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ORIGINAL ARTICLE

A phase I study of docetaxel with ketoconazole modulation in patients with advanced cancers

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Abstract
Purpose The aims were to determine the maximum tolerable dose (MTD) of docetaxel with CYP3A inhibition by ketoconazole, and to correlate the pharmacokinetics of docetaxel with midazolam phenotyping of CYP3A activity.
Methods Forty-one patients with refractory metastatic cancers were treated with an escalating dose of intravenous docetaxel once in every 3 week of 10 mg/m^2, concurrently with oral ketoconazole 200 mg twice daily for 3 days starting 2 days before the administration of docetaxel. Midazolam phenotyping test with ketoconazole modulation was performed before the first cycle of docetaxel. Docetaxel and midazolam pharmacokinetics were compared to our previous study of docetaxel treatment without ketoconazole modulation.
Results Neutropenia was the dose-limiting toxicity. The maximum tolerated dose was 70 mg with mean AUC at 70 mg similar to 75 mg/m^2 of docetaxel without ketoconazole. The plasma clearances of docetaxel and midazolam were reduced by 1.7- and 6-fold, respectively. The variability of midazolam AUC was reduced from 157 to 67%, but variability of docetaxel clearance was not reduced by CYP3A inhibition. Docetaxel clearance correlated with renal function and maximum concentration of ketoconazole, but not midazolam clearance or other variables of hepatic function.
Conclusion Fixed dosing was found to be feasible, without increased variability of clearance or neutrophil toxicity compared to BSA-based dosing. With ketoconazole modulation, docetaxel clearance correlated with renal function but not CYP3A phenotype.

Keywords CYP3A · Docetaxel · Ketoconazole · Midazolam · Pharmacokinetic

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Introduction

Body surface area (BSA) correlates poorly with systemic exposure to most anticancer agents [17, 26, 28]. Previous studies of single agent docetaxel showed that BSA correlated with docetaxel clearance [5, 6]. However, it has been reported that plasma clearance (CL) of docetaxel varied as much as 6-fold when dosed with BSA [18]. For drugs with a narrow therapeutic index, large interindividual differences in drug disposition may have an adverse impact on treatment toxicity and response on the phenotypic extremes.

Both CYP3A4 and CYP3A5 isoforms contribute to the majority of CYP3A activity in human adults. Docetaxel is biotransformed to its metabolites M1, M2, M3, and M4 by CYP3A isoenzymes, with only 10% eliminated unchanged through the renal route [8, 20, 27, 29, 30].

CYP3A activities are highly variable across human adults and largely account for the observed variability in CYP3A substrate pharmacokinetics. As the toxicity from docetaxel correlates with its plasma clearance, individualizing the dose of docetaxel based on estimated CYP3A activities may reduce interindividual variability in docetaxel

pharmacokinetics. Strategies such as the use of urinary metabolite of exogenous cortisol, erythromycin breath test, and in vivo probes of CYP3A activity such as midazolam have been demonstrated to predict for docetaxel clearance [14, 18, 25, 33]. Yamamoto et al. [34] has demonstrated that individualized dosing based on urinary 6-β-hydroxycortisol after cortisol administration reduced interindividual variability of docetaxel.

Ketoconazole is a potent but reversible inhibitor of CYP3A enzymes [22]. We hypothesized that potent CYP3A inhibition by ketoconazole may convert all patients into "poor metabolizers", thereby reducing interindividual variation in docetaxel clearance. In this phase I study, we sought to determine the feasibility of modulating docetaxel pharmacokinetics with ketoconazole and the predictive value of in vivo midazolam probe for docetaxel clearance in this setting.

