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Effects of Morin on the Bioavailability of Tamoxifen and its Main Metabolite, 4-Hydroxytamoxifen, in Rats

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Abstract. *This study examined the effect of morin on the bioavailability and pharmacokinetics of tamoxifen and its metabolite, 4-hydroxytamoxifen, in rats. A single dose of tamoxifen was administered to rats intravenously (2 mg/kg) or orally (10 mg/kg), with or without morin (3 or 10 mg/kg). The presence of morin significantly altered the pharmacokinetics of the orally administered tamoxifen. Compared with the oral control group (given tamoxifen alone), the total body clearance (CL/F) of tamoxifen in the presence of morin was significantly reduced (by 35.9-40.8%, $p<0.01$). The area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of tamoxifen significantly ($p<0.05$ for 3 mg/kg of morin, $p<0.01$ for 10 mg/kg of morin) increased by 50.6-68.9% and 65.1-80.9%, respectively. Consequently, the absolute bioavailability (AB) of tamoxifen in the presence of morin was 37.4-40.5%, which was enhanced significantly ($p<0.05$) compared with the oral control group (23.9%). The relative bioavailability (RB) of tamoxifen was 1.56 to 1.68 times higher than the control group. The increased bioavailability of tamoxifen is likely to be due to the decrease in the first-pass metabilism by the intestines and liver. Morin at a dose of 10 mg/kg significant increased the $AUC_{0-\infty}$ of 4-hydroxytamoxifen (by 50.9%, $p<0.05$) but the metabolite:parent ratio (MR) of 4-hydroxytamoxifen was not altered significantly, suggesting that the formation of 4-hydroxytamoxifen is not affected considerably by morin. The increased bioavailability of tamoxifen in the presence of morin should be taken into consideration for dosage regimens due to potential drug interaction.*

Tamoxifen is the agent of choice for treating and preventing breast cancer (1). Orally administered tamoxifen undergoes extensive hepatic metabolism and subsequent biliary excretion (2). The major primary metabolite, *N*-desmethyltamoxifen, is catalyzed by cytochrome P450 (CYP) 3A, and the minor metabolite, 4-hydroxytamoxifen, is catalyzed mainly by CYP2D6 as well as by CYP2C9 and CYP3A (3, 4). 4-Hydroxytamoxifen has 30- to 100-fold more potency than tamoxifen in suppressing estrogen-dependent cell proliferation (5, 6). A secondary metabolite of tamoxifen, endoxifen, exhibits similar potency to 4-hydroxytamoxifen (7, 8). Therefore, tamoxifen is referred to as a prodrug that requires activation to exert its effects. Tamoxifen is also a substrate for P-glycoprotein (P-gp) (9, 10). P-gp colocalizes with CYP3A in the polarized epithelial cells of the excretory organs, such as the intestine, liver and kidney (11, 12) to eliminate foreign compounds from the body. A substantial overlap in substrate specificity exists between CYP3A4 and P-gp (13). P-gp and CYP3A modulators might be able to improve the oral bioavailability of tamoxifen.

Morin (3, 5, 7, 2', 4'-pentahydroxyflavone) is a flavonoid constituent of many herbs and fruits. *In vitro* studies have shown morin to have a variety of beneficial activities, including antioxidation (14-16), anti-mutagenesis (17, 18) and anti-inflammation (19-21). Zhang and Morris (22) reported that morin can increase the level of daunomycin accumulation in P-gp-positive cell lines in a concentration- and P-gp expression level-dependent manner, which suggests that morin shows P-gp-mediated cellular efflux. The increase in daunomycin accumulation by morin in all the P-gp positive cells is comparable with that of verapamil, a potent P-gp inhibitor. In human liver microsomes, morin inhibited the formation of 6α-hydroxypaclitaxel (formed by CYP2C8), but had less effect on C_3'-hydroxypaclitaxel and C_2-hydroxypaclitaxel (formed by CYP3A4) (23). Buening *et al.* (24) also reported that morin could inhibit cytochrome P-450 reductase in human liver microsomes.

Orally administered morin, as P-gp and CYP3A inhibitors, may provide anticancer effects to improve the bioavailability of tamoxifen in combination therapy. Therefore, the purpose of this study examined the effect of morin on the bioavailability and pharmacokinetics of tamoxifen and 4-hydroxytamoxifen in rats.

