Effects of epigallocatechin gallate on the oral bioavailability and pharmacokinetics of tamoxifen and its main metabolite, 4-hydroxytamoxifen, in rats

Sang-Chul Shin[a] and Jun-Shik Choi[b]

The effects of epigallocatechin gallate (EGCG) on the oral bioavailability and pharmacokinetics of tamoxifen and its metabolite, 4-hydroxytamoxifen, were investigated in rats. A single dose of tamoxifen was administered intravenously (2 mg/kg) and orally (10 mg/kg) with or without epigallocatechin (0.5, 3 and 10 mg/kg) to rats. The presence of EGCG significantly altered the pharmacokinetics of orally administered tamoxifen. Compared with the oral control group (given tamoxifen alone), the area under the plasma concentration–time curve and the peak plasma concentration of tamoxifen significantly ($P<0.05$ for 3 mg/kg of EGCG, $P<0.01$ for 10 mg/kg of EGCG) increased 48.4–77.0 and 57.1–89.7%, respectively. Consequently, the absolute bioavailability of tamoxifen in the presence of EGCG (3 and 10 mg/kg) was 48.9–78.1%, which was significantly enhanced ($P<0.05$ for 3 mg/kg of EGCG, $P<0.01$ for 10 mg/kg of EGCG) compared with the oral control group (23.7%). Moreover, the relative bioavailability of tamoxifen was 1.48–1.77-fold greater than that of the control group. EGCG at a dose of 10 mg/kg significantly increased the area under the plasma concentration–time curve ($P<0.05$, 40.3%) of 4-hydroxytamoxifen, but the metabolite–parent ratio of 4-hydroxytamoxifen was also significantly altered ($P<0.05$ for 10 mg/kg of EGCG), implying that the formation of 4-hydroxytamoxifen was considerably affected by EGCG. The increase in bioavailability of tamoxifen is likely to be due to the decrease in first-pass metabolism in the intestine and liver by inhibition of P-glycoprotein and CYP3A by EGCG. The increase in oral bioavailability of tamoxifen in the presence of EGCG should be taken into consideration of potential drug interactions between tamoxifen and EGCG. *Anti-Cancer Drugs* 20:584–588 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

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 CYP3A, and the metabolite, 4-hydroxytamoxifen is mainly catalyzed by CYP3A, CYP2D6 and CYP2C9 [3,4]. 4-Hydroxytamoxifen has shown 30- to 100-fold greater potency than tamoxifen in suppressing estrogen-dependent cell proliferation [5,6]. A secondary metabolite of tamoxifen, endoxifen, exhibits potency similar to 4-hydroxytamoxifen [7,8]. Thus, tamoxifen is referred to as a prodrug that requires activation to exert its effects. Tamoxifen acts as a substrate for P-glycoprotein (P-gp) as well [9,10]. P-gp colocalized with CYP3A in the polarized epithelial cells of excretory organs such as the liver, kidney and intestine [11,12] to eliminate foreign compounds from the body. A substantial overlap in substrate specificity exists between CYP3A4 and P-gp [13]. Thus, a combined role of P-gp and CYP3A could decrease the oral bioavailability of drugs, which are substrates of P-gp and CYP3A, and so the P-gp and CYP3A modulators might be able to improve the oral bioavailability of tamoxifen.

Flavonoids represent a group of phytochemicals that are produced in high quantities by various plants [14]. These compounds exhibit a wide range of beneficial biological activities including antioxidative, radical scavenging, antiatherosclerotic, antitumor and antiviral effects [15]. Epigallocatechin gallate (EGCG), also known as catechins, is the major flavanoid found in green tea [16]. EGCG has a wide range of biological and pharmacological activities, including antioxidant [17], antimutagenic and anticarcinogenic activities [18]. EGCG inhibits human CYP3A4 [19] and P-gp in human Caco-2 cells [20]. Kitagawa *et al.* [21] reported that EGCG inhibited the efflux of P-gp substrates, verapamil and quercetin in KB-C2 cells. The plasma concentration–time profiles of EGCG in rats [22] and humans [23] have previously been reported. Green tea is widely consumed in many countries, particularly Korea, China, and Japan. Thus, it could be expected that EGCG would change the