Patients and methods

Eligibility criteria

Patients with histologically or cytologically proven metastatic or unresectable solid cancers refractory to standard treatment or for which docetaxel was indicated were eligible for this study. Additional eligibility criteria include the following: (1) the presence of measurable or evaluable disease, (2) life expectancy more than 3 months, (3) Karnofsky performance status (KPS) $\geq$ 70%, (4) adequate renal and liver functions (serum creatinine and bilirubin within normal institutional limits, AST (SGOT) and ALT (SGPT) $\leq$ 1.5 $\times$ upper limit of normal), (5) adequate marrow function (WBC $\geq$ 3,000 μl^{-1}, ANC $\geq$ 1,800 μl^{-1}, platelets $\geq$ 100,000 μl^{-1}), (6) adequate contraception for men and women of child-bearing potential, and (7) more than 4 weeks since surgery, radiotherapy, or chemotherapy with resolution of all acute toxic effects of grade $\leq$1 with the exception of alopecia, fatigue, nausea, and asthenia. Patients were excluded from this study if they were concurrently on antacid, proton pump inhibitors, H2-blockers, any other investigational agents, or medications that are inducers, inhibitors, or substrates of CYP3A4, had malabsorption syndromes, a known history of allergic reaction attributed to compounds of similar chemical or biological composition to agents used in this study, active central nervous system disease or severe intercurrent systemic disease. This study was approved by the Institutional Review Board (National University Hospital, Singapore) and written consent was obtained from all patients according to institutional and governmental guidelines. Data of control group (without ketoconazole) were based on our previous study on a cohort of Asian patients who were treated with docetaxel alone at doses of 75–100 mg/m^2 [14].

Study design

Eligible patients were received oral ketoconazole (Janssen Cilag, Italy) 200 mg twice daily for 3 days, each time beginning 2 days before and continuing 1 day after both midazolam (Hoffmann-La Roche, Basle, Switzerland) and docetaxel (Adventis Pharm Ltd, Singapore) administration, respectively. To ensure ketoconazole absorption, each dose of ketoconazole was administered with at least 100 ml of orange juice. Midazolam phenotyping test was performed at least 2 days before the first cycle of docetaxel treatment. An accelerated titration design, consisting of single patient cohort was used beginning with docetaxel 10 mg/m^2. Cohorts with minimum three patients were treated, once grade 2 or more toxicity was observed in the first cycle. If none of the three evaluable patients developed dose limiting toxicity (DLT), subsequent patients were treated at the next dose level. If one out of three patients in a cohort experienced DLT, an additional three patients were treated with the same dose. If no further DLT was observed, dose escalation continued until the maximum tolerable dose (MTD) is identified. MTD was defined as the dose level at which two or more patients out of the six experienced DLT. Intrapatient dose escalations were not permitted. Dose levels treated are shown in Table 1. The first four dose levels were based on BSA calculation and the final three dose levels were given as a flat dosing. BSA was calculated based on Dubois formula: BSA = 0.007184 $\times$ height (cm)$^{0.725}$ $\times$ weight (kg)$^{0.425}$.

DLT was defined as any grade 4 neutropenia lasting 7 days or more, grade 3–4 neutropenia with fever, grade 4 thrombocytopenia or grade 3 thrombocytopenia with active bleeding; failure to recover from toxicity despite a delay of more than 7 days from the next scheduled cycle, and grade 3 or greater non-hematologic toxicity with the exception of elevated serum AST or ALT, nausea, vomiting, and alopecia. Routine administration of colony-stimulating factors was not allowed with the exception of patients who experienced prolonged grade 4 neutropenia of greater than 7 days or neutropenic fever.

Patient evaluation

All patients were evaluated for toxicity. Toxicity was assessed weekly for the first cycle of treatment, and every 3 weeks for subsequent treatment cycles by history, physical examination and laboratory evaluation. Toxicities were graded according to the National Cancer Institute CTCAE version 2.0. Tumor response assessments were performed after two cycles of treatment using CT scans according to the RECIST criteria.

Table 1 Summary table of significant toxicities at each dosage level

Dose level	Docetaxel dose	Number of patients	Significant toxicities (grade; number of patients)
1	10 mg/m^2	1	Nil
2	20 mg/m^2	6	neutropenia (grade 3; $n = 2$), fatigue[a] (grade 3; $n = 1$)
3	25 mg/m^2	6	neutropenia with fever[a] (grade 4; $n = 1$)
4	30 mg/m^2	6	neutropenia (grade 4; $n = 1$), thrombocytopenia (grade 3; $n = 1$), fatigue[a] (grade 3; $n = 1$)
5	50 mg	6	neutropenia (grade 3; $n = 1$), fatigue[a] (grade 3; $n = 1$)[b], diarrhea [a] (grade 3; $n = 1$)[b]
6	60 mg	6	neutropenia (grade 3; $n = 1$)
7	70 mg	10	neutropenia (grade 3; $n = 2$) (grade 4; n = 2), neutropenia with fever [a] (grade 4; $n = 2$)

[a] Dose-limiting toxicities

[b] Both significant toxicities occurred in the same patient

Pharmacokinetic evaluation

Midazolam phenotyping test

Midazolam (1 mg) was administered as an intravenous (IV) bolus dose over 30 s. Blood samples were collected immediately before midazolam injection and at 15, 60, 120, and 300 min after the start of the midazolam injection. Blood samples were collected in heparinized tubes and centrifuged immediately to separate the plasma, which is then stored in plain tubes at −80°C until analysis.

