RESEARCH ARTICLE

Arch Pharm Res Vol 31, No 12, 1631-1636, 2008
DOI 10.1007/s12272-001-2161-7

Archives of Pharmacal Research
www.springer.com/12272

Enhanced Tamoxifen Bioavailability after Oral Administration of Tamoxifen in Rats Pretreated with Naringin

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(Received January 10, 2008/Revisee May 20, 2008/Accepted November 15, 2008)

The aim of this study was to investigate the effect of naringin on the bioavailability and pharmacokinetics of tamoxifen and of its metabolite, 4-hydroxytamoxifen in rats. The pharmacokinetic parameters of tamoxifen and 4-hydroxytamoxifen were determined by HPLC after pretreating with naringin (1.5, 7.5, and 15 mg/kg) 30 min before orally administering tamoxifen (10 mg/kg). Compared with the control group (treated with tamoxifen alone), naringin pretreated animals showed significantly ($p<0.01$) increased areas under the plasma concentration-time curves (AUC) and peak tamoxifen concentrations (C_{max}). The absolute bioavailabilities (AB%) of tamoxifen in naringin pretreated animals were enhanced versus control (from 32.8% to 47.1%), and the relative bioavailabilities (RB%) of tamoxifen in the naringin pretreated groups were 2.02-2.88 times higher than that in the control. No significant changes in the terminal half-life ($t_{1/2}$) or T_{max} of tamoxifen were observed in the naringin pretreated groups. The AUCs of 4-hydroxytamoxifen after pretreating naringin were also significantly elevated ($p<0.05$) versus the control. But metabolite ratios (MR; AUC of 4-hydroxytamoxifen to tamoxifen) were significantly lower. These results suggest that the enhanced bioavailability of tamoxifen in the presence of naringin might be due to the inhibition of CYP3A4 by naringin. If the results of this study are further confirmed by clinical trials, tamoxifen dosages should be adjusted to avoid potential drug interaction when tamoxifen is used clinically in combination with naringin-containing dietary supplements.

Key words: Tamoxifen, 4-Hydroxytamoxifen, Naringin, Bioavailability, Pharmacokinetics

INTRODUCTION

Tamoxifen as a nonsteroidal antiestrogen, is the agent of choice for the treatment and prevention of estrogen receptor-positive breast cancer (Stone, 1992; Powles, 1992; Jaiyesimi et al., 1995). Although, tamoxifen has relatively low toxicity as compared with other chemotherapeutic agents, the drug could increase the incidences of endometrial cancer and thromboembolic disease (Fornander et al., 1993; Meier et al., 1998).

Tamoxifen undergoes extensive hepatic metabolism and subsequently its metabolites are excreted in bile (Buckley and Goa, 1989). In humans, its major biotransformation pathway proceeds via *N*-demethylation catalyzed by CYP3A enzymes (Jacolot et al., 1991; Mani et al., 1993). Another important drug metabolite, 4-hydroxytamoxfen, is formed by CYP2D6, CYP2C9, CYP2E1, and CYP3A4 in humans (Mani et al., 1993; Crewe et al., 1997). Although plasma and tumor concentrations of 4-hydroxytamoxifen are only about 2% of those of the parent compound (Daniel et al., 1981), this metabolite is about 100 times more potent at inhibiting estrogen receptors than tamoxifen (Jordan et al., 1977). Tamoxifen and its metabolites are the substrates of P-glycoprotein (P-gp) (Gant et al., 1995; Rao et al., 1994), a membrane transporter that actively pumps xenobiotics from cells. P-gp is found in secretory epithelial tissues including the brush border of renal proximal tubules, the canalicular membranes in the liver, and the apical membranes lining the gut (Thiebaut et al., 1987). In the small intestine, P-gp is frequently observed to be co-localized at the apical membrane of cells with CYP3A4 (Gottesman et al., 1993). P-gp and CYP3A4 may act synergistically on the presystemic drug metabolism (Watkins, 1996; Wacher et al., 1998) and make the substrate of P-gp circulate between the lumen and epithelial cells, which lead to prolonged exposure of the substrate to CYP3A4 and finally reduced drug absorption.

