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Pharmacokinetics of piperine following single dose administration of benjakul formulation in healthy Thai subjects

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Benjakul formulation is a Thai traditional medicine preparation, has also been used as an adaptogenic drug for cancer patients. No toxicity, either acutely or chronically has been reported in experimental animals and humans. The study was to preliminarily investigate the pharmacokinetics of piperine, the major active component of Benjakul formulation, following the administration of single oral doses of 100 (group 1) and 200 (group 2) mg Benjakul tablets in 20 healthy Thai subjects. Venous blood samples were collected before and after dosing. Serum concentrations of piperine were measured using HPLC-UV method. Pharmacokinetic analysis was performed by using model-independent analysis approach. Benjakul formulation was well tolerated in all subjects, with no apparent adverse events. Piperine was rapidly absorbed following the administration of both dosage levels. The pharmacokinetics of piperine was dose–independent. The observed median first maximum concentration (Cmax-1st) of piperine of 1,078 ng/mL following the dose of 200 mg Benjakul was significantly higher than (p< 0.001) that of 100 mg dose (467 ng/mL). Median time to first maximum concentration (tmax-1st) was about 1 hours in both groups. The area under serum concentration-time curve (AUC 0-48hr) of 10,216 ng.hr/mL following 200 mg dose was also significantly greater than (p< 0.001) that of 100 mg dose (4,288 ng.hr/mL). It was noted however for the second maximum concentration (Cmax-2nd) of piperine at about 9 hours post-dosing observed in 9 (median: 203 ng/mL) and 7 (median: 499 ng/mL) subjects who received 100 and 200 mg Benjakul formulation, respectively.

Key words: Benjakul formulation, piperine, Piper chaba Hunter., Piper sarmentosum Roxb., Piper interruptum Opiz., Plumbago indica Linn., and Zingiber officinale Roscoe., pharmacokinetics.

INTRODUCTION

Cancer is one of the leading causes of death in Thailand, and the trend is continuously increasing (National Cancer Institute, 2012). Several approaches are currently being applied for treatment of cancer including chemotherapy, radiation, and surgery. The major problem of cancer chemotherapy is drug-associated toxicities due to non-selectivity of most chemotherapeutics to normal cells (Rebucci and Michiels, 2013). The use of herbs as complementary and alternative medicine has increased dramatically in the past decades. Plant-derived compounds or dietary phytochemicals have emerged as an accessible and promising approach to cancer control and management (Surh, 2003). A growing trend among cancer patients, especially those living in the rural areas

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is to combine conventional therapy with some forms of complementary therapy (Vapiwala et al., 2006). These products may act synergistically with other chemotherapeutics or other treatment approaches to kill tumor cells by reducing angiogenesis, inflammation, and/or metastasis.

Benjakul formulation is one Thai traditional medicine used as complementary medicine in conjunction with other herbal medicines for treatment of cancer. This therapeutic application is believed to be through balancing "Dhatu" (regulating body chemical and physical function) before cancer chemotherapy (Itharat et al., 1999; Sriyakul et al., 2010). Benjakul formulation is composed of parts from five plants, that is *Piper chaba* Hunter. (fruit), *Piper sarmentosum* Roxb. (root), *Piper interruptum* Opiz. (stem), *Plumbago indica* Linn. (root), and *Zingiber officinale* Roscoe. (rhizome). Bioassay guided fractionation of the ethanolic extract of this herbal formulation revealed active constituents, of which piperine from *P. chaba* Hunter., *P. sarmentosum* Roxb., and *P. interruptum* Opiz. were the major components (78.69%), followed by plumbagin from *P. indica* Linn. (17.05%), and 6-gingerol from *Z. officinale* Roscoe. (4.26%) (Itharat et al., 2010).

The formulation including their active constituents, particularly piperine, have been demonstrated to exhibit a wide range of pharmacological and biological activities. Apart from cytotoxic and anticancer activities against lung, breast and prostate cancers, Benjakul formulation and piperine also possess antioxidant, anti-inflammatory, analgesic and anti-pyretic, central nervous system depressant, antiplatelet, antihypertensive, hepatoprotective, antithyroid, immuno-stimulating (promoting natural killer cell activity) activities, as well as inhibitory activity on nitric oxide production (Bhitre et al., 2008; Vaghasiya et al., 2007; Veeru et al., 2009; Wu et al., 2004). Acute and chronic toxicity tests in animals and humans have demonstrated the preparation to be practically non-toxic and well-tolerated (Amorndoljai et al., 2011; Itharat et al., 2010). Nevertheless, there has been no information regarding its pharmacokinetics in humans.

