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Effects of curcumin on the pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen, in rats: possible role of CYP3A4 and P-glycoprotein inhibition by curcumin

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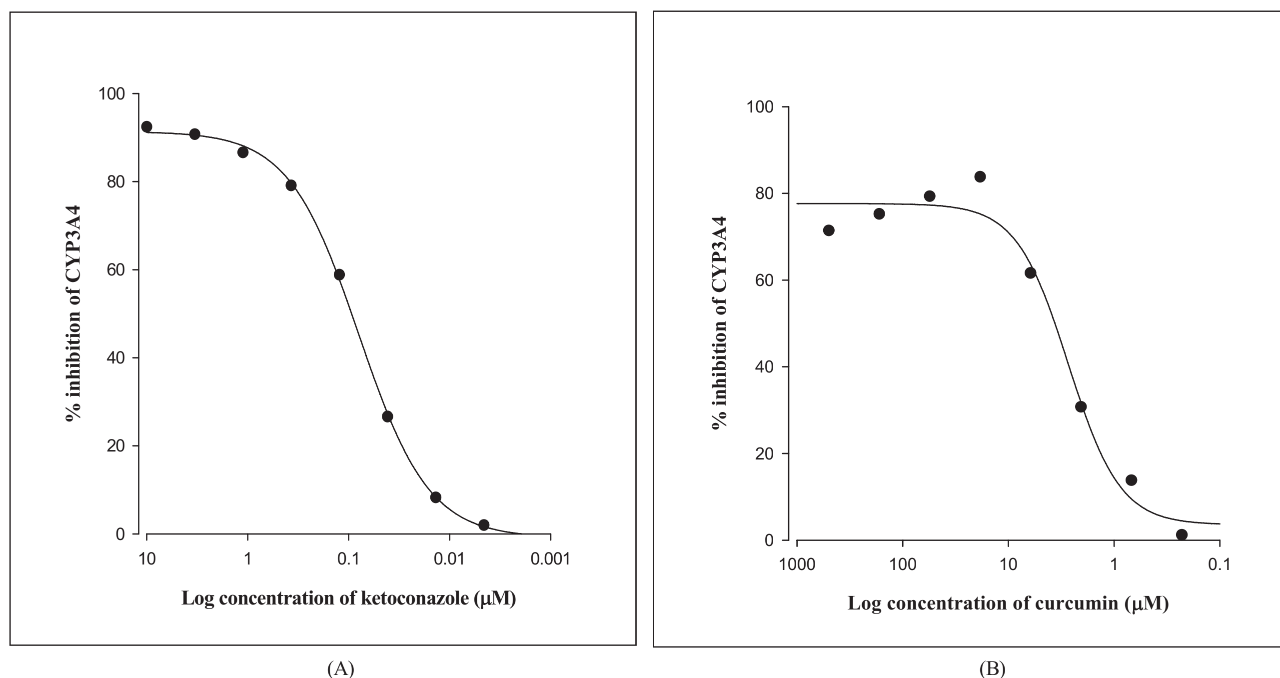
The effects of curcumin, a natural anti-cancer compound, on the bioavailability and pharmacokinetics of tamoxifen and its metabolite, 4-hydroxytamoxifen, were investigated in rats. Tamoxifen and curcumin interact with cytochrom P450 (CYP) enzymes and P-glycoprotein, and the increase in the use of health supplements may result in curcumin being taken concomitantly with tamoxifen as a combination therapy to treat or prevent cancer. A single dose of tamoxifen was administered orally ($9\,mg \cdot kg^{-1}$) with or without curcumin (0.5, 2.5 and $10\,mg \cdot kg^{-1}$) and intravenously ($2\,mg \cdot kg^{-1}$) with or without curcumin (2.5 and $10\,mg \cdot kg^{-1}$) to rats. The effects of curcumin on P-glycoprotein (P-gp) and CYP3A4 activity were also evaluated. Curcumin inhibited CYP3A4 activity with 50% inhibition concentration (IC_{50}) values of 2.7 μM. In addition, curcumin significantly ($P < 0.01$ at 10 μM) enhanced the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-gp in a concentration-dependent manner. This result suggested that curcumin significantly inhibited P-gp activity. Compared to 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 $2.5\,mg \cdot kg^{-1}$; $P < 0.01$ for $10\,mg \cdot kg^{-1}$) increased by 33.1–64.0% and 38.9–70.6%, respectively, by curcumin. Consequently, the absolute bioavailability of tamoxifen in the presence of curcumin (2.5 and $10\,mg \cdot kg^{-1}$) was 27.2–33.5%, which was significantly enhanced ($P < 0.05$ for $2.5\,mg \cdot kg^{-1}$; $P < 0.01$ for $10\,mg \cdot kg^{-1}$) compared to that in the oral control group (20.4%). Moreover, the relative bioavailability of tamoxifen was 1.12- to 1.64-fold greater than that in the control group. Furthermore, concurrent use of curcumin significantly decreased ($P < 0.05$ for $10\,mg \cdot kg^{-1}$) the metabolite-parent AUC ratio (MR), implying that curcumin may inhibit the CYP-mediated metabolism of tamoxifen to its active metabolite, 4-hydroxytamoxifen. The enhanced bioavailability of tamoxifen by curcumin may be mainly due to inhibition of the CYP3A4-mediated metabolism of tamoxifen in the small intestine and/or in the liver and to inhibition of the P-gp efflux transporter in the small intestine rather than to reduction of renal elimination of tamoxifen, suggesting that curcumin may reduce the first-pass metabolism of tamoxifen in the small intestine and/or in the liver by inhibition of P-gp or CYP3A4 subfamily.

