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Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats

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Abstract

Orally administered tamoxifen undergoes a first-pass metabolism and substrates for multidrug resistance (MDR) transporters efflux in the liver and intestines, which obstructs its systemic exposure. This study investigated the effect of quercetin, a dual inhibitor of CYP3A4 and P-gp, on the bioavailability and pharmacokinetics of tamoxifen and one of its metabolites, 4-hydroxytamoxifen, in rats. The pharmacokinetic parameters of tamoxifen and 4-hydroxytamoxifen in plasma were determined after orally administering tamoxifen (10 mg/kg) with or without quercetin (2.5, 7.5 and 15 mg/kg). The coadministration of quercetin (2.5 and 7.5 mg/kg) significantly ($p < 0.05$) increased the absorption rate constant (K_a), peak concentration (C_{max}) and the areas under the plasma concentration-time curve (AUC) of tamoxifen. The absolute bioavailability (AB%) of tamoxifen with 2.5 and 7.5 mg/kg quercetin ranged from 18.0% to 24.1%, which was significantly higher than the control group, 15.0% ($p < 0.05$). The relative bioavailability (RB%) of tamoxifen coadministered with quercetin was 1.20–1.61 times higher than the control group. The coadministration of quercetin caused no significant changes in the terminal half-life ($t_{1/2}$) and the time to reach the peak concentration (T_{max}) of tamoxifen.

Compared with the control group, the coadministration of 7.5 mg/kg quercetin significantly ($p < 0.05$) increased the AUC of 4-hydroxytamoxifen. However, the metabolite ratios (MR; AUC of 4-hydroxytamoxifen to tamoxifen) were significantly lower ($p < 0.05$). This suggests that quercetin inhibits the both MDR transporters efflux and first-pass metabolism of tamoxifen. The enhanced bioavailability of tamoxifen as a result of its coadministration with quercetin might be due to the effect of quercetin promoting the intestinal absorption and reducing the first-pass metabolism of tamoxifen. If the results are further confirmed in the clinical trials, the tamoxifen dosage should be adjusted when tamoxifen is administered with quercetin or quercetin-containing dietary supplements in order to avoid potential drug interactions.

Keywords: Tamoxifen; 4-Hydroxytamoxifen; Quercetin; Bioavailability; Pharmacokinetics

1. Introduction

Tamoxifen is a nonsteroidal antiestrogen and is the agent of choice for treating and preventing breast cancer (Powles, 1992; Stone, 1992; Jaiyesimi et al., 1995). 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 (Fornander et al., 1993; Meier and Jick, 1998). Oral tamoxifen undergoes extensive hepatic metabolism and the subsequent biliary excretion of its metabolites (Buckley and Goa, 1989). In humans, the main pathway in tamoxifen biotransformation proceeds via the *N*-demethylation catalyzed mostly by CYP3A4 enzymes (Jacolot et al., 1991; Mani et al., 1993). Another important drug metabolite, 4-hydroxytamoxfen, is produced in humans by CYP2D6, CYP2C9, CYP2E1 and CYP3A4 (Mani et al., 1993; Crewe et al., 1997). Although the plasma and tumor concentrations of 4-hydroxytamoxifen are only about 2% of those of the parent compound (Daniel et al., 1981), this metabolite has been reported to be about 100 times more potent as an estrogen antagonist than tamoxifen (Jordan et al., 1977). Tamoxifen and its metabolites, and 4-hydroxytamoxifen are substrates for the efflux of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance-associated protein (MRP) 2, the members of ATP-binding cassette (ABC) superfamily (Rao et al., 1994; Gant et

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al., 1995, Sugimoto et al., 2003; Kauffmann et al., 1998). ABC family of transport proteins play a central role in the defense of organisms against toxic compounds (Higgins, 1992; Borst et al., 2000). P-gp, MRP2 and BCRP located within the polarized apical membrane of the intestine, liver and kidney mediating the efflux of xenobiotics and toxins into the intestinal lumen, bile and urine (Chan et al., 2004). MRPs and P-gp are found to be expressed with CYP3A4, glutathione-*S*-transferases, UDP-glucuronosyltransferases (Sutherland et al., 1993; Turgeon et al., 2001), which may play the synergistic function in regulating the bioavailability of many orally ingested compounds.

