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Effect of *Jaeumkanghwatang* (JEKHT), a Polyherbal Formula on the Pharmacokinetics Profiles of Tamoxifen in Male SD Rats (2)

- Oral Combination Treatment of Tamoxifen 50 mg/kg with JEKHT 100 mg/kg on JEKHT 6-day Repeated Pretreated Rats with 8-day Repeated Co-administration -

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Abstract

Objectives : The effects of *Jaeumkanghwatang* (JEKHT) co-administration on the pharmacokinetics of tamoxifen were observed after oral combination treatment of tamoxifen 50 mg/kg with JEKHT 100 mg/kg on JEKHT 6-day repeated oral pretreated rats with 8-day repeated co-administration to confirm the effects of JEKHT co-administration on the pharmacokinetics of tamoxifen.

Methods : Six days after pretreatment of JEKHT 100 mg/kg, tamoxifen 50 mg/kg was co-administered with JEKHT 100 mg/kg, once a day for 8 days within 5 min. The blood were collected at 30 min before administration, 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of first and last 8th tamoxifen treatment, and plasma concentrations of tamoxifen were analyzed using LC-MS/MS methods. PK parameters of tamoxifen (T_{max}, C_{max}, AUC, $t_{1/2}$ and MRT_{inf}) were analysis as compared with tamoxifen single administered.

Results : Six-day repeated oral pretreatment of JEKHT and 8-day repeated oral co-administration of tamoxifen within 5 min did not influenced on the plasma concentrations and pharmacokinetic parameters of tamoxifen, oral bioavailability, as compared with tamoxifen single treated rats, except for some negligible effects.

Conclusions : It is concluded that JEKHT did not influenced on the plasma concentrations and pharmacokinetic parameters, the oral bioavailability of tamoxifen. Therefore, it is considered that co-administration of JEKHT and tamoxifen will be provide an effective novel treatment regimen on the comprehensive and integrative medicine for breast cancer patients, if they showed favorable synergic effects on the pharmacodynamics or

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reduce the tamoxifen treatment related toxicity and side effects in future studies.

Key words : *Jaeumkanghwatang*, Pharmacokinetics, Drug-drug interactions, Tamoxifen, Repeat oral dose, Repeated JEKHT pretreatment

I. Introduction

The research of combination therapies with other drugs to improve the side effects of tamoxifen or to achieve synergic effects, various drug-drug interactions of tamoxifen have been evaluated. Because tamoxifen was metabolized by a substrate of CYP3A, 2C9, 2D6[1),2)], it interacted with various drugs, namely, combinations containing any of the following medications, depending on the amount present, may also interact with aminoglutethimide - decreased plasma tamoxifen and N-desmethyltamoxifen concentrations[3)], anticoagulants - enhanced warfarin effects[4),5)], bromocriptine - increased plasma tamoxifen and N-desmethyltamoxifen concentrations[6)], letrozole - decreased plasma letrozole concentrationsa[7)], medroxyprogesterone - decreased plasma N-desmethyltamoxifen concentrations but did not reduce plasma tamoxifen concentrations[8)], phenobarbital - decreased plasma tamoxifen concentrations[9)], rifampin - decreased plasma tamoxifen and N-desmethyltamoxifen concentrations[10)], and cyclosporine, erythromycin, diltiazem and nifedipine - competitively inhibited formation of N-desmethyltamoxifen *in vitro*[11-13)], respectively. However, interactions with herbal products have not been established except for some restricted natural compounds; tamoxifen enhanced warfarin effects, and it is contraindicate that co-administration of tamoxifen and wafarin[4),5)].