1. Introduction

Tamoxifen belongs to a class of compounds known as selective estrogen receptor modulators which act as estrogen receptor agonists in some tissues and as antagonists in other tissues (Furr and Jordan 1984; Park and Jordan 2002). Tamoxifen is an estrogen receptor agonist in bone, the cardiovascular system, and the endometrium, but acts as an antagonist in breast tissue (Buchanan et al. 2007). Tamoxifen is clinically used for treating and preventing breast cancer (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 increased risks of endometrial cancer and thromboembolic diseases (Fornander et al. 1993; Meier and Jick 1998). Orally administered tamoxifen undergoes extensive hepatic metabolism with subsequent biliary excretion (Buckley and Goa 1989). In humans, the main pathway in tamoxifen biotransformation proceeds via N-demethylation catalyzed mostly by cytochrome P450 (CYP) 3A4 enzymes (Jacolot et al. 1991; Mani et al. 1993). Another important drug metabolite, 4-hydroxytamoxifen, is produced in humans by CYP2C9 and CYP3A4 (Crewe et al. 1997; Mani et al. 1993). 4-Hydroxytamoxifen has shown 30- to 100-fold greater potency than tamoxifen in suppressing estrogen-dependent cell proliferation (Borgna and Rochefort 1981; Coezy et al. 1982). A secondary metabolite of tamoxifen, endoxifen, exhibits a potency similar to 4-hydroxytamoxifen (Johnson et al. 2004; Stearns et al. 2003). Thus, tamoxifen is referred to as a prodrug that requires activation to exert its effects.

Tamoxifen also acts as a substrate for P-glycoprotein (P-gp) (Gant et al. 1995; Rao et al. 1994). P-gp co-localized with CYP3A in the polarized epithelial cells of excretory organs such as the liver, kidney and intestine (Sutherland et al. 1993;



(A) (B)

Fig. 1: Inhibitory effects of ketoconazole (A) and curcumin (B) on CYP3A4 activity All experiments were done in duplicate, and results are expressed as the percent of inhibition

Turgeon et al. 2001) to eliminate foreign compounds out of the body. A substantial overlap in substrate specificity exists between CYP3A4 and P-gp (Wacher et al. 1995). P-gp and CYP3A modulators might be able to affect 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.

Curcumin is the major yellow pigment in turmeric, curry, and mustard and has been widely used medicinally (Govindarajan 1980). Studies on the chemopreventive efficacy of curcumin have shown that it possesses both antiinitiating and antipromoting activities in several experimental systems (Deshpande et al. 1998; Huang et al. 1994). Curcumin inhibited carcinogenesis in various tissues, including skin (Huang et al. 1997), colorectal (Rao et al. 1995), oral (Tanaka et al. 1994), forestomach (Singh et al. 1998) and mammary (Singletary et al. 1998) cancers. *In vitro* and animal studies have suggested that curcumin may have antitumor (Aggarwal and Shishodia 2006; Choi et al. 2006), antioxidant, anti-ischemic (Shukla et al. 2008) and anti-inflammatory properties (Srivastava et al. 1995).