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pharmacokinetics of drugs, substrate of P-gp and/or CYP3A4, if they were concomitantly used. It has been reported that EGCG dose not impair non-heme iron absorption in humans [24], and that tea inhibits the uptake of [^{3}H]-folic acid and [^{3}H]-methotrexate in caco-2 cells [25]. Green tea is a widely consumed beverage in many countries, but mainly in Korea, China, and Japan. A prospective cohort study of Japanese population revealed that the daily intake of EGCG in green tea in these individuals was calculated to be 500–700 mg [19], which was about 10 mg/kg body weight.

As a dual inhibitor of CYP3A4 and P-gp, EGCG might affect the bioavailability and pharmacokinetics of tamoxifen when EGCG and tamoxifen are used concomitantly for the prevention or therapy of cancer as a combination therapy. However, the possible effects of EGCG on the oral bioavailability and pharmacokinetics of tamoxifen have not been reported *in vivo*. Therefore, the aim of this study was to investigate the oral bioavailability and pharmacokinetics of tamoxifen in the presence of EGCG in rats.

Materials and methods

Materials

Tamoxifen, 4-hydroxytamoxifen, EGCG and butylparaben (*p*-hydroxybenzoic acid n-butyl ester) were purchased from the Sigma-Aldrich (St. Louis, Missouri, USA). High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were acquired from the Merck (Darmstadt, Germany). All other chemicals for this study were of reagent grade and were used without further purification. Apparatus used in this study were an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler and a Waters 474 scanning fluorescence detector (Waters Co., Milford, Massachusetts, USA), an HPLC column temperature controller (Phenomenex Inc., California, USA), a Bransonic Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, Connecticut, USA), a vortex-mixer (Scientific Industries Co., New York, USA), and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments

Male Sprague-Dawley rats (weighing 270–300 g) were purchased from the Dae Han Laboratory Animal Research (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 before the experiments and each animal was anaesthetized lightly with ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, inside diameter 0.58, outside diameter 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and intravenous injection, respectively.

Drug administration

The rats were divided into four groups ($n = 6$, each); an oral control group (10 mg/kg of tamoxifen dissolved in distilled water, 3.0 ml/kg) without or with 0.5, 3, and 10 mg/kg of EGCG (mixed in distilled water, 3.0 ml/kg), and a IV group (2 mg/kg of tamoxifen, dissolved in 0.9% NaCl solution, 1.5 ml/kg). Oral tamoxifen was administered intragastrically using a feeding tube, and epigallocatechin was administered in the same manner 30 min before the oral administration of tamoxifen. Tamoxifen for intravenous administration was injected through the femoral vein within 0.5 min. A 0.45 ml aliquot of blood sample was collected into heparinized tubes from the femoral artery at 0 (to serve as control), 0.017, 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 HPLC analysis.

High-performance liquid chromatography analysis

The plasma concentrations of tamoxifen and 4-hydroxytamoxifen were determined by HPLC using a method reported by Fried *et al.* [26] 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 20 mmol/l dipotassium hydrogen phosphate (pH 3.0, adjusted with phosphoric acid)–acetonitrile (60 : 40, v/v). The flow-rate of the mobile phase was maintained at 1.0 ml/min. Chromatography was performed at a temperature of 30°C that was regulated by an HPLC column temperature controller. The fluorescence detector was operated at an excitation wavelength of 254 nm with an emission wavelength of 360 nm. A homemade postcolumn photochemical reactor was supplied with a bactericidal ultraviolet lamp (Sankyo Denki Co., Tokyo, Japan), and a Teflon tubing (inside diameter 0.01″, outside diameter 1/16″, 2 m long) was crocheted and fixed horizontally with a stainless steel frame under the lamp at a 10 cm distance to convert tamoxifen and 4-hydroxytamoxifen to the fluorophors to increase the 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 and 0.5 ng/ml. The coefficients of variation of tamoxifen and 4-hydroxytamoxifen were below 4.5 and 1.5%, respectively.

