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## Co-Administration of Piperine and Docetaxel Results in Improved Anti-Tumor Efficacy via Inhibition of CYP3A4 Activity

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### Abstract

**Background**—Docetaxel is the mainline treatment approved by the FDA for castration-resistant prostate cancer (CRPC) yet its administration only increases median survival by two to four months. Docetaxel is metabolized in the liver by hepatic CYP3A4 activity. Piperine, a major plant alkaloid/amide, has been shown to inhibit the CYP3A4 enzymatic activity in a cell-free system. Thus, we investigated whether the co-administration of piperine and docetaxel could increase docetaxel's pharmacokinetic activity *in vitro* and *in vivo*.

**Methods**—Liver CYP3A4 enzymatic activity was measured by fluorescence. *In vivo* docetaxel pharmacokinetic activity was analyzed by liquid chromatography. An *in vivo* xenograft model of human CRPC was utilized to assess the anti-tumor effect of docetaxel when co-administered with piperine.

**Results**—Inhibition of hepatic CYP3A4 activity resulted in an increased area under the curve (AUC), half-life and maximum plasma concentration of docetaxel when compared to docetaxel alone administration. The synergistic administration of piperine and docetaxel significantly improved the anti-tumor efficacy of docetaxel in a xenograft model of human CRPC.

**Conclusions**—Docetaxel is one of the most widely used cytotoxic chemotherapeutic agents and is currently the mainstay treatment for metastatic CRPC. Dietary constituents are important agents modifying drug metabolism and transport. In our studies, dietary consumption of piperine increases the therapeutic efficacy of docetaxel in a xenograft model without inducing more adverse effects on the treated mice.

### Keywords

piperine; docetaxel; prostate; cancer

### Introduction

Prostate cancer is the most common solid malignancy in men, accounting for an estimated 217,000 new cases and 32,000 deaths in 2010 [1]. Median survival for men with castration-resistant prostate cancer (CRPC) is 16.5 months, and treatment options for are limited. Docetaxel-based combination chemotherapy is the mainline therapy approved by the FDA for treatment of CRPC, extending survival by 2–4 months, however toxicities, especially

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hematopoietic side-effects, are significant and dose-limiting [2]. Docetaxel is primarily metabolized by the hepatic cytochrome P450 family of enzymes. Specifically, a member of the cytochrome P450 mixed-function oxidase system CYP3A4 is responsible for the breakdown of docetaxel, in particular isoforms CYP3A4 and CYP3A5, the latter of which has a 10-fold lower affinity for docetaxel [3].

Hepatic metabolism is the primary elimination pathway of docetaxel with urinary excretion accounting for less than 10% of the clearance of each administered dose. [4,5] Furthermore, metabolites of docetaxel are substantially less active than the parent drug and contribute very little to docetaxel activity. [3] Thus, manipulation of the hepatic metabolic pathway can considerably alter the pharmacokinetics of docetaxel. For example, docetaxel's relative bioavailability was increased from 4% to 183% by the co-administration of the CYP3A4 inhibitor ritonavir, increasing systemic exposure by 50-fold [6]. Although studies using another potent CYP3A4 inhibitor ketoconazole demonstrated an inconsistent effect on docetaxel's systemic levels [7,8], this inconsistency can potentially be explained by the large inter- and intra-individual variability in absorption seen with this drug. [7,9] Nevertheless, manipulation of CYP3A4 hepatic metabolism represents a potential therapeutic avenue to augment docetaxel's efficacy.

Natural products are the most consistent source of drug development. Furthermore, new insights suggest that dietary constituents may play an important role in cancer prevention as well as in cancer management/therapy. These products may act synergistically with radiotherapy and chemotherapy to kill tumor cells by reducing angiogenesis, inflammation and the induction of metastasis. Recent studies demonstrate that piperine, a major plant alkaloid/amide present in black (Piper nigrum Linn) and long (P. longum Linn) peppers, inhibits the activity of the drug-metabolizing enzyme CYP3A4 in a cell-free system [10,11]. Also, by acting on hepatic enzymatic breakdown, piperine can enhance the bioavailability of several therapeutic compounds, including phenytoin, propranolol and theophylline in healthy volunteers and rifampin in patients with tuberculosis [12]. Furthermore, preliminary studies suggest that piperine itself can inhibit the development of breast cancer by inhibiting stem cell renewal [13] as well as inhibit antioxidant, anti-inflammatory, and anti-cancer activities in cancer cell models [14]. Taken together, these findings indicate that piperine not only can alter the metabolism and thereby the therapeutic effectiveness of many pharmaceutical compounds that are processed by the CYP3A4 pathway, including chemotherapeutic agents such as docetaxel, but also may promote anti-tumor effect either individually or in concert with traditional therapies.