Jaeumkanghwatang (JEKHT) is a traditional yin-tonifying herbal medicine has been used for various oriental obstetrical and gynecological fields and it comprises of 12 kinds of herbs like Glycyrrhizae Radix et Rhizoma, Angelicae Gigantis Radix, Zizyphi Fructus, Liriopis Tuber, Atractylodis Rhizoma Alba, Paeoniae Radix, Anemarrhenae Rhizoma, Rehmanniae Radix Crudus, Citri Unshii Pericarpium, Phellodendri Cortex, Zingiberis Rhizoma Crudus and Asparagi Tuber[14)]. It is widely used in China, Japan, and Korea to treat bronchitis and tuberculosis[15)] with in some immune stimulation effects[14)]. JEKHT has been demonstrated anti-allergic properties *in vitro* study and they include suppression of secretion of inflammatory cytokines through blockade of NF-κb activation[16)]. In addition, it has been reported that JEKHT has beneficial effects in the treatment of patients with bronchial asthma[15)] and the relieving hot flush with JEKHT, representative side effect in tamoxifen treated patients with breast cancer, was reported recently[17)].

Although co-administration of JEKHT with tamoxifen within 5 min did not influences on the absorption and excretion, the oral bioavailability of tamoxifen in our other study[18)], the effects of JEKHT co-administration on the pharmacokinetics of tamoxifen were observed after 8-day repeated co-administration of tamoxifen 50 mg/kg with JEKHT 100 mg/kg to confirm the effects of JEKHT co-administration on the pharmacokinetics of tamoxifen, in this study.

II. Materials & methods

1. Animals and husbandry

Total ten male Sprague-Dawley (SD) rats (6-wk old upon receipt, SLC, Japan) were used after

acclimatization for 23 days. Animals were allocated five per polycarbonate cage in a temperature (20-25℃) and humidity (40-45%) controlled room. Light : dark cycle was 12 hr : 12 hr and feed (Samyang, Korea) and water were supplied free to access. All animals were marked by picric acid, and overnight fasted (about 18 hrs; water was not restricted) before first and last treatment of test materials, and further fasted during 3 hrs after end of treatment. All laboratory animals were treated according to the national regulations of the usage and welfare of laboratory animals, and approved by the Institutional Animal Care and Use Committee in Daegu Haany University (Gyeongsan, Gyeongbuk, Korea) prior to animal experiment.

2. Test articles and formulation

JEKHT, prepared and purchase from Hanzung Pharm. Co. (Daejeon, Korea), and tamoxifen (Hangzhou Tacon Co., Ltd, Hangzhou, China) was used as control drug as listed follows. Individual compositions of 12 kinds of herbs in JEKHT were listed in Table 1. Tamoxifen and powders of JEKHT extracts were stored in a refrigerator at 4℃ to protect from light and degeneration until use. Both drugs are well dissolved (up to 20 mg/ml solutions in JEKHT and up to 10 mg/ml solutions in tamoxifen) in distilled water as vehicle, respectively.

3. Groupings and administration

Five rats per group (two groups) were used in this study. The doses of test materials were selected based on their toxicity and pharmacodynamics with the results of previous single co-administration[18] - 50 mg/kg of tamoxifen with 100 mg/kg of JEKHT. Six days after pretreatment of JEKHT 100 mg/kg (once a day for 6 days), tamoxifen 50 mg/kg was co-administered with JEKHT 100 mg/kg, once a day for 8 days within 5 min. In Tamoxifen single treated rats, only distilled water 5 ml/kg was orally administered during 6 days of JEKHT pretreatment periods; after that 50 mg/kg of tamoxifen was administered with distilled water, instead of JEKHT, once a day for 8 days. Each tamoxifen or JEKHT was orally administered, in a volume of 5 ml/kg, dissolved in distilled water, and bloods were sampled after first and last 8th

