

Effects of Silybinin on the Pharmacokinetics of Tamoxifen and Its Active Metabolite, 4-Hydroxytamoxifen in Rats

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Abstract. *The effects of silybinin, an antioxidant, on the pharmacokinetics of tamoxifen and its metabolite, 4-hydroxytamoxifen, were investigated in rats. A single dose of tamoxifen was administered intravenously (2 mg/kg) and orally (10 mg/kg) without or with silybinin (0.5, 2.5 and 10 mg/kg) to rats. Silybinin significantly altered the pharmacokinetics of orally administered tamoxifen. Compared to those in the oral control group (given tamoxifen alone), the area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of tamoxifen were significantly ($p < 0.05$ for 2.5 mg/kg, $p < 0.01$ for 10 mg/kg) increased by 40.2-71.3% and 45.2-78.6%, respectively, with silybinin. Consequently, the absolute bioavailability (AB) of tamoxifen in the presence of silybinin (2.5 and 10 mg/kg) was 31.1-38.1%, which was significantly enhanced ($p < 0.05$) compared to that in the oral control group (22.2%). Moreover, the relative bioavailability (RB) of tamoxifen was 1.40- to 1.72-fold greater than that in the control group. Silybinin (10 mg/kg) significantly increased the $AUC_{0-\infty}$ ($p < 0.05$, 40.0%) of 4-hydroxytamoxifen, but the metabolite-parent ratio (MR) of 4-hydroxytamoxifen was significantly altered ($p < 0.05$ for 10 mg/kg), implying that the formation of 4-hydroxytamoxifen was considerably affected by silybinin. The enhanced bioavailability of tamoxifen by silybinin might be due to the promotion of intestinal absorption in the small intestine and the reduction of first-pass metabolism of tamoxifen in the small intestine and in the liver. If these results are confirmed in clinical trials, the tamoxifen dosage should be adjusted when tamoxifen is administered with silybinin or silybinin-containing dietary supplements.*

Tamoxifen is a member of a class of compounds known as selective estrogen receptor modulators which acts as estrogen receptor agonists in some tissues and as antagonists in other tissues (1, 2). Tamoxifen is an estrogen receptor agonist in bone, the cardiovascular system, and the endometrium, but acts as an antagonist in breast tissue (3). Tamoxifen is used clinically as the agent of choice for treating and preventing breast cancer (4). Tamoxifen has a relatively low toxicity and is less harmful than most chemotherapeutics. The main adverse effects of tamoxifen in humans are that there might be an increased risk of endometrial cancer and thromboembolic diseases (5, 6). Orally administered tamoxifen undergoes extensive hepatic metabolism and subsequent biliary excretion (7). In humans, the main pathway in tamoxifen biotransformation proceeds via the *N*-demethylation catalyzed mostly by cytochromes P450 (CYP) 3A4 enzymes (8, 9). Another important drug metabolite, 4-hydroxytamoxifen, is produced in humans by CYP2D6, CYP2C9 and CYP3A4 (9, 10). 4-hydroxytamoxifen has shown 30- to 100-fold greater potency than tamoxifen in suppressing estrogen-dependent cell proliferation (11, 12). A secondary metabolite of tamoxifen, endoxifen, exhibits potency similar to 4-hydroxytamoxifen (13, 14). Thus, tamoxifen is referred to as a prodrug that requires activation to exert its effects.

Tamoxifen acts as a substrate for P-glycoprotein (P-gp) as well (15, 16). P-gp co-localized with CYP3A in the polarized epithelial cells of excretory organs such as the liver, kidney and intestine (17, 18) to eliminate foreign compounds out of the body. A substantial overlap in substrate specificity exists between CYP3A4 and P-gp (19). The P-gp and CYP3A modulators might be able to improve the oral bioavailability of tamoxifen. The low bioavailability of oral tamoxifen is mainly due to first-pass metabolism in the intestine or in the liver and P-gp mediated efflux in the intestine.

Flavonoids represent a group of phytochemicals that are produced in high quantities by various plants (20). These compounds exhibit a wide range of beneficial biological activities including antioxidative, radical scavenging, antiatherosclerotic, antitumor and antiviral effects (21).

