

Effect of Jaeumkanghwatang (JEKHT), a Polyherbal Formula on the Pharmacokinetics Profiles of Tamoxifen in Male SD Rats (1) - Single Oral Combination Treatment of Tamoxifen 50 mg/kg with JEKHT 100 mg/kg within 5 min -

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Objectives: The objective of this study was to elucidate the effect of Jaeumkanghwatang (JEKHT) on the plasma concentration and pharmacokinetics of tamoxifen in combination therapy as a process of the comprehensive and integrative medicine against breast cancer.

Methods: After 50 mg/kg of tamoxifen treatment, JEKHT 100 mg/kg was orally administered within 5 min. The plasma were collected at 30 min before administration, 30min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of JEKHT 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 rats.

Results: JEKHT did not influenced on the plasma concentrations and pharmacokinetics of tamoxifen after single oral co-administration, within 5min except for some negligible effects on plasma concentration. The T_{max} , C_{max} , AUC, $t_{1/2}$ and MRT_{inf} of tamoxifen in co-administered rats were quite similar to those of tamoxifen single treated rats.

Conclusions: Based on the results of the present study, JEKHT did not influenced on the oral bioavailability of tamoxifen, when they were single co-administered within 5min. However, more detail pharmacokinetic studies should be tested to conclude the possibilities that can be used as comprehensive and integrative therapy with JEKHT and tamoxifen for breast cancers, when they were co-administered, like the effects on the pretreatment of JEKHT and after repeat co-administrations.

Key Words : Jaeumkanghwatang, Pharmacokinetics, Drug–drug interactions, Rat, Tamoxifen, Nolvadex™

• Received : 4 February 2016 • Revised : 18 April 2016 • Accepted : 19 April 2016

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Introduction

Tamoxifen (NolvadexTM) is a nonsteroidal estrogen agonist-antagonist antineoplastic agent has been used for breast cancer¹. It is the usual endocrine (anti-estrogen) therapy for hormone receptor-positive breast cancer in pre-menopausal women, and is also a standard in post-menopausal women although aromatase inhibitors are also frequently used in that setting^{2,3}. In addition, tamoxifen also used to treat infertility in women with anovulatory disorders^{4,5} and prevention for gynecomastia^{6,7} and bipolar disorder^{8,9} as anti-angiogenesis¹⁰, control of gene expression¹¹, and treat Riedel thyroiditis^{12,13} and Albright's syndrome^{14,15}. Tamoxifen competitively binds to estrogen receptors on tumors and other tissue targets, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects. It is a nonsteroidal agent with potent antiestrogenic properties which compete with estrogen for binding sites in breast and other tissues. Tamoxifen causes cells to remain in the G0 and G1 phases of the cell cycle. Because it prevents (pre)cancerous cells from dividing but does not cause cell death, tamoxifen is cytostatic rather than cytotoxic¹⁶⁻¹⁸. However, various side effects related to tamoxifen treatment also have been arise as bone loss in premenopausal women who continue to menstruate after adjuvant chemotherapy¹⁹, endometrial changes, including cancer, are among tamoxifen's side effects²⁰, increased risk of thromboembolism²¹, cause of fatty liver²², reduced cognition²³, semantic memory scores²⁴ and libido^{25,26}, and premature growth plate fusion²⁷. Tamoxifen also depress the immune response^{28,29}, and it also known that hypersensitivity to tamoxifen or any ingredient in the formulation^{30,31}.

As results 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^{32,33}, 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 concentrations³⁴, anticoagulants - enhanced warfarin effects^{35,36}, bromocriptine - increased plasma tamoxifen and N-desmethyltamoxifen concentrations³⁷, letrozole - decreased plasma letrozole concentrations³⁸, medroxyprogesterone - decreased plasma N-desmethyltamoxifen concentrations but did not reduce plasma tamoxifen concentrations³⁹, phenobarbital - decreased plasma tamoxifen concentrations⁴⁰, rifampin - decreased plasma tamoxifen and N-desmethyltamoxifen concentrations⁴¹, and cyclosporine, erythromycin, diltiazem, erythromycin and nifedipine - competitively inhibited formation of N-desmethyltamoxifen *in vitro*⁴²⁻⁴⁴, 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^{35,36}.