Docetaxel assay

Docetaxel was administered as an IV infusion over 1 h, once in every 3 week with oral dexamethasone 8 mg for 3 days beginning a day before docetaxel infusion. Plasma concentrations of docetaxel were obtained during the first course of chemotherapy at the following sampling times: 0, 0.5, 1, 2, 5, 7, and 24 h. Blood samples were collected in heparinized tubes and centrifuged immediately to separate the plasma, which is then stored in plain tubes at −80°C until analysis.

Pharmacokinetic analysis

Analytical grade midazolam and docetaxel reference standards were gifts from Hoffmann-La Roche Ltd. (Basel, Switzerland) and Aventis Pharmaceuticals SA (Antony Cedex, France). Pure ketoconazole was purchased from Sigma-Aldrich Co. (Steinheim, Germany). All reagents used were of high performance liquid chromatography grade.

Midazolam and its 1-hydroxy metabolite concentrations were analyzed by reversed-phase liquid chromatography with tandem mass spectrometry (LC–MS–MS) using the API-2000 triple quadrupole mass spectrometer (Applied Biosystems, MDS SCIEX, ON, Canada) based on a modified form of the detailed method description reported by Toyo'oka et al. [31]. The linear correlation coefficient of analysis was >0.995 while the linear range was 1–100 ng/ml. The assay accuracy and coefficient of variation (CV) for inter-day precision of midazolam were 88–112 and <9%, respectively.

Ketoconazole plasma concentrations were measured to assess if plasma exposure was adequate to achieve CYP3A inhibition. Plasma samples of ketoconazole, taken based on docetaxel sampling time points, were analyzed by LC–MS–MS as described in detail by Chen et al. [7] with minor modifications. The standard curve was linear over the range 5–1,000 ng/ml and its linear correlation coefficient was >0.995. The assay accuracy and coefficient of variation (CV) for inter-day precision were 89–111 and <10%, respectively.

Docetaxel and its main hydroxylated metabolites (M1, M2, M3, and M4) were measured using an isocratic liquid chromatography–tandem mass spectrometry (LC–MS–MS) method described previously [16]. This assay was performed on plasma samples in this study as well as on 31 patients on our previous study who were treated with docetaxel alone.

Compartmental analyses for docetaxel and midazolam were carried out using ADAPT II [9]. Noncompartmental analysis for docetaxel and midazolam was performed using Kinetica 4.3 (InnaPhase Corp., Philadelphia, PA). Area under the concentration–time curve (AUC) was estimated using the log-linear trapezoidal method from time 0 to ∞. Derivation of the elimination rate constants for the terminal phase (k) was done with extrapolation of the last measured concentration to ∞, by including the final three sampling time points. The other pharmacokinetic parameters studied were the peak concentration (C_{max}), CL (CL = dose/AUC), half-life of the terminal disposition phase ($t_{1/2} = \ln 2/k$), and volume of distribution at steady state (Vss = CL/k).

Statistical analysis

Data were expressed as mean with standard deviation (SD). A two-tailed Mann–Whitney test was used for 2-group comparisons. Coefficient of variation (SD/mean) was expressed in percent. *F*-test was used to compare variances. Pearson's correlation was used to determine the correlation between docetaxel CL and various clinical variables. Multiple linear regression analysis using stepwise regression was used to determine the significant covariates of docetaxel CL, selecting variables stepwise according to their contribution to the model. Collinearity between variables was checked for using variance inflation factor calculations. A *P* value of <0.05 was considered to be statistically significant. All statistical calculations were computed using SPSS version 13.0 (SPSS Inc., Chicago, IL).