The aim of the present study was therefore to preliminarily investigate the pharmacokinetics of Benjakul formulation in healthy Thai subjects, using piperine as a pharmacological marker.

MATERIALS AND METHODS

Study subjects

Twenty healthy Thai subjects (10 males and 10 females), aged between 20 and 38 years, weighing 42 to 84 kg, with body mass index (BMI) between 17.9 and 25.9 kg/m^2 participated in the study. Inclusion criteria included: non-lactating and non-pregnant verified by urine β-hCG urine pregnancy test (females), normal laboratory tests (haematology, serum biochemistry, and urinalysis), no significant abnormal findings on physical and clinical examination particularly liver, kidney, cardiovascular diseases, or peripheral neuropathy, no previous consumption of foods containing the five composition of Benjakul formulation within two weeks, and no previous administration of other drugs within three months. None was a smoker, alcohol drinker, or was on regular medication. Written informed consent for participation was obtained from each volunteer before initiation of the study. The study was approved by the Human Ethics Committee of the Faculty of Medicine, Thammasat University, Thailand.

At enrollment, a medical history was taken, including a full physical examination; each volunteer had a thorough physical examination and routine laboratory investigations (haematology, serum biochemsitry, and urinalysis).

Preparation of Benjakul formulation

Plant materials, that is fruits of *P. chaba* Hunter. (from Thongphaphoom District, Kanchanaburi Province, voucher number SKP 146160301), roots of *P. sarmentosum* Roxb. (from Hadyai District, Sonkhla Province, voucher number SKP 146161901), stems of *P. interruptum* Opiz. (Mae-rim District, Chaingmai Province, voucher number SKP 146160901), roots of *P. indica* Linn. (Bankoknoi District, Bangkok, voucher number SKP148160901), and rhizomes of *Z. officinale* Roscoe. (Khaokho District, Petchaboon Province, voucher number SKP206261501) were collected from all parts of Thailand during January to March, 2006. Authentication of plant materials was carried out at the herbarium of the Department of Forestry, Bangkok, Thailand, where the herbarium vouchers have been kept. The plant materials were rinsed thoroughly with tap water to remove extraneous contaminants and cut into small pieces, oven-dried at 50°C until stability of dry weight was observed, and then ground into powder with an electric-grinder.

Extraction was carried out by macerating the powdered plant materials (100 g) in a flask containing 500 ml of 95% ethanol at room temperature (25 to 30°C) for 7 days. The extracted solvent was separated and filtered through Whatman no. 1 filter paper. After filtration, the extracts were evaporated under reduced pressure by rotary evaporation. The crude extracts were weighed and stored at -20°C until use. Standardization of the extract was performed using activity-guided fractionation. Piperine, the major component of Benjakul formulation was used as a marker for standardization of the formulation using high-performance liquid chromatography. Acceptable content of piperine was 27 ± 1 mg/g of dried weight plant materials with IC_{50} (concentration that inhibits cell growth by 50%) value in lung cancer cell of 30 ± 1 µg/ml. Chromatographic separation condition used was as follow: Phenonemex^{TM} Luna C18 column (5 µm particle size); mobile phase: a mixture of water and acetonitrile with gradient elution 0 min (60:40), 30 min (50:50), 50 min (5:95), and 60 min (0:100) at a flow rate of 1 ml/min.

Bajakul tablet was prepared and tested according to the United States Pharmacopeia USP Standard (weight variation, content uniformity, disintegration, and dissolution), as well as those not given in the USP Standard (hardness, thickness, and friability). The dried extract was formulated in tablets; each tablet contained 60 mg/g of total content (100 mg Benjakul formulation contained 6 mg piperine) (Itharat and Sakpakdeejaroen, 2010; Sakpakdeejaroen, 2009).

Drug administration

The subjects were randomly divided into two groups (5 males and 5 females in each group) to receive a single oral dose of 100 (group 1) or 200 (group 2) mg Benjakul formulation (100 and 200 mg tablets, respectively). Compliance with all drug intake was under investigators' supervision. No food was allowed until 2 h after drug intake. Volunteers were hospitalized at Thammasat Chalermprakiet Hospital, Pathumtanee Province one day prior to, and on the day of

pharmacokinetic study. No other concurrent drugs or alcohol were allowed during the study period. Meals with no composition of the five plant materials were provided to all subjects during the investigation period.