Quercetin is a member of an extensive class of polyphenolic flavonoid compounds that is widely distributed mainly as glycosides in components of the daily diet such as onions, apples, berries, tea and red wine (Hertog et al., 1992, 1995). Quercetin is nontoxic and displays a variety of biological actions such as antioxidation (Takahama, 1985), antivirus (Ohnishi and Bannai, 1993), antiulcer (Alarcon de la Lastra et al., 1994), antiallergic (Murray, 1998), and anticancer (Lamson and Brignall, 2000), etc. Currently, it is in clinical trial as an anticancer therapy and is a potential drug of the future (Ferry et al., 1996). Epidemiological studies in the U.S., Europe, and Asia have estimated that the daily dietary intake of quercetin by an individual ranges from 4 to 68 mg (Hertog et al., 1993, 1995; Rimm et al., 1996; Knekt et al., 1997), and even can be as high as several 100 mg in dietary supplements and several grams in anticancer therapy (Lamson and Brignall, 2000). It was reported that quercetin could competitively inhibit the members of MDR family, P-gp, MRP1 and BCRP (Scambia et al., 1994; van Zanden et al., 2005; Cooray et al., 2004), and the metabolizing enzyme, CYP3A4 (Guengerich and Kim, 1990; Miniscalco et al., 1992). Dupuy et al. (2003) reported that the AUC and plasma concentration of moxidectin (a substrate for P-gp and CYP3A) increased when used concomitantly with 10 mg/kg of quercetin in lambs.

The oral bioavailability of tamoxifen is mainly affected by the first-pass metabolism and P-gp pump efflux in the liver and intestine. As a dual inhibitor of the metabolizing enzyme CYP3A and MDR transporter, quercetin might alter the pharmacokinetics of tamoxifen when used concomitantly with tamoxifen. The aim of this study was to examine the bioavailability and pharmacokinetic changes of tamoxifen and 4-hydroxytamoxifen after the oral administration of tamoxifen with quercetin in rats. Because the potent metabolite, 4-hydroxytamoxifen, presented in lower concentrations in rat and humans, and in higher concentrations in mice (DeGregorio et al., 1989; Robinson et al., 1989), the rat may be the preferable rodent animal model for studying the pharmacokinetics of tamoxifen and 4-hydorxytamoxifen for further extrapolate to humans. Therefore, rats were chosen as the animal model for investigating the pharmacokinetics of tamoxifen and its metabolite, 4-hydroxytamoxifen in this study.

2. Materials and methods

2.1. Materials and apparatus

Tamoxifen citrate salt, 4-hydroxytamoxifen, quercetin and butylparaben (*p*-hydroxybenzoic acid *n*-butyl ester) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol were acquired from Merck Co. (Darmstadt, Germany). All other chemicals in this study were of reagent grade and were used without further purification.

A high performance liquid chromatograph (HPLC, Waters 1515 isocratic HPLC Pump, Waters 717 plus autosampler, Waters™ 474 scanning fluorescence detector, Waters Co., Milford, MA, USA), a microcentrifuge (National Labnet Co., NY, USA), a sonicator (Bransonic Ultrasonic, CT, USA), a HPLC column temperature controller (Phenomenex Inc., CA, USA) and a vortex-mixer (Scientific Industries Co., NY, USA) were used in this study.

