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Original Article

Effects of mixed formulation of tamoxifen and blue honeysuckle on the pharmacokinetics profiles of tamoxifen after single oral administration

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Objectives: Here, we investigated the effects of concentrated and lyophilized powders Blue honeysuckle (BH) on the PK of tamoxifen, to establish the pharmacokinetics (PK) profiles as one of essential process in new drug development.
Methods: After single oral treatment of 0.4 mg/ml of tamoxifen or tamoxifen 0.4 with BH 40, 20 and 10 mg/ml, the plasma were collected at 0.5 hr before administration, 0.5, 1, 2, 3, 4, 6, 8 and 24 hr after end of single or mixed formula treatment. Plasma concentrations of tamoxifen were analyzed using LC-MS/MS methods. Tmax, Cmax, AUC, t1/2 and MRTinf were analyzed using noncompartmental PK data analyzer programs.
Results: Tamoxifen and BH 40 mg/ml did not induce any significant change on the plasma tamoxifen concentrations, while significant decreases were observed in tamoxifen and BH 10 mg/ml from 2 to 8 hr as compared with tamoxifen only, respectively. Furthermore, significant increases of Tmax in tamoxifen and BH 40 mg/ml, significant decreases of Cmax in tamoxifen and BH 20 mg/ml, significant decreases of AUC0-t, AUC0-inf and MRTinf in tamoxifen and BH 10 mg/ml were demonstrated as compared with tamoxifen only.
Conclusion: Taken together, tamoxifen and BH 10 mg/ml induced significant decrease of the oral bioavailability of tamoxifen, while tamoxifen and BH 40 or 20 mg/ml did not critically influenced, suggesting formulated BH concentration-independencies. It, therefore, seems to be needed that pharmacokinetic study after repeated administration should be tested to conclude the effects of BH on the pharmacokinetics of tamoxifen.

Key Words : Blue honeysuckle, Tamoxifen, Mixed formulation, Pharmacokinetics, Drug-drug interactions, Rat

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Introduction

Pharmacokintics (PK) is a branch of pharmacology dedicated to the determination of the fate of substances administered externally to a living organism. PK includes the study of the mechanisms of absorption and distribution of an administered drug, the rate at which a drug action begins and the duration of the effect, the chemical changes of the substance in the body[1,2]. PK of drugs in animals, especially rodents have been provided a valuable data for absorption, distribution, metabolism and elimination of drugs *in vivo*, directly related with their pharmacodynamics. Therefore, the PK profiles have been observed as one of essential process in new drug development including natural products[1-4]. In addition, they also provided constantly reliable data on the possible potential drug-drug interactions, when drugs were co-administered[2-4].

Tamoxifen is a nonsteroidal estrogen agonist -antagonist antineoplastic agent has been used for breast cancer[5]. 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[6,7]. 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 cytocidal[8,9]. However, various side effects related to tamoxifen treatment also have been arise as bone loss in premenopausal women who continue to menstruate after adjuvant chemotherapy[10], endometrial changes, including cancer, are among tamoxifen's side effects[11], increased risk of thromboembolism[12], cause of fatty liver[13], reduced cognition[14], semantic memory scores[15] and libido[16,17], and premature growth plate fusion[18]. Tamoxifen also depress the immune response[19,20], and it also known that hypersensitivity to tamoxifen or any ingredient in the formulation[21,22].

Tamoxifen is contraindicated, when used in women with ductal carcinoma in situ and women at high risk for breast cancer, concurrent anticoagulant therapy with a warfarin derivative[23], and be used with caution in patients with leukopenia or thrombocytopenia[24] and pregnant[25,26]. Hot flashes, vaginal discharge, menstrual irregularities and weight loss are common side effects related with tamoxifen treatment[25,27].