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Flavonoids represent a group of phytochemicals and are found in many plants (Dixon et al., 1999). They exhibit a wide range of biological activities, i.e., they act as antioxidants, radical scavengers, and anti-inflammatory agents (Nijveldt et al., 2001). Grapefruit is normal dietary component in many countries, where it is usually consumed as juice, and naringin (4',5,7-trihydroxy-flavanone-7-rhamnoglucoside) is the predominant flavonoid in grapefruit juice. Naringin is rapidly transformed into naringenin by bacteria to produce α-rhamnosidase and β-glucosidase or endo-b-glucosidase in the human intestine (Kim et al., 1998). Moreover, the oral bioavailability of quinine was found to be significantly increased after pretreating rats with naringin, and this was associated with the inhibition of CYP3A4 (Zhang et al., 2000). Naringin or naringenin reduced the apical efflux of vinblastine, a substrate of P-gp in Caco-2 cells (Takanaga et al., 1998), and also inhibited CYP3A4-mediated saquinavir metabolism and modulated its P-gp transport in Caco-2 cells (Eagling et al., 1999). Hence, naringin is believed to be a natural inhibitor of CYP3A4 and P-gp.

The oral bioavailability of tamoxifen is mainly affected by CYP3A4, a first-pass metabolizing enzyme, and P-gp may obstruct the absorption of tamoxifen in the intestinal membrane. Hence, it might be expected that the pharmacokinetics of tamoxifen could be changed by naringin. The purpose of this study was to investigate pharmacokinetic changes of tamoxifen and 4-hydroxytamoxifen after the oral administration of tamoxifen in rats pretreated with naringin.

MATERIALS AND METHODS

Materials and apparatus

Tamoxifen citrate salt, 4-hydroxytamoxifen, naringin (4',5,7-trihydroxy-flavanone-7-rhamnoglucoside), and butyl paraben (p-hydroxybenzoic acid n-butyl ester) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol were acquired from Merck Co. (Darmstadt, Germany). All other chemicals were of reagent grade and were used without further purification.

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

Animal experiments and drug administration

The female Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Eumsung, Korea), and were given access to a normal standard chow diet (JaeIIChow, Korea) and tap water *ad libitum*. Throughout the experiment, the animals were housed, three per cage, in laminar flow cages maintained at 22±2°C, 50-60% relative humidity, under a 12 h light-dark cycle. The animals were kept in these facilities for at least one week prior to the experiment. All experiments were 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; control group (tamoxifen 10 mg/kg, oral), three pretreatment groups (1.5, 7.5 or 15 mg/kg of naringin, where animals were orally pretreated 0.5 h prior to oral tamoxifen administration), and i.v. group (intravenous administration of 2.0 mg/kg tamoxifen).

Rats were fasted for at least 24 h prior to experiments and given free access to water. Each rat was anaesthetized with diethyl ether. Right femoral arteries were cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, USA) for blood sampling.

A tamoxifen dose of 10 mg/kg was chosen to keep plasma concentrations above the limit of HPLC detection. As naringin is the predominant constituent in grapefruit juice, and is present at concentrations up to 1,200 mg/L (Bailey et al., 1998; Ho et al., 2000), 7.5 mg/kg of naringin was taken as a normal human daily consumption level. Thus, naringin dosages were selected at the lower (1.5 mg/kg), normal (7.5 mg/kg), and higher (15 mg/kg) levels. Naringin solutions were prepared by dissolving naringin (1.5, 7.5, 15 mg/kg) in distilled water (1 mL), and administered *p.o.* 0.5 h prior to tamoxifen administration. Tamoxifen was dissolved in 1.5 mL of distilled water for oral administration. Tamoxifen saline solution (for i.v. injection; 0.2 mL) was injected through the femoral vein in 1 min.

Blood samples (0.5 mL) were withdrawn from the femoral artery either 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, and 36 h after i.v. injection of tamoxifen or 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, and 36 h after oral administration of tamoxifen, and centrifuged at 13,000 rpm for 5 min. Plasma samples (0.2 mL) so obtained were stored at -40°C until analyzed by HPLC.