Adverse reaction monitoring

Following the administration of Benjakul formulation, all subjects were physically examined and adverse reactions during the study were recorded with the date and time at which they occurred and disappeared. These included gastrointestinal, central nervous, cardiovascular, dermatological effects, as well as other changes possibly attributable to the study drugs. Adverse effects were assessed on the basis of non-suggestive questioning by the study investigators. Routine blood investigations (haematology and biochemistry) and urinalysis were performed at baseline and 14 days after last drug administration (Amorndoljai et al., 2011).

Sample collection

An indwelling catheter was inserted into the antecubital vein of one arm of the patient, and patency maintained with heparin saline. Whole blood (5 ml) was collected from each patient and placed into sterile heparin tubes at 0.5, 1, 2, 4, 6, 9, 12, 18, 24, and 48 h after drug administration. Serum samples were prepared through centrifugation at 1,000 ×*g* for 15 min and stored at -80°C until analysis.

Drug analysis

Serum piperine concentrations were measured according to the methods of Bajad et al. (2002) and Sethi et al. (2009), with modifications. Piperine and the internal standard β-17-estradiol acetate (99% pure) were purchased from Merck Co. Ltd. (Darmstadt, Germany) and Sigma-Aldrich (CA, USA). Acetonitrile, methanol, ethyacetate, and propan-1-ol were of HPLC grade, which were purchased from Labscan Co. Ltd., Bangkok, Thailand. The following chemicals and solvents were obtained in the highest purity available: phosphoric acid and di-potassium hydrogen phosphate-3 hydrate obtained from Analytical Sciences Co. Ltd., Bangkok, Thailand. Deionized double distilled water was used for the preparation of working nicotine standard solutions. Serum from healthy volunteers used for standard curves was provided from the blood bank of Thammasat Chalermprakiet Hospital.

Standard stock solutions of both piperine and internal standard were prepared in methanol at a concentration of 1 mg/ml. Working solutions of 1 mg/ml for piperine and 3,000 ng/µl for internal standard were prepared and stored at -80°C. Calibration curves were constructed by analysis of 500 µl serum samples spiked with various concentrations of piperine (0, 25, 50, 100, 250, 500, and 1,000 ng/ml). The internal standard was used at a concentration of 15,000 ng/ml. Serum samples (500 µl) were spiked with internal standard, and the resultant mixture extracted with distilled water (500 µl), 12 mM phosphate buffer pH 3.4 (100 µl), and a mixture of ethyl acetate and propanol (9:1 v:v) (6 ml).

After centrifugation at 2000 ×*g* for 10 min, the clear organic layer was evaporated to dryness with nitrogen gas. The residue was then dissolved in 100 µl methanol and an aliquot of 40 µl was injected onto the HPLC system. Piperine and β-17-estradiol acetate were analyzed by HPLC using SpectraSystem™ P4000 Quaternary Solvent Delivery/Controller equipped with SpectraSystem SCM1000 Solvent Degasser, a SpectraSystem AS3500 Autosampler, and a SpectraSystem UV/Vis 3000 Detector (Thermo Fisher Scientific, CA, USA). The wavelength of UV-Vis detector was operated at 340 and 280 nm for piperine and β -17-estradiol acetate, respectively. Piperine and the internal standard were separated on a ZORBAX Eclipse XDB-C18 (4.6 × 250 mm, 5 µm particle size) (Agilent Technologies™, CA, USA).

The HPLC system was operated under an isocratic mode at a flow-rate of 1 ml/min. The mobile phase was a mixture of 25 mM dipotassiumphosphate (pH 4.5, adjusted with orthophosphoric acid) and acetonitrile at the ratio of 35:65 (v:v). The retention times of piperine and β-17-estradiol acetate were 4.6 and 10.0 min, respectively. Piperine was quantified by using the ratio of the peak area of analyte to that of internal standard. Serum analysis was calibrated using concentration range of 25 to 4,000 ng/ml. All calibration ranges yielded linear relationships with correlation coefficients ($r^2 \geq 0.999$) or better. The limit of quantification (LOQ) for piperine was 25 ng/ml. The intra- and inter-day coefficients of variation (CV) of piperine were 3.0 versus 19.11, 13.59 versus 4.79, and 10.78 versus 10.79% at concentrations of 25, 250, and 500 ng/ml, respectively.

