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Research Paper

Effect of Silymarin Supplement on the Pharmacokinetics of Rosuvastatin

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Objectives. To evaluate the effect of silymarin on the pharmacokinetics of rosuvastatin in systems overexpressing OATP1B1 or BCRP transporters and in healthy subjects.
Materials and Methods. The concentration-dependent transport of rosuvastatin and the inhibitory effect of silymarin were examined *in vitro* in OATP1B1-expressing oocytes and MDCKII-BCRP cells. For *in vivo* assessment, eight healthy male volunteers, divided into two groups, were randomly assigned to receive placebo or silymarin (140 mg) three times per day for 5 days. On day 4, all subjects received rosuvastatin (10 mg, 8 AM) 1 h after the placebo or silymarin administration. A series of blood samples were collected for 72 h, and the plasma concentration of rosuvastatin was determined using LC-MS/MS.
Results. Based on the concentration dependency of rosuvastatin transport in the OATP1B1 and BCRP overexpression systems, rosuvastatin is a substrate for both transporters. Silymarin inhibited both OATP1B1- and BCRP-mediated rosuvastatin transport *in vitro* (K_i 0.93 μM and 97 μM, respectively). However, no significant changes in AUC, half-life, V_d/F, or Cl/*F* of rosuvastatin were observed in human subjects following pretreatment with silymarin.
Conclusions. Silymarin does not appear to affect rosuvastatin pharmacokinetics *in vivo*, suggesting that silymarin, administered according to a recommended supplementation regimen, is not a potent modulator of OATP1B1 or BCRP *in vivo*.

KEY WORDS: BCRP; OATP1B1; pharmacokinetics; rosuvastatin; silymarin.

INTRODUCTION

Flavonoids are present in fruits, vegetables, plant-derived beverages, and many herbal products. The average daily intake of total flavonoids in the U.S. diet is estimated to be 0.2–1 g (1). Given the increasing availability of flavonoid-containing dietary supplements and herbal products, possible flavonoid–drug and flavonoid–flavonoid interactions may become more likely.

Silymarin is the polyphenolic fraction from the seeds of milk thistle (*Silybum marianum*) and is composed of various flavonoids, including silybin (major component), silydianin, and silychristine (2,3). Silymarin has been used as a supplementary treatment to protect the liver and to treat liver diseases such as gallbladder disorders, hepatitis, cirrhosis, and jaundice (3,4).

Previous experiments have demonstrated that silymarin is a potential human organic anion transporting polypeptide 1B1 (OATP1B1) modulator *in vitro* (5) and OATP1B1 is a liver-specific uptake transporter that is important for hepatic drug disposition. Previous studies have shown that silymarin significantly decreases uptake of dehydroepiandrosterone 3-sulfate (DHEAS) in OATP1B1-expressing cells; its effect was comparable to or greater than that caused by rifampin (5). In addition, the cellular accumulation of breast cancer resistance protein (BCRP) substrates significantly increased in the presence of flavonoids such as silymarin, hesperetin, quercetin, and daidzein (6,7). Daidzein and silybin, known BCRP inhibitors, significantly increased mitoxanthrone accumulation in MCF-7/MX100 cells, whereas no significant effect was observed in MCF-7/sensitive cells (8). The additive effects of multiple flavonoids in BCRP inhibition indicate the possibility of BCRP-mediated flavonoid–drug interactions (8,9). Numerous studies have shown that flavonoids also interact with other efflux transporters such as P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1) (9–12).

Rosuvastatin (Crestor; AstraZeneca, Wilmington, DE) is a new and highly effective statin used to treat hypercholesterolemia. This drug was shown to reduce low-density lipoprotein cholesterol more effectively than pravastatin, atorvastatin, or simvastatin in hypercholesterolemic patients

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ABBREVIATIONS: BCRP, breast cancer resistance protein; DHEAS, dehydroepiandrosterone 3-sulfate; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MRP1, multidrug resistance-associated protein 1; OATP1B1, organic anion transporting polypeptide 1B1; P-gp, P-glycoprotein; SNP, single nucleotide polymorphisms.

(13,14). The results of previous *in vitro* and *in vivo* experiments demonstrated that rosuvastatin is a substrate for the OATP1B1 transporter, which plays an important role in hepatic uptake of rosuvastatin (15,16). Rosuvastatin is also a good substrate for BCRP. An Eadie–Hofstee analysis indicated that there are two transport sites in the BCRP transporter, with apparent K_m values of 10.8 μM for the high-affinity site and 307 μM for the low-affinity site (17). These results suggest that functional changes in BCRP and OATP1B1, owing to genetic polymorphism or the addition of specific inhibitors, could modulate the disposition of rosuvastatin. For example, a functional single nucleotide polymorphism (SNP) of BCRP, c.421C>A (Lys141Gln), which results in decreased protein expression (18), was associated with an increase in the C_{max} and AUC for rosuvastatin (19). Similarly, the OATP1B1 polymorphism c.521T>C (Val174Ala) is associated with decreased function and with increased C_{max} and AUC values (20,21). Moreover, co-administration of gemfibrozil, an OATP1B1 inhibitor, increased the plasma concentration of rosuvastatin approximately two-fold (15).