Appiah-Opong et al. (2008) reported that curcumin inhibits human CYP3A4 and 2C9, while Thapliyal et al. (2001) found that curcumin inhibits human CYP1A1 and 1A2. Thus, the inhibitory effects of curcumin against human CYP enzymes remain somewhat controversial. Curcumin is an inhibitor of P-gp in the KB/MDR cell line (Efferth et al. 2002), but the inhibitory effect of curcumin against P-gp is ambiguous elsewhere. Therefore, we re-evaluated the inhibition of CYP enzyme activity and P-gp activity by curcumin using CYP inhibition assays and rhodamine-123 retention assays in P-gp-overexpressing MCF-7/ADR cells. Tamoxifen and curcumin interact with CYP enzymes and P-gp, and the increase in the use of health supplements may result in curcumin being taken concomitantly with tamoxifen to treat or prevent cancer. It is important to assess the potential pharmacokinetic interactions after the concurrent use of tamoxifen and curcumin in order to ensure the effectiveness and safety of the drug therapy. However, there a few studies investigated the effect of some flavonoids on the bioavailability of tamoxifen in rats (Kim et al. 2010; Shin et al. 2006; Shin and Choi 2009). Consequently, it could be expected that curcumin would change the pharmacokinetics of drugs, substrates of P-gp and/or CYP3A4, if they are concomitantly used. Curcumin and tamoxifen could be prescribed for the treatment or prevention of cancer as a combination therapy. However, the possible effects of curcumin on the bioavailability of tamoxifen have not been studied *in vivo*.

Therefore, the aim of this study was to investigate effect of curcumin, herbal anti-cancer compound, on inhibitory effect of P-gp, CYP3A4 activity, bioavailability, and pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen, after oral and intravenous administration of tamoxifen in rats.

2. Investigations and results

2.1. *Inhibitory effect of CYP3A4 activity*

The inhibitory effect of curcumin on CYP3A4 activity is shown in Fig. 1. Curcumin inhibited CYP3A4 activity and the 50% inhibition concentration (IC_{50}) value of curcumin on CYP3A4 activity is 2.7 μM.

2.2. *Rhodamine-123 retention assay*

As shown in Fig. 2, accumulation of rhodamine-123, a P-gp substrate, was raised in MCF-7/ADR cells over-expressing P-gp compared with that in MCF-7 cells lacking P-gp. The concurrent use of curcumin enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner and showed a statistically significant ($P < 0.01$) increase over the concentration range of 10 μM. This result suggested that curcumin significantly inhibited P-gp activity.

2.3. *Effect of curcumin on the pharmacokinetics of oral tamoxifen*

Mean arterial plasma concentration-time profiles of tamoxifen following oral administration of tamoxifen ($9\,mg \cdot kg^{-1}$) to rats in the presence or absence of curcumin (0.5, 2.5 and $10\,mg \cdot kg^{-1}$) are shown in Fig. 3, the corresponding pharmacokinetic parameters are shown in Table 1. The presence of curcumin significantly altered the pharmacokinetic parameters of tamoxifen.

Table 1: Mean (± S.D.) pharmacokinetic parameters of tamoxifen after the oral administration of tamoxifen ($9\,mg \cdot kg^{-1}$) to rats in the presence or absence of curcumin

Parameter	Control	Tamoxifen + Curcumin		
		$0.5\,mg \cdot kg^{-1}$	$2.5\,mg \cdot kg^{-1}$	$10\,mg \cdot kg^{-1}$
$AUC_{0-\infty}$ ($ng{\cdot}h{\cdot}ml^{-1}$)	1832 ± 359	2051 ± 418	2439 ± 494*	3004 ± 558**
C_{max} ($ng{\cdot}ml^{-1}$)	126 ± 22	146 ± 29	175 ± 33*	215 ± 41**
T_{max} (h)	1.17 ± 0.41	1.33 ± 0.52	1.33 ± 0.52	1.33 ± 0.52
$t_{1/2}$ (h)	11.3 ± 2.76	11.7 ± 2.86	11.9 ± 2.84	12.4 ± 3.12
A.B. (%)	20.4 ± 4.52	22.9 ± 4.77	27.2 ± 5.02*	33.5 ± 5.80**
R.B. (%)	100	112	133	164