The intra- and inter-day accuracy values were 19.68 versus 0.48, 0.74 versus 11.98, and -4.43 versus -2.18% for the concentrations of 25, 250, and 1,000 ng/ml, respectively. The mean recoveries for piperine at 50, 500 and 1,000 ng/ml were 90.13, 90.06 and 92.18%, respectively. The recovery of β-17-estradiol acetate at the concentration of 3,000 ng/ml was 99.47 ± 0.15%. Quality control (QC) samples for piperine were made up in serum using a stock solution separated from that used to prepare the calibration curve, at the concentrations of with 50 (low), 500 (medium), and 1,000 ng/ml (high) piperine and 3,000 ng/ml internal standard. Samples were aliquoted into cryovials and stored frozen at -20°C for use with each analytical run. The results of the QC samples provided the basis of accepting or rejecting the run. At least four of the six QC samples had to be within ±20% of their respective nominal values. Two of the six QC samples could be outside the ±20% of their respective nominal value, but not at the same concentration.

Pharmacokinetic and statistical analysis

Serum concentration-time profile of piperine for each subject was plotted and the pharmacokinetic analysis was performed using model-independent analysis approach. Maximum concentration (the first and second: $C_{max\text{-}1st}$ and $C_{max\text{-}2nd}$) and time to maximum concentration (the first and second: $t_{max\text{-}1st}$ and $t_{max\text{-}2nd}$) were obtained directly from the concentration-time profile of each individual. The area under the curve from zero time to forty-eight hours of dosing ($AUC_{0\text{-}48\ h}$) was deduced using the linear trapezoidal rule for ascending data points and the log trapezoidal rule for descending data points. Data are presented as median (range) values. Comparison of the pharmacokinetic parameters obtained from subjects following administration of the two dosage regimens of Benjakul formulation was performed using Mann-Whitney U test for data not conforming to normal distribution (SPSS for windows Release 13, NY, USA.) at statistical significance level of $\alpha = 0.05$.

RESULTS

Tolerability

All volunteers were healthy, verified by laboratory results, physical examination, and vital sign monitoring with no significant difference between the two dosing groups. The demographic characteristics (age, body weight, height, and BMI), vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate) and laboratory investigations (haematology, biochemistry, and urinalysis) were comparable in both groups of subjects ($p > 0.05$) (Table 1).

Table 1. Baseline characteristics of healthy subjects in group 1 (100 mg Benjakul formulation) and group 2 (200 mg Benjakul formulation). Data are presented as median (range) values.

Parameter	Group 1	Group 2
Age (years)	23.5 (20-25)	24 (20-38)
Weight (kg)	52.5 (42-83)	54 (44-84)
Height (cm)	165.50 (148-178)	161.00 (155-180)
BMI (kg/m^2)	19.25 (17.95-26.20)	19.74 (17.95-25.92)
Haematology		
WBC (μl^{-1})	5.74 (4.27-7.31)	6.01 (4.45-8.04)
Neutrophil (%)	56 (38-64)	59 (43-73)
Lymphocyte (%)	35 (29-54)	31 (21-45)
Monocyte (%)	3.8 (2.9-7.2)	4.2 (2.3-7.6)
Eosinophil (%)	3.7 (1.3-13.3)	2.7 (0.6-8.1)
Basophil (%)	0.4 (0.2-0.6)	0.35 (0.2-0.7)
RBC ($\times 10^6/\mu l$)	4.86 (3.89-6.00)	4.91 (4.14-5.57)
Haemoglobin (g/dl)	13.0 (11.4-16.1)	13.9 (11.3-15.3)
Haematocrit (%)	38.1 (34.1-46.9)	40.8 (34.5-44.6)
Platelets (μl^{-1})	234 (179-338)	226 (163-326)
Biochemistry		
Glucose (mg/dl)	83 (73-89)	80 (76-95)
Blood urea nitrogen (mg/dl)	11.2 (7.0-15.4)	11.6 (7.7-13.5)
Creatinine (mg/dl)	1.00 (0.70-1.20)	0.95 (0.80-1.20)
Total cholesterol (mg/dl)	195 (150-208)	196 (157-208)
Triglyceride (mg/dl)	69 (44-109)	74 (30-125)
HDL-cholesterol (mg/dl)	55 (34-75)	51 (42-72)
LDL-cholesterol (mg/dl)	101 (92-106)	101 (91-108)
Globulin (mg/dl)	3.15 (2.90-3.80)	3.35 (2.70-3.70)
Albumin (mg/dl)	4.40 (4.10-4.70)	4.3 (4.00-4.60)
Total protein (mg/dl)	7.55 (7.20-8.30)	7.7 (7.00-8.90)
Total bilirubin (mg/dl)	0.95 (0.90-1.60)	0.95 (0.50-1.60)
Conjugated bilirubin (mg/dl)	0.20 (0.10-0.40)	0.2 (0.10-0.40)
Aspartate aminotranferase (mg/dl)	18 (14-23)	17.5 (12-30)
Alanine aminotranferase (mg/dl)	11 (6-24)	11.5 (9-18)
Alkaline Phosphatase (mg/dl)	63 (40-78)	59.5 (16-85)