2.2. Animal experiments and drug administration

Female Sprague–Dawley rats (270–300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea), and given a normal standard chow diet (No. 322-7-1) purchased from Superfeed Co. (Gangwon, Korea) and tap water ad libitum. Throughout the experiment, the animals were housed in laminar flow cages, three per cage, which was maintained at $22 \pm 2\,^{\circ}C$, 50–60% relative humidity, under a 12 h light–dark cycle. The animals were allowed to acclimatize for at least 1 week prior to the experiment. This experiment was performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The animal care committee at our institution (Chosun University) approved this study.

Rats were divided into five groups of six each: the control group (tamoxifen 10 mg/kg, oral), three coadministration groups (1.5, 7.5 or 15 mg/kg of quercetin orally coadministered with 10 mg/kg of tamoxifen, respectively) and IV group (intravenous administration of 2.0 mg/kg tamoxifen). The rats were fasted for at least 24 h prior to the experiment but given free access to water. Each rat was anaesthetized with diethyl ether. The right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, USA) to allow for blood sampling. Tamoxifen dose (10 mg/kg) was chosen in order to keep the plasma concentrations above the detection limit. The drug used in the control group was prepared by adding tamoxifen to distilled water (1.5 ml). The quercetin suspensions were prepared by dissolving the required quercetin dose (2.5, 7.5, and 15 mg/kg) in distilled water (1 ml). Tamoxifen saline solution (0.5 ml) was injected through the femoral vein within 1 min. Blood samples (0.5 ml) were withdrawn from the femoral artery at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24 and 36 h time-point after administering tamoxifen, and centrifuged at 13,000 rpm for 5 min. The plasma samples (0.2 ml) were stored at $-40\,^{\circ}C$ until analyzed by HPLC.

2.3. HPLC assay

The plasma concentrations of tamoxifen were determined by a HPLC assay and a modification of the method reported by Fried and Wainer (1994). Briefly, 0.05 ml of butylparaben

(8 μg/ml, dissolved in methanol), as an internal standard, and 0.2 ml of acetonitrile were added to 0.2 ml of the plasma sample. The resulting mixture was then vortex-mixed vigorously for 2 min and centrifuged at 13,000 rpm for 10 min. 0.05 ml of the supernatant was injected into the HPLC system.

The detector was operated at an excitation wavelength of 254 nm with an emission cut-off filter of 360 nm. A Symmetry® C_{18} column (4.6 mm × 150 mm, 5 μm, Waters Co., Milford, MA, USA) was used at a temperature of 30 °C set by HPLC column temperature controller. Mobile phase consisted 20 mM dipotassium hydrogen phosphate (pH 3.0, adjusted with phosphoric acid)-acetonitrile (60:40, v/v). The flow rate was maintained at 1.0 ml/min. A homemade post-column photochemical reactor, which was supplied with a bactericidal ultraviolet lamp (Sankyo Denki Co., Japan), and a Teflon® tubing (0.01 in. i.d. × 1/16 in. o.d., 2 m long), was crocheted and fixed horizontally with a stainless steel frame under the lamp at a 10 cm distance in order to convert the tamoxifen and 4-hydroxytamoxifen to the fluorophors. A six-point standard curve was prepared by adding known concentrations of tamoxifen and 4-hydroxytamoxifen to drug-free rat plasma in the range anticipated in this study. The 4-hydroxytamoxifen concentrations used were 0.5, 1, 2, 5, 10 and 20 ng/ml, whereas the tamoxifen concentration used were 10, 20, 50, 100, 200 and 500 ng/ml.