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[28], 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[29], anticoagulants - enhanced warfarin effects[30,31], bromocriptine - increased plasma tamoxifen and N-desmethyltamoxifen concentrations[32], letrozole - decreased plasma letrozole concentrations[33], medroxyprogesterone - decreased plasma N-desmethyltamoxifen concentrations but did not reduce plasma tamoxifen concentrations[34], phenobarbital − decreased plasma tamoxifen concentrations[35], rifampin - decreased plasma tamoxifen and N-desmethyltamoxifen concentrations[36], and cyclosporine, erythromycin, diltiazem, erythromycin and nifedipine - competitively inhibited formation of N-desmethyltamoxifen *in vitro*[37-39], respectively.

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However, interactions with herbal products have not been established except for some restricted natural compounds[30,31].

Until now, we have observed the possible interactions with Korean traditional polyherbal formulas; oral co-administration of *Jaeumkanghwa-tang*, a traditional yin-tonifying herbal medicine used for various oriental obstetrical and gynecological fields within 5 min did not critically influenced on the pharmacokinetics profiles of tamoxifen after single[40] and repeated[41] co-administration at dosage levels of 50 mg/kg in tamoxifen and 100 mg/kg in *Jaeumkanghwa-tang*, respectively. It was also demonstrated that single co-administration of *Gamiondam-tang* within 5 min and with 2.5 hr-intervals critically influenced on the oral bioavailability of tamoxifen through variable influences on the absorption and excretion of tamoxifen at dosage levels of GMODT 100 mg/kg and tamoxifen 50 mg/kg, can be influenced on the toxicity or pharmacodynamic of tamoxifen in our previous studies[42,43].

Blue honeysuckle (Berries of *Lonicera caerulea* L., Caprifoliaceae) is a traditional shrub used in folk medicine in northern Russia, China, and Japan, but its fruits are little known as edible berries in North America and Europe, and also in Korea[44]. The berries are a rich source of ascorbic acid and phenolic components, particularly anthocyanins, flavonoids and low molecular weight phenolic acids[44]. These compounds have been reported to have multiple biological activities including strong antioxidant activity[44]. Recently, orally administered blue honeysuckle was reported to protect mice against ionizing radiation[45], ameliorates abnormal lipid and glucose metabolism in rats[46], hepatoprotective effects[47], anti-inflammatory effects[48]. Especially blue honeysuckle extracts have been showed the strongest antioxidant potent among 12 types of colored berries[49], and phenolic rich extract of BH has been shown to possess anti-inflammatory and wound-healing effects *in vitro* and *in vivo*[48] and skin protective effects to ultraviolet-induced damages[50] with less toxicity[51].

In the present study, the effects of BH on the pharmacokinetics of tamoxifen were examined in rats after mixed formulation as tamoxifen 0.4 and BH 40, 20 and 10 mg/ml concentrations. After single oral treatment of 4 mg/kg of tamoxifen single formula or tamoxifen 0.4 with BH 40, 20 and 10 mg/ml concentration mixed formulas, the plasma were collected at 30 min before administration, 30 min, 1, 2, 3, 4, 6, 8 and 24 hr after end of single or mixed formula treatment. Plasma concentrations of tamoxifen were analyzed using LC-MS/MS methods. PK parameters of tamoxifen (Tmax, Cmax, AUC, $t_{1/2}$ and MRT_{inf}) were analysis as compared with tamoxifen single formula administered rats.

Materials & methods

1. Animals and husbandry

A total of fifty-three male SPF/VAF Outbred Crl:CD [SD] rats (OrientBio, Seungnam, Korea) were used after acclimatization for 15 days. Animals were allocated five per polycarbonate cage in a temperature (20-25ºC) and humidity (40-45%) controlled room. Light : dark cycle was 12hr : 12hr and feed (Samyang, Korea) and water were supplied free to access. After 15 days of acclimatization, four groups of 5 rats each were selected based on the body weights, and used further experiments, in the present study. All animals were marked by picric acid, and overnight

fasted (about 18 hr; water was not restricted) before treatment, and further fasted during 3 hr after end of treatment. Animal experiments were conducted 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) [Approval Approval No DHU2014-081].