Median plots of serum concentration-time profiles of piperine following the administration of the two dosage regimen of Benjakul formualtion in both groups of subjects are shown in Figure 1. Piperine was rapidly absorbed from gastrointestinal tract after oral dose administration with marked inter-individual variation. In most cases, the drug was detectable in plasma within 0.5 h of dosing, with median $t_{max\text{-}1st}$ of 1 h. It disappeared thereafter from systemic circulation within 48 h. The pharmacokinetic parameters of piperine calculated based on model-independent approach are summarized in Table 2. Large inter-individual variation among the pharmacokinetic parameters was noted, particularly with $C_{max\text{-}1st}$ and $AUC_{0\text{-}48\ h}$ as reflected by the values of coefficients of variation (CVs) for both parameters ($_{Cmax\text{-}1st}$ 44 to 52%, and $AUC_{0\text{-}48\ h}$ 63 to 91%). Median $C_{max\text{-}1st}$ and $AUC_{0\text{-}48\ h}$ values were found to be about twice in the group receiving 200 mg dose compared with that of 100 mg dose (median $C_{max\text{-}1st}$ 1,078 versus 467 ng/ml; $AUC_{0\text{-}48\ h}$ 10,216 versus 4,288 ng h/ml; $p < 0.05$). It was noted however for the second maximum serum concentration ($C_{max\text{-}2nd}$) of piperine at about 9 h post-dosing observed in 9 (median: 203 ng/ml) and 7 (median: 499 ng/ml) subjects who received 100 and 200 mg Benjakul formulation, respectively.