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, Liriopsis Tuber, Atractylodis Rhizoma Alba, Paeoniae Radix, Anemarrhenae Rhizoma, Rehmanniae Radix Crudus, Citri Unshii Pericarpium, Phellodendri Cortex, Zingiberis Rhizoma Crudus and Asparagi Tuber⁴⁵ (Table 1). It is widely used in China, Japan, and Korea to treat bronchitis and tuberculosis⁴⁶ with in some immune stimulation effects⁴⁵. JEKHT has been demonstrated anti-allergic properties *in vitro* study and they include suppression of secretion of inflammatory cytokines through blockade of NF- κ b activation⁴⁷. In addition, It has been reported that JEKHT has

beneficial effects in the treatment of patients with bronchial asthma⁴⁶⁾ and the relieving hot flash with JEKHT, representative side effect in tamoxifen treated patients with breast cancer, was reported recently⁴⁸⁾.

In the present study, the effects of JEKHT co-administration on the plasma concentration and pharmacokinetics of tamoxifen were observed as a process of the comprehensive and integrative medicine, against breast cancers.

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 16 days. Animals were allocated five per polycarbonate cage in a temperature (20-25 °C) 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 treatment, 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. 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 °C 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 - 50 mg/kg of tamoxifen with 100 mg/kg of JEKHT. After 50 mg/kg of tamoxifen treatment, JEKHT 100 mg/kg was orally administered, within 5 min. In tamoxifen single treated rats, 50 mg/kg of tamoxifen was orally administered and 5 min after treatment, only distilled water 5 ml/kg was orally administered, instead of JEKHT solutions. Each tamoxifen or JEKHT was single orally administered, in a volume of 5 ml/kg, dissolved in distilled water.

4. Plasma collections

All rats were slightly anesthesia under ethyl ether (Duksan Pure Chemical, Seoul, Korea) and blood samples (0.5 ml) were collected into 50IU 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 oral 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 °C deep freezer until analysis of tamoxifen.

5. 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 °C 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 μ l of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000 rpm for 10 min at 4 °C. The clear supernatants were transferred to injection vials and the aliquot was injected into the LC-MS/MS system. In addition, 100 μ l of sample plasma and internal standard working solution were mixed with 200 μ l of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000 rpm for 10 min at 4 °C. Clear supernatants (5.0 μ l) were directly transferred to injection vials and the aliquot was injected into the LC-MS/MS system.

6. 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 \times 50 mm, 3.5 μ m) (Waters Corp., MA, USA) at column oven 30 °C. 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-quadrupole mass-spectrometric 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 °C. 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.

7. 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)^{49,50}. 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 rule⁵¹. 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} .

8. Statistical analyses

All the means are presented with their standard deviation of five rats (Mean \pm SD of five rat plasma concentrations of tamoxifen). 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.

Results

1. Changes on the plasma concentrations of tamoxifen

Tamoxifen was detected from 30 min to 24 hrs after end of administration in the both tamoxifen single and co-administered rats with JEKHT,

respectively. 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 single co-administration of tamoxifen 50 mg/kg with JEKHT 100mg/kg, except for non-significant slight increases of plasma tamoxifen levels detected at 30 min and 1 hr after administration in JEKHT co-administered rats as compared with tamoxifen single treated rats (Fig 1). The plasma tamoxifen concentrations at 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of treatment were changed as 26.53, 49.94, -6.49, -3.76, -6.59, -13.04, -15.36 and -8.81% in tamoxifen + JEKHT treated rats as compared with

