanigawara, Y., Tanaka, K., Yasuhara, M., Ueda, K., Komano, T., Hori, R. (1993) Digoxin-cyclosporin A interaction: modulation of the multidrug transporter P-glycoprotein in the kidney. *J. Pharmacol. Exp. Ther.* **266**: 1614–1619

Quion, J. A. V., Jones, P. H. (1994) Clinical pharmacokinetics of pravastatin. *Clin. Pharmacokinet.* **27**: 94–103

Serajuddin, A. T. M., Ranadive, A. S., Mahoney, E. M. (1991) Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin. *J. Pharm. Sci.* **80**: 830–834

Singhvi, S. M., Pan, H. Y., Morrison, R. A., Willard, D. A. (1990) Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. *Br. J. Clin. Pharmacol.* **29**: 239–243

Takara, K., Tanigawara, Y., Komada, F., Nishiguchi, K., Sakaeda, T., Okumura, K. (1999) Cellular pharmacokinetic aspects of reversal effect of itraconazole on P-glycoprotein-mediated resistance of anticancer drugs. *Biol. Pharm. Bull.* **22**: 1355–1359

Takara, K., Tanigawara, Y., Komada, F., Nishiguchi, K., Sakaeda, T., Okumura, K. (2000) Nicardipine and itraconazole inhibited cellular transport of digoxin. *Pharm. Pharmacol. Commun.* **6**: 167–171

Tanigawara, Y., Okamura, N., Hirai, M., Yasuhara, M., Ueda, K., Kioka, N., Komano, T., 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, M. M., Pastan, I., Willingham, M. C. (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl Acad. Sci. USA* **84**: 7735–7738

Ueda, K., Okamura, N., Hirai, M., Tanigawara, Y., Saeki, T., Kioka, N., Komano, T., Hori, R. (1992) Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.* **267**: 24248–24252

Ueda, K., Taguchi, Y., Morishima, M. (1997) How does P-glycoprotein recognize its substrates? *Semin. Cancer Biol.* **8**: 151–159

Wacher, V. J., Wu, C. Y., Benet, L. Z. (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol. Carcinog.* **13**: 129–134

Wacher, V. J., Silverman, J. A., Zhang, Y., Benet, L. Z. (1998) Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J. Pharm. Sci.* **87**: 1322–1330

Wandel, C., Kim, R. B., Kajiji, S., Guengerich, P., Wilkinson, G. R., Wood, A. J. (1999) P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. *Cancer Res.* **59**: 3944–3948

Wang, E., Casciano, C. N., Clement, R. P., Johnson, W. W. (2001) HMG-CoA reductase inhibitors (statins) characterized as direct inhibitors of P-glycoprotein. *Pharm. Res.* **18**: 800–806

Wu, X., Whitfield, L. R., Stewart, B. H. (2000) Atorvastatin transport in the Caco-2 cell model: contributions of P-glycoprotein and the proton-monocarboxylic acid co-transporter. *Pharm. Res.* **17**: 209–215