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach; $t_{1/2}$: terminal half-life; A.B.: absolute bioavailability; R.B.: relative bioavailability
* $P < 0.05$, ** $P < 0.01$, significant difference compared to the control

Compared to the control group (given oral tamoxifen alone), curcumin significantly ($P < 0.05$ for $2.5\,mg \cdot kg^{-1}$; $P < 0.01$ for $10\,mg \cdot kg^{-1}$) increased the $AUC_{0-\infty}$ and the C_{max} of tamoxifen by 33.1–64.0% and 38.9–70.6%, respectively. Consequently, the absolute bioavailability (A.B.) of tamoxifen in the presence of curcumin (2.5 and $10\,mg \cdot kg^{-1}$) was 27.2–33.5%, which was significantly enhanced ($P < 0.05$ for $2.5\,mg \cdot kg^{-1}$; $P < 0.01$ for $10\,mg \cdot kg^{-1}$) compared to that in the oral control group (20.4%). The relative bioavailability (R.B.) of tamoxifen was 1.12- to 1.64-fold greater than that in the control group. However, there were no significant differences in T_{max} and the $t_{1/2}$ of tamoxifen in the presence of curcumin.

2.4. *Effect of curcumin on the pharmacokinetics of 4-hydroxytamoxifen*

Mean plasma concentration-time profiles of 4-hydroxytamoxifen after oral administration of tamoxifen ($9\,mg \cdot kg^{-1}$) to rats in the presence or absence of curcumin (0.5, 2.5 and $10\,mg \cdot kg^{-1}$) are shown in Fig. 4, while the correlated pharacokinetic parameters are shown in Table 2. The metabolite-parent AUC ratio (MR) was significantly ($P < 0.05$ for $10\,mg \cdot kg^{-1}$ of curcumin) decreased in the presence of curcumin compared with that in the control group, indicating that curcumin may effectively inhibit the CYP3A4-mediated metabolism of tamoxifen in the small intestine and/or in the liver. These results suggest that the production of 4-hydroxytamoxifen was considerably inhibited by curcumin. The AUC, C_{max}, $t_{1/2}$ and T_{max} of 4-hydroxytamoxifen were not significantly altered by the presence of curcumin.

2.5. *Effects of curcumin on the pharmacokinetics of intravenous tamoxifen*

The mean arterial plasma concentration–time profiles of tamoxifen following the intravenous administration of tamoxifen ($2\,mg \cdot kg^{-1}$) in the absence and presence of curcumin (2.5 and $10\,mg \cdot kg^{-1}$) are shown in Fig. 5. The relevant pharmacokinetic parameters are listed in Table 3. The plasma concentrations of tamoxifen declined in a poly-exponential fashion in all rats studied. The pharmacokinetic parameters of intravenous tamoxifen listed in Table 3 were comparable among three groups of rats, suggesting that the effects of oral curcumin on the pharmacokinetics of intravenous tamoxifen were almost negligible.

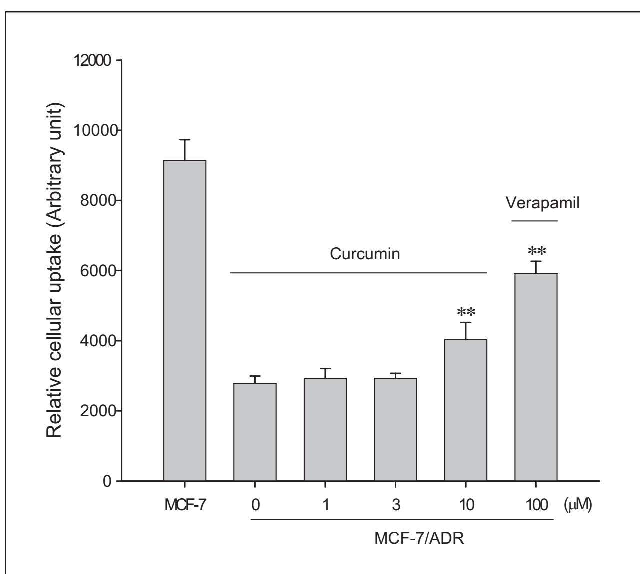


Fig. 2: Rhodamine-123 retention. MCF-7/ADR cells were preincubated with curcumin for 30 min and incubation of MCF-7/ADR cells with 20 µM R-123 for 90 min. Verapamil (100 µM) was used as a positive control. The values were divided by total protein contents of each sample. Data represents mean ± SD of 6 separate samples (significantly different from control MCF-7, ** $P < 0.01$)