Table 1. Composition of JEKHT used in this study

Herbs	Scientific Names/ Produce Region	Amounts (g)
Glycyrrhizae Rhizoma	*Glycyrrhiza uralensis* Fisch	0.50
Angelicae Gigantis Radix	*Angelica gigas* N.	0.83
Zizyphi Fructus	*Zizyphus jujuba var. inermis* (Bunge) Rehder	0.33
Liriopis Tuber	*Liriope platyphylla* Wang et Tang	0.83
Atractylodis Rhizoma Alba	*Atractylodes ovata* (Thunb.) DC.	1.00
Paeoniae Radix	*Paeonia lactiflora* Pall.	0.83
Anemarrhenae Rhizoma	*Anemarrhena asphodeloides* Bunge	0.50
Rehmanniae Radix Crudus	*Rehmannia glutinosa var. purpurea* (Makino) Makino & Nemoto	0.83
Citri Unshii Pericarpium	*Citrus unshiu* S.Marcov.	0.83
Phellodendri Cortex	*Phellodendron amurense* Ruprecht	0.50
Zingiberis Rhizoma Crudus	*Zingiber officinale* Roscoe	0.33
Asparagi Tuber	*Asparagus cochinchinensis* (Lour.) Merr.	0.83
Total	12 types	8.14

JEKHT, *Jaeumkanghwatang* aqueous extracts were purchase from Hanzung Pharm. Co. (Daejeon, Korea)

tamoxifen treatment, respectively.

4. Changes in body weights

Changes of body weight were daily measured from 1 day before initiation of JEKHT administration to the last 8th co-administration of tamoxifen and JEKHT using an automatic electronic balance (Precisa Instrument, Switzland). At initiation of JEKHT pretreatment and first and last 8th co-administration, all experimental animals were overnight fasted (water was not; about 12 hr) to reduce the differences from feeding. In addition, body weight gains during JEKHT pretreatment, co-administration and total 14 days of experiment days were calculated.

5. Plasma collections

All rats were slightly anesthesia under ethyl ether (Duksan Pure Chemical, Seoul, Korea) and blood samples (0.5 ml) were collected into 50 IU heparinized tubes via the orbital plexus at 30 min before treatment (as a control), 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of first and last 8th co-administration. Blood samples were immediately centrifuged for 10 min at 13,000 rpm and about 0.3 ml aliquots of plasma were stored in a −70℃ deep freezer until analysis of tamoxifen.

6. Sample preparation and calibrations

Primary stock solution, 1.0 mg/ml of tamoxifen in 50% acetonitrile (Sigma, MO, USA) mixtures with distilled water and internal standard working solution, carbamazepine (Sigma, MO, USA) 500 ng/ml in acetonitrile were prepared. Working standard solutions were prepared by dilution with acetonitrile. All standard solutions were stored at −20℃ in the dark when not in use, and calibrated the standard samples as 100 μl of blank plasma, working standard solutions and internal standard working solution were mixed with 100 μl of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000 rpm for 10 min at 4℃. The clear supernatants were transferred to injection vials and the aliquot was injected into the LC-MS/MS system. In addition, 100 μl of sample plasma and internal standard working solution were mixed with 200 μl of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000 rpm for 10 min at 4℃. Clear supernatants (5.0 μl) were directly transferred to injection vials and the aliquot was injected into the LC-MS/MS system.

7. LC-MS/MS conditions

Concentrations of tamoxifen in the rat plasma samples were determined LC-MS/MS method. Chromatographic analysis was performed using an Agilent 1100 Series HPLC (Agilent Technologies, CA, USA) equipped with on-line degasser, binary pump, autosampler and column compartment. Separation of the analyte from potentially interfering material was achieved at ambient temperature using Waters Xterra MS C18 columns (2.1×50 mm, 3.5 μm) (Waters Corp., MA, USA) at column oven 30℃. The mobile phase used for the chromatographic separation was composed of 5% acetonitrile/95% distilled water (0.1% formic acid) to 95% acetonitrile/5% distilled water (0.1% formic acid), and was delivered isocratically at a flow rate of 0.35 ml/min. The column effluent was monitored using an API 2000 triple-quadruple mass-spectometric detector (Applied Biosystems, CA, USA). The instrument was equipped with an electrospray interface in positive ion mode, and controlled by the Analyst version 1.4.2 software (Applied Biosystems, CA, USA). Samples were introduced to the interface through a Turbo IonSpray with the temperature set at 400℃. A