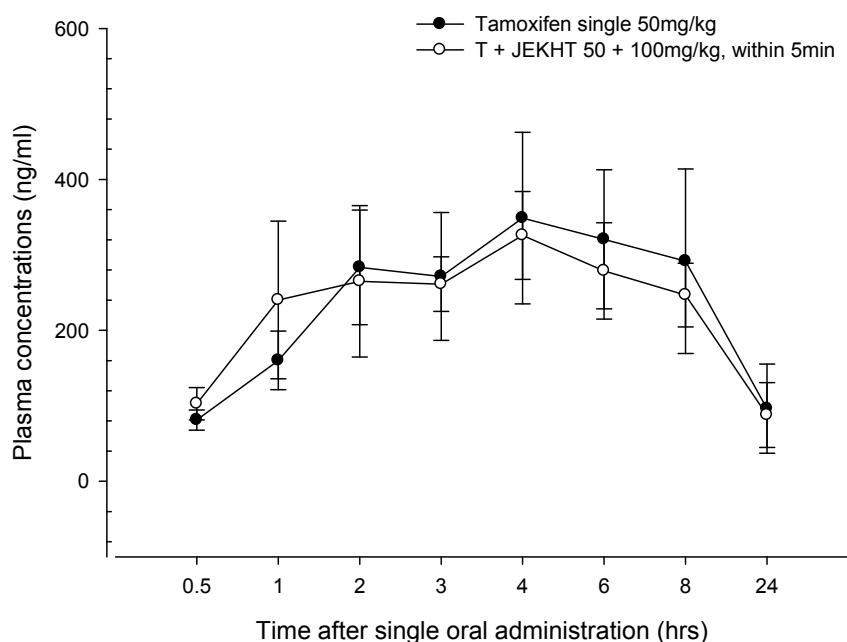


Fig 1. Plasma concentrations of tamoxifen with and without JEKHT co-administration in male rats. Tamoxifen was detected from 30 min to 24 hrs after end of administration in the both tamoxifen single and co-administered rats with JEKHT, respectively. No meaningful and no-significant changes on the plasma tamoxifen concentrations were detected in JEKHT co-administered rats as compared with as compared with tamoxifen single treated rats after single co-administration of tamoxifen 50 mg/kg with JEKHT 100 mg/kg, except for non-significant slight increases of plasma tamoxifen levels detected at 30 min and 1 hr after administration in JEKHT co-administered rats as compared with tamoxifen single treated rats, in the present study. Values are expressed as mean \pm SD of five rats (ng/ml). T, tamoxifen, JEKHT: Jaumkanghwatang aqueous extracts (Hanzung Pharm, Co., Daejeon, Korea).

Table 1. Composition of JEKHT Used in This Study

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

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

Table 2. Pharmacokinetic Parameters of Tamoxifen with and without JEKHT Co-Administration in Male Rats

Parameters	Tamoxifen (50 mg/kg)	
	Without JEKHT co-administration (Distill water)	With JEKHT co-administration (100mg/kg)
T _{max} (hrs)	5.60 ± 2.19	4.80 ± 1.10
C _{max} (ng/ml)	364.20 ± 121.77	345.00 ± 46.69
AUC _{0-t} (hr · μg/ml)	5.27 ± 1.79	4.73 ± 0.85
AUC _{0-inf} (hr · μg/ml)	6.86 ± 2.92	6.30 ± 2.07
t _{1/2} (hr)	9.56 ± 4.16	10.91 ± 3.99
MRT _{inf} (hr)	14.87 ± 6.29	16.02 ± 5.60

Values are expressed as mean ± SD of five rats, JEKHT: Jaeumkanghwatang aqueous extracts (Hanzung Pharm, Co., Daejeon, Korea), C_{max}: The peak plasma concentration, T_{max}: Time to reach C_{max}, 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

tamoxifen single treated rats, respectively.

2. Changes on the T_{max} of tamoxifen

The T_{max} of tamoxifen were slight and non-significantly decreased as -14.29% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg (4.80±1.10 hr) as compared with tamoxifen single treated rats (5.60±2.19 hr), in the present study (Table 2).

3. Changes on the C_{max} of tamoxifen

The C_{max} of tamoxifen were slight and non-significantly decreased as -5.27% in co-administrated rats with

tamoxifen 50 mg/kg and JEKHT 100 mg/kg (345.00±46.69 ng/ml) as compared with tamoxifen single treated rats (364.20±121.77 ng/ml), in the present study (Table 2).