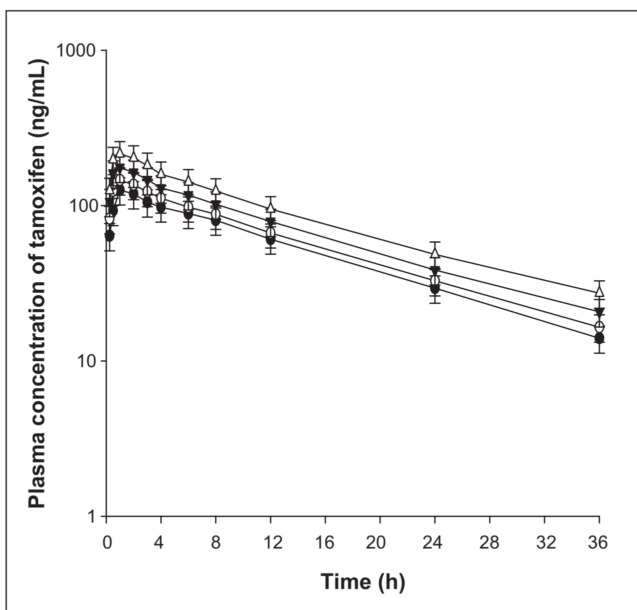


Fig. 3: Mean plasma concentration-time profiles of tamoxifen after oral ($9\,mg \cdot kg^{-1}$) administration of tamoxifen to rats in the presence or absence of curcumin (0.5, 2.5 and $10\,mg \cdot kg^{-1}$) ($n = 6$, each). Bars represent the standard deviation. (●) Oral administration of tamoxifen ($9\,mg \cdot kg^{-1}$); (○) the presence of $0.5\,mg \cdot kg^{-1}$ of curcumin; (▼) the presence of $2.5\,mg \cdot kg^{-1}$ of curcumin; (△) the presence of $10\,mg \cdot kg^{-1}$ of curcumin

Table 2: Mean (± S.D.) pharmacokinetic parameters of 4-hydroxytamoxifen after the oral administration of tamoxifen (9 mg · kg^{-1}) to rats in the presence or absence of curcumin

Parameter	Control	Tamoxifen + Curcumin		
		0.5 mg · kg^{-1}	2.5 mg · kg^{-1}	10 mg · kg^{-1}
$AUC_{0-\infty}$ (ng · h · ml^{-1})	284 ± 62	301 ± 66	315 ± 70	334 ± 74
C_{max} (ng · ml^{-1})	13.3 ± 2.74	13.5 ± 3.49	13.7 ± 3.51	13.9 ± 3.52
T_{max} (h)	2.17 ± 0.42	2.33 ± 0.52	2.33 ± 0.52	2.33 ± 0.52
$t_{1/2}$ (h)	15.3 ± 3.66	15.9 ± 4.18	16.4 ± 4.21	16.8 ± 4.23
MR (%)	15.5 ± 3.16	14.7 ± 3.05	12.9 ± 2.41	11.1 ± 2.14*

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

Table 3: Mean (± S.D.) pharmacokinetic parameters of tamoxifen after an intravenous administration of tamoxifen (2 mg · kg^{-1}) to rats in the presence or absence of curcumin

Parameters	Control	Tamoxifen + Curcumin	
		2.5 mg · kg^{-1}	10 mg · kg^{-1}
$AUC_{0-\infty}$ (ng · h · ml^{-1})	1792 ± 326	1898 ± 371	1998 ± 406
CL_t (mL · min^{-1} · kg^{-1})	17.9 ± 3.32	16.9 ± 3.19	16.2 ± 3.10
$t_{1/2}$ (h)	9.0 ± 1.56	9.1 ± 1.63	9.2 ± 1.68
K_{el}(h^{-1})	0.077 ± 0.015	0.076 ± 0.013	0.075 ± 0.013
R.B. (%)	100	106	111

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; CL_t: total body clearance; $t_{1/2}$: terminal half-life; K_{el}: elimination rat constant; R.B.: relative bioavailability

3. 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 (Cummins et al. 2002; Wolozin et al. 2000). 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 herbal products-drug interactions when one agent decreases or increases the bioavailability and absorption rate constant of a concurrently administered drug (Kaminsky and Fasco 1991; Kim et al. 2010; Shin et al. 2006; Shin and Choi 2009).