These studies indicate that, if silymarin inhibits both OATP1B1- and BCRP-mediated transport of rosuvastatin, silymarin-induced inhibition of OATP1B1-mediated uptake of rosuvastatin into the liver and of BCRP-mediated efflux of rosuvastatin from hepatocytes, brain capillaries, and the apical membrane of intestine (16) may increase the plasma concentration of rosuvastatin and consequently influence its efficacy and dose-dependent side effects.

Therefore, our objectives were to analyze the effect of silymarin on OATP1B1- and BCRP-mediated rosuvastatin transport and to assess the effects of silymarin on the pharmacokinetics of rosuvastatin in healthy volunteers.

MATERIALS AND METHODS

Materials

Rosuvastatin was purchased from Toronto Research Chemicals (Toronto, ON, Canada). All other chemicals, including silymarin, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Inhibitory Effect of Silymarin on OATP1B1-mediated Rosuvastatin Uptake

Complementary ribonucleic acid (cRNA) synthesis and uptake measurements were performed as previously described (22). The capped cRNAs were synthesized *in vitro* using T7 RNA polymerase with linear OATP1B1 cDNA. Defolliculated oocytes were injected with 25 ng of the capped cRNA and incubated at 18°C in Barth's solution, containing 50 μg/mL gentamicin. After incubation for 2 days, the uptake reaction was initiated by replacing the ND96 solution [96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES (pH 7.4)] with various concentrations (1–100 μM) of rosuvastatin. The inhibition experiments were initiated by replacing the ND96 solution with rosuvastatin (0.1 or 10 μM) in the presence of various concentrations of silymarin (0.5–50 μM). After incubation for 30 min, the reactions were terminated by the addition of ice-cold ND96 solution and washed five times.

To determine the rosuvastatin concentration, oocytes were sonicated in 50 μL of distilled water and 50 μL of acetonitrile containing atorvastatin (50 ng, internal standard), followed by centrifugation. The supernate was evaporated, and the residue was reconstituted in 100 μL of mobile phase solution [acetonitrile: distilled water: formic acid, 70:30:0.1 (v/v/v)]. After centrifugation, a 10 μL aliquot was injected into a Qtrap 4000 LC-MS/MS system (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization interface used to generate positive ions. The compounds were separated on a reversed-phase column Luna C18 column (100×2.0 mm, 5-μm particle size, Phenomenex, Torrance, CA) at a flow rate of 0.2 mL/min. The turbo ion spray interface was operated in the negative ion mode at 4500 eV and 350°C. The operating conditions were optimized by flow injection of a mixture of all analytes and were determined as follows: nebulizing gas flow, 8 L/min; curtain gas flow, 12 L/min; collision gas (nitrogen) pressure, medium; collision energy, 40 eV. Quantitation was performed by multiple reaction monitoring (MRM) of the deprotonated precursor ion and the related product ion for rosuvastatin using the internal standard method with peak area ratios and a weighting factor of $1/x$. The mass transition used for rosuvastatin and internal standard were m/z 482.2→258.2 and 559.2→440.2, respectively, (dwell time 200 ms). The analytical data were processed by Analyst software (ver. 1.41, Applied Biosystems). The lower limit of quantification was 0.05 ng/mL, and the coefficient of variation for assay precision was less than 12.4%.

To confirm the functionality of OATP1B1-expressing oocytes, the uptake of [^{3}H]estrone-3-sulfate, a representative substrate of OATP1B1, was monitored for 30 min. After dissolving the oocytes in 200 μL of 1% SDS, radioactivity was quantified by liquid scintillation counting.

Inhibitory Effect of Silymarin on BCRP-mediated Rosuvastatin Transport

MDCKII-BCRP cells were kindly provided by Dr. Alfred H. Schinkel (Division of Experimental Therapy, Netherlands Cancer Institute, Amsterdam, The Netherlands). MDCKII-BCRP cells were grown on permeable polycarbonate inserts (1 cm^2, 0.2 μm pore size; Corning Costar Co., Cambridge, MA) in 12-Transwell plates (4 cm^2, Corning Costar Co.) at a density of 1×10^6 cells/cm^2. The medium was changed at 2-day intervals. After the seeded cells reached a transepithelial electrical resistance (TEER) value of 250–300 Ω cm^2, vectorial transport of 1 μM [^{3}H]methotrexate was determined to confirm the functionality of the MDCKII-BCRP transport system.