2.4. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was carried out using the LAGRAN computer program (Rocci and Jusko, 1983), which uses the LARGAN method to calculate the AUC of the plasma concentration (C_p) as a function of time (t). The maximum plasma concentration (C_{max}) and the time needed to reach the maximum plasma concentration (T_{max}) were determined by a visual inspection of the experimental data. The elimination rate constant (K_{el}) was obtained from the terminal slope using regression analysis, and the half-life ($t_{1/2}$) of the drug was calculated by $0.693/K_{el}$. The absolute bioavailability of tamoxifen was calculated using the following equation:

$$\text{absolute bioavailability (AB\%)} = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{IV}}} \times \frac{\text{IV dose}}{\text{oral dose}} \times 100$$

The relative bioavailability of tamoxifen was calculated as follows:

$$\text{relative bioavailability (RB\%)} = \frac{\text{AUC}_{\text{coadmin}}}{\text{AUC}_{\text{control}}} \times 100$$

2.5. Statistical analysis

The data is presented as a mean ± S.D. The pharmacokinetic parameters were compared using a one-way analysis of variance, followed by posteriori testing using the Dunnett correction. A P-value of < 0.05 was considered significant.

3. Results

Chromatograms of rat's blank plasma and the plasma spiked with tamoxifen, 4-hydroxytamoxifen and butylparaben are shown in Fig. 1. The peaks of tamoxifen, 4-hydroxytamoxifen and the internal standard separated clearly. The retention times of 4-hydroxytamoxifen, internal standard and tamoxifen were 7.3, 14.5 and 26.1 min, respectively. The typical equation describing the calibration curve in rat plasma was $y = 0.018x + 0.096$ for tamoxifen and $y = 0.024x + 0.004$ for 4-hydroxytamoxifen, where y is the peak area ratio of tamoxifen or 4-hydroxytamoxifen against butylparaben and x is the concentration of tamoxifen or 4-hydroxytamoxifen, with a mean correlation coefficient of 0.9998 for both of them. The coefficients of intra-day ($n = 5$) and inter-day ($n = 5$) variation were less than 2.9% for tamoxifen, 4.5% for 4-hydroxytamoxifen and 1.5% for internal standard.

The plasma concentration-time profiles of tamoxifen were characterized in Fig. 2 after the oral administration of tamoxifen

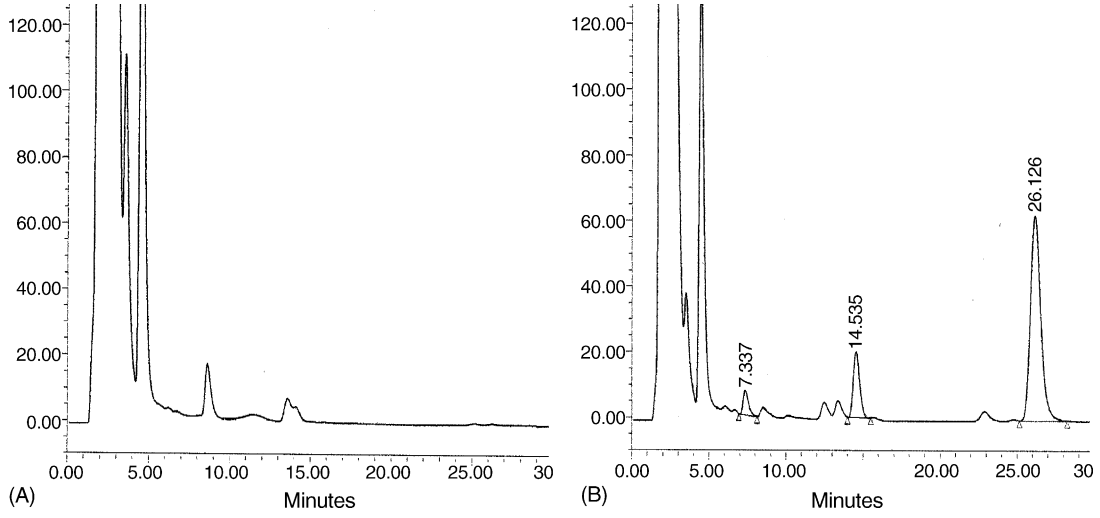

Fig. 1. Chromatogram of the rat's blank plasma (A), and the plasma (B) spiked with tamoxifen (26.1 min), 4-hydroxytamoxifen (7.3 min) and butylparaben (internal standard, 14.5 min).