Tamoxifen and its primary metabolites undergo extensive oxidation, principally by CYP3A4 and CYP2C9 (Crewe et al. 1997; Mani et al. 1993). Tamoxifen and its metabolites, N-desmethyltamoxifen and 4-hydroxytamoxifen, are substrates for the efflux of P-gp as well (Gant et al. 1995; Rao et al. 1994).

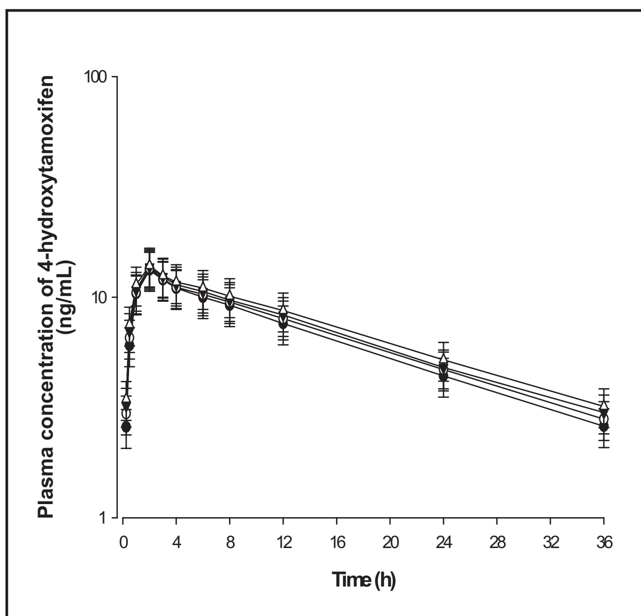


Fig. 4: Mean plasma concentration-time profiles of 4-hydroxytamoxifen after an oral (9 mg · kg^{-1}) administration of tamoxifen to rats in the presence or absence of curcumin (0.5, 2.5 and 10 mg · kg^{-1}) ($n = 6$, each). Bars represent the standard deviation. (●) Oral administration of 4-hydroxytamoxifen (9 mg · kg^{-1}); (○) the presence of 0.5 mg · kg^{-1} of curcumin; (▼) the presence of 2.5 mg · kg^{-1} of curcumin; (Δ) the presence of 10 mg · kg^{-1} of curcumin

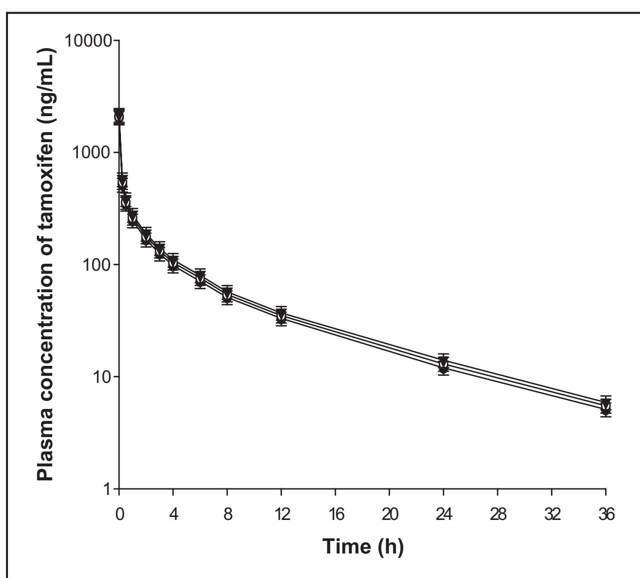


Fig. 5: Mean plasma concentration-time profiles of tamoxifen after an intravenous (2 mg · kg^{-1}) administration of tamoxifen to rats in the presence or absence of curcumin (2.5 and 10 mg · kg^{-1}) ($n = 6$, each). Bars represent the standard deviation. (●) Intravenous administration of tamoxifen (2 mg · kg^{-1}); (○) the presence of 2.5 mg · kg^{-1} of curcumin; (▼) the presence of 10 mg · kg^{-1} of curcumin