2. Test articles

Deep purple colored solution of concentrated blue honeysuckle, about 63 brix, were supplied by H&K Bioscience Co., Ltd. (Seoul, Korea) and used as same as our previous single oral dose toxicity test[51] in this experiment. The brief process for making BH was as follows. 200 g of 63 brix concentrated blue honeysuckle solutions supplied by H&K Bioscience were diluted into 25 brix using distilled water, and then completely lyophilized by programmable freeze dryer (Operon FDB-5503, Kimpo, Korea). Total 124.40 g (yield = 62.2%) of BH were acquired. At proximate analysis of BH by Association of Official Analytical Chemists (AOAC)[52] methods, BHcL contains energy 380 kcal/100 ml, carbohydrate 93 g/100 ml, sugar 41 g/100 ml, protein 2 g/100 ml, sodium 20 mg/100 ml, but it did not contains total lipids (0 mg/100 ml), saturated lipids (0 mg/100 ml), trans-fat (0 mg/100 ml), cholesterol (0 mg/100 ml), respectively. In addition, phytochemical analysis of BH reveals that it contains 4.54 ± 0.09% of betaine by high performance liquid chromatography (HPLC), 210.63 ± 23.65 mg gallic acid equivalents (GAE)/g of total phenols by Folin-Ciocalteu colorimetric method[53], 159.30 ± 12.51 mg catechin equivalents (CE)/g of total flavonoids by a modified colorimetric method[54] and 133.57 ± 4.06 mg malvidin-3-O-glucoside equivalents (M3GE)/g of total antocyanins by a modified pH differential method[55], respectively. In addition, white crystalline powders of tamoxifen (Kunshan SanYou Pharmaceutical Material Co., Ltd., Suzhou, China) was used as control drug as listed follows. Tamoxifen and BH were stored in a refrigerator at 4ºC and -20 ºC to protect from light and degeneration until use. Both drugs are well dissolved (up to 40 mg/ml solutions in BH and upto 0.4 mg/ml solutions in tamoxifen) in distilled water as vehicle, respectively.

3. Groupings, test article formulation and administration

Four groups of 5 rats each were used in this study as follows. The dosages of tamoxifen 4 mg/kg were selected, considering the clinical dosage in human and body surface of rats. Single formula of tamoxifen was prepared as dissolved in distilled water (0.4 mg/ml concentration), and each mixed formula consisted of tamoxifen and BH was prepared by dissolved of approximate amounts of tamoxifen (0.4 mg/ml) and BH (40, 20 or 10 mg/ml) in distilled water. Tamoxifen single formula and all three types of mixed formula consisted of tamoxifen and BH were once orally administered, in a volume of 10 ml/kg, respectively.

4. Plasma collections

All rats were anesthetized with 2 to 3% isoflurane (Hana Pharm. Co., Hwasung, Korea) in the mixture of 70% N_2O and 28.5% O_2, and blood samples (0.5 ml) were collected into 50 IU heparinized 0.7 ml Eppendorf tubes through the orbital plexus at 30 min before treatment (as a control), 30 min, 1, 2, 3, 4, 6, 8 and 24 hr after

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end of single oral administration of tamoxifen single or mixed formula with three different dosages of BH, respectively. Blood samples were immediately centrifuged for 10 min at 13,000 rpm and about 0.3 ml aliquots of plasma were stored in a -150 ºC deep freezer until analysis of tamoxifen.

5. Sample preparation and calibrations

Primary stock solution, 1.0 mg/ml of tamoxifen in 50% acetonitrile (Sigma-Aldrich, St Louise, MO, USA) mixtures with distilled water and internal standard working solution, carbamazepine (Sigma-Aldrich, St Louise, MO, USA) 500 ng/ml in acetonitrile were prepared. Working standard solutions were prepared by dilution with acetonitrile. All standard solutions were stored at -20ºC 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. 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 4ºC. Clear supernatants (150 μ l) were directly transferred to injection vials and the aliquot (5.0 μl) 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, Santa Clara, 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 Symmetry^{TM} C_{18} (2.1×50mm, 3.5μm) (Waters Corp., Milford, MA, USA) at column oven 30ºC. The mobile phase used for the chromatographic separation was composed of 50% distilled water (0.1% formic acid) / 50% acetonitrile 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, Foster City, CA, USA). The instrument was equipped with an electrospray interface in positive ion mode, and controlled by the Analyst version 1.4.1 software (Applied Biosystems, Foster City, CA, USA). Samples were introduced to the interface through a Turbo Ion Spray with the temperature set at 500°C. A high positive voltage of 4.0 kV was applied to the ion spray. Nitrogen was used as the nebulizer gas, curtain gas, and collision gas with the settings of 70, 20, and 7, 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: 0.74 min), tamoxifen: 372>178 (Retention time: 0.55 min). Calibration curves of tamoxifen were linear over the ranges studied with r^2>0.994. The lower limit of quantification of the tamoxifen in the rat plasma was 0.5 ng/ml.