Table 1
Pharmacokinetic parameters of tamoxifen following the oral administration of tamoxifen (10 mg/kg) with or without quercetin (2.5, 7.5 or 15 mg/kg) in rats

Parameters	Tamoxifen control	Quercetin coadministration (mg/kg)			i.v. (2 mg/kg)
		2.5	7.5	15	
AUC (ng/ml h)	1802 ± 507	2430 ± 634*	2891 ± 728*	2161 ± 572	2402 ± 617
C_{max} (ng/ml)	73 ± 18.1	90 ± 24.1*	99 ± 28.6*	85 ± 24.0	
T_{max} (h)	2	2	2	2	
K_a (h^{-1})	17.8 ± 5.0	24.4 ± 6.6*	29.4 ± 8.0*	21.5 ± 5.4	
$t_{1/2}$(h)	19.9 ± 5.1	22.5 ± 7.0	24.4 ± 7.3	21.2 ± 7.1	15.9 ± 4.6
AB(%)	15.0 ± 4.1	20.2 ± 5.4*	24.1 ± 6.5*	18.0 ± 4.6	100
RB(%)	100	135	161	120	

Mean ± S.D. ($n = 6$). AUC: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak concentration; T_{max}: time to reach peak concentration; K_a: absorption rate constant; $t_{1/2}$: terminal half-life; AB(%): absolute bioavailability; RB(%): relative bioavailability; compared $AUC_{coadmin}$ to $AUC_{control}$.
* Statistically significant at $p < 0.05$ when compared with the control.

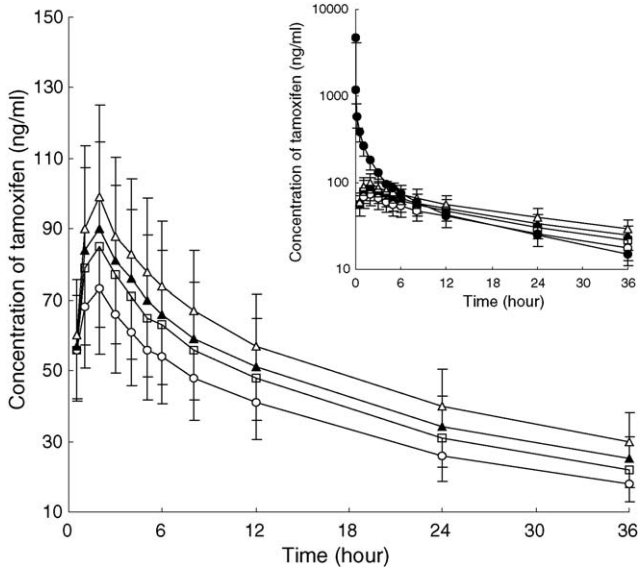

Fig. 2. Mean plasma concentration-time profiles of tamoxifen after the oral administration of tamoxifen (10 mg/kg) with or without quercetin to rats. Bars represent the standard deviation ($n = 6$). (◯) Tamoxifen control; (▲) coadministered with quercetin 2.5 mg/kg; (△) coadministered with quercetin 7.5 mg/kg; (□) coadministered with quercetin 15 mg/kg; (●) tamoxifen i.v. 2.0 mg/kg.

with or without of quercetin to the rats. Table 1 summarizes the pharmacokinetic parameters of tamoxifen. Compared with the control group (given tamoxifen alone), the coadministration of quercetin (2.5 and 7.5 mg/kg) significantly ($p < 0.05$) increased