HPLC assay

The plasma concentrations of tamoxifen were determined by HPLC assay using a modification of the method described by Fried et al. (1994). Briefly, 0.05 mL of butyl paraben (8 μg/mL, dissolved in methanol), as the internal standard, and 0.2 mL of acetonitrile were added to 0.2 mL of a plasma sample and then vortexed for 2 min. The

samples were then centrifuged at 13,000 rpm for 10 min, and supernatants (0.05 mL) were 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; and the column (Symmetry® C_{18}; 4.6×150 mm, 5 mm, Waters Co., Ireland) was operated at a temperature of 30ºC. The mobile phase consisted of 20 mM of 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 self-made post-column photochemical reactor containing a bactericidal ultraviolet lamp (Sankyo Denki Co, Japan) was used to convert tamoxifen and 4-hydroxytamoxifen to fluorophors. Standard curves were prepared by adding known concentrations of tamoxifen and 4-hydroxytamoxifen to drug-free rat plasma using 4-hydroxytamoxifen concentrations of 0.5, 1, 2, 5, 10, and 20 ng/mL, and tamoxifen concentrations of 10, 20, 50, 100, 200, and 500 ng/mL. Tamoxifen, 4-hydroxytamoxifen and internal standard peaks were clearly resolved, and no endogenous peak interfered with these emissions. The retention times of 4-hydroxytamoxifen, internal standard and tamoxifen were 7.3, 14.7, and 26.6 min, respectively.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using the LAGRAN computer program (Yamaoka et al., 1981), which uses the LARGAN method to calculate AUCs of plasma concentration (C_p) versus time (t). Maximum plasma concentrations (C_{max}) and times to reach maximum plasma concentrations (T_{max}) were determined by visually inspecting the experimental data. Elimination rate constant (K_{el}) was calculated from slopes by regression analysis, and the half-life ($t_{1/2}$) of the drug was obtained using $0.693/K_{el}$. The absolute bioavailability of tamoxifen after the oral administration (10 mg/kg) compared to the intravenous administration (2.0 mg/kg) was calculated as follows:

$$\text{Absolute bioavailability (AB\%)} = \frac{AUC_{oral}}{AUC_{iv}} \times \frac{\text{IV dose}}{\text{Oral dose}} \times 100$$

The relative bioavailability of tamoxifen administered orally was calculated as follows:

$$\text{Relative bioavailability (RB\%)} = \frac{AUC_{coadmin.}}{AUC_{control}} \times 100$$

Statistical analysis

Data are presented as means and standard deviations (mean±S.D.). Pharmacokinetic parameters were compared by one-way analysis of variance, followed by a posteriori testing using the Dunnett's correction. P values of < 0.05 or 0.01 were considered statistically significant.

RESULTS AND DISCUSSION

Orally administered tamoxifen is metabolized by CYP3A4 both in the human liver and small intestine (Jacolot et al., 1991; Mani et al., 1993; Crewe et al., 1997), and the absorption of tamoxifen in intestinal mucosa is inhibited by the P-gp efflux pump (Gant et al., 1995; Rao et al., 1994). Both CYP3A4, the major phase I drug metabolizing enzyme in human, and the multidrug efflux pump, P-gp, are present at high levels in the small intestine, the primary site of absorption for orally administered drugs. Moreover, these proteins have a broad substrate overlap, suggesting that they act synergistically during the first-pass metabolism (Watkins, 1996; Wacher et al., 1998). Hence, dual inhibitors against both CYP3A4 and P-gp should have a great impact on the bioavailabilities of many drugs. Since naringin inhibits the activities of P-gp and CYP3A4 (Ho et al., 2001; Takanaga et al., 1998; Eagling et al., 1999), we investigated the influence of naringin, a naturally occurring flavonoid, on the pharmacokinetics of tamoxifen in rats to examine potential drug interactions between naringin and tamoxifen.