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Silymarin, a flavonoid complex, is extracted from seeds of the milk thistle (*Silybum marianum* L.) which is a medicinal plant widely used in traditional European medicine (22). Silymarin has a strong antioxidant activity (23), exhibits cytoprotective, anti-inflammatory and anticarcinogenic effects (24). Silybinin is the major and most active component in silymarin about 60-70% (25). Kosina *et al.* (2005) (26) reported that silybinin could inhibit human CYP 1A2 and 3A4, however, Zuber *et al.* (2002) (27) found that silybinin inhibits human CYP 2D6 and 3A4, the assay results of inhibition CYP enzymes activities on human is partially doubtful. Silybinin is the inhibitor of P-gp in cell line KB/MDR system (28,29), but the effect of silybinin, its inhibition on P-gp is ambiguous. So we attempted to re-evaluate CYP enzyme activities and P-gp activity about silybinin using CYP inhibition assay and rhodamine-123 retention assay in P-gp-overexpressed MCF-7/ADR cells.

There were a few papers about effect of some flavonoids on the bioavailability of tamoxifen in rats (30-32). Consequently, it could be expected that silybinin would change the pharmacokinetics of drugs, substrate of P-gp and/or CYP3A4, if they are concomitantly used. Silybinin and tamoxifen could be prescribed for the treatment or prevention of cancer as a combination therapy. However, the possible effects of silybinin on the bioavailability and pharmacokinetics of tamoxifen have not been reported *in vivo*.

Therefore, the aim of this study was to investigate effect of silybinin on the bioavailability and pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen, in rats.

Materials and Methods

Chemicals and apparatus. Tamoxifen, 4-hydroxytamoxifen, silybinin and butylparaben (p-hydroxybenzoic acid n-butyl ester) were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were acquired from the Merck Co. (Darmstadt, Germany). All other chemicals for this study were of reagent grade and were used without further purification.

Apparatus used in this study were an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler and a Waters™ 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), an HPLC column temperature controller (Phenomenex Inc., CA, USA), a Branson® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA), and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments. Male Sprague-Dawley rats (weighing 270-300 g) were purchased from the Dae Han Laboratory Animal Research Co. (Choongbuk, Korea), and were given access to a commercial rat chow diet (No. 322-7-1, Superfeed Co., Gangwon, Korea) and tap water. The animals were housed, two per cage, and maintained at 22±2°C and 50-60% relative humidity, under a 12:12 h light-dark cycle. The experiments were initiated after acclimation under these conditions for at least 1 week. The Animal Care Committee of Chosun University

(Gwangju, Korea) approved the design and the conduct of this study. The rats were fasted for at least 24 h prior to the experiments and each animal was anesthetized lightly with ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, i.d. 0.58 mm, o.d. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and *i.v.* injection, respectively.

Drug administration. The rats were divided into four groups (n=6, each); an oral control group (10 mg/kg of tamoxifen dissolved in 0.9% NaCl solution containing 10% tween 80, 3.0 ml/kg) without or with 0.5, 2.5 and 10 mg/kg of silybinin (mixed in distilled water, 3.0 ml/kg), and an IV group (2 mg/kg of tamoxifen, dissolved in 0.9% NaCl solution containing 10% of tween 80, 1.5 ml/kg). Oral tamoxifen was administered intragastrically using a feeding tube, and silybinin was administered in the same manner 30 min prior to the oral administration of tamoxifen. Tamoxifen for IV administration was injected through the femoral vein within 1 min. A 0.4 ml-aliquot of blood sample was collected into heparinized tubes from the femoral artery at 0 (to serve as control), 0.017 (only for IV group), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 36 h (only for oral group) after tamoxifen administration. The blood samples were centrifuged at 13,000 rpm for 5 min, and the plasma samples were stored at -40°C until HPLC analysis.