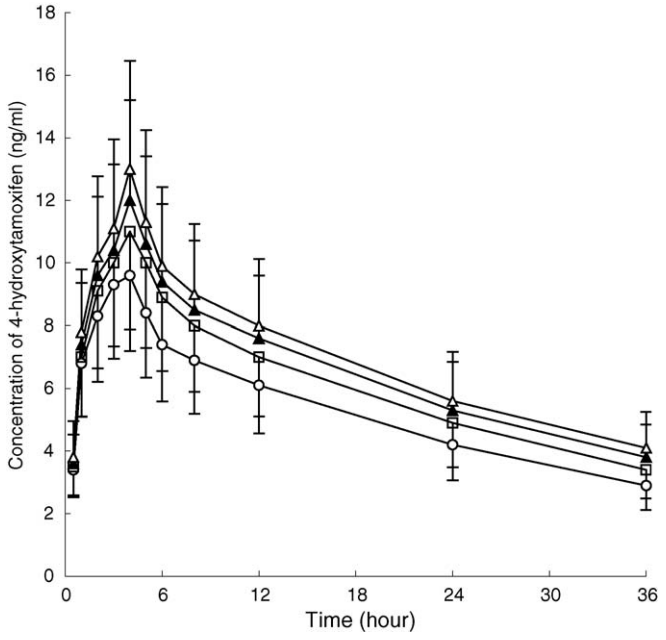

Fig. 3. Mean plasma concentration-time profiles of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) with or without quercetin to rats. Bars represent the standard deviation ($n = 6$): (◯) tamoxifen control, (▲) coadministered with quercetin 2.5 mg/kg, (△) coadministered with quercetin 7.5 mg/kg, (□) coadministered with quercetin 15 mg/kg.

Table 2
Pharmacokinetic parameters of 4-hydroxytamoxifen following the oral administration of tamoxifen (10 mg/kg) with or without quercetin (2.5, 7.5 or 15 mg/kg) in rats

Parameter	Tamoxifen control	Quercetin coadministration (mg/kg)		
		2.5	7.5	15
AUC (ng/ml h)	298 ± 82	347 ± 95	383 ± 104*	321 ± 87
C_{max} (ng/ml)	9.6 ± 2.4	12.0 ± 3.2	13.0 ± 3.4*	11.0 ± 3.0
T_{max} (h)	3.6	4.4	4.4	3.8
$t_{1/2}$(h)	23 ± 5.8	24 ± 6.1	25 ± 6.3	24 ± 6.2
RB(%)	100	116	128	108
MR	0.17 ± 0.03	0.14 ± 0.02*	0.13 ± 0.02*	0.15 ± 0.03

Mean ± S.D. ($n = 6$). AUC: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak concentration; T_{max}: time to reach peak concentration; $t_{1/2}$: terminal half-life; RB(%): relative bioavailability; compared $AUC_{coadmin}$ to $AUC_{control}$; MR: metabolite ratio; compared $AUC_{4\text{-hydoxytamoxifen}}$ to $AUC_{tamoxifen}$.
* Statistically significant at $p < 0.05$ when compared with the control.

the absorption rate constant (K_a), peak concentrations (C_{max}) and area under the plasma concentration-time curve (AUC) of tamoxifen. The terminal half-life ($t_{1/2}$) and the time to reach the peak concentration (T_{max}) were not altered significantly. Moreover, the absolute bioavailability (AB%) of tamoxifen with quercetin is significantly higher (20.2% by 2.5 mg/kg and 24.1% by 7.5 mg/kg, $p < 0.05$) than the control (15.0%), and the relative bioavailability (RB%) of tamoxifen increased 1.35-fold by 2.5 mg/kg of quercetin, and 1.61-fold by 7.5 mg/kg. An amount of 15 mg/kg of quercetin has induced no significant change of the parameters of tamoxifen.