After tamoxifen had been orally administered to rats in the presence or absence of naringin, we characterized its plasma concentration-time profiles (Fig. 1). As shown in

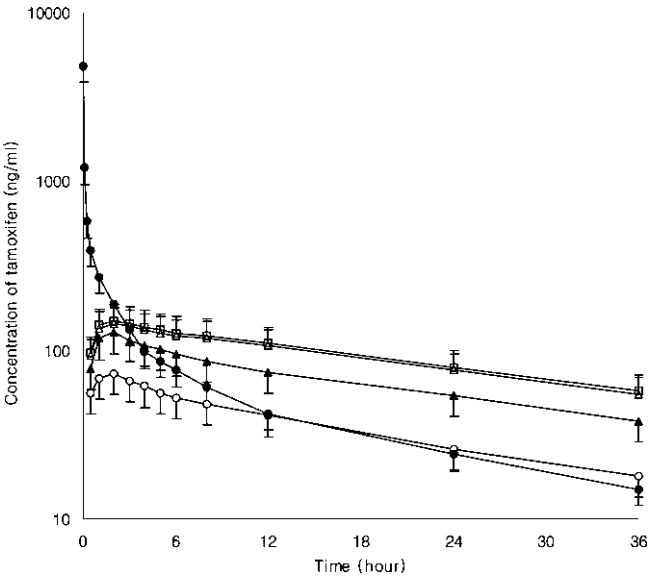

Fig. 1. Mean plasma concentration-time profiles of tamoxifen following the oral administration of tamoxifen (10 mg/kg) with/without naringin pretreatment in rats. Bars represent standard deviations. (n=6). (○) Tamoxifen control, (▲) Pretreated with naringin 1.5 mg/kg, (△) Pretreated with naringin 7.5 mg/kg, (□) Pretreated with naringin 15 mg/kg, (●) Tamoxifen I.V. 2.0 mg/kg.

Table I, the areas under the plasma concentration-time curve (AUC) and the peak concentrations (C_{max}) of tamoxifen in the naringin (1.5-15 mg/kg)-treated groups were significantly ($p<0.01$) higher than in the control group. This result appeared to be consistent with that of a previous study by Zhang et al., in which naringin led to an increase in the C_{max} and AUC of quinine, the substrate of CYP3A4 and P-gp (Zhang et al., 2000). Although naringin obviously enhanced the AUC and C_{max} of tamoxifen when the animals were treated with various doses of naringin (from 1.5 mg/kg to 15 mg/kg), we failed to detect dose-dependency. 7.5 mg/kg of naringin seems to be saturated to exert its inhibition potency against CYP3A4 and p-glycoprotein. We recently reported that verapamil has the same pattern of pharmacokinetics as tamoxifen by concomitant administration of naringin to rabbits (Yeum and Choi, 2006).

The terminal half-life ($t_{1/2}$) and the time required to reach the peak concentration (T_{max}) were not significantly altered by naringin. Consequently, the absolute bioavailability (AB%) of tamoxifen in the presence of naringin is remarkably enhanced (32.8-47.1%) compared to the control (16.3%), and the relative bioavailability (RB%) of tamoxifen was also increased by 2.02- to 2.88-fold by naringin pretreatment. In the present study, we found that the $t_{1/2}$ of orally administered tamoxifen was longer than that of i.v. injected tamoxifen (Table I). Longer half-life after oral administration was maybe due to flip-flop phenomenon between k_a and k_e. Considering poor solubility of tamoxifen in water, delayed absorption in gastrointestinal lumen might be the reason for flip-flop phenomenon, and this caused the longer half-life after oral administration compared with that after iv administration. Buchanan et al. also recently reported longer half-life after oral administration in rats (Buchanan et al., 2006). We also previously showed that terminal half-life of tamoxifen in the oral administered group was longer than the i.v. group (19.9 h vs 15.9 h) (Shin et al., 2006).

The plasma concentration-time profiles of 4-hydroxytamoxifen are shown in Fig. 2, and the pharmacokinetic parameters of 4-hydroxytamoxifen are summarized in Table II. The AUC of 4-hydroxytamoxifen in naringin pretreated animals were significantly ($p<0.05$) higher than in the control group, RB%'s of 4-hydroxytamoxifen were

Table I. Pharmacokinetic parameters of orally administered tamoxifen (10 mg/kg) in the presence or absence of naringin (1.5, 7.5 or 15 mg/kg) in rats

Parameters	Tamoxifen Control	Naringin			i.v. (2 mg/kg)
		1.5 mg/kg	7.5 mg/kg	15 mg/kg	
AUC (ng/mL·h)	1879±468	3708±927*	5307±1361*	5421±763*	2304±551
C_{max} (ng/mL)	73±18	127±32*	143±35*	149±38*	
T_{max} (h)	2	2	2	2	
$t_{1/2}$ (h)	20.9±5.11	24.3±6.2	25.3±6.4	25.0±6.3	11.0±2.8
AB (%)	16.3	32.8	46.0	47.1	100
RB (%)	100	202	282	288	

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; $t_{½}$: terminal half-life; AB(%): absolute bioavailability; RB(%): relative bioavailability; compared $AUC_{pretreated}$ to $AUC_{control}$.
* $p<0.01$ significant difference compared to control.