high positive voltage of 5.0 kV was applied to the ion spray. Nitrogen was used as the nebulizer gas, curtain gas, and collision gas with the settings of 12, 6, and 8, respectively. The multiple reaction monitoring (MRM) detection method was employed for the detection of tamoxifen; the transitions monitored were carbamazepine (IS): m/z 237〉194 (Retention time: 2.4 min), tamoxifen: 372〉72 (Retention time: 2.3 min). Calibration curves of tamoxifen were linear over the ranges studied with r^2>0.999. The lower limit of quantification of the tamoxifen in the rat plasma was 1 ng/ml.

8. Pharmacokinetic analysis

The plasma concentration data were analyzed using a noncompartmental method on commercial pharmacokinetics data analyzer programs (PK solutions 2.0; Summit, CO, USA)[19),20)]. The elimination rate constant (K_{el}) was calculated by the log-linear regression of tamoxifen concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by 0.693/K_{el}. The peak concentration (C_{max}) and time to reach the peak concentration (T_{max}) of tamoxifen in the plasma were obtained by visual inspection of the data in the concentration-time curve. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of the last measured concentration (C_{last}) was calculated using the linear trapezoidal rule[21)]. The AUC zero to infinity (AUC_{0-inf}) was obtained by adding AUC_{0-t} and the extrapolated area was determined by C_{last}/K_{el}. The mean residence time infinity (MRT_{inf}) was calculated by dividing the first moment of AUC ($AUMC_{0-inf}$) by AUC_{0-inf}.

9. Statistical analyses

All the means are presented with their standard deviation of five rats (Mean ± SD of five rats). The pharmacokinetic parameters were compared using a non-parametric comparison test, Mann-Whitney U (MW) test, on the SPSS for Windows (Release 14.0K, SPSS Inc., USA). A p-value 〈 0.05 was considered statistically significant. In addition, the percent changes between tamoxifen single treated rats and tamoxifen with JEKHT co-administered rats were calculated to help the understanding of the effects of co-administration.

III. Results

1. Changes on the plasma concentrations of tamoxifen

No meaningful changes on the body weight and gains were detected in JEKHT and tamoxifen co-administered rats as compared with tamoxifen single treated rats throughout experimental periods, respectively (Table 2, Fig 1).

2. Changes on the plasma concentrations of tamoxifen

Tamoxifen was detected from 30 min to 24 hrs after end of first and last 8th co-administration in the both tamoxifen single and co-administered rats with JEKHT, regardless of JEKHT pretreatment. No meaningful and no-significant changes on the plasma tamoxifen concentrations were detected in JEKHT co-administered rats as compared with tamoxifen single treated rats after first and last 8th co-administration of tamoxifen 50 mg/kg with JEKHT 100 mg/kg, regardless of JEKHT pretreatment except for non-significantly and slightly increased plasma tamoxifen concentrations at 30 min or 1 hr after end of first or last 8th co-administration, detected in JEKHT co-administered rats as compared with tamoxifen single treated rats, respectively (Fig 2A, B).

Table 2. Body weight gains during administration (from first to last treatment) of JEKHT with and without tamoxifen in male rats

Groups	Tamoxifen (50 mg/kg)	
	Without JEKHT co-administration (Distilled water)	With JEKHT co-administration (100 mg/kg)
Body weights		
At first JEKHT treatment [A]	310.20 ± 7.26	309.80 ± 5.76
At first co-administration [B]	330.20 ± 12.38	328.80 ± 6.61
At last 8th co-administration [C]	318.40 ± 12.56	315.00 ± 7.78
Body weight gains during		
JEKHT pretreatment [B] - [A]	20.00 ± 5.70	19.00 ± 2.92
Co-administration [C] - [B]	- 11.80 ± 3.77	- 13.80 ± 4.38
Experimental periods [C] - [A]	8.20 ± 7.19	5.20 ± 4.87

Values are expressed as mean ± SD of five rats. JEKHT : *Jaeumkanghwatang* aqueous extracts.