HPLC analysis. The plasma concentrations of tamoxifen and 4-hydroxytamoxifen were determined by HPLC using a method reported by Fried *et al.* (33) after a slight modification. Briefly, a 50- μ l aliquot of 8 μ g/ml butylparaben, as an internal standard, and a 0.2-ml aliquot of acetonitrile were mixed with a 0.2-ml aliquot of the plasma sample. The resulting mixture was then vortex-mixed vigorously for 2 min and centrifuged at 13,000 rpm for 10 min. A 50- μ l aliquot of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a Symmetry® C₁₈ column (4.6×150 mm, 5 μ m, Waters Co.), and a μ Bondapak™ C₁₈ HPLC Precolumn (10 μ m, Waters Co.). The mobile phase consisted of 20 mM dipotassium hydrogen phosphate (pH 3.0, adjusted with phosphoric acid)-acetonitrile (60:40, v/v). The flow-rate of the mobile phase was maintained at 1.0 ml/min. Chromatography was performed at a temperature of 30°C that was regulated by an HPLC column temperature controller. The fluorescence detector was operated at an excitation wavelength of 254 nm with an emission wavelength of 360 nm. A homemade post-column photochemical reactor was supplied with a bactericidal ultraviolet lamp (Sankyo Denki Co, Japan), and a Teflon® tubing (i.d. 0.01", o.d. 1/16", 2 m long) was crocheted and fixed horizontally with a stainless steel frame under the lamp at a 10 cm-distance. Tamoxifen, 4-hydroxytamoxifen and butylparaben were eluted with retention times at 26.1, 7.3 and 14.5 min, respectively. The lower limit of quantification for tamoxifen and 4-hydroxytamoxifen in the rat plasma was 5 ng/ml and 0.5 ng/ml. The coefficients of variation of tamoxifen and 4-hydroxytamoxifen were below 4.5 and 1.5 %, respectively.

Pharmacokinetic analysis. The plasma concentration data were analyzed by non-compartmental method using WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of tamoxifen or 4-hydroxytamoxifen concentration data during the elimination phase. The terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak plasma concentration (C_{max}) and

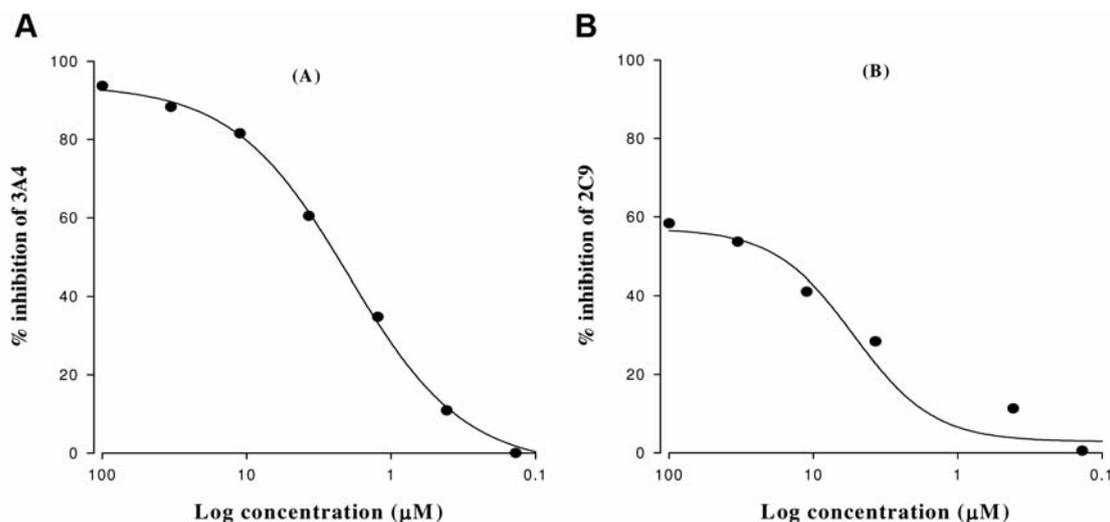


Figure 1. Inhibitory effect of silybinin on CYP3A4 (A) and 2C9 (B) activity. All experiments were carried out in duplicate, and results are expressed as the percentage of inhibition.

time to reach peak plasma concentration (t_{max}) of tamoxifen or 4-hydroxytamoxifen in plasma were obtained by visual inspection of the data from the concentration-time curve. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el} . The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{IV} \times Dose_{IV}/Dose_{oral}$, and the relative bioavailability (RB) was calculated by $AUC_{control}/AUC_{with\ silybinin}$. The metabolite-parent ratio (MR) was estimated by $(AUC_{4-hydroxytamoxifen}/AUC_{tamoxifen}) \times 100$.

CYP inhibition assay. The assays of inhibition on human CYP3A4 and 2C9 enzyme activities were performed in a multiwell plate using CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously (34). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates (7-BFC and 7-MFC for CYP3A4 and 2C9, respectively) were incubated with or without tested compounds in the enzyme/substrate contained buffer consisting of 1 pmol of P450 enzyme and NADPH generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM $MgCl_2$) in a potassium phosphate buffer (pH 7.4). Reactions were terminated by adding a stop solution after 45 min incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA) set at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole and 2 μM sulfaphenazole for CYP3A4 and 2C9, respectively) was run on the same plate producing 99% inhibition. All experiments were done in duplicate, and results are expressed as the percent of inhibition.