Fig. 3 shows the plasma concentration-time profiles of 4-hydroxytamoxifen, and Table 2 summarizes the pharmacokinetic parameters of 4-hydroxytamoxifen. The AUC of 4-hydroxytamoxifen was increased significantly ($p < 0.05$) by coadministration of 7.5 mg/kg quercetin. However, the metabolite ratios (MR; AUC of 4-hydroxytamoxifen to tamoxifen) were significantly lower ($p < 0.05$). This suggests that quercetin can effectively to inhibit the MDR transporters efflux and CYP3A4-mediated metabolism of tamoxifen. There were no apparent changes in the T_{max} and $t_{1/2}$ of 4-hydroxytamoxifen by coadministration of quercetin.

4. Discussion

Orally administered tamoxifen is metabolized by CYP3A in both the human liver and small intestine (Jacolot et al., 1991; Mani et al., 1993; Crewe et al., 1997), and it substrates for the MDR family, P-gp, MRP2 and BCPR (Rao et al., 1994; Gant et al., 1995; Sugimoto et al., 2003; Kauffmann et al., 1998). The phase I and phase II metabolizing enzymes, especially CYP3A4, are expressed with MDR family, P-gp, BCRP and MRP2 in the liver, kidney and intestine (Sutherland et al., 1993; Turgeon et al., 2001), regulating the bioavailability of many orally ingested compounds. Therefore, the inhibitors against both metabolizing enzyme CYP3A4 and MDR family should have a large impact on the pharmacokinetics of those compounds. Since quercetin can competitively inhibit P-gp and BCRP efflux pump function (Scambia et al., 1994; van Zanden et al., 2005; Cooray et al., 2004) and CYP3A metabolizing enzyme, this study examined the effect of quercetin on the pharmacokinetics of tamoxifen.

As shown in Table 1, the coadministration of quercetin significantly increased the K_a, AUC and C_{max} of tamoxifen ($p < 0.05$) with the largest effect being observed with 7.5 mg/kg of quercetin. Dupuy et al. (2003) reported that the subcutaneous coadministration of quercetin (10 mg/kg) with moxidectin (substrate for P-gp and CYP3A) significant increased the plasma AUC of moxidectin in lambs. Wang et al. (2004) reported that the oral coadministration of 40 mg/kg quercetin increased the C_{max} and the AUC of digoxin (substrate for P-gp) in pigs by 413% and 170%. However, the increased parameters of oral tamoxifen do not appear to be dose dependent because 15 mg/kg of quercetin had a lower effect on the pharmacokinetics of tamoxifen than 7.5 mg/kg. Hsiu et al. (2002) reported that the oral coadministration of quercetin (50 mg/kg) with cyclosporine in pigs and rats significantly decreased the bioavailability of cyclosporine, which is also a substrate for CYP3A4 and P-gp, even though quercetin significantly inhibited the function of intestinal P-gp in rats. One of the MDR family member, MRP1 is localized to the basolateral membranes of polarized epithelial cells including the intestinal crypt, renal tubules, and liver (Chan et al., 2004), and it is suggested to extrude its substrates into blood in the normal tissues. Quercetin as MRP1 inhibitor (van Zanden et al., 2005) competitively inhibited MRP1 at therapeutic non-toxic concentrations. The decreased bioavailability of tamoxifen made us to speculate that it might be due to the contrast direction of MRP1 with P-gp and BCRP at the polarized cells although there are no evidence to demonstrate this speculation now.

As shown in Table 2, although the coadministration of quercetin increased the AUC and C_{max} of 4-hydroxytamoxifen, but significantly decreased the MR of 4-hydroxytamoxifen. This indicates that quercetin can effectively inhibit the first-pass metabolism of tamoxifen.

5. Conclusion

The coadministration of quercetin enhanced the oral bioavailability of tamoxifen decreasing the efflux by MDR transporters in intestine, liver and kidney as well as reducing the first-pass metabolism of tamoxifen. However, further studies using clinical trials will be needed to determine if the results obtained in this study can be extrapolated to humans. If the results obtained from the rats' model is confirmed in the clinical trials, the tamoxifen dose should be adjusted for potential drug interactions when tamoxifen is used with quercetin or the quercetin-containing dietary supplements.

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