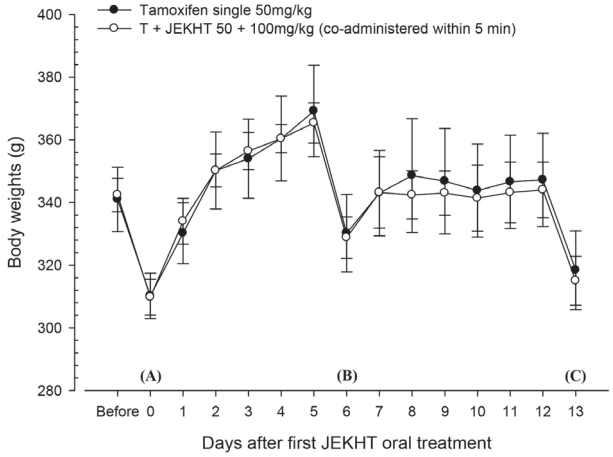

Figure 1. Changes on the body weights during co-administration of JEKHT with and without tamoxifen in male rats from 6-days repeated oral pretreatment of JEKHT. Values are expressed as mean ± SD of five rats (g). All animals were overnight fasted before first and last treatment of test materials. Before means 1 day before first JEKHT treatment. (A) At first JEKHT treatment. (B) At first co-administration. (C) At last 8th co-administration. JEKHT: *Jaeumkanghwatang* aqueous extracts, T: tamoxifen.

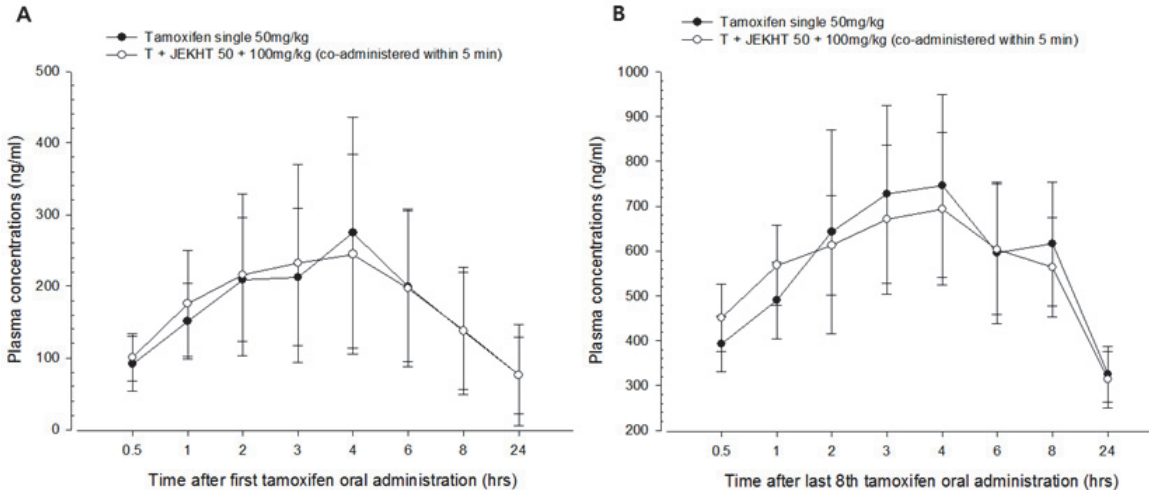

Figure 2. Plasma concentrations of JEKHT with and without tamoxifen after first co-administration (A) and last 8th co-administration (B) within 5 min in male rats from 6-days repeated oral pretreatment of JEKHT. Values are expressed as mean ± SD of five rats (ng/ml). JEKHT: *Jaeumkanghwatang* aqueous extracts, T: tamoxifen.