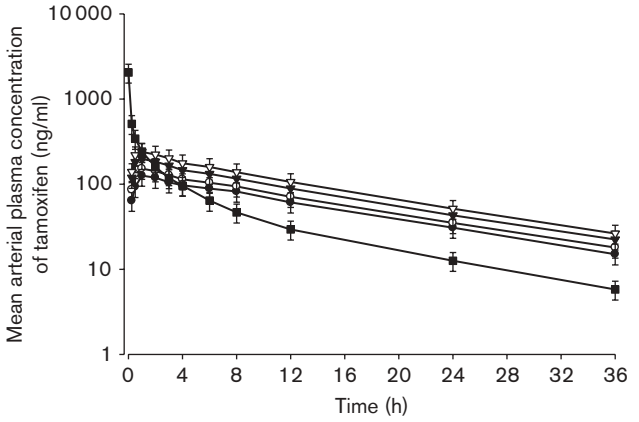
Pharmacokinetic analysis

The plasma concentration data were analyzed by non-compartmental method using WinNonlin software Version 4.1 (Pharsight Co., Mountain View, California, USA). The elimination rate constant (K_{el}) was calculated by log–linear regression of tamoxifen or 4-hydroxytamoxifen concentration data during the elimination phase. The terminal half life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}) of tamoxifen or 4-hydroxytamoxifen in plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration–time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. The absolute bioavailability was calculated by $(AUC_{oral}/AUC_{IV} \times Dose_{iv}/Dose_{oral}) \times 100$ and the relative bioavailability was calculated by $(AUC_{control}/AUC_{with\ EGCG}) \times 100$. The metabolite–parent ratio (MR) was estimated by $(AUC_{4\text{-hydroxytamoxifen}}/AUC_{tamoxifen}) \times 100$.

Statistical analysis

Statistical analysis was conducted using a one-way analysis of variance followed by a posteriori testing with the use of the Dunnett correction. Differences were considered to be significant at a level of P value less than 0.05. All mean values are presented with their standard deviation (mean ± standard deviation).

Fig. 1



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

Results and discussion

Tamoxifen and its primary metabolites undergo extensive oxidation, principally by CYP3A and CYP2D6 [3,4]. Tamoxifen and its metabolites, *N*-desmethyltamoxifen and 4-hydroxytamoxifen, are substrates for the efflux pump of P-gp as well [9,10]. P-gp is found to be expressed with CYP3A4, uridine diphosphate-glucuronosyltransferases [11], which may play the synergistic function in regulating the bioavailability of many orally ingested compounds. EGCG inhibited human CYP3A4 with an half maximal inhibitory concentration value of 10 μmol/l [19], and has an inhibitory effect on P-gp in human Caco-2 cells [20]. It is possible that the concomitant administration of EGCG might affect the oral bioavailability and pharmacokinetics of orally administered tamoxifen.

Effect of epigallocatechin gallate on the pharmacokinetics of tamoxifen

Mean arterial plasma concentration–time profiles of tamoxifen after an intravenous administration of tamoxifen (2 mg/kg), and an oral administration of tamoxifen

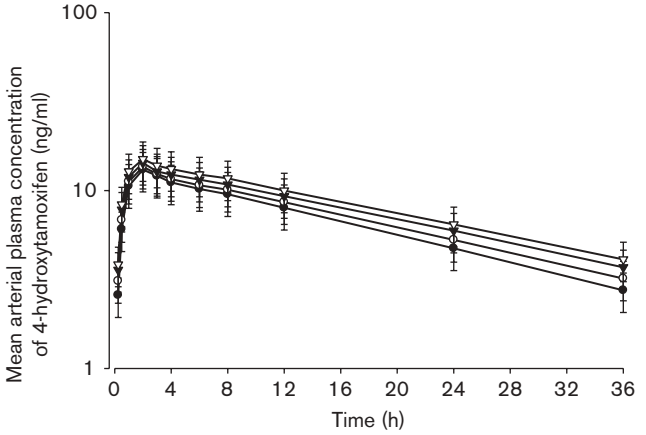
Table 1 Mean (± SD) pharmacokinetic parameters of tamoxifen after the intravenous (2 mg/kg) and oral administration of tamoxifen (10 mg/kg) to the rats in the presence or absence of EGCG (0.5, 3 and 10 mg/kg) ($n=6$, each)