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Materials and Methods

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

The apparatus used in this study was a high-performance liquid chromatograph 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 Bransonic® ultrasonic cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA), and a high-speed microcentrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments. Male Sprague-Dawley rats (weighing 270-300 g) were purchased from the Daehan 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 *ad libitum*. The animals were housed, two per cage, maintained at 22±2˚C and a 50-60% relative humidity, under a 12:12 h light/dark cycle. The animals were allowed one week for acclimation. The Animal Care Committee of Chosun University (Gwangju, Korea) approved the design and conduct of this study. The rats were fasted for at least 24 h before the experiments and each animal was anaesthetized 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) to allow for blood sampling and the *i.v.* injection, respectively.

Drug administration. The rats were divided into the following four groups (n=6, each): three groups (10 mg/kg of tamoxifen dissolved in 0.9% NaCl solution containing 10% of Tween 80, 3.0 ml/kg) (oral control) or with 3 or 10 mg/kg of morin (mixed in distilled water, 3.0 ml/kg), and an *i.v.* 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, while morin was administered intragastrically 30 min before the oral administration of tamoxifen. Tamoxifen for *i.v.* administration was injected through the femoral vein within 1 min. A 0.45-ml aliquot of blood 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 (only for oral group), 4, 6, 8, 12, 24 and 36 h (only for oral group) after administering the tamoxifen. The blood samples were centrifuged at 13,000 rpm for 5 min and the plasma samples were stored at –40˚C until analyzed by HPLC.

HPLC analysis. The plasma concentrations of tamoxifen and 4-hydroxytamoxifen were determined using a slight modification of the HPLC method reported by Fried and Wainer (25). Briefly, a 50-μl aliquot of 8-μg/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 for 2 min and centrifuged at 13,000 rpm for 10 min. A 50-μl aliquot of the supernatant was injected into the HPLC system. The chromatographic separations were achieved using a Symmetry® C_{18} column (4.6x150 mm, 5 μm; Waters Co.) and a μBondapak™ C_{18} HPLC Precolumn (10 μm, 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 30˚C, which was set by an HPLC column temperature controller. The fluorescence detector was operated at an excitation and emission wavelength of 254 nm and 360 nm, respectively. A homemade post-column photochemical reactor was supplied with a bactericiadal ultraviolet lamp (Sankyo Denki Co, Japan), and Teflon® tubing (i.d. 0.01'', o.d. 1/16'', 2 m long) was crocheted and fixed horizontally with a stainless steel frame under a lamp at a 10 cm-distance in order to convert the tamoxifen and 4-hydroxytamoxifen to the fluorophors for increased detection sensitivity. 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, respectively. The coefficients of the variation of tamoxifen and 4-hydroxytamoxifen were <4.5% and 1.5%, respectively.

Pharmacokinetic analysis. The plasma concentration data were analyzed using a noncompartmental method on WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by the log-linear regression of tamoxifen and 4-hydroxytamoxifen concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and time to reach the peak concentration (t_{max}) of tamoxifen and 4-hydroxytamoxifen in the plasma were obtained by a 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 the last measured concentration (C_{last}) was calculated using the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by adding AUC_{0-t} and the extrapolated area was determined by C_{last}/K_{el}. The total body clearance (CL/F) was calculated from the Dose/AUC. The absolute bioavailability (AB) was calculated by AUC_{oral}/AUCIV x $Dose_{IV}/Dose_{oral}$, and the relative bioavailability (RB) was calculated by $AUC_{control}/AUC_{with\ morin}$. The metabolite:parent ratio (MR) was estimated from ($AUC_{4\text{-}hydroxytamoxifen}/AUC_{tamoxifen}$) x100.

Statistical analysis. Statistical analysis was carried out using a one-way ANOVA followed by a posteriori testing with a Dunnett correction. The differences were considered significant at a level of $p<0.05$. All the mean values are presented with their standard deviation (Mean±S.D.).