Rhodamine-123 retention assay. MCF-7/ADR cells were seeded in 24-well plates. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was changed to Hanks' balanced

Table I. IC_{50} values of silybinin on CYP 3A4 and CYP2C9-catalysed substrates oxidation.

Silybinin	IC_{50} (μM)	
	CYP3A4	CYP2C9
	1.97	5.25

salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 for 90 min, the medium was completely removed. The cells were then washed three times with an ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the ratio to controls.

Statistical analysis. Statistical analysis was conducted using a one-way ANOVA followed by a posteriori testing with the use of the Dunnett correction. Differences were considered to be significant at a level of $p < 0.05$. All mean values are presented with their standard deviation (mean \pm S.D.).

Results

Inhibition of CYP3A4 and 2C9. The inhibitory effects of silybinin on CYP3A4 and CYP2C9 activity are shown in Figure 1. The IC_{50} values of silybinin on CYP3A4 and 2C9 activity is listed in Table I. Higher concentration of silybinin inhibited CYP3A4 and CYP2C9 enzyme activity in a concentration-dependent manner.

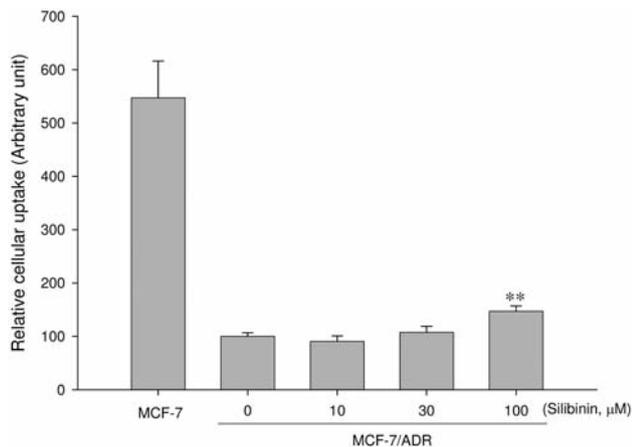


Figure 2. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with silybinin for 30 min. After incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min, the R-123 fluorescence values in cell lysates were measured using the excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by total protein contents of each sample. Data represents mean±SD of 6 separate samples (significantly different from the control MCF-7 cells, ** $p<0.01$).

Rhodamine-123 retention assay. In this study, the cell-based P-gp activity test using rhodamine-123 also showed that silybinin (100 μM, $p<0.01$) significantly inhibited P-gp activity (Figure 2).

Effect of silybinin on the pharmacokinetics of tamoxifen. Mean arterial plasma concentration-time profiles of tamoxifen following an intravenous administration of tamoxifen (2 mg/kg), and an oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of silybinin (0.5, 2.5 and 10 mg/kg) are shown in Figure 3; the corresponding pharmacokinetic parameters are shown in Table II. The presence of silybinin significantly altered the pharmacokinetic parameters of tamoxifen. Compared to the control group (given oral tamoxifen alone), the presence of silybinin significantly ($p<0.05$ at 2.5 mg/kg of silybinin, $p<0.01$ at 10 mg/kg of silybinin) increased area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of tamoxifen by 40.2-71.3% and 45.2-78.6%, respectively. The absolute bioavailability (AB) of tamoxifen in the presence of silybinin (2.5 and 10 mg/kg) was 31.1-38.1%, which was enhanced significantly ($p<0.05$) compared to that in the oral control group (22.2%), and the relative bioavailability (RB) of tamoxifen was 1.40- to 1.72-fold greater than the control group. There were no significant differences in the time to reach peak plasma concentration (T_{max}) and the terminal half-life ($t_{1/2}$) of tamoxifen in the presence of silybinin.