In this study, we investigated the pharmacokinetic and anti-cancer effects of piperine when given in combination with docetaxel. We demonstrate that treatment with piperine inhibits hepatic CYP3A4 activity *in vivo* which coincides with an increased area under the curve (AUC), half-life, and maximum plasma concentration of docetaxel when administered in combination with piperine versus docetaxel alone. Importantly, the co-administration of docetaxel and piperine resulted in a statistically significant synergistic anti-tumor effect in a xenograft animal model of castration-resistant human prostate cancer.

## Materials and Methods

### Reagents

Piperine was obtained from Sigma (St. Louis, MO). Docetaxel was obtained from LC Laboratories (Woburn, MA).

### Analysis of CYP3A4 activity

Six week-old male C.B17/Icr-scid mice were given piperine (100 mg/kg by mouth in vegetable oil) or vegetable oil only. The mice were sacrificed by CO<sub>2</sub> asphyxiation one hour after piperine administration. After homogenizing mice livers, CYP3A4 activity was measured using CYP3A4 assay kit (Promega, Madison, WI) and normalized according to wet tissue weight. According to manufacturer's instructions, CYP3A4 activity of control and piperine treated mice was determined by relative fluorescence and quantified by a Synergy HT Microplate Reader (Bio Tek, Winooski, VT).

### Pharmacokinetic analysis

Six week-old male C.B17/Icr-scid mice were given piperine (100 mg/kg p.o. in vegetable oil) or vegetable oil only followed by intravenous administration of docetaxel (12.5 mg/kg in 5% ethanol, 5% TWEEN 80 and 5% glucose in PBS). At the termination of the experiment, blood was collected from the retro-orbital plexus under anesthesia from both experimental and control animals. The mice were then sacrificed by CO<sub>2</sub> asphyxiation. Docetaxel was extracted from study plasma samples by liquid-liquid extraction using methyl tert-butyl ether (MTBE) fortified with 10 ng/mL paclitaxel as an internal standard (IS). Briefly, a 50 µL volume of plasma was diluted with 50 µL of water. An 800 µL volume of MTBE (with paclitaxel) was added and the sample was mixed for approximately 10 minutes. After mixing, the samples were centrifuged at 3,220 rcf for 10 minutes and approximately 500 µL of the MTBE was removed and dried under nitrogen. The residue was re-suspended in 100 µL of 50% methanol with 0.1% acetic acid. The re-suspension was gently vortexed for approximately five minutes and stored at 10°C. Docetaxel calibration standards in plasma were prepared by diluting a 10 µg/mL docetaxel stock solution in plasma to obtain a final range of standards from 10 to 5,000 ng/mL. The docetaxel plasma standards were extracted in duplicate using the same procedure as the study samples. A HPLC/MS/MS method utilizing electrospray ionization in positive mode was used for the analysis of docetaxel in mouse plasma using paclitaxel as IS. Docetaxel's extraction from plasma samples and pharmacokinetic analysis were performed at Wolfe Laboratories (Watertown, MA).

### Assessment of *in vivo* tumor growth

$1 \times 10^6$  castration-resistant prostate cancer PC-3 cells were inoculated s.c. in the flank region of 6 week-old male C.B17/Icr-scid mice using a 27-gauge needle. All animal procedures were done in accordance with local guidelines on animal care and with appropriate institutional certification. Animals were fed an autoclaved AIN-93M diet (Harlan Teklad, Madison, WI) and water *ad libitum*. Two weeks after injection of tumor cells animals were randomly assigned to the control and experimental groups (n=5 mice/group). Mice were given piperine (100 mg/kg via oral gavage in vegetable oil) or vegetable oil only followed by intravenous administration of docetaxel (12.5 mg/kg in 5% ethanol, 5% TWEEN 80 and 5% glucose in PBS) or solvent solution 2 hours after ingestion of piperine. Administration of piperine with or without docetaxel occurred on a weekly basis. Tumors were measured twice weekly and their volumes were calculated by the formula: [volume =  $0.52 \times (\text{width})^2 \times \text{length}$ ]. Animals were sacrificed 14 days after treatment initiation.