Results

Effect of morin on the pharmacokinetics of tamoxifen. Figure 1 shows the mean arterial plasma concentration-time profiles of tamoxifen after the intravenous administration of tamoxifen (2 mg/kg), and the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of morin. The corresponding pharmacokinetic parameters are shown in Table I. The presence of morin significantly altered the pharmacokinetic parameters of tamoxifen. Compared with the control group (given oral tamoxifen alone), the presence of morin significantly reduced the CL/F

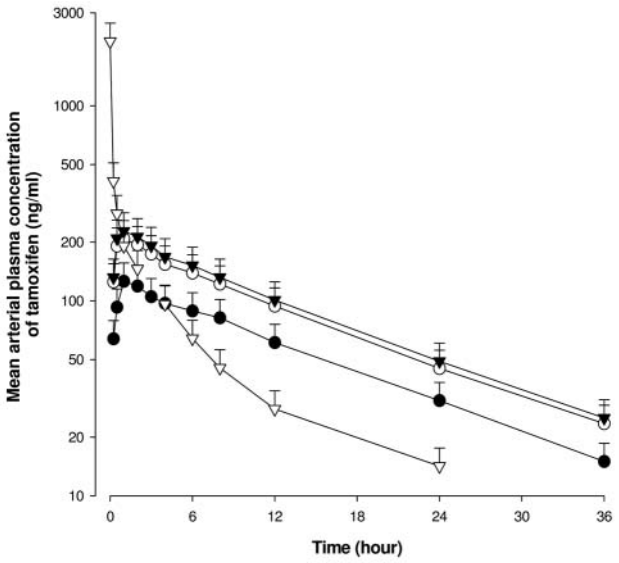

Figure 1. *Mean plasma concentration-time profiles of tamoxifen after the intravenous administration of tamoxifen (2 mg/kg), and the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of morin (3 or 10 mg/kg) (n=6, each). The bars represent the standard deviation. (▽) Intravenous administration of tamoxifen (2 mg/kg); (●) Oral administration of tamoxifen (10 mg/kg); (○) the presence of 3 mg/kg of morin; (▼) the presence of 10 mg/kg of morin.*

Table I. *Mean (± S.D.) pharmacokinetic parameters of tamoxifen after the intravenous administration of tamoxifen (2 mg/kg) and the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of morin (3 or 10 mg/kg) (n=6, each).*

Parameter	Control	Morin		*i.v.* tamoxifen (2 mg/kg)
		3 mg/kg	10 mg/kg	
$AUC_{0-\infty}$ (ng h/ml)	2137±513	3334±802*	3610±867**	1783±428
C_{max} (ng/ml)	126±30.2	208±49.9*	228±54.7**	
T_{max} (h)	1	1	1	
CL/F (ml/min/kg)	78.0±18.7	50.0±12.1**	46.2±11.1**	18.7±4.49
$t_{1/2}$ (h)	11.6±2.78	11.8±2.83	11.7±2.81	8.80±2.11
AB (%)	23.9±5.74	37.4±8.98*	40.5±9.72*	100
RB (%)	100	156	168	

*$p<0.05$, **$p<0.01$ compared to control. $AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach Cmax; CL/F: total body clearance; $t_{1/2}$: terminal half-life; AB: absolute bioavailability; RB: relative bioavailability.

of tamoxifen ($p<0.01$, 35.9-40.8%), and significantly increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen by 56.0-68.9% and 65.1-80.9%, respectively ($p<0.05$ at 3 mg/kg of morin, $p<0.01$ at 10 mg/kg of morin). The AB of tamoxifen was elevated significantly ($p<0.05$) by 37.4-40.5%, compared with the control group (23.9%). The RB of tamoxifen in the presence of morin was 1.56 to 1.68 times higher. There was no significant difference in the T_{max} or the $t_{1/2}$ of tamoxifen in the presence of morin.

Effect of morin on the pharmacokinetics of 4-hydroxytamoxifen. Figure 2 shows the mean plasma concentration-time profiles of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) to the rats in the presence or absence of morin (3 or 10 mg/kg). Table II shows the corresponding pharmacokinetic parameters. Compared with the control group, the presence of morin at 10 mg/kg increased the $AUC_{0-\infty}$ (50.9%) of 4-hydroxytamoxifen significantly ($p<0.05$). Although the metabolite:parent ratio (MR) of tamoxifen was lower in the presence of morin, the reduction was not significant. These results suggest that the production of 4-hydroxytamoxifen is not affected considerably by the addition of morin. The C_{max}, $t_{1/2}$ and T_{max} of 4-hydroxytamoxifen were not affected by morin.

Discussion

Tamoxifen and its primary metabolites undergo extensive oxidation, principally by CYP3A and CYP2D6 (26). Tamoxifen and its metabolites, *N*-desmethyltamoxifen and 4-hydroxytamoxifen, are also substrates for the efflux of P-gp as well (9, 10). CYP3A and P-gp inhibitors might interact with tamoxifen and its metabolites and contribute to the substantial alteration of their pharmacokinetic fate. Morin was suggested to inhibit P-gp-mediated efflux of daunomycin, which was comparable to verapamil, a potent P-gp inhibitor (22, 24). It is possible that the concomitant administration of morin might affect the bioavailability or pharmacokinetics of orally administered tamoxifen.