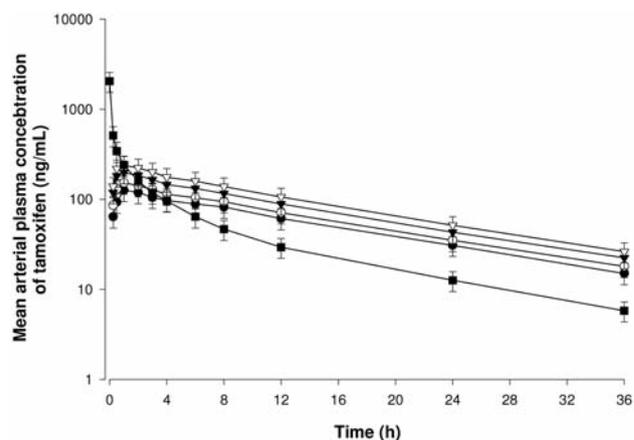


Figure 3. Mean plasma concentration-time profiles of tamoxifen after an intravenous (2 mg/kg) and oral (10 mg/kg) administration of tamoxifen to rats in the presence or absence of silybinin (0.5, 2.5 and 10 mg/kg) ($n=6$, each). Bars represent the standard deviation. (■) Intravenous administration of tamoxifen (2 mg/kg); (●) oral administration of tamoxifen (10 mg/kg); (○) the presence of 0.5 mg/kg of silybinin; (▼) the presence of 2.5 mg/kg of silybinin; (▽) the presence of 10 mg/kg of silybinin.

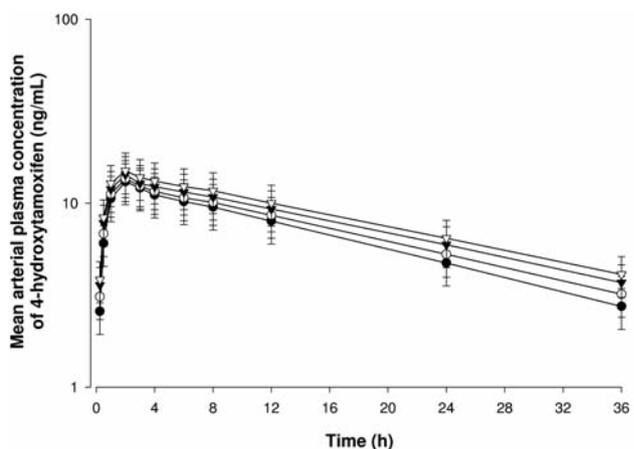


Figure 4. Mean plasma concentration-time profiles of 4-hydroxytamoxifen after an oral (10 mg/kg) administration of tamoxifen to rats in the presence or absence of silybinin (0.5, 2.5 and 10 mg/kg) ($n=6$, each). Bars represent the standard deviation. (●) Oral administration of tamoxifen (10 mg/kg); (○) the presence of 0.5 mg/kg of silybinin; (▼) the presence of 2.5 mg/kg of silybinin; (▽) the presence of 10 mg/kg of silybinin.

Effect of silybinin on the pharmacokinetics of 4-hydroxytamoxifen. Mean plasma concentration-time profiles of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of silybinin (0.5, 2.5 and 10 mg/kg) are shown in Figure 4, while the correlated pharmacokinetic parameters are shown in

Table II. Mean (\pm S.D.) pharmacokinetic parameters of tamoxifen after the intravenous (2 mg/kg) and oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of silybinin (0.5, 2.5 and 10 mg/kg) ($n=6$, each).

Parameter	Control	Tamoxifen + Silybinin			<i>i.v.</i> (2 mg/kg)
		0.5 mg/kg	2.5 mg/kg	10 mg/kg	
AUC _{0-∞} (ng/ml • h)	2060±501	2380±796	2888±815*	3539±823**	1860±435
C _{max} (ng/ml)	126±30.2	147±47.3	183±52.6*	225±61.8**	
T _{max} (h)	1.17±0.41	1.33±0.52	1.33±0.52	1.33±0.52	
t _{1/2} (h)	11.2±2.73	11.6±2.83	11.7±2.80	12.0±2.99	9.0±2.05
AB (%)	22.2±5.69	25.6±8.68	31.1±9.12*	38.1±9.81**	100
RB (%)	100	116	140	172	

* $p<0.05$, ** $p<0.01$, significant difference from the control. AUC_{0-∞}: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; t_{1/2}: terminal half-life; AB: absolute bioavailability; RB: relative bioavailability.

Table III. Mean (\pm S.D.) pharmacokinetic parameters of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of silybinin (0.5, 2.5 and 10 mg/kg) ($n=6$, each).