CYP3A and P-gp inhibitors might interact with tamoxifen and its metabolites and thus contribute to substantial alteration of their pharmacokinetics. Curcumin is a popular herbal product marketed to treat or prevent cancer in various tissues (Singh et al. 1998; Singletary et al. 1998; Rao et al. 1995; Tanaka et al. 1994). Despite its popularity, limited information is available on the safety, interactions with other drugs, or the mechanisms of interactions of curcumin. As shown in Fig. 1, curcumin inhibited CYP3A4 activity with an IC_{50} value of 2.7 μM. The cell-based P-gp activity test using rhodamine-123 also showed that curcumin (10 μM, $P < 0.01$) significantly inhibited P-gp activity (Fig. 2). The phase I and phase II metabolizing enzymes are expressed with P-gp 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 P-gp may have a large impact on the pharmacokinetics of those compounds. Since curcumin may competitively inhibit P-gp and CYP3A4 this study examined the effect of curcumin on the bioavailability and pharmacokinetics of tamoxifen.

As CYP3A9 in rats is corresponds to the ortholog of CYP3A4 in humans (Kelly et al. 1999), rat CYP3A2 is similar to human CYP3A2 (Bogaards et al. 2000; Guengerich et al. 1986). Human CYP2C9 and 3A4 and rat CYP2C11 and 3A1 have 77 and 73% protein homology, respectively (Lewis 1996). Rats were selected as an animal model in this study to evaluate the potential pharmacokinetic interactions mediated by CYP3A4, although there should be some difference in enzyme activity between rat and human (Cao et al. 2006). The presence of curcumin (2.5 and 10 mg · kg^{-1}) significantly increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen (Table 1). Since orally administered tamoxifen is a substrate for CYP3A4-mediated metabolism and P-gp-mediated efflux in the intestine and/or in the liver, curcumin might be effective to obstruct this metabolic pathway. These results are consistent with a report by Shin et al. (2006) where the oral coadministration of quercetin increased the C_{max} and the AUC of tamoxifen in rats and with a report by Kim et al. (2010) where the presence of silybinin significantly increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen, a P-gp and CYP3A substrate, in rats. This is also supported by the finding that the presence of epigallocatechin gallate significantly enhanced the oral bioavailability of tamoxifen in rats (Shin and Choi 2009).

Curcumin did not increase the $AUC_{0-\infty}$ of 4-hydroxytamoxifen compared to the control group. However, these results are not consistent with reports by Kim et al. (2010) and Shin et al. (2009) showing that silybinin significantly increased AUC of 4-hydroxytamoxifen in rats. The metabolite-parent AUC ratio (MR) was significantly reduced in the presence of curcumin (Table 2), this result suggested that curcumin was capable of altering the production of 4-hydroxytamoxifen, which is mainly formed by CYP3A4 (Crewe HK et al. 1997; Mani et al. 1993). These results are consistent with Shin et al. and Kim et al. who reported that quercetin, silybinin and epigallocatechin gallate significantly decreased MR of tamoxifen, a P-gp and CYP3A substrate, in rats. (Kim et al. 2010; Shin et al. 2006; Shin and Choi 2009)

We selected two concentrations (2.5 and 10 mg · kg^{-1} of curcumin) because those significantly increased the AUC of oral tamoxifen. In Table 3, the pharmacokinetic parameters of intravenous tamoxifen are compared among three groups of rats data, suggesting that the effects of oral curcumin on the pharmacokinetics of intravenous tamoxifen were almost negligible.

Those studies in conjunction with our present findings suggest that the combination of tamoxifen and CYP3A4 and P-gp inhibitors may result in a significant pharmacokinetic drug interaction. Therefore, the enhanced bioavailability of tamoxifen may be mainly due to inhibition of the CYP3A4-mediated metabolism in the liver and/or in the intestine and to inhibition of the P-gp efflux transporter in the small intestine by curcumin. Although being potentially an adverse effect, this interaction may provide a therapeutic benefit whereby it enhances bioavailability and lowers the dose administered. The present study raises awareness about potential drug interactions with concomitant use of curcumin and tamoxifen, but further evaluation in clinical studies is necessary.