To measure the concentration dependent basal-to-apical (B–A) transport of rosuvastatin, 1.5 mL of Dulbecco's modified Phosphated Buffered Saline (DPBS) containing various concentration of rosuvastatin (1–100 μM) were added to the basal side of the insert and 0.5 mL of fresh DPBS was added to the apical side of the insert. The transport medium on the apical side of the insert was replaced with 0.35 mL of fresh medium every 15 min for 1 h. For the measurement of inhibitory effect of silymarin on the apical-to-basal (A–B) transport of rosuvastatin, 0.5 mL of DPBS containing various concentration of rosuvastatin (0.1 and 5 μM) was added to

the basal side of the insert in the absence or presence of silymarin (1–100 μM), and 1.5 mL of DPBS without rosuvastatin were added to the basal side of the insert. An insert was transferred to a well containing fresh DPBS medium every 15 min for 1 h. To measure B–A transport of rosuvastatin in the absence or presence of silymarin (1–100 μM), 1.5 mL of DPBS containing rosuvastatin (0.1 and 5 μM) were added to the basal side of the insert in the absence or presence of silymarin (1–100 μM), and 0.5 mL of DPBS without rosuvastatin was added to the apical side. The concentration of rosuvastatin of the apical or basal side was measured using LC-MS/MS after each medium replacement.

In Vitro Kinetic Analyses

To evaluate the kinetic parameters of OATP1B1-mediated uptake of rosuvastatin, all curve fitting and rate constant determinations were performed after subtracting the rosuvastatin uptake into water-injected oocytes from the rosuvastatin uptake measured in oocytes overexpressing OATP1B1. The uptake rate of rosuvastatin was then plotted against the initial concentration of rosuvastatin, and the resulting profile was fitted to the Michaelis–Menten equation (Eq. 1) using WinNonlin (ver. 5.2, Pharsight, Mountain View, CA) to estimate the kinetic parameters V_{max} and K_m.

$$v = \frac{V_{max} \times [S]}{K_m + [S]} \quad (1)$$

To evaluate the kinetic characteristics of BCRP-mediated rosuvastatin transport, the total amount of rosuvastatin transported across the cell monolayer was plotted against time, and the transport rate (nmole/cm^2/h) was calculated from the linear portion of the curve (slope of the plot). The B–A transport rate of rosuvastatin was then plotted against the initial concentration of rosuvastatin, and the resulting profile was known to follow the Michaelis–Menten equation with two binding sites (Eq. 2). From the slope of the Eadie–Hofstee transformation, K_{m1} value was calculated.

$$v = \frac{V_{max\,1} \times [S]}{K_{m1} + [S]} + \frac{V_{max\,2} \times [S]}{K_{m2} + [S]} \quad (2)$$

The estimation of the 50% inhibitory concentration (IC_{50}) and curve fitting were achieved by nonlinear regression analysis using Inhibitory Effect model (Eq. 3).

$$v = V_{max} \times \left(1 - \frac{[S]}{[S] + IC_{50}}\right) \quad (3)$$

To determine the K_i value, the results from the inhibition study were transformed to generate a Dixon plot and the K_i was calculated using the following Eq. 4.

$$\frac{1}{v} = \frac{K_m}{V_{max}[S]K_i}[I] + \frac{1}{V_{max}}\left(1 + \frac{K_m}{[S]}\right) \quad (4)$$

In Vivo Pharmacokinetic Study

This study was approved by the Institutional Review Board at the Busan Paik Hospital (Busan, Korea), and written informed consent was obtained from all participants. Eight healthy Korean male subjects were selected based on their medical histories, physical examinations, and routine laboratory tests. The subjects were not permitted to take any medications or herbal supplements during the study procedure and were required to abstain from alcohol, caffeine, grapefruit juice, citrus fruits, and cruciferous vegetables for 72 h prior to each drug administration and throughout the study.

A randomized two-way crossover study design was used to compare the pharmacokinetic parameters determined after oral administration of rosuvastatin. In brief, healthy male subjects were divided into two groups, which were treated with silymarin (Legalon 140 capsule, Bukwang Pharm., Seoul, Korea) or placebo three times per day (7 AM, 1 PM, and 7 PM) for 5 days. On day 4, all subjects received a single oral dose of rosuvastatin (10 mg; Crestor, Astra Zeneca) 1 h after the placebo or silymarin administration (8 AM). To avoid possible carryover effects, each phase was separated by a 2-week washout period. Serial blood samples (8 mL) were drawn via indwelling venous catheters immediately before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h after the administration of rosuvastatin. Plasma samples were stored at −80°C until analysis.

Quantification of Plasma Rosuvastatin

Plasma samples (0.5 mL) were spiked with an 50 μL internal standard (20 ng/mL atorvastatin) and extracted with 5 mL of dichlomethane/ether. The upper organic phase was transferred to a glass test tube, evaporated for 1 h, and reconstituted in 100 μL of the mobile phase. The plasma concentration of rosuvastatin was determined using a Qtrap 4000 LC-MS/MS system, identical to the analysis of rosuvastatin in oocytes. The lower limit of quantification was 0.05 ng/mL, and the calibration curves were linear over the range of 0.1 to 10 ng/mL (r>0.99). The accuracy of this assay was within 95 to 113%, and the inter-batch coefficient of variation was less than 6.5% over the calibrated range.