### Statistical analysis

Statistical analysis was performed using a two-sided Student t test. Results are expressed as the mean  $\pm$  SEM. Non-compartmental pharmacokinetic analysis was performed using WinNonlin.

## Results

### The effect of piperine administration on the hepatic activity of CYP3A4 *in vivo*

Hepatic metabolism by CYP3A4 is the primary elimination pathway of docetaxel [4,5]. Given that piperine is able to inhibit CYP3A4 activity in a cell-free system [10,11] we examined the effect of piperine on CYP3A4 hepatic activity in an animal model. Piperine was administered through oral gavage in a homogeneous suspension mixed in vegetable oil at a concentration of 50 mg/kg/dose. Control animals received an equal volume of vegetable oil only. One hour after piperine administration animals were euthanized by CO<sub>2</sub> asphyxiation, and CYP3A4 activity was measured from liver homogenates obtained from control and experimental animals and normalized according to wet tissue weight. As displayed in Figure 1, CYP3A4 activity was significantly reduced in hepatic cells obtained from mice treated with piperine compared with control animals, confirming piperine's ability to inhibit hepatic CYP3A4 enzymatic activity.

### Pharmacokinetic studies

Next, we investigated the effect of piperine-mediated CYP3A4 inhibition on docetaxel's pharmacokinetics *in vivo*. The administered doses of docetaxel selected were based on previously published results in animal studies showing that the maximally tolerated dose (MTD) of docetaxel ranges from 15 to 33 mg/kg/dose [15]. A summary plot comparing average docetaxel concentration in mice given piperine and mice not given piperine over time is presented in Figure 2. Administration of piperine significantly increased and prolonged the mouse plasma levels of docetaxel compared with mice which received docetaxel alone. Table 1 displays the corresponding mean docetaxel concentrations between the two groups over five time points. In mice that were fed piperine, mean docetaxel concentrations were uniformly almost double the mean docetaxel concentrations of mice not fed piperine. Most notably, the difference in docetaxel plasma levels was most pronounced between 1 and 2 hours after docetaxel administration, i.e., plasma docetaxel levels in the piperine/docetaxel group was 7-fold higher than in docetaxel only group.

Table 2 compares the pharmacokinetics of docetaxel with and without administration of piperine. The area under AUC for docetaxel increased by 230% in the piperine/docetaxel group compared with the docetaxel alone group. Furthermore, docetaxel's half-life increased from 0.44 hr to 1.14 hr when piperine was co-administered. Similarly, the mean values of docetaxel's maximum concentration (C<sub>max</sub>) given in combination with piperine versus given alone were 11,380 ng/ml and 6,808 ng/ml, respectively. These pharmacokinetic data clearly demonstrate that inhibition of hepatic CYP3A4 activity by piperine results in meaningful prolongation of docetaxel's half-life with an expected increase and prolongation of docetaxel's serum concentration.

### Piperine enhances anti-tumor efficacy of docetaxel *in vivo*

Given that docetaxel is metabolized by hepatic CYP3A4 enzymatic activity and that piperine inhibits this activity resulting in a meaningful increase and prolongation of docetaxel's serum levels, we examined the effect of the addition of piperine to docetaxel on anti-tumor efficacy using a xenograft model of human prostate cancer. Castration-resistant PC-3 xenograft tumors were established in 6-week old male C.B17/Icr-scid mice. Two weeks after tumor cell implantation, at a time when baseline tumor volumes reached 200–400 mm<sup>3</sup> across all mice, animals were randomly selected to the following groups (n=5 mice/group): (i) control; (ii) piperine p.o. weekly at 100 mg/kg/dose; (iii) docetaxel intravenously weekly at 12.5 mg/kg/dose; or (iv) piperine/docetaxel combination. There was no statistically significant difference in tumor volumes among these groups. Piperine was administered one hour prior to docetaxel injection.