4. Changes on the AUC of tamoxifen

The AUC_{0-t} of tamoxifen were slight and non-significant decreased as -10.37% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg (4.73±0.85 hr · μg/ml) as compared with tamoxifen single treated rats (5.27±1.79 hr · μg/ml). In addition, AUC_{0-inf} of tamoxifen were also slight and non-significantly

decreased as -8.16% in co-administrated rats with tamoxifen and JEKHT ($6.30 \pm 2.07 \text{ hr} \cdot \mu\text{g/ml}$) as compared with tamoxifen single treated rats ($6.86 \pm 2.92 \text{ hr} \cdot \mu\text{g/ml}$), in the present study (Table 2).

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

The $t_{1/2}$ of tamoxifen were non-significantly increased as 14.16% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg ($10.91 \pm 3.99 \text{ hr}$) as compared with tamoxifen single treated rats ($9.56 \pm 4.16 \text{ hr}$), in the present study (Table 2).

6. Changes on the MRT_{inf} of tamoxifen

The MRT_{inf} of tamoxifen were slight and non-significantly increased as 7.75% in co-administrated rats with tamoxifen 50 mg/kg and JEKHT 100 mg/kg ($16.02 \pm 5.60 \text{ hr}$) as compared with tamoxifen single treated rats ($14.87 \pm 6.29 \text{ hr}$), in the present study (Table 2).

Discussion

In the present study, the effects of JEKHT co-administration on the pharmacokinetics of tamoxifen were observed as a process of the comprehensive and integrative medicine, combination therapy of tamoxifen with JEKHT to achieve synergic pharmacodynamics and reduce toxicity on breast cancers. After 50 mg/kg of tamoxifen treatment, JEKHT 100 mg/kg was administered within 5 min. The plasma were collected at 30min before administration, 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of JEKHT 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. JEKHT did not influenced on the plasma

concentrations and pharmacokinetics of tamoxifen after single oral co-administration, within 5 min except for some negligible effects on plasma concentration. The T_{max} , C_{max} , AUC, $t_{1/2}$ and MRT_{inf} of tamoxifen in co-administered rats were quite similar to those of tamoxifen single treated rats, respectively.

Tamoxifen was absorbed slowly following oral administration and T_{max} of tamoxifen occur about 3-6 hrs after a single dose⁵²⁻⁵⁴) 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^{55,56}. Steady-state concentrations of tamoxifen are attained after 3-4 weeks and those of N-desmethyltamoxifen, an active metabolite, are attained after 3-8 weeks⁵⁷. Tamoxifen excreted principally in feces as polar conjugates⁵⁸) with about 5-7 days of $t_{1/2}$ in tamoxifen and 9-14 days in N-desmethyltamoxifen⁵³). Clearance of tamoxifen is higher in female children 2-10 years of age than in women^{59,60}). In the present study, T_{max} of tamoxifen in tamoxifen single oral treated rats was detected as $5.60 \pm 2.19 \text{ hr}$, and C_{max} , AUC_{0-t} , $\text{AUC}_{0-\text{inf}}$, $t_{1/2}$ and MRT_{inf} were detected as $364.20 \pm 121.77 \text{ ng/ml}$, $5.27 \pm 1.79 \text{ hr} \cdot \mu\text{g/ml}$, $6.86 \pm 2.92 \text{ hr} \cdot \mu\text{g/ml}$, $9.56 \pm 4.16 \text{ hr}$ and $14.87 \pm 6.29 \text{ hr}$, respectively. In tamoxifen with JEKHT co-administered rats, T_{max} , C_{max} , AUC_{0-t} , $\text{AUC}_{0-\text{inf}}$, $t_{1/2}$ and MRT_{inf} of tamoxifen were detected as $4.80 \pm 1.10 \text{ hr}$, $345.00 \pm 46.69 \text{ ng/ml}$, $4.73 \pm 0.85 \text{ hr} \cdot \mu\text{g/ml}$, $6.30 \pm 2.07 \text{ hr} \cdot \mu\text{g/ml}$, $10.91 \pm 3.99 \text{ hr}$ and $16.02 \pm 5.60 \text{ hr}$; changed as -14.29, -5.27, -10.37, -8.16, 14.16 and 7.75% as compared with tamoxifen 50 mg/kg single oral treated rats. These results are considered as direct evidences that JEKHT did not influenced on the absorption and excretion of tamoxifen, when they were single orally co-administered within 5 min, at least.