Pharmacokinetic and Statistical Analyses

The maximum drug concentration in the plasma (C_{max}) was determined from the observed values. The area under the plasma concentration-time curve (AUC) was calculated using a combination of the trapezoidal rule and extrapolation to infinity using the elimination rate constant. The oral clearance of rosuvastatin (Cl/F) was calculated as Cl/F= Dose/AUC. All pharmacokinetic data for rosuvastatin are presented as the mean±SD. Statistical differences in various parameters between the groups were determined using the Wilcoxon signed rank sum test, and P<0.05 indicated statistical significance.

RESULTS

Effect of Silymarin on OATP1B1-mediated Uptake of Rosuvastatin

To confirm the functionality of our OATP1B1-overexpressing system, the uptake of [^{3}H]estrone-3-sulfate, a

representative substrate of OATP1B1, was measured in oocytes after injecting cRNA encoding OATP1B1. The uptake of estrone-3-sulfate into OATP1B1-expressing oocytes was 19.3-fold that into control oocytes injected with water (OATP1B1, 537.4±34 fmol/h/oocyte; water, 27.8±3.4 fmol/h/oocyte), which is consistent with previous results (23). The uptake of rosuvastatin at all concentrations was greater into OATP1B1-overexpressing oocytes than into water-injected oocytes. For example, at 1 μM rosuvastatin, the uptake into OATP1B1-expressing oocytes was 22.3-fold that into water-injected oocytes (OATP1B1, 34.8±5.9 fmol/min/oocyte; water, 1.56±0.6 fmol/min/oocyte). OATP1B1-mediated rosuvastatin uptake showed concentration dependency, and a nonlinear least squares regression analysis of the data yielded an apparent K_m value of 4.26 μM and V_{max} of 159.5 fmol/min/oocytes (Fig. 1A).

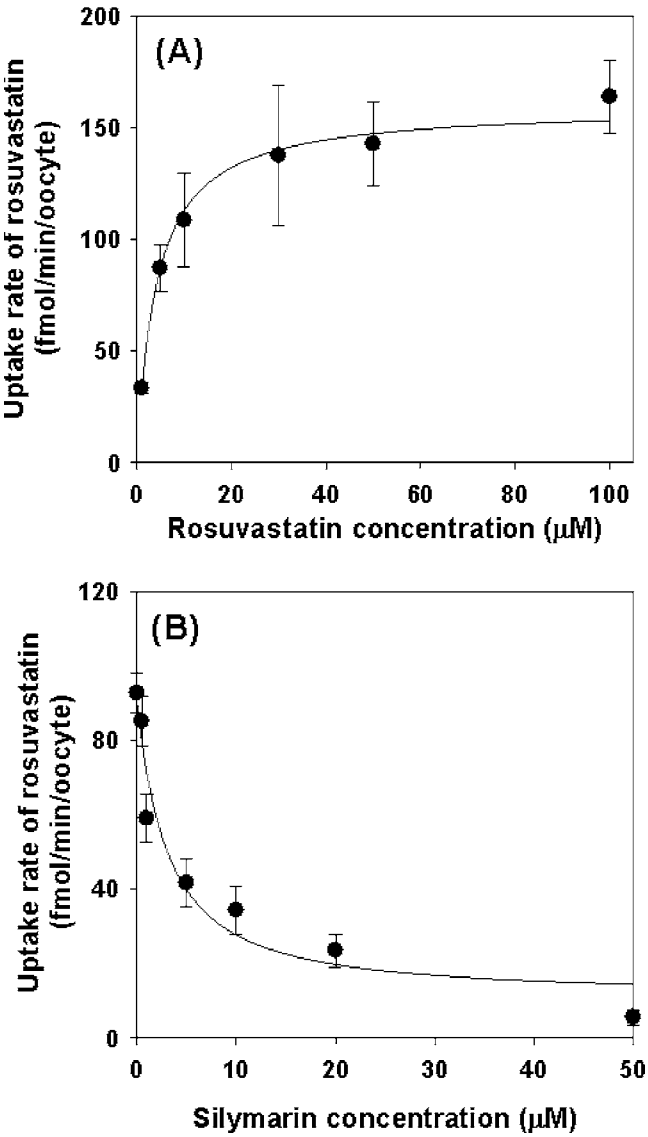

Fig. 1. A Concentration dependent uptake of rosuvastatin (1–100 μM) into OATP1B1-expressing oocytes. A nonlinear least squares regression analysis yielded an apparent association constant (K_m) of 4.26 μM. **B** Effect of silymarin (0.5–50 μM) on the kinetics of OATP1B1-mediated uptake at 10 μM rosuvastatin. The 50% inhibitory concentration (IC_{50}) of silymarin was 3.23 μM. Each data point represents the mean±SD of ten oocytes per treatment group.

OATP1B1-mediated uptake of rosuvastatin was shown concentration dependent inhibition with the increasing silymarin concentration of silymarin (Fig. 1B), indicating that silymarin is an effective inhibitor of OATP1B1-mediated rosuvastatin uptake with an IC_{50} of 3.23 μM.