Two weeks after the initiation of treatment, mice treated only with docetaxel showed a significant decrease in the growth of their xenograft tumors (313% vs. 461%,  $P=0.039$ ). Furthermore, as Figure 3 demonstrates, treatment with piperine alone also caused an observable decrease in tumor growth (373% vs. 461%), however this difference did not achieve statistical significance ( $P=0.201$ ). Co-administration of piperine and docetaxel resulted in the most significant inhibition of tumor growth (188% vs. 461%,  $P=0.003$ ) among the experimental groups. Importantly, the resulting increase in the mean plasma concentrations of docetaxel in mice fed piperine did not translate into an observed increase in mice toxicity during treatment. Also, none of the mice in the treatment groups died prematurely. Thus, by inhibiting hepatic clearance of docetaxel, piperine results in increased docetaxel serum levels without resulting in an increase in docetaxel-mediated toxicities. These findings indicate that piperine may represent a therapeutic strategy to enhance docetaxel's efficacy in treating castration-resistant prostate cancer.

## Discussion

Docetaxel is one of the most widely used cytotoxic chemotherapeutic agents and has currently been approved for the treatment of breast cancer, non-small-cell lung cancer, squamous cell carcinoma of the head and neck, gastric adenocarcinoma, and CRPC). Despite its efficacy, docetaxel administration is limited by its toxicities, specifically myelosuppression, which can result in cessation of therapy. The pharmacokinetic profile of docetaxel is characterized by substantial inter-individual variability, with up to 10-fold differences in drug clearance even in patients with normal hepatic function. The importance of docetaxel's clearance is highlighted by a previous study documenting that a 50% decrease in docetaxel clearance is associated with a greater than 430% increase in the odds of developing severe-grade 3 or 4-neutropenia [16]. Furthermore, a reduced AUC was associated with shorter survival in patients with non-small-cell lung cancer receiving docetaxel chemotherapy [17].

Dietary constituents are important agents modifying drug metabolism and transport and thereby can contribute to great variability in drug disposition among individuals [18,19]. The induction or inhibition of drug-metabolizing enzymes or transporters could contribute to the underlying mechanism of altered drug concentrations [10,20]. Piperine, a major constituent of black pepper, has been shown to inhibit human P-glycoprotein transporters and CYP3A4 enzymatic activity in a cell-free system [10]. It has been well established that CYP3A4-mediated metabolism is the major route of docetaxel inactivation in humans [3]. Our present study demonstrates that treatment with piperine inhibits hepatic CYP3A4 activity *in vivo* which correlated with an increased AUC, half-life and maximum plasma concentration of docetaxel. Moreover, synergistic administration of piperine and docetaxel significantly enhanced the anti-tumor efficacy of docetaxel in an animal model of castration-resistant human prostate cancer.

Prior work has shown an anti-neoplastic effect of piperine [14]. The antitumor activity of piperine appears to be related to its immunomodulatory properties, which involves the activation of cellular and humoral immune responses [21]. In fact, piperine possesses only weak cytotoxic activity [21,22], which indicates that its anti-tumor activity is indirect and not related to anti-proliferative or pro-apoptotic effects on tumor cells. Some authors have shown that piperine's anti-cancer effects may be due to its ability to inhibit stem cell renewal [13]. Although our data indicate piperine itself demonstrated a minor anti-tumor efficacy *in vivo*, dietary consumption of piperine might increase the therapeutic efficacy of drugs that are substrates for CYP3A4.

Polypharmacy is a very effective regimen to increase the therapeutic efficacy of docetaxel for the treatment of certain solid malignancies such as breast cancer [23]. In a clinical study, the combination of docetaxel and adriamycin showed a better cure rate against metastatic breast cancer than other regimens [24]. However, the combination of docetaxel and adriamycin induces severe dose-limiting toxicity [24]. Therefore, increased antitumor effect of docetaxel could be compromised by adverse effects. The increased serum half-life of docetaxel due to the inactivation of CYP3A4 might potentially result in undesirable toxicity *in vivo*.

Daily dietary consumption of black pepper varies considerably within the population [25]. In the United States, average daily intake of black pepper has been estimated at 359 mg [26]. Piperine accounts for between 5% and 9% of black pepper content, implying a daily intake of approximately 60 to 110  $\mu\text{mol}$  of piperine. Results of our study demonstrate that such an amount of piperine could potentially modulate pharmacokinetics of docetaxel in cancer patients. Information on dietary consumption of black pepper should be taken into consideration in patients receiving docetaxel and treatment outcome should be considered and assessed prospectively.