7. Pharmacokinetic analysis

The plasma concentration data were analyzed using a noncompartmental method on commercial pharmacokinetics data analyzer programs (PK solutions2.0; Summit, Montrose, CO, USA)[56]. 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 (Cmax) and time to reach the peak concentration (Tmax) 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[57]. 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 (SD) of five rats (Mean±SD of five rat plasma tamoxifen concentrations). 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., Chicago, IL, USA). A p-value <0.05 was considered statistically significant. In addition, the percent-point changes between tamoxifen single formula treated rats and tamoxifen with BH 40, 20 or 10 mg/ml mixed formula administered rats were calculated to help the understanding of the effects after mix formulations, according to our previous study[58]: Percentage-point changes as compared with tamoxifen 0.4 mg/ml single formula treated mice (%) = [((Data of mixed formula administrated rats − data of tamoxifen single formula treated rats)/Data of tamoxifen single formula treated rats) × 100].

Results

1. Changes on the plasma concentrations of tamoxifen

Tamoxifen was detected from 30 min to 24 hr after end of administration in tamoxifen single formula and all three different concentrations of mixed formulas with BH administered rats, respectively. Although single oral administration of mixed formulas consisted of tamoxifen and BH 40 mg/ml did not induced any significant changed on the plasma tamoxifen concentrations, but significant ($p<0.01$ or $p<0.05$) decreases of plasma tamoxifen concentrations were demonstrated in tamoxifen and BH 20 mg/ml mixed formula treated rats at 1, 2 and 6 hr after administration, and also in BH 10 mg/ml mixed formula treated rats from 2 to 8 hr after end of treatment as compared with tamoxifen 0.4 mg/ml single formula treated rats, in the current result (Fig 1).

2. Changes on the Tmax of tamoxifen

The Tmax of tamoxifen were significantly ($p<0.05$) increased as 172.73% points in tamoxifen and BH 40 mg/ml mixed formula treated rats (3.00±1.00 hr) as compared with tamoxifen single formula treated rats (1.10±0.55 hr). The Tmax of tamoxifen were non-significantly changed as 27.27 and 9.09% points in tamoxifen and BH 20 or 10 mg/ml mixed formula treated rats as compared with tamoxifen single formula treated rats; they were detected as 1.40±0.89 and 1.20±0.45 hr in tamoxifen and BH 20 or 10 mg/ml mixed formula treated rats, respectively (Table 1).

3.3. Changes on the Cmax of tamoxifen

The Cmax of tamoxifen were significantly

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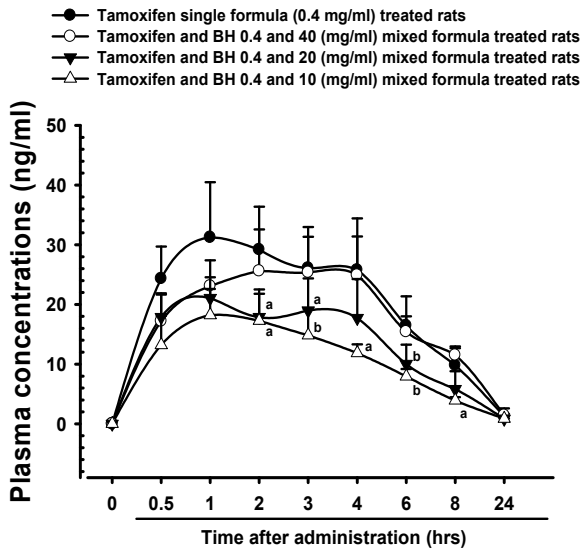