Parameter	Control	Tamoxifen + Silybinin		
		0.5 mg/kg	2.5 mg/kg	10 mg/kg
AUC _{0-∞} (ng/ml • h)	283±64.1	301±75.3	318±78.3	337±84.8*
C _{max} (ng/ml)	13.3±2.79	13.5±3.05	13.7±3.19	13.9±3.37
T _{max} (h)	2.17±0.41	2.33±0.52	2.33±0.52	2.33±0.52
t _{1/2} (h)	15.3±3.62	15.9±3.98	16.2±4.11	16.3±4.22
MR (%)	13.7±2.82	12.6±2.52	11.0±2.32	9.5±2.05*

* $p<0.05$, significant difference from the control. AUC_{0-∞}: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; t_{1/2}: terminal half-life; MR: metabolite-parent ratio.

Table III. Compared to the control group, the presence of silybinin at a dose of 10 mg/kg significantly ($p<0.05$) increased the AUC_{0-∞} (40.0%) of 4-hydroxytamoxifen. The metabolite-parent ratio (MR) of 4-hydroxytamoxifen was reduced significantly ($p<0.05$ for 10 mg/kg of silybinin). These results suggest that the production of 4-hydroxytamoxifen was considerably inhibited by silybinin. The C_{max}, t_{1/2} and T_{max} of 4-hydroxytamoxifen were not significantly altered by the presence of silybinin.

Discussion

Based on the broad overlap in substrate specificities as well as co-localization in the small intestine, the primary site of absorption for orally administered drugs, CYP3A4 and P-gp, are recognized as a concerted barrier to drug absorption (35, 36). CYP enzymes contribute significantly to first-pass metabolism and oral bioavailability of many drugs. The first-pass metabolism of compounds in the intestine limits the absorption of toxic xenobiotics and may ameliorate side-effects. Moreover, induction or inhibition of intestinal CYPs may be

responsible for significant drug-drug interactions when one agent decreases or increases the bioavailability and absorption rate constant of a concurrently administered drug (37).

Tamoxifen and its primary metabolites undergo extensive oxidation, principally by CYP3A and CYP2C9 (9, 10). Tamoxifen and its metabolites, N-desmethyltamoxifen and 4-hydroxytamoxifen, are substrates for the efflux of P-gp as well (15, 16). CYP3A and P-gp inhibitors might interact with tamoxifen and its metabolites and thus contribute to substantial alteration of their pharmacokinetic fate. Silymarin is a popular herbal product marketed to treat liver disorders. Despite its popularity, limited information is available on the safety, interactions with other drugs, or the mechanisms of interactions of silymarin. As shown in Table I and Figure 1, silybinin inhibited respectively human CYP3A4 and 2C9 with an IC₅₀ value of 1.97 and 5.25 μ M, respectively. The cell-based P-gp activity test using rhodamine-123 also showed that silybinin (100 μ M, $p<0.01$) significantly inhibited P-gp activity (Figure 2). The phase I and phase II metabolizing enzymes are expressed with P-gp in the liver, kidney and intestine (17, 18), regulating the bioavailability

of many orally ingested compounds. Therefore, the inhibitors against both metabolizing enzyme CYP3A4 and P-gp should have a large impact on the pharmacokinetics of those compounds. Since silybinin can competitively inhibit P-gp and CYP3A and 2C9 metabolizing enzymes, this study examined the effect of silybinin on the bioavailability and pharmacokinetics of tamoxifen.

The presence of silybinin (2.5 and 10 mg/kg) significantly increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen (Table II). Since orally administered tamoxifen is a substrate for CYP3A-mediated metabolism and P-gp-mediated efflux in the intestine and liver, the presence of silybinin might obstruct this metabolic pathway. Shin *et al.* (30) reported that the oral coadministration of quercetin (a kind of flavonoid which can inhibit CYP3A4 and P-gp) increased the C_{max} and the AUC of tamoxifen in rats. Shin *et al.* (31) reported that the presence of morin significantly increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen, a P-gp and CYP 3A substrate, in rats and Shin and Choi (32) also reported that the presence of epigallocatechin gallate, a flavonoid, significantly increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen in rats. Rajnarayana *et al.* (38) demonstrated that pretreatment with silymarin led to significant changes in the disposition of metronidazole and its active metabolite, hydroxymetronidazole. These findings are consistent with the results of our study.