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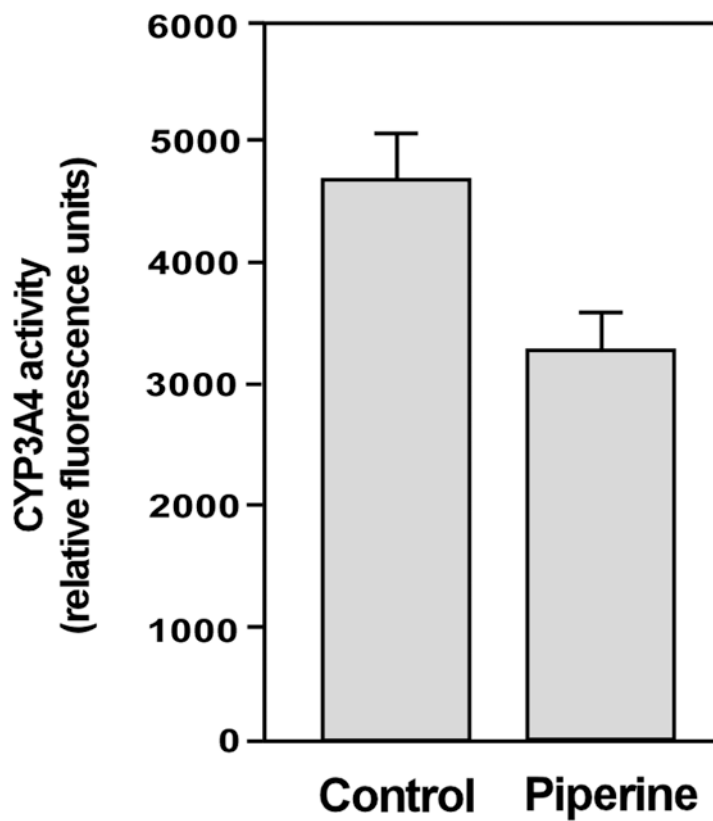
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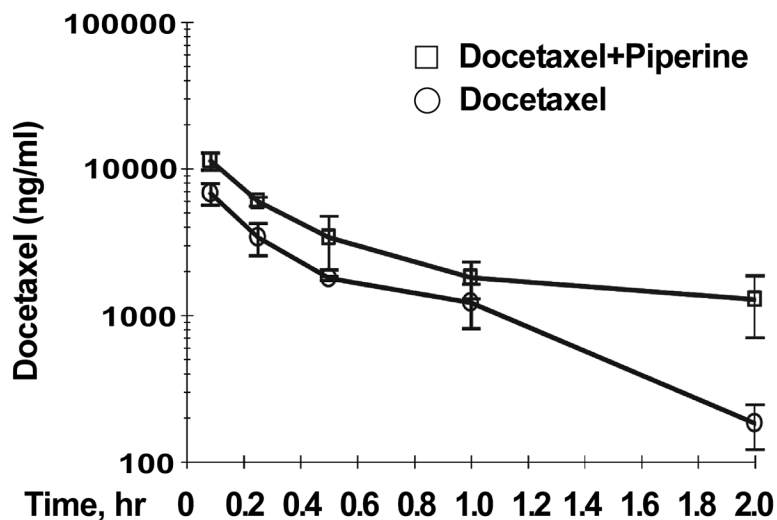