Effect of Silymarin on BCRP-mediated Transport of Rosuvastatin

To confirm the functionality of our BCRP-overexpressing system, the vectorial transport of [^{3}H]methotrexate, a representative substrate of BCRP (24), was measured in MDCK-BCRP cell monolayers. The B–A transport of methotrexate was 3.1-fold the A–B transport (58.2±1.0 pmol/h/10^6 cells *vs*. 18.8±0.3 pmol/h/10^6 cells, respectively). The B–A transport at 5 μM rosuvastatin was 4.7-fold the corresponding A–B transport (Fig. 2C), indicating that BCRP is involved in the efflux of rosuvastatin. The mechanism of BCRP-related B–A transport of rosuvastatin was further investigated. Rosuvastatin transport was concentration-dependent, and an Eadie–Hofstee trans- formation revealed two K_m values for this transport system (Fig. 2A, B), which is consistent with previous results (17). The high-affinity K_{m1} was 10.1 μM, based on the Eadie–Hofstee transformation.

The presence of silymarin inhibited B–A transport of rosuvastatin in a concentration-dependent manner, with an apparent IC_{50} of 46 μM (Fig. 2C), indicating that silymarin also inhibits BCRP-mediated rosuvastatin transport.

In Vitro Inhibitory Effect of Silymarin on the Uptake of Rosuvastatin

To assess the inhibitory effect of silymarin in the range of plausible plasma concentrations, we used a low concentration of rosuvastatin (100 nM) in OATP1B1- and BCRP-overexpressing systems. In the OATP1B1 overexpression system, 1 μM silymarin significantly inhibited the uptake of 100 nM rosuvastatin (2.15±0.2 fmol/min/oocyte in the absence of silymarin, 1.15±0.4 fmol/min/oocyte in the presence of silymarin). In contrast, the B–A transport of rosuvastatin mediated by BCRP transporter was not affected in the presence of 10 μM silymarin, but a significant decrease was observed by the addition of 100 μM silymarin (5.48±0.2 pmol/h/10^6 cells in the absence of silymarin, 5.06±0.5 pmol/h/10^6 cells, 5.61±0.7 pmol/h/10^6 cells, 2.80±0.3 pmol/h/10^6 cells in the presence of 1 μM, 10 μM, and 100 μM silymarin, respectively). The A–B transport of rosuvastatin was not altered by the presence of silymarin (data not shown).

To calculate the K_i values of silymarin for the inhibition of OATP1B1- and BCRP-mediated rosuvastatin transport, the data from inhibition studies using two different rosuvastatin concentrations were transformed into a Dixon plot, and the K_i was calculated according to Eq. 4. The K_i values of silymarin for the inhibition of OATP1B1 and BCRP were 0.93 μM and 97.9 μM, respectively, (Fig. 3). The K_i for BCRP was 105-fold that for OATP1B1, suggesting that silymarin is a much more potent inhibitor of OATP1B1-mediated uptake of rosuvastatin. Therefore, an interaction between rosuvastatin and silymarin would be more likely to occur via OATP1B1 than via BCRP.