The presence of silybinin at 10 mg/kg significantly ($p < 0.05$) increased the $AUC_{0-\infty}$ of 4-hydroxytamoxifen compared to those in the control group. The metabolite-parent ratio (MR) of 4-hydroxytamoxifen was significantly altered in the presence of silybinin (Table III), this result suggested that silybinin was capable of altering the production of 4-hydroxytamoxifen, which is mainly formed by CYP3A and CYP2C9 (9, 10). These results are consistent with Shin *et al.* (30, 32) who reported that quercetin and epigallocatechin gallate significantly reduced the MR of tamoxifen, a P-gp and CYP 3A substrate, in rats. However, these results are not consistent with reports by Shin *et al.* (31) showing that morin did not significantly reduced the MR of tamoxifen in rats. Tamoxifen might be more potent by coadministration of silybinin, since 4-hydroxytamoxifen is much more effective than tamoxifen in cancer therapy (11, 12).

Conclusion

The presence of silybinin enhanced the oral bioavailability of tamoxifen, which might be mainly attributable to the promotion of intestinal absorption and reduction of first-pass metabolism of tamoxifen in the intestine and liver in rats by silybinin. If the results obtained from the rats' model are confirmed in clinical trials, tamoxifen dose should be adjusted for potential drug interactions when tamoxifen is used with silybinin or silybinin-containing dietary supplements.

References

- 1 Furr BJA and Jordan VC: The pharmacology and clinical uses of tamoxifen. *Pharmacol Ther* 25: 127-205, 1984.
- 2 Park WC and Jordan VC: Selective estrogen receptor modulators (SERMS) and their roles in breast cancer prevention. *Trends Mol Med* 8: 82-88, 2002.
- 3 Buchanan CM, Buchanan NL, Edgar KJ, Little JL, Malcolm MO, Ruble KM, Wacher VJ and Wempe MF: Pharmacokinetics of tamoxifen after intravenous and oral dosing of tamoxifen-hydroxybutenyl-beta-cyclodextrin formulations, *J Pharm Sci* 96: 644-660, 2007.
- 4 Jaiyesimi IA, Buzdar AU, Decker DA and Hortobagyi GN: Use of tamoxifen for breast cancer: twenty-eight years later. *J Clin Oncol* 13: 513-529, 1995.
- 5 Fornander T, Hellstrom AC and Moberger B: Descriptive clinicopathologic study of 17 patients with endometrial cancer during or after adjuvant tamoxifen in early breast cancer. *J Nat Cancer Inst* 85: 1850-1855, 1993.
- 6 Meier CR and Jick H: Tamoxifen and risk of idiopathic venous thromboembolism. *Br J Clin Pharmacol* 45: 608-612, 1998.
- 7 Buckley MT and Goa KL: Tamoxifen: a reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Clin Pharmacokin* 37: 451-490, 1989.
- 8 Jacolot F, Simon I, Dreano I, Beaune P, Riche C and Berthou F: Identification of the cytochrome P450 IIIA family as the enzymes involved in the *N*-demethylation of tamoxifen in human liver microsomes. *Biochem Pharmacol* 41: 1911-1919, 1991.
- 9 Mani C, Gelboin HV, Park SS, Pearce R, Parkinson A and Kupfer D: Metabolism of the antimammmary cancer antiestrogenic agent tamoxifen. I. Cytochrome P450-catalysed *N*-demethylation and 4-hydroxylation. *Drug Metab Dispos* 21: 645-656, 1993.
- 10 Crewe HK, Ellis SW, Lennard MS and Tucker GT: Variable contribution of cytochromes P450 2D6 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. *Biochem Pharmacol* 53: 171-178, 1997.
- 11 Borgna JL and Rochefort H: Hydroxylated metabolites of tamoxifen are formed *in vivo* and bound to estrogen receptor in target tissues *J Biol Chem* 256: 859-868, 1981.
- 12 Coezy E, Borgna JL and Rochefort H: Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. *Cancer Res* 42: 317-323, 1982.
- 13 Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P, Hayes DF, Desta Z and Flockhart DA: Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J Natl Cancer Inst* 95: 1758-1764, 2003.
- 14 Johnson MD, Zu OH, Lee KH, Trebley JP, Rae JM, Weatherman RV, Desta Z, Flockhart DA and Skaar TC: Pharmacological characterization of 4-hydroxy-*N*-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat* 85: 151-159, 2004.
- 15 Rao US, Fine RL and Scarborough GA: Antiestrogens and steroid hormones: substrates of the human P-glycoprotein. *Biochem Pharmacol* 48: 287-292, 1994.
- 16 Gant TW, O'Connor CK, Corbitt R, Thorgeirsson U and Thorgeirsson SS: *In vivo* induction of liver P-glycoprotein expression by xenobiotics in monkeys. *Toxicol Appl Pharmacol* 133: 269-276, 1995.