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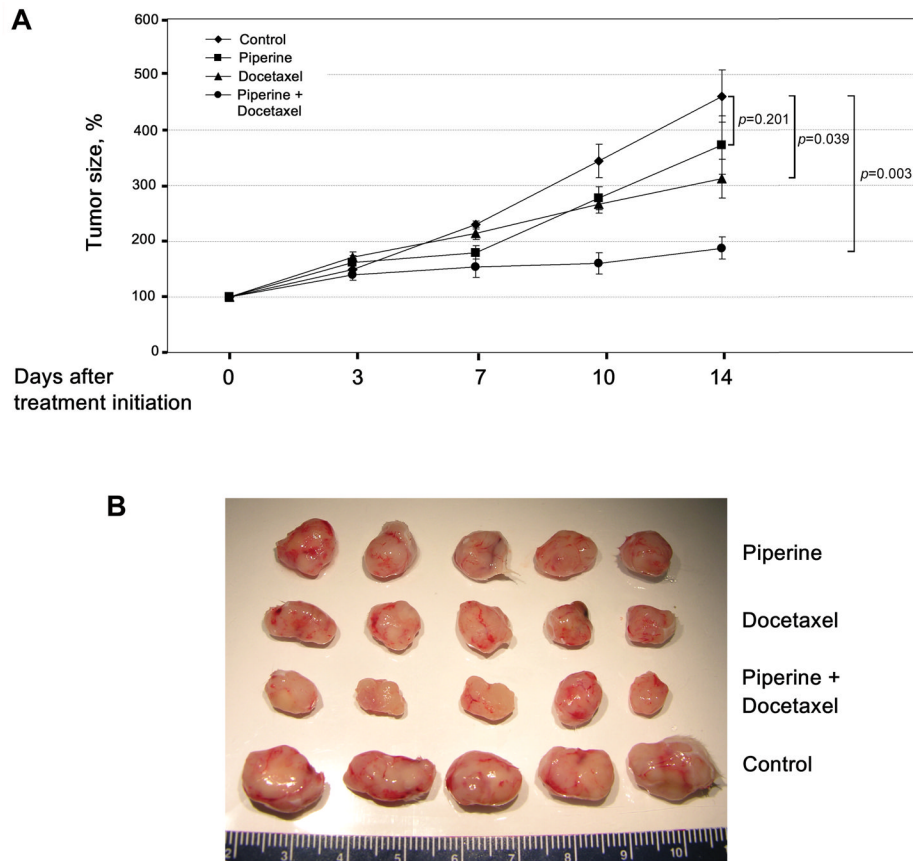


**Figure 1.** Piperine inhibits CYP3A4 hepatic activity *in vivo*. Six week-old male C.B17/Icr-scid mice were given piperine (50 mg/kg p.o.) or vegetable oil only. CYP3A4 activity was measured using the CYP3A4 assay kit (Promega, Madison, WI). Values represent means (n=3)  $\pm$  SD.





**Figure 2.** Effect of piperine co-administration on docetaxel plasma levels. Six week-old male C.B17/Icr-scid mice were given piperine (50 mg/kg p.o. in vegetable oil) or vegetable oil only followed by intravenous administration of docetaxel (12.5 mg/kg in 5% ethanol, 5% TWEEN 80 and 5% glucose in PBS). Blood was collected from the retro-orbital plexus under anesthesia from both experimental and control animals, and docetaxel plasma levels were examined as described above. Values represent means ( $n=3$ )  $\pm$  SD.



**Figure 3.**

Piperine enhances docetaxel's anti-tumor efficacy *in vivo*. (A) PC-3 subcutaneous xenografts were established in 6 week old male C.B17/Icr-scid mice. Two weeks after tumor implantation animals were randomly assigned to the control and experimental groups (n=5 mice/group). Mice were given piperine (50 mg/kg p.o in vegetable oil) or vegetable oil only followed by intravenous administration of docetaxel (12.5 mg/kg) or solvent solution 2 hours after piperine ingestion. Administration of piperine with or without docetaxel occurred on a weekly basis. Tumors were measured twice weekly and their volumes were calculated by the formula: [volume =  $0.52 \times (\text{width})^2 \times \text{length}$ ]. Values are means (n=5)  $\pm$  SEM. (B) Photographs of tumors from each group showing grossly a marked tumor response in mice treated with piperine and docetaxel compared to other experimental groups and control group.

**Table 1**

Docetaxel levels in animal plasma samples

Time (minutes)	Mean docetaxel concentration (ng/ml)		<i>p</i> value (two tail)
	Docetaxel	Docetaxel/Piperine	
5	6808±1140	11380±1528	0.0013
15	3403±841	5993±1969	0.0039
30	1793±55	3400±410	0.0003
60	1221±411	1810±508	0.0357
120	183±62	1284±580	0.0358

**Table 2**

Summary of calculated PK parameters

RoA	IV	IV	Units
<b>Compound</b>	<b>Docetaxel</b>	<b>Docetaxel + Piperine</b>	
<b>Dose (docetaxel)</b>	<b>12.5</b>	<b>12.5</b>	<b>mg/kg</b>
<b>Tmax</b>	0.083	0.083	hr
<b>Cmax</b>	6808*	11380*	ng/mL
<b>Half-life</b>	0.440	1.14	hr
<b>AUC</b>	3756	8715	hr*ng/mL
<b>Vz</b>	2114	2368	mL/kg
<b>Cl</b>	3328	1434	mL/hr/kg

\*  
p-value=0.028