Tamoxifen rapidly and extensively metabolized in the liver, through a substrate of CYP3A, 2C9, 2D626 to active major metabolite, N-desmethyltamoxifen

^{55,56)} and, therefore, tamoxifen can be interacted with various drugs like aminoglutethimide³⁴⁾, anticoagulants^{35,36)}, bromocriptine³⁷⁾, letrozole³⁸⁾, medroxyprogesterone³⁹⁾, phenobarbital⁴⁰⁾ and rifampin⁴¹⁾. In addition the possibilities that tamoxifen competitively interacted with cyclosporine, erythromycin, diltiazem, erythromycin and nifedipine were also suggested in vitro experiments⁴²⁻⁴⁴⁾. The severities of various side effects arise from tamoxifen treatment, especially bone loss¹⁹⁾, endometrial cancer²⁰⁾, thromboembolism²¹⁾, fatty liver²²⁾, reduced cognition²³⁾, semantic memory scores²⁴⁾ and libido^{25,26)}, premature growth plate fusion²⁷⁾, immune suppression^{28,29)} and hypersensitivity^{30,31)} are considered as directly co-related with absorption and excretion of tamoxifen or pharmacodynamics. In the present study, co-administration of JEKHT did not influenced on the plasma concentrations and pharmacokinetics of tamoxifen. It means that JEKHT didn't interact with tamoxifen.

Based on the results of the present study, JEKHT did not influenced on the oral bioavailability of tamoxifen, when they were single co-administered within 5 min. However, more detail pharmacokinetic studies should be tested to conclude the possibilities that can be used as comprehensive and integrative therapy with JEKHT and tamoxifen for breast cancers, when they were co-administered, like the effects on the pretreatment of JEKHT and after repeat co-administrations.

Conflicts of interest

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

Acknowledgments

This study was supported by grant of Korea of Health & Welfare, Republic of Korea (Project No: 20-11-0-090-091-3000-3033-320).

References

1. The BIG 1-98 Collaborative Group. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. *N Engl J Med.* 2009;361(8):766-776.
2. Jordan VC. Fourteenth Gaddum Memorial Lecture. A current view of tamoxifen for the treatment and prevention of breast cancer. *Br J Pharmacol.* 1993;110(2):507-517.
3. Jordan VC. Tamoxifen (ICI46,474) as a targeted therapy to treat and prevent breast cancer. *Br J Pharmacol.* 2006;147 Suppl 1:S269-276.
4. Steiner AZ, Terplan M, Paulson RJ. Comparison of tamoxifen and clomiphene citrate for ovulation induction: a meta-analysis. *Hum Reprod.* 2005;20(6):1511-1515.
5. van Bommel EF, Hendriksz TR, Huiskes AW, Zeegers AG. Brief communication: tamoxifen therapy for nonmalignant retroperitoneal fibrosis. *Ann Intern Med.* 2006;144(2):101-106.
6. Boccardo F, Rubagotti A, Battaglia M, Di Tonno P, Selvaggi FP, Conti G, et al. Evaluation of tamoxifen and anastrozole in the prevention of gynecomastia and breast pain induced by bicalutamide monotherapy of prostate cancer. *J Clin Oncol.* 2005;23(4):808-815.
7. Fradet Y, Egerdie B, Andersen M, Tammela TL, Nachabe M, Armstrong J, et al. Tamoxifen as prophylaxis for prevention of gynecomastia and breast pain associated with bicalutamide 150 mg monotherapy in patients with prostate cancer: a randomised, placebo-controlled, dose-response study. *Eur Urol.* 2007;52(1):106-114.
8. Einat H, Yuan P, Szabo ST, Dogra S, Manji HK. Protein kinase C inhibition by tamoxifen antagonizes manic-like behavior in rats: implications for the development of novel therapeutics for bipolar disorder. *Neuropsychobiology.* 2007;55(3-4):123-131.