Fig. 1. Plasma concentrations of tamoxifen after single oral administration of tamoxifen single or mixed formulas with BH 40, 20 or 10 mg/ml in male rats. Values are expressed as mean ± S.D. of five rats (ng/ml). BH = Blue honeysuckle (fruit parts of *Lonicera caerulea* L., Caprifoliaceae) concentrated and lyophilized powder. [a] $p<0.01$ and [b] $p<0.05$ as compared with tamoxifen single formula administered rats.

($p<0.05$) decreased as -32.90% points in tamoxifen and BH 20 mg/ml mixed formula treated rats (21.46±4.01 ng/ml) as compared with tamoxifen single formula treated rats (31.98±8.98 ng/ml). The Cmax of tamoxifen were non-significantly changed as -15.13 and -33.77% points in tamoxifen and BH 40 or 10 mg/ml mixed formula treated rats as compared with tamoxifen single formula treated rats; they were detected as 27.14±7.24 and 21.18±7.61 ng/ml in tamoxifen and BH 20 or 10 mg/ml mixed formula treated rats, respectively (Table 1).

Table 1. Pharmacokinetic parameters of tamoxifen after single oral administration of tamoxifen single or mixed formulas with BH 40, 20 or 10 mg/ml in male rats.

Treatment PK Parameters	Tamoxifen single formula	Tamoxifen mixed formulas with BH		
	0.4 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml
Tmax (hr)	1.10±0.55	3.00±1.00 [b]	1.40±0.89	1.20±0.45
Cmax (ng/ml)	31.98±8.98	27.14±7.24	21.46±4.01 [b]	21.18±7.61
AUC_{0-t} (hr · ng/ml)	244.44±85.90	260.11±46.59	162.10±65.97	106.01±37.79 [b]
AUC_{0-inf} (hr · ng/ml)	258.74±81.02	271.99±56.50	169.01±66.94	119.45±33.45 [a]
$t_{1/2}$ (hr)	4.44±0.98	4.98±1.20	4.50±1.28	3.13±1.23
MRT_{inf} (hr)	6.15±0.63	7.10±1.32	5.84±1.16	4.83±0.91 [b]

Values are expressed as mean ± S.D. of five rats. BH = Blue honeysuckle (fruit parts of *Lonicera caerulea* L., Caprifoliaceae) concentrated and lyophilized powder. Cmax: The peak plasma concentration; Tmax: 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. [a] $p<0.01$ and [b] $p<0.05$ as compared with tamoxifen single formula administered rats.

4. Changes on the AUC of tamoxifen

The AUC_{0-t} of tamoxifen were non-significantly changed as 6.41 and -33.69% points in tamoxifen and BH 40 or 20 mg/ml mixed formula treated rats as compared with tamoxifen single formula treated rats, respectively. They were detected as 260.11±46.59 and 162.10±65.97 hr·ng/ml in tamoxifen and BH 40 or 20 mg/ml mixed formula treated rats, and as 244.44±85.90 hr·ng/ml in tamoxifen single formula treated rats, respectively. However, the AUC_{0-t} of tamoxifen was significantly ($p<0.05$) decreased as -56.63% points in tamoxifen and BH 10 mg/ml mixed formula treated rats (106.01±37.79 hr·ng/ml) as compared with tamoxifen single formula treated rats, in this experiment. In addition, the AUC_{0-inf} of tamoxifen were non-significantly changed as 5.12 and -34.68% points in tamoxifen and BH 40 or 20 mg/ml mixed formula treated rats as compared with tamoxifen single formula treated rats, respectively. They were detected as 271.99±56.50 and 169.01±66.94 hr·ng/ml in tamoxifen and BH 40 or 20 mg/ml mixed formula treated rats, and as 258.74±81.02 hr·ng/ml in tamoxifen single formula treated rats, respectively. However, the AUC_{0-inf} of tamoxifen was significantly ($p<0.01$) decreased as -53.83% points in tamoxifen and BH 10 mg/ml mixed formula treated rats (119.45± 33.45 hr·ng/ml) as compared with tamoxifen single formula treated rats, in the current result (Table 1).