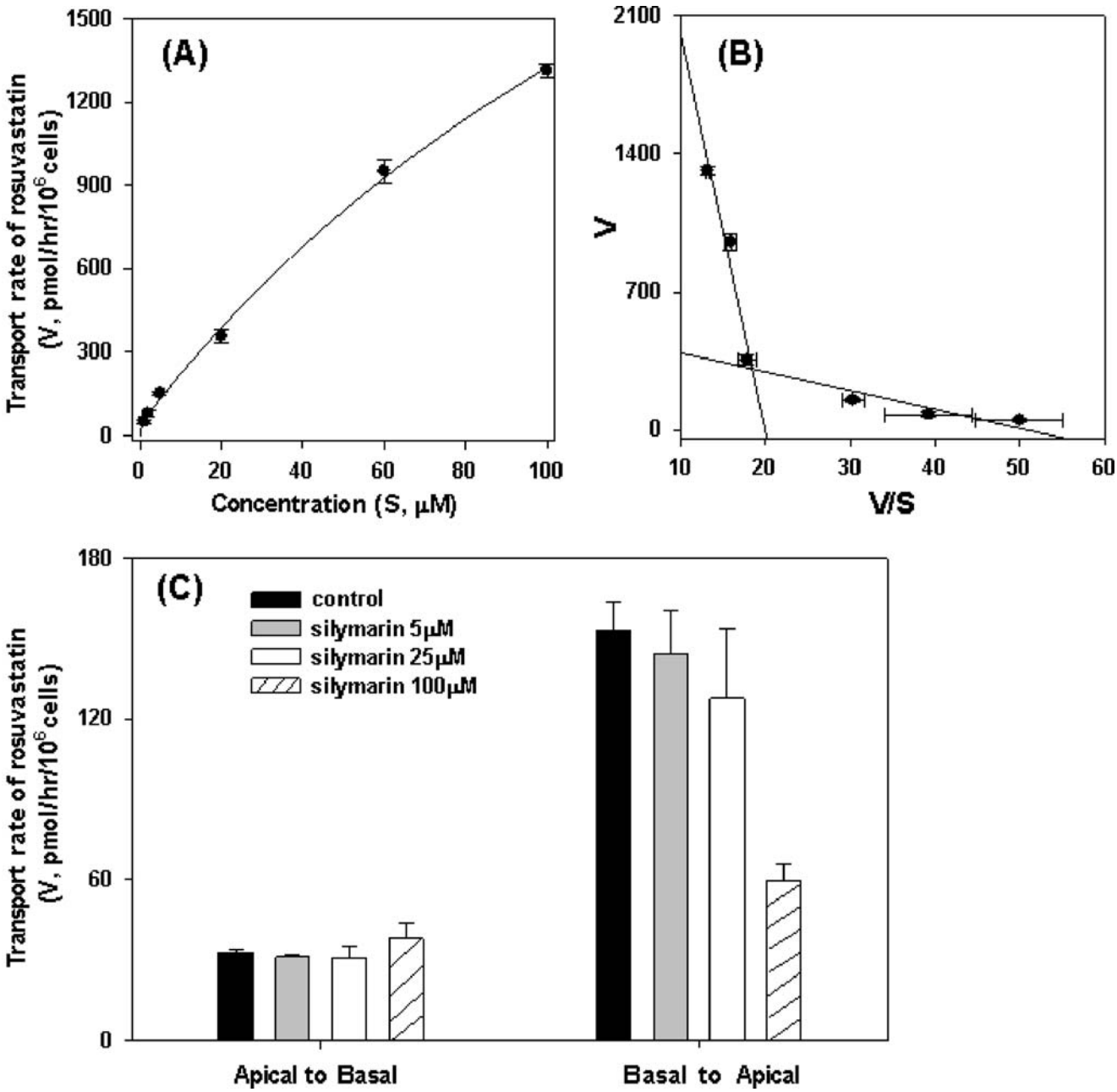

Fig. 2. **A** Concentration-dependent rosuvastatin transport (1–100 μM) in a BCRP-overexpressing MDCKII cell line. **B** Eadie–Hofstee transformation of the observed rosuvastatin transport data. **C** Inhibitory effect of silymarin (5, 25, 100 μM) on the transport rate of rosuvastatin (5 μM) in a MDCKII-BCRP cells. Each data point represents the mean±SD of three independent experiments.

In Vivo Effect of Silymarin on the Pharmacokinetics of Rosuvastatin

As silymarin may modulate the transport function of both OATP1B1 and BCRP, the pharmacokinetics of rosuvastatin was assessed in healthy male subjects after pretreatment with silymarin (420 mg/day) for 3 days. At 1 h after the silymarin dose (140 mg), each subject received a single dose of rosuvastatin (10 mg), and the plasma rosuvastatin concentration was measured over time. During the plasma sampling, subjects were taken silymarin or placebo capsules according to the recommended regimen (three times per day for 2 days). The plasma concentration–time profile of rosuvastatin showed no significant effect of pre-treatment with silymarin (Fig. 4). The C_{max} values (mean±SD) for rosuvastatin were 13.7±4.7 ng/mL in the presence of silymarin supplement and 14.8±5.3 ng/mL in the absence of silymarin supplement. The AUC values (mean±SD) for rosuvastatin with and without silymarin treatment were 134.6±42.3 and 144.0±38.7 ng h/mL, respectively. Thus, silymarin treatment did not significantly affect any pharmacokinetic parameter of rosuvastatin (Table I).

DISCUSSION

Transporters play key roles in the absorption, disposition, toxicity, and efficacy of drugs, and thus transporter-based drug interactions can lead to pharmacokinetic changes, including increased toxicity and lack of efficacy, in co-