Table II. Pharmacokinetic parameters of 4-hydroxytamoxifen after administering tamoxifen (10 mg/kg, *p.o.*) with/without naringin (1.5, 7.5 or 15 mg/kg) pretreatment

Parameters	Tamoxifen Control	Naringin		
		1.5 mg/kg	7.5 mg/kg	15 mg/kg
AUC (ng/mL·h)	298±82	407±129*	437±135*	454±137*
C_{max} (ng/mL)	9.6±2.4	13.0±3.4	13.9±3.6*	14.5±3.1*
T_{max} (h)	3.6	4.4	4.4	4.5
$t_{1/2}$ (h)	24±5.8	25±6.1	26±6.3	25±6.4
RB (%)	100	136	146	152
MR	0.16±0.038	0.11±0.029*	0.08±0.022*	0.08±0.023*

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; $t_{½}$: terminal half-life; RB(%): relative bioavailability; compared $AUC_{pretreated}$ to $AUC_{control}$; MR: metabolite Ratio; compared $AUC_{4\text{-hydoxytamoxifen}}$ to $AUC_{tamoxifen}$.
* $p < 0.05$ versus the control.

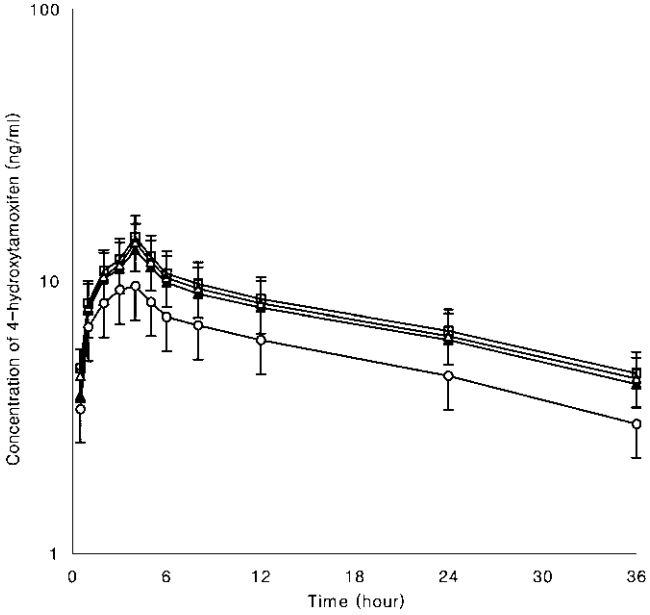

Fig. 2. Mean plasma concentration-time profiles of 4-hydroxytamoxifen following the oral administration of tamoxifen (10 mg/kg) with/without naringin pretreatment in rats. Bars represent the standard deviation (n=6). (○) Tamoxifen control, (▲) Pretreated with naringin 1.5 mg/kg, (△) Pretreated with naringin 7.5 mg/kg, (□) Pretreated with naringin 15 mg/kg.

1.36- to 1.52-fold higher, and the C_{max} of 4-hydroxytamoxifen were also significantly increased ($p<0.05$) in the 7.5 mg/kg and the 15 mg/kg groups. But, the metabolite ratios (MR; AUC of 4-hydroxytamoxifen to tamoxifen) were significantly ($p<0.05$) lower in the naringin pretreated groups. This may have been because orally absorbed naringin exerted its dual inhibitory effect more effectively when pretreated 30 min prior to tamoxifen administration. In summary, naringin pretreatment significantly enhance the bioavailability of tamoxifen presumably via its dual inhibition of CYP3A4 and P-gp. 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 rat model is confirmed in the clinical trials, the tamoxifen dose should be adjusted for potential drug interactions when tamoxifen is used with naringin-containing dietary supplements.

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