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

The $t_{1/2}$ of tamoxifen were non-significantly changed as 12.31, 1.32 and -29.50% points in tamoxifen and all three different concentrations of BH mixed formula treated rats as compared with tamoxifen single formula treated rats, respectively. They were detected as 4.98±1.20, 4.50±1.28 and 3.13±1.23 hr in tamoxifen and BH 40, 20 or 10 mg/ml mixed formula treated rats, and as 4.44±0.98 hr in tamoxifen single formula treated rats, in our experiment (Table 1).

6. Changes on the MRT_{inf} of tamoxifen

The MRT_{inf} of tamoxifen were significantly ($p<0.05$) decreased as -21.43% points in tamoxifen and BH 10 mg/ml mixed formula treated rats (4.83±0.91 hr) as compared with tamoxifen single formula treated rats (6.15±0.63 hr). The MRT_{inf} of tamoxifen were non-significantly changed as 15.40 and -5.00% points in tamoxifen and BH 40 or 20 mg/ml mixed formula treated rats as compared with tamoxifen single formula treated rats; they were detected as 7.10±1.32 and 5.84±1.16 hr in tamoxifen and BH 40 or 10 mg/ml mixed formula treated rats, in this study (Table 1).

Discussion

Although single oral administration of mixed formulas consisted of tamoxifen and BH 40 mg/ml did not induced any significant changed on the plasma tamoxifen concentrations, but significant decreases of plasma tamoxifen concentrations were demonstrated in tamoxifen and BH 20 mg/ml mixed formula treated rats at 1, 2 and 6 hr after administration, and also in BH 10 mg/ml mixed formula treated rats from 2 to 8 hr after end of treatment as compared with tamoxifen 0.4 mg/ml single formula treated rats, respectively. In addition, significant increases of Tmax in tamoxifen and BH 40 mg/ml mixed formula treated rats, significant decreases of Cmax in tamoxifen and BH 20 mg/ml mixed formula treated rats, and significant decreases of AUC_{0-t}, AUC_{0-inf} and MRT_{inf} in tamoxifen and

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BH 10 mg/ml mixed formula treated rats were demonstrated as compared with tamoxifen 0.4 mg/ml single formula treated rats, respectively. These findings are considered as direct evidences that single oral administration of mixed formulation with BH 10 mg/ml concentration significantly decreased the oral bioavailability of tamoxifen through decrease of the absorptions, but not in tamoxifen and BH 40 and 20 mg/ml mixed formulas, suggesting formulated BH concentration -independencies in the current study. It, therefore, seems to be need that pharmacokinetic study after repeated administration of mixed formulas for considerable periods should be tested to conclude the effects of mixed formulation with BH on the pharmacokinetics of tamoxifen, and can be used as potent comprehensive and integrative medicine for the mammary cancer.