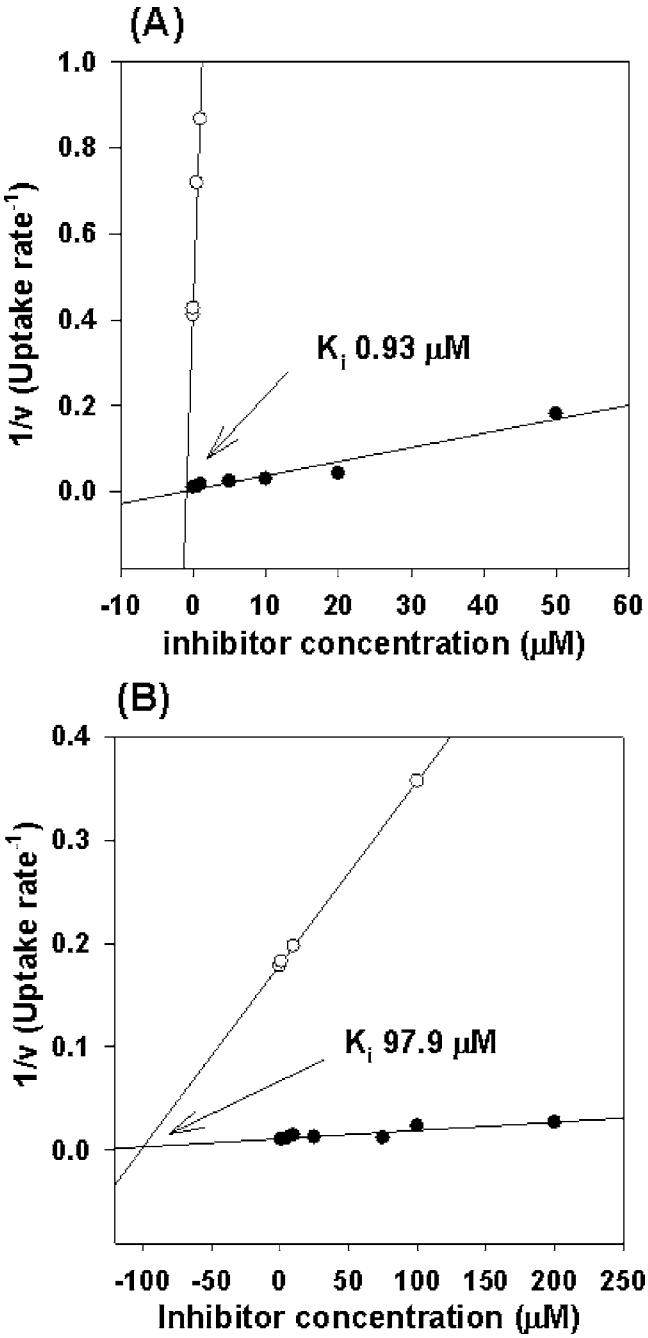

Fig. 3. **A** The OATP1B1- and **B** BCRP-mediated transport data for rosuvastatin in the presence of silymarin were transformed into a Dixon plot, and the K_i values of silymarin for OATP1B1- and BCRP-mediated transport were calculated according to Eq. 1 (described in the "Materials and Methods" section).

administered substrates (25). For example, the absorption of orally administered rosuvastatin is limited by the intestinal BCRP transporter, and the plasma concentration of rosuvastatin is modulated by hepatic uptake via OATP1B1 and biliary excretion via hepatic BCRP, without significant metabolism (17,19). Therefore, the cholesterol-lowering effect of rosuvastatin may be associated with the functions of OATP1B1 and BCRP (26), and thus an interaction of rosuvastatin and another drug through OATP1B1 or BCRP

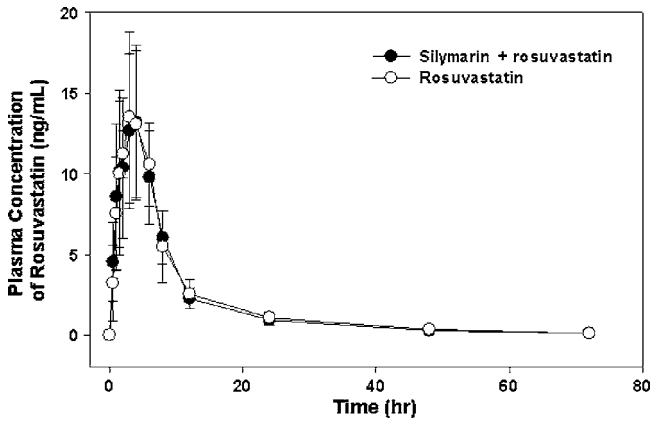

Fig. 4. Mean concentration–time curve for plasma rosuvastatin. The subjects received a single dose of rosuvastatin (10 mg) on fourth day during the silymarin supplementation (420 mg/day) for 5 days, and serial blood samples were collected for analysis for 72 h.

could potentially alter the pharmacokinetics, efficacy, or toxicity of rosuvastatin.

Rosuvastatin is a substrate for OATP1B1 and BCRP with high affinity (i.e., the K_m values of 4.26 μM and 10.1 μM, respectively). However, inhibitory potential of silymarin for OATP1B1 and BCRP-mediated rosuvastatin transport was not identical. The IC_{50} value of silymarin for OATP1B1-mediated uptake (3.23 μM) is much smaller than that for BCRP-mediated rosuvastatin transport (46 μM), suggesting that silymarin is a more potent inhibitor for OATP1B1-mediated rosuvastatin uptake rather than BCRP-mediated transport. For the estimation of IC_{50} value of silymarin, we used high concentration of rosuvastatin (10 μM) because of the detection limit of rosuvastatin. This suggested that the IC_{50} value of silymarin may be overestimated, which accounts for the reason why the K_i value of silymarin is much lower than IC_{50} value.