3. Changes on the T_{max} of tamoxifen

The T_{max} of tamoxifen was non-significantly decreased as −22.22% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg (2.80±1.30 hr) as compared with tamoxifen single treated rats (3.60±0.00 hr) after end of first co-administration of tamoxifen 50 mg/kg with JEKHT 100 mg/kg from 6-days repeated oral pretreatment of JEKHT. However, the T_{max} of tamoxifen after end of last 8th co-administration of tamoxifen 50 mg/kg with JEKHT 100 mg/kg from 6-days repeated oral pretreatment of JEKHT was slight and non-significantly increased as 11.76% in JEKHT co-administrated rats (3.80± 0.45 hr) as compared with tamoxifen single treated rats (3.40±0.89 hr), in the present study (Table 3 and 4).

4. Changes on the C_{max} of tamoxifen

The C_{max} of tamoxifen in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg (0.32±0.17 μg/ml) were non-significantly changed as 17.39% as compared with tamoxifen single treated rats (0.28±0.16 μg/ml) after end

Table 3. Pharmacokinetic parameters of JEKHT with and without tamoxifen after first co-administration within 5min in male rats from 6-days repeated oral pretreatment of JEKHT

Parameters	Tamoxifen (50 mg/kg)	
	Without JEKHT co-administration (Distilled water)	With JEKHT co-administration (100 mg/kg)
T_{max}(hrs)	3.60 ± 0.89	2.80 ± 1.30
C_{max}(μg/ml)	0.28 ± 0.16	0.32 ± 0.17
AUC_{0-t}(hr • μg/ml)	3.23 ± 1.79	3.70 ± 2.22
AUC_{0-inf}(hr • μg/ml)	4.86 ± 2.87	4.88 ± 4.09
$t_{1/2}$(hr)	13.11 ± 5.77	12.47 ± 5.01
MRT_{inf}(hr)	18.12 ± 5.71	17.16 ± 8.35

Values are expressed as mean ± SD of five rats. JEKHT : *Jaeumkanghwatang* aqueous extracts, C_{max} : The peak plasma concentration, T_{max} : Time to reach Cmax, AUC_{0-t} : The total area under the plasma concentration-time curve from time zero to time measured, AUC_{0-inf} : The total area under the plasma concentration-time curve from time zero to time infinity, $t_{1/2}$: half life, MRT_{inf} : mean residence to time infinity

Table 4. Pharmacokinetic parameters of JEKHT with and without tamoxifen after last 8th co-administration within 5 min in male rats from 6-days repeated oral pretreatment of JEKHT

Parameters	Tamoxifen (50 mg/kg)	
	Without JEKHT co-administration (Distilled water)	With JEKHT co-administration (100 mg/kg)
T_{max}(hrs)	3.40 ± 0.89	3.80 ± 0.45
C_{max}(μg/ml)	0.77 ± 0.22	0.70 ± 0.17
AUC_{0-t}(hr • μg/ml)	12.39 ± 2.66	12.11 ± 3.24
AUC_{0-inf}(hr • μg/ml)	21.50 ± 3.04	19.60 ± 2.60
$t_{1/2}$(hr)	19.62 ± 5.84	18.68 ± 7.30
MRT_{inf}(hr)	28.48 ± 8.53	26.85 ± 10.69

Values are expressed as mean ± SD of five rats. JEKHT : *Jaeumkanghwatang* aqueous extracts, C_{max} : The peak plasma concentration, T_{max} : Time to reach Cmax, AUC_{0-t} : The total area under the plasma concentration-time curve from time zero to time measured, AUC_{0-inf} : The total area under the plasma concentration-time curve from time zero to time infinity, $t_{1/2}$: half life, MRT_{inf} : mean residence to time infinity.

of first co-administration from 6-days repeated oral pretreatment of JEKHT. In addition, the C_{max} of tamoxifen was non-significantly decreased as -9.54% in JEKHT co-administrated rats (0.70±0.17 μg/ml) as compared with tamoxifen single treated rats (0.77±0.22 μg/ml) after end of last 8th co-administration within 5min (Table 3 and 4).