As shown in Table I, the presence of morin significantly reduced the CL/F and increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen. These results suggest that presence of morin might inhibit the CYP3A and the P-gp pathway because orally administered tamoxifen is a substrate for CYP3A-catalyzed metabolism and the P-gp-mediated efflux in the intestine and liver. Like its isomer quercetin, orally administered morin is absorbed easily in the intestine of rodents but it is mainly metabolized as glucuronides and sulfates (27, 28). These results are consistent with the report by Choi and Han (29) in that the presence of morin significantly increased the AUC_{0-}

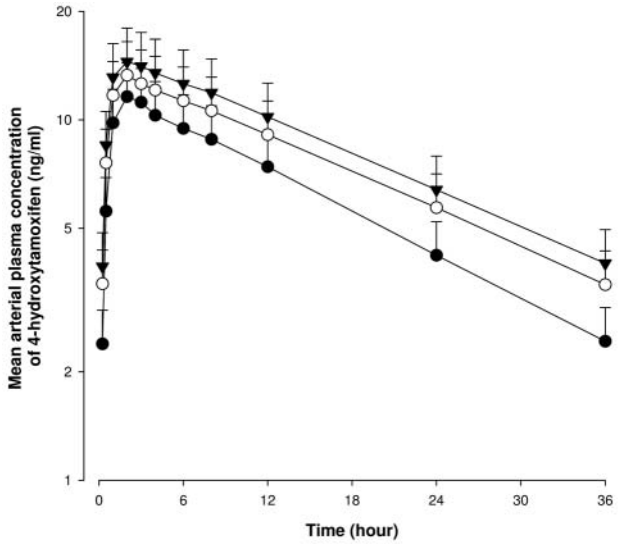

Figure 2. *Mean plasma concentration-time profiles of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of morin (3 or 10 mg/kg) (n=6, each). The bars represent the standard deviation. (●) Oral administration of tamoxifen (10 mg/kg); (○) the presence of 3 mg/kg of morin; (▼) the presence of 10 mg/kg of morin.*

$_{\infty}$ and C_{max} of diltiazem, a P-gp and CYP3A4 substrate, in rats, and the report by Choi *et al.* (30) that a pretreatment of morin significantly increased the $AUC_{0-\infty}$ of paclitaxel in rats. Shin *et al.* (31) also reported that the presence of the morin analogue quercetin at doses of 2.5 and 7.5 mg/kg to rats increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen significantly.

The presence of 10 mg/kg of morin increased the $AUC_{0-\infty}$ of 4-hydroxytamoxifen significantly compared with the control group ($p<0.05$). However, the MR of 4-hydroxytamoxifen was not altered significantly (Table II), which suggests that morin does not affect the production of 4-hydroxytamoxifen, which is formed mainly by CYP2D6 and is also catalyzed by CYP2C9 and CYP3A (3, 4). The unaltered MR of 4-hydroxytamoxifen suggests that morin does not affect the CYP2D6-mediated metabolism. The anticancer effect of tamoxifen might be increased by the presence of morin; furthermore enhanced bioavailability of 4-hydroxitamoxifen may increase its anticancer effects, because it is much more effective in anticancer therapy than tamoxifen (5, 6).

Conclusion

The presence of morin enhanced the oral bioavailability of tamoxifen, which might be due to the promotion of intestinal absorption and a reduction of the first-pass metabolism of tamoxifen. Therefore, the dose of tamoxifen should be readjusted when coadministered with morin or the dietary complement of morin for a rational dosage regimen.

Table II. *Mean (±S.D.) pharmacokinetic parameters of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of morin (3 or 10 mg/kg) (n=6, each).*

Parameter	Control	Morin	
		3 mg/kg	10 mg/kg
$AUC_{0-\infty}$ (ng h/ml)	271±65.0	361±86.7	409±98.1*
C_{max} (ng/ml)	11.6±2.78	13.3±3.19	14.5±3.48
T_{max} (h)	2	2	2
$t_{1/2}$ (h)	15.0±3.60	17.5±4.20	17.8±4.27
MR (%)	12.2±0.290	10.4±2.51	11.0±2.83

*$p<0.05$ compared to control. $AUC_{0-\infty}$: 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.

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