		Tamoxifen + EGCG						
Parameter	Control	0.5 mg/kg	*P* value	3 mg/kg	*P* value	10 mg/kg	*P* value	Intravenous (2 mg/kg)
$AUC_{0-\infty}$ (ng h/ml)	2137 ± 513	2518 ± 802	>0.05	3171 ± 867*	<0.05	3783 ± 898**	<0.01	1799 ± 428
C_{max} (ng/ml)	126 ± 30	152 ± 49.9	>0.05	198 ± 55*	<0.05	239 ± 65**	<0.01	–
T_{max} (h)	1.2 ± 0.4	1.3 ± 0.5	>0.05	1.3 ± 0.5	>0.05	1.3 ± 0.5	>0.05	–
$t_{1/2}$ (h)	11.4 ± 2.8	11.6 ± 2.8	>0.05	11.8 ± 2.8	>0.05	11.9 ± 2.9	>0.05	8.4 ± 2.1
AB (%)	23.7 ± 5.7	28.0 ± 9.0	>0.05	35.3 ± 9.7*	<0.05	42.2 ± 10.2**	<0.01	100
RB (%)	100	118		148		177		–

AB, absolute bioavailability; $AUC_{0-\infty}$, area under the plasma concentration–time curve from 0 h to infinity; C_{max}, peak plasma concentration; EGCG, epigallocatechin gallate; RB, relative bioavailability; SD, standard deviation; T_{max}, time to reach peak plasma concentration; $t_{1/2}$, terminal half life.
*$P<0.05$ and **$P<0.01$, significant difference compared with control.

(10 mg/kg) to rats in the presence or absence of EGCG (0.5, 3 and 10 mg/kg) are shown in Fig. 1; the corresponding pharmacokinetic parameters are shown in Table 1. The presence of EGCG significantly altered the pharmacokinetic parameters of tamoxifen. Compared with the control group (given oral tamoxifen alone), the presence of EGCG significantly ($P < 0.05$ at 3 mg/kg of EGCG, $P < 0.01$ at 10 mg/kg of EGCG) increased area under the plasma concentration–time curve ($AUC_{0-\infty}$) and the C_{max} of tamoxifen 48.4–77.0 and 57.1–89.7%, respectively. The absolute bioavailability of tamoxifen in the presence of EGCG (3 and 10 mg/kg) was 48.9–78.1%, which was significantly enhanced ($P < 0.05$ for 3 mg/kg of EGCG, $P < 0.01$ for 10 mg/kg of EGCG) compared with the oral control group (23.7%), and the relative bioavailability of tamoxifen was 1.48–1.77-fold greater than the control group. There were no significant differences in the T_{max} and the $t_{1/2}$ of tamoxifen in the presence of EGCG.

Fig. 2



Mean plasma concentration–time profiles of 4-hydroxytamoxifen after an oral (10 mg/kg) administration of tamoxifen to the rats in the presence or absence of epigallocatechin gallate (EGCG) (0.5, 3, and 10 mg/kg) ($n = 6$, each). Bars represent the standard deviation; (●) oral administration of tamoxifen (10 mg/kg); (○) the presence of 0.5 mg/kg of EGCG; (▼) the presence of 3 mg/kg of EGCG; (▽) the presence of 10 mg/kg of EGCG.

Consequently, EGCG significantly increased the $AUC_{0-\infty}$ and C_{max} of oral tamoxifen in rats. As orally administered tamoxifen is a substrate for CYP3A-mediated metabolism and P-gp-mediated efflux in the intestine and liver, the presence of EGCG might be effective to obstruct this metabolic pathway. These results are consistent with a report by Li and Choi [27] showing that the presence of EGCG significantly increased the $AUC_{0-\infty}$ and C_{max} of diltiazem, a P-gp and CYP3A substrate, in rats, and a report by Chung *et al.* [28] showing that EGCG significantly increased the $AUC_{0-\infty}$ of verapamil by inhibition of P-gp and CYP3A in rats. Shin *et al.* [29,30] and Piao *et al.* [31] also reported that the presence of flavonoids (quercetin, morin and kaempferol) significantly increased the $AUC_{0-\infty}$ and C_{max} of tamoxifen by inhibition of P-gp and CYP3A in rats.