5. Changes on the AUC of tamoxifen

The AUC_{0-t} and AUC_{0-inf} of tamoxifen were non-significantly changed as 14.32 and 0.40% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg (3.70±2.22 and 4.88±4.09 hr • μg/ml) as compared with tamoxifen single treated rats (3.23±1.79 and 4.86±2.87 hr • μg/ml) after end of first co-administration from 6-days repeated oral pretreatment of JEKHT. In addition, the AUC_{0-t} and AUC_{0-inf} of tamoxifen were non-significant and slightly decreased as -2.26 and -8.83% in co-administrated rats with tamoxifen and JEKHT (12.11±3.24 and 19.60±2.60 hr • μg/ml) as compared with tamoxifen single treated rats (12.39±2.66 and 21.50±3.04 hr • μg/ml) after end of last 8th co-administration (Table 3 and 4).

6. Changes on the $t_{1/2}$ of tamoxifen

The $t_{1/2}$ of tamoxifen was slightly and non-significantly decreased as -4.86% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg (12.47±5.01 hr) as compared with tamoxifen single treated rats (13.11±5.77 hr) after end of first co-administration from 6-days repeated oral pretreatment of JEKHT. In addition, the $t_{1/2}$ of tamoxifen was also non-significantly decreased as -4.79% in co-administrated rats with tamoxifen and JEKHT (18.86±7.30 hr) as compared with tamoxifen single treated rats (19.62±5.84 hr) after end of last 8th co-administration (Table 3 and 4).

7. Changes on the MRT_{inf} of tamoxifen

The MRT_{inf} of tamoxifen slightly and non-significantly decreased as -5.30% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg (17.16±8.35 hr) as compared with tamoxifen single treated rats (18.12±5.71 hr) after end of first co-administration from 6-days repeated oral pretreatment of JEKHT. In addition, the MRT_{inf} of tamoxifen was also slightly and non-significantly decreased as -5.75% in co-administrated rats with tamoxifen and JEKHT (26.85±10.69 hr) as compared with tamoxifen single treated rats (28.48±8.53 hr) after end of last 8th co-administration (Table 3 and 4).

IV. Discussion

Six-day repeated oral pretreatment of JEKHT and 8-day repeated oral co-administration of tamoxifen within 5 min did not influenced on the plasma concentrations and pharmacokinetic parameters of tamoxifen, oral bioavailability, as compared with tamoxifen single treated rats except for some negligible effects as quite similar to the results of the previous single co-administration within 5 min study[18]. These findings are considered as direct evidences that JEKHT co-administration did not influenced on the oral bioavailability of tamoxifen, even if after repeated co-administered within 5 min. It therefore, is considered that co-administration of JEKHT and tamoxifen will be provide an effective novel treatment regimen on the comprehensive and integrative medicine for breast cancer patients, if they showed favorable synergic effects on the pharmacodynamics or reduce the tamoxifen treatment related toxicity and side effects in future studies. All rats used in this study, showed

normal body weight increases ranged in normal age-matched rats regardless of treatment in the present study[22),23)]. In addition, no meaningful changes on the body weight and gains were detected in JEKHT and tamoxifen co-administered rats as compared with tamoxifen single treated rats throughout experimental periods, in the present study.