9. Amrollahi Z, Rezaei F, Salehi B, Modabbernia AH, Maroufi A, Esfandiari GR, et al. Double-blind, randomized, placebo-controlled 6-week study on the efficacy and safety of the tamoxifen adjunctive to lithium in acute bipolar mania. *J Affect Disord.* 2011;129(1-3):327-331.
10. Blackwell KL, Haroon ZA, Shan S, Saito W, Broadwater G, Greenberg CS, et al. Tamoxifen inhibits angiogenesis in estrogen receptor-negative animal models. *Clin Cancer Res.* 2000;6(11):4359-4364.
11. Feil R, Brocard J, Mascrez B, LeMeur M, Metzger D, Chambon P. Ligand-activated site-specific recombination in mice. *Proc Natl Acad Sci U S A.* 1996;93(20):10887-10890.
12. Dabelic N, Jukic T, Labar Z, Novosel SA, Matesa N, Kusic Z. Riedel's thyroiditis treated with tamoxifen. *Croat Med J.* 2003;44(2):239-241.
13. Fatourechhi MM, Hay ID, McIver B, Sebo TJ, Fatourechhi V. Invasive fibrous thyroiditis (Riedel thyroiditis): the Mayo Clinic experience, 1976-2008. *Thyroid.* 2011;21(7):765-772.
14. Eugster EA, Shankar R, Feezle LK, Pescovitz OH. Tamoxifen treatment of progressive precocious puberty in a patient with McCune-Albright syndrome. *J Pediatr Endocrinol Metab.* 1999;12(5):681-686.
15. Sawathiparnich P, Osuwanaratana P, Santiprabhob J, Likitmaskul S. Tamoxifen improved final height prediction in a girl with McCune-Albright syndrome: patient report and literature review. *J Pediatr Endocrinol Metab.* 2006;19(1):81-86.
16. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell.* 2000;103(6):843-852.
17. Massarweh S, Osborne CK, Creighton CJ, Qin L, Tsimelzon A, Huang S, et al. Tamoxifen resistance in breast tumors is driven by growth factor receptor signaling with repression of classic estrogen receptor genomic function. *Cancer Res.* 2008;68(3):826-833.
18. Hurtado A, Holmes KA, Geistlinger TR, Hutcheson IR, Nicholson RI, Brown M, et al. Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen. *Nature.* 2008;456(7222):663-666.
19. Vehmanen L, Elomaa I, Blomqvist C, Saarto T. Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status. *J Clin Oncol.* 2006;24(4):675-680.
20. Grilli S. Tamoxifen (TAM): the dispute goes on. *Ann Ist Super Sanita.* 2006;42(2):170-173.
21. Decensi A, Maisonneuve P, Rotmensz N, Bettega D, Costa A, Sacchini V, et al. Effect of tamoxifen on venous thromboembolic events in a breast cancer prevention trial. *Circulation.* 2005;111(5):650-656.
22. Osman KA, Osman MM, Ahmed MH. Tamoxifen-induced non-alcoholic steatohepatitis: where are we now and where are we going? *Expert Opin Drug Saf.* 2007;6(1):1-4.
23. Paganini-Hill A, Clark LJ. Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen. *Breast Cancer Res Treat.* 2000;64(2):165-176.
24. Eberling JL, Wu C, Tong-Turnbeaugh R, Jagust WJ. Estrogen- and tamoxifen-associated effects on brain structure and function. *Neuroimage.* 2004;21(1):364-371.
25. Mortimer JE, Boucher L, Baty J, Knapp DL, Ryan E, Rowland JH. Effect of tamoxifen on sexual functioning in patients with breast cancer. *J Clin Oncol.* 1999;17(5):1488-1492.
26. Cella D, Fallowfield L, Barker P, Cuzick J, Locker G, Howell A, et al. Quality of life of postmenopausal women in the ATAC ("Arimidex", tamoxifen, alone or in combination) trial after completion of 5 years' adjuvant treatment for early breast cancer. *Breast Cancer Res Treat.*