To determine whether plasma concentrations of rosuvastatin and silymarin might be sufficient to cause drug interactions or whether other confounding effects might exist, we investigate the possibilities for OATP1B1- and BCRP-mediated drug interactions within a plausible range of rosuvastatin and silymarin plasma concentrations. As a result, 1 μM silymarin inhibited OATP1B1-mediated rosuvastatin uptake (100 nM). However, BCRP-mediated transport of rosuvastatin (100 nM) was not affected by the presence of 1 μM and 10 μM silymarin. A significant decrease was

Table I. Pharmacokinetic Parameters of Rosuvastatin after Receiving a Single Dose of Rosuvastatin (10 mg) on Fourth Day During the Silymarin Supplementation (420 mg/day) for 5 days

PK Parameters	Silymarin + Rosuvastatin	Rosuvastatin	*P*
AUC(ng/mL h)	135±42.3	144±38.7	0.327
C_{max}(ng/mL)	13.7±4.7	14.8±5.3	0.484
T_{max}(h)	3.5±1.5	3.4±1.3	1.000
$T_{1/2}$(h)	13.9±4.4	13.6±2.8	0.889
Cl/*F*(L/h/kg)	1.28±0.8	1.10±0.4	0.233
V_d/F(L/kg)	24.0±12.5	21.5±8.4	0.727

Data represent means±S.D. from eight healthy subjects.
P values are determined by Wilcoxon signed rank sum test.

observed in BCRP-mediated transport of rosuvastatin with the addition of 100 μM silymarin.

Our clinical trial results, however, were not consistent with our *in vitro* findings. Pretreatment with silymarin produced no effects on the pharmacokinetic behavior of rosuvastatin plasma concentrations *in vivo*, indicating that silymarin, administered according to a recommended supplementation regimen, is not a potent modulator of OATP1B1 or BCRP activity *in vivo*. This suggests that there is no risk for a drug interaction between silymarin and rosuvastatin *in vivo*.

The C_{max} values for rosuvastatin and silybin (a major component of the silymarin) were 28.4±9.8 nM (this study) and 2.76±1.1 μM (27), respectively, after a separate oral administration of 10 mg rosuvastatin (Crestor) and 140 mg silymarin (Legalon 140). The OATP1B1 transporter is expressed primarily in the sinusoidal membrane of hepatocytes, and it is therefore reasonable to assume that any interaction between rosuvastatin and silymarin might occur during hepatic OATP1B1-mediated uptake of rosuvastatin, making the unbound plasma concentration and K_i of silymarin critical. Since plasma protein binding of silybin was 95.7±2.2% (n=5) when measured using 5 μg/mL silybin by equilibrium dialysis method (our unpublished data), unbound C_{max} concentration of silybin might be expected to be 0.12 μM. The K_i of silymarin for OATP1B1-mediated rosuvastatin transport was 0.93 μM in this study, and the $C_{max,unbound}/K_i$ ratio was 0.13, indicating the low possibility of an OATP1B1-mediated interaction between silymarin and rosuvastatin according to the guidance for industry (i.e., Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling, released from FDA, September 2006). Furthermore, the $C_{max,unbound}/K_i$ of silymarin for BCRP-mediated rosuvastatin transport was 0.001. These results indicate that a BCRP-mediated interaction between silymarin and rosuvastatin is unlikely to occur *in vivo*, even though BCRP is localized in the epithelial membrane of the intestine and the canalicular membrane of hepatocytes and the concentration of silymarin in the gut lumen or hepatocytes may be higher than that of plasma.

Another possibility is the potential involvement of sodium-dependent taurocholate cotransporting polypeptide (NTCP). In human hepatocytes, approximately 35% of hepatic rosuvastatin uptake is sodium-dependent, suggesting a role for NTCP in rosuvastatin disposition *in vivo* (16). However, the true contribution of NTCP to the pharmacokinetics of rosuvastatin is unclear and other uptake transporters such as OATP1B3 or OATP2B1 may also mediate the *in vivo* uptake of rosuvastatin.

Alternatively, the lack of an *in vivo* drug interaction may be because the short half-life (3.42±0.7 h) and low bioavailability of silymarin in the human body (27–30) prevented the accumulation of an effective plasma concentration, despite the 3-day pretreatment and 2-day of additional supplementation. Even if the plasma concentration of silymarin had reached a level sufficient for interaction with rosuvastatin, this level would have been maintained for only a limited time.

Further complicating matters, the silymarin content of milk thistle extracts can vary from 40 to 80% (3). There are several difficulties inherent to the scientific assessment of herbal remedies such as silymarin. Herbs contain many substances, and each may function via one or more mechanisms. The combination of these mechanisms can produce a different overall effect *in vivo* from that observed *in vitro* (4).

In conclusion, pretreatment with silymarin did not produce a significant change in the pharmacokinetics of rosuvastatin even though, according to our data, rosuvastatin is a good substrate for both OATP1B1 and BCRP, and both transporters are inhibited by silymarin *in vitro*. Given the wide range of bioavailability and solubility of silymarin-containing products (29,30), our results should be interpreted carefully, especially when silymarin products with improved bioavailability are used with rosuvastatin.

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