DISCUSSION

This study is the first investigation of the pharmaco-

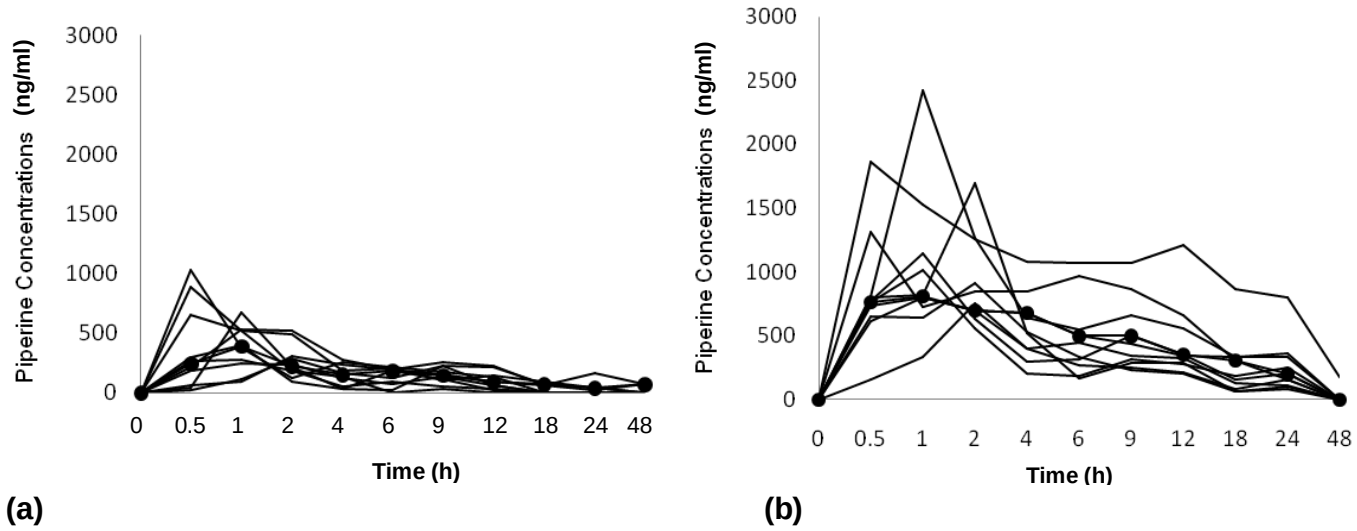

Figure 1. Median (filled circle) and individual serum concentration-time profiles (lines) of piperine following an oral administration of a single dose of (a) 100 mg (Group 1, n = 10), and (b) 200 mg (Group 2, n = 10) Benjakul formulation in healthy subjects.

kinetics of piperine following the administration of Benjakul formulation. The formulation was well tolerated with no apparent adverse drug reaction. The two dosage regimens, that is 100 and 200 mg Benjakul formulation were selected based on the doses used in phase I clinical study in 23 healthy Thai subjects. No significant adverse reaction except reversible gastrointestinal irritation was observed following the administration of 100 or 200 mg dose given three times daily (after meal) for 14 days (Amorndoljai et al., 2011).

Piperine was well absorbed from the gastrointestinal tract after oral dose of Benjakul formulation with median $t_{max\text{-}1st}$ of about 1 h. Pharmacokinetics of piperine following a single oral dose of 200 (12 mg piperine) or 100 (6 mg piperine) mg exhibited dose linearity as ascribed by the proportional increase in both dose-dependent pharmacokinetic parameters; $C_{max\text{-}1st}$ and $AUC_{0\text{-}48\ h}$ were increased by about 2-fold when the dose was doubled from 100 to 200 mg. Unfortunately, other pharmacokinetic parameters including terminal phase elimination half-life ($t_{1/2z}$), total clearance (CL/f) and apparent volume of distribution (V_z/F) could not be determined with accuracy due to limitation of blood sampling schedule during distribution and elimination phases.

The pharmacokinetic study in rats following a single oral dose of 100 mg/kg body weight alkaloids from *P. longum L*. (equivalent to 54.1 mg/kg bodyweight or approximately 12 mg piperine per 200 mg body weight rat) showed that the compound was rapidly absorbed and slowly eliminated (Liu et al., 2011). Mean C_{max}, t_{max}, $AUC_{0\text{-}\alpha}$, and $t_{1/2z}$ were 4,292 ng/ml, 2.45 h, 23.10 µg h/ml, and 4.10 h, respectively. The observed C_{max} ($C_{max\text{-}1st}$) in humans was thus only one-fourth of that reported in rats following an equivalent dose in rat (12 mg piperine). The absorption appeared however to be more rapid in humans (median of 1 versus mean of 2.45 h).

A previous *in vitro* study has shown that piperine, as a weak base and highly lipophilic molecule, is absorbed very fast across the intestinal barrier through passive diffusion with short absorption clearance and high apparent permeability co-efficient (Khajuria et al., 1998). Investigation of tissue distribution and elimination of piperine in rats showed its absorption to be approximately 96% of the administered dose (170 mg/kg body weight) (Suresh and Srinivasan, 2010). Furthermore, pharmacokinetics of piperine in lipid nanospheres in mice showed a biexponential decline with a significantly high AUC, a lower rate of clearance and a smaller volume of distribution than piperine (Veerareddy and Vobalaboina, 2008).

Following absorption, piperine distributed throughout various tissues including liver, kidney, spleen, stomach, small intestine (Suresh and Srinivasan, 2010; Bhat and Chandrasekhara, 1986). It was noted for the observation of the second peak of piperine in almost all subjects (9 subjects in group 1 and 7 subjects in group 2) at about 6 h ($t_{max\text{-}2nd}$). This was not observed in any of the previous pharmacokinetic investigations in rats (Suresh and Srinivasan, 2010). The phenomenon is a common characteristics of drugs or compounds that undergo hepatic metabolism through phase II glucuronidation reaction, which is likely to exhibit entero-hepatic recycling. Urinary excretion and biliary excretion happen to be the main routes of piperine excretion (Bhat and Chandrasekhara, 1986). Glucuronidation and sulfation appeared to be the major pathways in the disposition of piperine, as glucuronides and conjugated sulfates were found in the urinary excretions of rats after oral administration of piperine (Bhat and Chandrasekhara,

Table 2. Pharmacokinetic parameters of piperine in individual subjects following an oral administration of a single dose of 100 mg (group 1, n = 10), and 200 mg (group 2, n = 10) Benjakul formulation in healthy subjects.