- 2006;100(3):273-284.
27. Karimian E, Chagin AS, Gjerde J, Heino T, Lien EA, Ohlsson C, et al. Tamoxifen impairs both longitudinal and cortical bone growth in young male rats. *J Bone Miner Res.* 2008; 23(8):1267-1277.
 28. Wilson SC, Knight PG, Cunningham FJ. Evidence for the involvement of central conversion of testosterone to oestradiol-17b in the regulation of luteinizing hormone secretion in the cockerel. *J Endocrinol.* 1983;99(2):301-310.
 29. Nalbandian G, Paharkova-Vatchkova V, Mao A, Nale S, Kovats S. The selective estrogen receptor modulators, tamoxifen and raloxifene, impair dendritic cell differentiation and activation. *J Immunol.* 2005;175(4):2666-2675.
 30. Berstein LM, Wang JP, Zheng H, Yue W, Conaway M, Santen RJ. Long-term exposure to tamoxifen induces hypersensitivity to estradiol. *Clin Cancer Res.* 2004;10(4):1530-1534.
 31. Rousset-Jablonski C, Thalabard JC, Gompel A. Tamoxifen contraindicated in women with hereditary angioedema? *Ann Oncol.* 2009;20(7): 1281-1282.
 32. Kim SY, Suzuki N, Santosh Laxmi YR, Rieger R, Shibutani S. a-hydroxylation of tamoxifen and toremifene by human and rat cytochrome P450 3A subfamily enzymes. *Chem Res Toxicol.* 2003;16(9):1138-1144.
 33. Notley LM, Crewe KH, Taylor PJ, Lennard MS, Gillam EM. Characterization of the human cytochrome P450 forms involved in metabolism of tamoxifen to its a-hydroxy and a,4-dihydroxy derivatives. *Chem Res Toxicol.* 2005;18(10): 1611-1618.
 34. Lien EA, Anker G, Lønning PE, Solheim E, Ueland PM. Decreased serum concentrations of tamoxifen and its metabolites induced by aminoglutethimide. *Cancer Res.* 1990;50(18): 5851-5857.
 35. Ritchie LD, Grant SM. Tamoxifen-warfarin interaction: the Aberdeen hospitals drug file. *BMJ.* 1989;298(6682):1253.
 36. Tenni P, Lalich DL, Byrne MJ. Life threatening interaction between tamoxifen and warfarin. *BMJ.* 1989;298(6666):93.
 37. Lamberts SW, Verleun T, Hofland L, Oosterom R. Differences in the interaction between dopamine and estradiol on prolactin release by cultured normal and tumorous human pituitary cells. *J Clin Endocrinol Metab.* 1986;63(6):1342-1347.
 38. Dowsett M, Pfister C, Johnston SR, Miles DW, Houston SJ, Verbeek JA, et al. Impact of tamoxifen on the pharmacokinetics and endocrine effects of the aromatase inhibitor letrozole in postmenopausal women with breast cancer. *Clin Cancer Res.* 1999;5(9):2338-2343.
 39. Reid AD, Horobin JM, Newman EL, Preece PE. Tamoxifen metabolism is altered by simultaneous administration of medroxyprogesterone acetate in breast cancer patients. *Breast Cancer Res Treat.* 1992;22(2):153-156.
 40. Dehal SS, Brodie AM, Kupfer D. The aromatase inactivator 4-hydroxyandrostenedione (4-OH-A) inhibits tamoxifen metabolism by rat hepatic cytochrome P-450 3A: potential for drug-drug interaction of tamoxifen and 4-OH-A in combined anti-breast cancer therapy. *Drug Metab Dispos.* 1999;27(3):389-394.
 41. West CM, Reeves SJ, Brough W. Additive interaction between tamoxifen and rifampicin in human biliary tract carcinoma cells. *Cancer Lett.* 1990;55(2):159-163.
 42. Mani C, Gelboin HV, Park SS, Pearce R, Parkinson A, Kupfer D. Metabolism of the antimammary cancer antiestrogenic agent tamoxifen. I. Cytochrome P-450-catalyzed N-demethylation and 4-hydroxylation. *Drug Metab Dispos.* 1993;21(4):645-656.
 43. Mani C, Pearce R, Parkinson A, Kupfer D. Involvement of cytochrome P4503A in catalysis of tamoxifen activation and covalent binding to