- 17 Sutherland L, Ebner T and Burchell B: The expression of UDP-glucuronosyltransferases of the UGT1 family in human liver and kidney and in response to drugs. *Biochem Pharmacol* 45: 295-301, 1993.
- 18 Turgeon D, Carrier JS, Lévesque E, Hum DW and Bélanger A: Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members. *Endocrinology* 142: 778-787, 2001.
- 19 Wachter VJ, Wu CY and Benet LZ: 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, 1995.
- 20 Dixon RA and Steel CL: Flavonoids and isoflavonoids – a gold mine for metabolic engineering. *Trend Plant Sci* 4: 394-400, 1999.
- 21 Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K and van Leeuwen PA: Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr* 74: 418-425, 2001.
- 22 Morazzoni P and Bombardelli E: *Silybum marianum* (fitoterapia). *Fitoterapia* 66: 3-42, 1995.
- 23 Zhao J, Lahiri-Chatterjee M, Sharma Y and Agarwal R: Inhibitory effect of a flavonoid antioxidant silymarin on benzoyl peroxide-induced tumor promotion, oxidative stress and inflammatory responses in SENCAR mouse skin. *Carcinogenesis* 21: 811-816, 2000.
- 24 Katiyar SK, Korman NJ, Mukhtar H and Agarwal R: Protective effects of silymarin against photocarcinogenesis in a mouse skin model. *JNCI* 89: 556-566, 1997.
- 25 Saller R, Meier R and Brignoli R: The use of silymarin in the treatment of liver diseases. *Drugs* 61: 2035-2063, 2001.
- 26 Kosina P, Maurel P, Ulrichová J and Dvorák Z: Effect of silybin and its glycosides on the expression of cytochromes P450 1A2 and 3A4 in primary cultures of human hepatocytes. *J Biochem Mol Toxicol* 19: 149-153, 2005.
- 27 Zuber R, Modrianský M, Dvorák Z, Rohovský P, Ulrichová J, Simánek V and Anzenbacher P: Effect of silybin and its congeners on human liver microsomal cytochrome P450 activities. *Phytother Res* 16: 632-638, 2002.
- 28 Wu JW, Lin LC, Hung SC, Lin CH, Chi CW and Tsai TH: Hepatobiliary excretion of silybinin in normal and liver cirrhotic rats. *Drug Metab Dispos* 36: 589-596, 2008.
- 29 Dzubák P, Hajdúch M, Gazák R, Svobodová A, Psotová J, Walterová D, Sedmera P and Kren V: New derivatives of silybin and 2,3-dehydrosilybin and their cytotoxic and P-glycoprotein modulatory activity. *Bioorg Med Chem* 14: 3793-3810, 2006.
- 30 Shin SC, Choi JS and Li X: Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats. *Int J Pharm* 313: 144-149, 2006.
- 31 Shin SC, Piao YJ and Choi JS: Effects of morin on the bioavailability of tamoxifen and its main metabolite, 4-hydroxytamoxifen, in rats. *In Vivo* 22: 391-396, 2008.
- 32 Shin SC and Choi JS: Effects of epigallocatechin gallate on the oral bioavailability and pharmacokinetics of tamoxifen and its main metabolite, 4-hydroxytamoxifen, in rats. *Anti-Cancer Drugs* 20: 584-588, 2009.
- 33 Freid KM and Wainer IW: Direct determination of tamoxifen and its four major metabolites in plasma using coupled column high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 655: 261-268, 1994.
- 34 Crespi CL, Miller VP and Penman BW: Microtiter plate assays for inhibition of human, drug-metabolizing cytochromes P450. *Anal Biochem* 248: 188-190, 1997.
- 35 Cummins CL, Jacobsen W and Benet LZ: Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. *J Pharmacol Exp Ther* 300: 1036-1045, 2002.
- 36 Wolozin B, Kellman W, Rousseau P, Celesia GG and Siegel G: Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arch Neurol* 10: 1439-1443, 2000.
- 37 Kaminsky LS and Fasco MJ: Small intestinal cytochromes P450. *Crit Rev Toxicol* 21: 407-422, 1991.
- 38 Rajnarayana K, Reddy MS, Vidyasagar J and Krishna DR: Study on the influence of silymarin pretreatment on metabolism and disposition of metronidazole. *Arzneimittelforschung* 54: 109-113, 2004.

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