Tamoxifen was absorbed slowly following oral administration and Tmax of tamoxifen occur about 3-6 hr after a single dose[6,61] 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[60,61]. 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[62]. Tamoxifen excreted principally in feces as polar conjugates[63] with about 5-7 days of $t_{1/2}$ in tamoxifen and 9-14 days in N-desmethyltamoxifen[59]. Clearance of tamoxifen is higher in female children 2-10 years of age than in women[64,65]. In the present study, Tmax of tamoxifen in tamoxifen 0.4 mg/ml single formula oral treated rats was detected as 1.10±0.55 hr, and Cmax, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $t_{1/2}$ and MRT_{inf} were detected as 31.98±8.98 ng/ml, 244.44±85.90 hr·ng/ml, 258.74±81.02 hr·ng/ml, 4.44±0.98 hr and 6.15±0.63 hr, respectively. In tamoxifen and BH 40 mg/ml mixed formula administrated rats, Tmax, Cmax, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $t_{1/2}$ and MRT_{inf} of tamoxifen were detected as 3.00±1.00 hr, 27.14±7.24 ng/ml, 260.11±46.59 hr·ng/ml, 271.99±56.50 hr·ng/ml, 4.98±1.20 hr and 7.10±1.32 hr as changed as 172.73, -15.13, 6.41, 5.12, 12.31 and 15.40% points as compared with tamoxifen 0.4 mg/ml single formula treated rats, in the present study. They showed significant ($p<0.05$) decreases of Tmax, but not in $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ as compared with tamoxifen 0.4 mg/ml single formula treated rats, respectively. In addition, tamoxifen and BH 20 mg/ml mixed formula administrated rats also showed significant ($0<0.01$) reduced of Cmax, but not in $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ as compared with tamoxifen 0.4 mg/ml single formula treated rats, respectively. They showed 27.27, -32.90, -33.69, -34.68, 1.32 and -5.00% points of Tmax, Cmax, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $t_{1/2}$ and MRT_{inf} as compared with tamoxifen 0.4 mg/ml single formula treated rats, and detetced as 1.40±0.89 hr, 21.46±4.01 ng/ml, 162.10±65.97 hr·ng/ml, 169.01±66.94 hr·ng/ml, 4.50±1.28 hr and 5.84±1.16 hr hr, in our result. However, Tmax, Cmax, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $t_{1/2}$ and MRT_{inf} of tamoxifen in tamoxifen and BH 10 mg/ml mixed formula administrated rats were detected as 1.20±0.45 hr, 21.18±7.61 ng/ml, 106.01± 37.79 hr·ng/ml, 119.45±33.45 hr·ng/ml, 3.13±1.23 hr and 4.83±0.91 hr as changed as 9.09, -33.77, -56.63, -53.83, -29.50 and -21.43% points as compared with tamoxifen 0.4 mg/ml single formula treated rats; They showed significant ($p<0.01$ or $p<0.05$) decreases of $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and MRT_{inf} as compared with tamoxifen 0.4 mg/ml single formula treated rats, in the current study.

Tamoxifen rapidly and extensively metabolized in the liver, through a substrate of CYP3A, 2C9, 2D626 to active major metabolite, N-desmethyltamoxifen[6,61] and, therefore, tamoxifen can be interacted with various drugs[29-36]. In addition the possibilities that tamoxifen competitively interacted with cyclosporine, erythromycin, diltiazem, erythromycin and nifedipine were also suggested *in vitro* experiments[37-39]. The severities of various side effects arise from tamoxifen treatment, especially bone loss[10], endometrial cancer[11], thromboembolism[12], fatty liver[13], reduced cognition[14], semantic memory scores[15] and libido[16,17], premature growth plate fusion[18], immune suppression[19,20] and hypersensitivity[21,22] are considered as directly co-related with absorption and excretion of tamoxifen or pharmacodynamics. In the present study, it is demonstrated that single oral administration of mixed formulation with BH 10 mg/ml concentration significantly decreased the oral bioavailability of tamoxifen through decrease of the absorptions, but not in tamoxifen and BH 40 and 20 mg/ml mixed formulas, suggesting formulated BH concentration-independencies and repeated administration of mixed formulas for considerable periods seems to be need to conclude the effects of mixed formulation with BH on the pharmacokinetics of tamoxifen and whether can be adjusted to mammary cancer patient as potent comprehensive and integrative medicine, at least in a condition of this experiment.

Conclusions

Taken together, single oral administration of tamoxifen 0.4 mg/ml and BH 10 mg/ml mixed formulation induced significant decrease of the oral bioavailability of tamoxifen through inhibition of absorptions. However, tamoxifen 0.4 mg/ml and BH 40 or 20 mg/ml mixed formulation did not critically influenced on the oral bioavailability (AUCs) of tamoxifen, suggesting formulated BH concentration-independencies. It, therefore, seems to be need that pharmacokinetic study after repeated administration of mixed formulas for considerable periods should be tested to conclude the effects of mixed formulation with BH on the pharmacokinetics of tamoxifen. It can be used as potent comprehensive and integrative medicine for the mammary cancer.

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