Subjects	Group 1					Subjects	Group 2				
	$c_{max\text{-}1st}$ (ng/ml)	$t_{max\text{-}1st}$ (h)	$AUC_{0\text{-}48hr}$ (ng × h/ml)	$c_{max\text{-}2nd}$ (ng/ml)	$t_{max\text{-}2nd}$ (h)		$c_{max\text{-}1st}$ (ng/ml)	$t_{max\text{-}1st}$ (h)	$AUC_{0\text{-}48hr}$ (ng × h/ml)	$c_{max\text{-}2nd}$ (ng/ml)	$t_{max\text{-}2nd}$ (h)
1 (male)	531	1	7902	225	9	1 (male)	1015	1	9881	312	9
2 (male)	654	0.5	4524	203	6	2 (male)	909	2	7263	-	-
3 (male)	891	0.5	1498	37	9	3 (male)	749	2	8230	440	6
4 (male)	251	1	1750	139	9	4 (male)	2423	1	18262	662	9
5 (male)	404	1	9811	184	6	5 (male)	1690	2	9021	289	9
6 (female)	283	1	1309	99	6	6 (female)	797	1	14936	-	-
7 (female)	289	2	3846	224	9	7 (female)	1141	1	10552	499	9
8 (female)	1030	0.5	8704	256	4	8 (female)	803	1	7907	-	-
9 (female)	674	1	5190	226	9	9 (female)	1862	1	57644	1212	12
10 (female)	310	2	4051	-	-	10 (female)	1314	0.5	29165	963	6
Median	467	1	4,288	203	9	Median	1,078*	1	10216*	499*	9
(Range)	(251-1,030)	(0.5-2)	(1,309-9,811)	(37-256)	(4-9)	(Range)	(749-2,423)	(0.5-2)	(7,263-57,644)	(289-1,212)	(6-12)

*Statistically significantly higher in group 2 compared with group 1 (p-value < 0.01, Mann-Whitney U Test).

1986). After oral administration of piperine at the dose of 170mg/kg body weight to rats, four metabolites of piperine, that is piperonylic acid, piperonyl alcohol, piperonal, and vanillic acid were identified in the free form in 0 to 96 h urine, whereas only piperic acid was detected in 0 to 6 h bile (Bhat and Chandrasekhara, 1987).

The most common concern in clinical application of piperine or herbal preparation containing piperine has been its inhibitory influence on hepatic and intestinal metabolic enzymes including cytochrome P450 (CYP)-mediated pathways (phase I) and phase II metabolism which results in potentially toxic concentrations of a number of concurrently administered drugs. Inhibitory effect of piperine on human CYP3A4 activity has been shown with phenytoin, propranolol, theophylline, and rifampin (Bano et al., 1987, 1991; Hu et al., 2005; Velpandian et al., 2001). In addition, previous studies revealed that piperine could modulate the functional activity or gene expression of the efflux protein P-glycoprotein (P-gp: multidrug resistance protein 1 or ATP-binding cassette sub-family B member 1) (Bhardwaj et al., 2002; Han et al., 2008; Han, 2011; Lin and Han, 2010). The inhibition of this P-gp-mediated transportation was reported with digoxin, cyclosporine, fexofenadine (Bhardwaj et al., 2002).

Conclusion

The current pharmacokinetic study provides preliminary information on the absorption and disposition characteristics of piperine in humans, which would form the basis for further well-design pharmacokinetic-pharmacodynamic studies for dose optimization of Benjakul formulation in healthy subjects and cancer patients. In addition, the impact of piperine on disposition of co-administered drugs which are substrates of CYP3A4, P-gp, and possibly other drug-metabolizing enzymes should be clarified to avoid any clinically relevant pharmacokinetic drug interactions in cancer patients who are likely to receive multiple drug therapy for cancer and concurrent complications.

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