In conclusion, the presence of curcumin enhanced the oral bioavailability of tamoxifen in rats. The enhanced bioavalability of tamoxifen may be mainly due to inhibition of the CYP3A4-mediated metabolism of tamoxifen in the intestinal and/or in the liver and to inhibition of the P-gp efflux transporter in the small intestine rather than to reduction of renal elimination of tamoxifen by curcumin. If the results obtained from the rats' model is confirmed in clinical trials, the tamoxifen dose should be adjusted for potential drug interactions when tamoxifen is used with curcumin.

4. Experimental

4.1. Chemicals and apparatus

Tamoxifen, 4-hydroxytamoxifen, curcumin and butylparaben (*p*-hydroxybenzoic acid *n*-butyl ester) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were acquired from Merck Co. (Darmstadt, Germany). All other chemicals in this study were of reagent grade and were used without further purification. Rhodamine was from Calbiochem (USA), the CYP inhibition assay kit was from GENTEST (Woburn, MA, US).

Apparatus used in this study included 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 Bransonic® 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).

4.2. 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.

4.3. Drug administration

The rats were divided into seven groups ($n = 6$ each group): an oral control group (9 mg · kg^{-1} of tamoxifen, dissolved in distilled water, 3.0 ml · kg^{-1}) without or with 0.5, 2.5 or 10 mg · kg^{-1} of curcumin (mixed in distilled water, 3.0 ml · kg^{-1}), and an i.v. group (2 mg · kg^{-1} of tamoxifen, dissolved in 0.9% NaCl solution, 1.5 ml · kg^{-1}) without or with 2.5 or 10 mg · kg^{-1} of curcumin (mixed in distilled water, 3.0 ml · kg^{-1}). Oral tamoxifen was administered intragastrically using a feeding tube, and curcumin was administered in the same manner 30 min prior to the oral administration of tamoxifen. Tamoxifen for i.v. administration was injected through the femoral vein within 1 min and curcumin was administered in the same manner 30 min prior to the i.v. administration of tamoxifen. 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 the i.v. group), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 36 h 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 analysed by HPLC.

4.4. HPLC Analysis

The plasma concentrations of tamoxifen and 4-hydroxytamoxifen were determined by HPLC using a method reported by Fried et al. (1994) after

a slight modification. 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 vigorously 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. Chromatographic separations were achieved using a Symmetry® C_{18} column (4.6 × 150 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^{-1}. Chromatography was performed at a temperature of 30 °C regulated by an HPLC column temperature controller. The fluorescence detector was operated at 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 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 of 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^{-1} and 0.5 ng · ml^{-1}, with coefficients of variation below 4.5 and 1.5%, respectively.

4.5. CYP Inhibition assay

The assays of inhibition on human CYP3A4 enzyme activities were performed in multiwell plates using CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously (Crespi et al. 1997). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates (7-BFC for CYP3A4) were incubated with or without curcumin in enzyme/substrate 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^{-1} glucose 6-phosphate dehydrogenase and 3.3 mM $MgCl_2$) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding a stop solution after the 45-min incubation. Metabolite concentrations were measured by a spectrofluorometer (Molecular Device, Sunnyvale, CA, USA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 µM ketoconazole for CYP3A4) was run on the same plate and produced 99% inhibition. All experiments were carrid out in duplicate, and the results are expressed as the percentage of inhibition.

4.6. 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 salt solution and the cells were incubated at 37 °C for 30 min. After the cells were incubated with 20 µM in the presence of curcumin (0, 1, 3 and 10 µM) for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured at excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and are presented as the ratio to controls.

4.7. 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 and 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 time to reach peak plasma concentration (T_{max}) of tamoxifen and 4-hydroxytamoxifen in plasma was 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 infinity ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. The absolute bioavailability (A.B.) was calculated by $AUC_{oral}/AUC_{i.v.} \times Dose_{i.v.}/Dose_{oral}$, and the relative bioavailability (R.B.) was calculated by $AUC_{control}/AUC_{with\ curcumin}$. The metabolite-parent ratio (MR) was estimated by ($AUC_{4\text{-hydroxytamoxifen}}/AUC_{tamoxifen}$) × 100.

4.8. Statistical analysis

Statistical analysis was conducted using 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 ± S.D.).

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