Effect of epigallocatechin gallate on the pharmacokinetics of 4-hydroxytamoxifen

Mean plasma concentration–time profiles of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) to rats in the presence or absence of EGCG (0.5, 3 and 10 mg/kg) are shown in Fig. 2, while the correlated pharmacokinetic parameters are shown in Table 2. Compared with the control group, the presence of EGCG at a dose of 10 mg/kg significantly ($P < 0.05$) increased the $AUC_{0-\infty}$ (40.3%) of 4-hydroxytamoxifen. MR of 4-hydroxytamoxifen was decreased significantly ($P < 0.05$ for 10 mg/kg of EGCG). These results suggest that the production of 4-hydroxytamoxifen was considerably affected by addition of EGCG. The C_{max}, $t_{1/2}$, and T_{max} of 4-hydroxytamoxifen were not significantly altered by the presence of EGCG.

This result suggested that EGCG was capable of altering the production of 4-hydroxytamoxifen, which is mainly formed by CYP3A and CYP2C9 [3,4]. These results are consistent with Shin *et al.* [29] who reported that quercetin significantly decreased MR of tamoxifen, a P-gp and CYP3A substrate, in rats. However, these results are not consistent with reports by Shin *et al.* [30] and Piao *et al.* [31], showing that morin and kaempferol did

Table 2 Mean (± SD) pharmacokinetic parameters of 4-hydroxytamoxifen after the oral administration of tamoxifen (10 mg/kg) to the rats in the presence or absence of EGCG (0.5, 3 and 10 mg/kg) ($n = 6$, each)

		Tamoxifen + EGCG					
Parameter	Control	0.5 mg/kg	*P* value	3 mg/kg	*P* value	10 mg/kg	*P* value
$AUC_{0-\infty}$ (ng h/ml)	300 ± 65	340 ± 87	>0.05	391 ± 87	>0.05	421 ± 95*	<0.05
C_{max} (ng/ml)	12.5 ± 2.8	12.9 ± 3.2	>0.05	13.6 ± 3.3	>0.05	14.2 ± 3.5	>0.05
T_{max} (h)	2.2 ± 0.4	2.3 ± 0.5	>0.05	2.3 ± 0.5	>0.05	2.3 ± 0.5	>0.05
$t_{1/2}$ (h)	15.6 ± 3.6	17.0 ± 4.1	>0.05	17.5 ± 4.2	>0.05	17.7 ± 4.3	>0.05
MR (%)	14.1 ± 2.9	13.5 ± 2.6	>0.05	12.3 ± 2.5	>0.05	11.1 ± 2.3*	<0.05

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

not significantly decrease MR of tamoxifen in rats. In the small intestine, P-gp is colocalized at the apical membrane of the cells with CYP3A4 [32]. P-gp and CYP3A4 might act synergistically to the first-pass metabolism [27,28,33–36]. CYP3A and P-gp inhibitors might interact with tamoxifen and its metabolites and thus contribute to substantial alteration of their pharmacokinetic fate. The metabolites of tamoxifen are also dependent on CYP2D6 [3,4] and uridine diphosphate-glucuronosyltransferases [11], but those are not mainly metabolic pathway. It might be considered that CYP3A and P-gp have more effect on the bioavailability of tamoxifen than CYP2D6 and glucuronidation. EGCG could affect the efficacy and toxicity of tamoxifen in rats, but EGCG and tamoxifen interaction on the pharmacokinetics need to be further evaluated in humans.

Conclusion

The presence of EGCG enhanced the oral bioavailability of tamoxifen, which might be mainly attributable to the promotion of intestinal absorption and reduction of first-pass metabolism of tamoxifen in the intestine and liver in rats by EGCG. The increase in oral bioavailability of tamoxifen in the presence of EGCG might be taken into consideration of potential drug interactions between tamoxifen and EGCG.

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