Tamoxifen was absorbed slowly following oral administration and T_{max} of tamoxifen occur about 3-6 hrs after a single dose[24-26)] but it rapidly and extensively metabolized in the liver, through a substrate of CYP3A, 2C9, 2D626 including an active major metabolite, N-desmethyltamoxifen has biologic activity similar to that of the parent drug[27),28)]. Steady-state concentrations of tamoxifen are attained after 3-4 weeks and those of N-desmethyltamoxifen, an active metabolite, are attained after 3-8 weeks[29)]. Tamoxifen excreted principally in feces as polar conjugates[30)] with about 5-7 days of $t_{1/2}$ in tamoxifen and 9-14 days in N-desmethyltamoxifen[25)]. Clearance of tamoxifen is higher in female children 2-10 years of age than in women[31),32)]. In the present study, T_{max} of tamoxifen in tamoxifen single oral treated rats was detected as 3.60±0.89 hr after first co-administration from 6-days repeated oral pretreatment of JEKHT, and C_{max}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} were detected as 0.28±0.16 μg/ml, 3.23±1.79 hr • μg/ml, 4.86±2.87 hr • μg/ml, 13.11±5.77 hr and 18.12±5.17 hr after first co-administration, respectively. In tamoxifen with JEKHT co-administered rats, T_{max}, C_{max}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} of tamoxifen were detected as 2.80±1.30 hr, 0.32±0.17 μg/ml, 3.70±2.22 hr • μg/ml, 4.88±4.09 hr • μg/ml, 12.47±5.01 hr and 17.16± 8.35 hr; changed as −22.22, 17.39, 14.32, 0.40, −4.86 and −5.30% as compared with tamoxifen 50 mg/kg single oral treated rats after first co-administration, respectively. These are means that repeated JEKHT pretreatment did not influenced on the absorption and excretion of tamoxifen, when they were single co-administered within 5 min, quite similar to the results of the previous single co-administration within 5 min study[18)]. In addition, T_{max} of tamoxifen in tamoxifen single oral treated rats was detected as 3.40±0.89 hr after last 8th co-administration from 6-days repeated oral pretreatment of JEKHT, and C_{max}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} were detected as 0.77±0.22 μg/ml, 12.39±2.66 hr • μg/ml, 21.50±3.04 hr • μg/ml, 19.62±5.84 hr and 28.48±8.53 hr after last 8th co-administration, respectively. In tamoxifen with JEKHT co-administered rats, T_{max}, C_{max}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} of tamoxifen were detected as 3.80±0.45 hr, 0.70±0.17 μg/ml, 12.11±3.24 hr • μg/ml, 19.60±2.60 hr • μg/ml, 18.68±7.30 hr and 26.85±10.69 hr; changed as 11.76, −9.54, −2.26, −8.83, −4.79 and −5.75% as compared with tamoxifen 50 mg/kg single oral treated rats after last 8th co-administration, as direct evidences that repeated co-administration of JEHKT within 5 min also did not influenced on the oral bioavailability of tamoxifen, quite similar from the results of the previous single co-administration[18)] and pretreatment rats in this study.

Tamoxifen rapidly and extensively metabolized in the liver, through a substrate of CYP3A, 2C9, 2D626 to active major metabolite, N-desmethyltamoxifen[27),28)] and, therefore, tamoxifen can be interacted with various drugs like aminoglutethimide[3)], anticoagulants[4),5)], bromocriptine[6)], letrozole[7)], medroxyprogesterone[8)], phenobarbital[9)] and rifampin[10)]. In addition the possibilities that tamoxifen competitively interacted with cyclosporine, erythromycin, diltiazem and nifedipine were also suggested *in vitro* experiments[11-13)]. The severities of various side effects arise from tamoxifen treatment, especially bone loss[33)], endometrial cancer[34)], thromboembolism[35)], fatty liver[36)], reduced cognition[37)], semantic memory scores[38)] and libido[39),40)], premature growth plate

fusion[41], immune suppression[42),43)] and hypersensitivity[44),45)] are considered as directly co-related with absorption and excretion of tamoxifen or pharmacodynamics. In the present study JEKHT co-administration did not influenced on the oral bioavailability of tamoxifen, even if after repeated co-administered within 5min.

V. Conclusions

Based on the present and previous single co-administration study of JEKHT and tamoxifen, it is concluded that JEKHT did not influenced on the plasma concentrations and pharmacokinetic parameters, the oral bioavailability of tamoxifen. Therefore, it is considered that co-administration of JEKHT and tamoxifen will be provide an effective novel treatment regimen on the comprehensive and integrative medicine for breast cancer patients, if they showed favorable synergic effects on the pharmacodynamics or reduce the tamoxifen treatment related toxicity and side effects in future studies.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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