- rat and human liver microsomes. *Carcinogenesis*. 1994;15(12):2715-2720.
44. Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, et al. Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos*. 2002;30(8):883-891.
 45. Jung DY, Ha HK, Lee HY, Lee JA, Lee JK, Huang DS, et al. Stimulation of the immune response by Yin-Tonifying formula. *J Korean Oriental Med*. 2010;31:112-123.
 46. Sekiya N, Hikiami H, Sakai S, Kainuma M, Goto H, Shibahara N, et al. Experimental Application of Jiin-koka-to to Cases of Bronchial Asthma. *Kampo Medicine*. 2003;54(6):1097-1101.
 47. Kim YK, Kim HJ, Kim WS, Park HJ, Moon G, Kim DW, et al. Inhibitory effect of Jaemganhwa-tang on allergic inflammatory reaction. *Korean J Orien Inter Med*. 2004;25:174-182.
 48. Zheng H, Lee Y, Yoo H, Cho C. Case Study of a Breast Cancer Patient Accompanying with Hot Flush by Tamoxifen Whose Condition Was Improved by Jayeumganhwa-tang. *Korean J Orien Inter Med*. 2010;6:395-400.
 49. Gibaldi M, Perrier D. *Pharmacokinetics*. 2nd ed. Oxford: Taylor & Francis; 1982.
 50. Bailer AJ. Testing for the equality of area under the curves when using destructive measurement techniques. *J Pharmacokinet Biopharm*. 1988;16(3):303-309.
 51. Chiou WL. Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level-time curve. *J Pharmacokinet Biopharm*. 1978;6(6):539-546.
 52. Adam HK, Gay MA, Moore RH. Measurement of tamoxifen in serum by thin-layer densitometry. *J Endocrinol*. 1980;84(1):35-42.
 53. Adam HK, Patterson JS, Kemp JV. Studies on the metabolism and pharmacokinetics of tamoxifen in normal volunteers. *Cancer Treat Rep*. 1980;64(6-7):761-764.
 54. Fabian C, Sternson L, Barnett M. Clinical pharmacology of tamoxifen in patients with breast cancer: comparison of traditional and loading dose schedules. *Cancer Treat Rep*. 1980;64(6-7):765-773.
 55. Jordan VC, Bain RR, Brown RR, Gosden B, Santos MA. Determination and pharmacology of a new hydroxylated metabolite of tamoxifen observed in patient sera during therapy for advanced breast cancer. *Cancer Res*. 1983;43(3):1446-1450.
 56. Murphy C, Fotsis T, Pantzar P, Adlercreutz H, Martin F. Analysis of tamoxifen and its metabolites in human plasma by gas chromatography-mass spectrometry (GC-MS) using selected ion monitoring (SIM). *J Steroid Biochem*. 1987;26(5):547-555.
 57. Jordan VC. Metabolites of tamoxifen in animals and man: identification, pharmacology, and significance. *Breast Cancer Res Treat*. 1982;2(2):123-138.
 58. Sun D, Sharma AK, Dellinger RW, Blevins-Primeau AS, Balliet RM, Chen G, et al. Glucuronidation of active tamoxifen metabolites by the human UDP glucuronosyltransferases. *Drug Metab Dispos*. 2007;35(11):2006-2014.
 59. AstraZeneca Pharmaceuticals. Nolvadex (tamoxifen citrate) prescribing information. Wilmington, DE: 2003 June.
 60. Hall WA, Doolittle ND, Daman M, Bruns PK, Muldoon L, Fortin D, et al. Osmotic blood-brain barrier disruption chemotherapy for diffuse pontine gliomas. *J Neurooncol*. 2006;77(3):279-284.