Results

Patient characteristics

Forty-five patients were accrued for this study, and their characteristics are shown in Table 2. Of these patients, 41 had complete toxicity assessments after receiving a total of 70 cycles of docetaxel with ketoconazole. Among the four who were not assessable, one died before docetaxel was administered, two withdrew consent for participation in the study prior to commencement of treatment, and one patient received an erroneous administration of a lower dose of docetaxel (12.5 mg/m^2) instead of 20 mg/m^2.

Patient toxicity profile

Grade 3 neutropenia and fatigue was first observed at 20 mg/m^2, necessitating an expansion of this cohort of patients to six. As only one DLT was seen, further dose escalations proceeded at 5 mg/m^2 increments. Further DLTs of neutropenic fever and fatigue of grade 3 were experienced in one patient each at dose levels of 25 and 30 mg/m^2, respectively. Analysis of the plasma docetaxel clearance of 19 patients accrued till then showed no correlation with the BSA (see below). Therefore, further dose escalations of docetaxel were in fixed 10 mg steps beginning with a fixed dose of 50 mg, which was approximately 30 mg/m^2, taking into account the mean BSA of our patient population.

The most commonly observed toxicities that occurred during the first cycle of chemotherapy are listed in Table 1. The frequency of grade 3–4 neutropenia increased with increasing doses. Other commonly observed non-hematological DLTs included fatigue and diarrhea, which occurred

Table 2 Patient characteristics

Characteristic	Number of subjects
Patients enrolled	45
Patients evaluated	41
Gender	
Male	19
Female	22
Age	58.2 ± 11.3
Race	
Chinese	33
Malay	6
Indian	1
Eurasian	1
BSA (m^2)	1.57 ± 0.14
KPS (%)	
100–90	9
80	28
70	4
Prior chemotherapy	
0–1 regimens	22
> 1 regimens	19
Diagnosis	
Lung–NSCLC	10
SCLC	6
Breast	8
Nasopharynx	5
Colon	3
Adenocarcinoma (unknown origin)	2
Esophagus	2
Gastric	2
Prostate	2
Bladder	1
Mesothelioma	1
Neuroendocrine	1
Pancreas	1
Ovary	1
Liver/renal function tests	
ALT (IU/L)	27.8 ± 16.9
AST (IU/L)	33.9 ± 17.3
ALP (IU/L)	176.8 ± 226.8
Albumin (g/dL)	36.8 ± 6.5
Bilirubin (mg/dL)	7.2 ± 5.1
AAG (g/L)	1.0 ± 0.35
Creatinine (mmol/L)	77.8 ± 18.6

BSA body surface area, *KPS* Karnofsky performance status, *NSCLC* non-small cell lung cancer; *SCLC* small cell lung cancer, *ALT* alanine aminotransferase, *AST* aspartate aminotrasnferase, *ALP* alkaline phosphatase, *AAG* α_1-acid glycoprotein

in one patient at 20 mg/m^2, 30 mg/m^2, and 50 mg, respectively. Febrile neutropenia occurred in one of six initial patients treated at the 70 mg dose level. As the mean AUC at this level had reached levels comparable to single agent docetaxel at 75 mg/m^2 in our previous study [14], dose escalation was stopped and an additional four patients were enrolled at 70 mg dose level to determine the tolerability of this dose. Of these four patients, only one experienced neutropenic fever. The median cycle 1 ANC in this dose level was 0.58×10^9 L^{-1} (range 0.08–1.76×10^9 L^{-1}). Therefore, we considered, 70 mg dose the recommended safe dose based on this study. The overall incidence of grades 3 and 4 neutropenia in this study was 29% (12 patients). Half of these were observed in the 70 mg group. At 70 mg, 60% of patients experienced grades 3–4 neutropenia. No toxicities attributable to ketoconazole were observed.

Plasma pharmacokinetics

Ketoconazole pharmacokinetics

Ketoconzole at 200 mg twice a day achieved mean minimal concentrations of 0.8 ± 0.9 mg/L (1.5 μmol/L) during and up to 24 h after docetaxel infusions. This gave concentrations several log-fold higher than the in vitro Ki (0.0037–0.015 μmol/L) of ketoconazole for CYP3A4 inhibition [2, 13]. Ketoconazole C_{max} was significantly correlated with docetaxel CL ($r = -0.346$, $P = 0.029$).

Midazolam pharmacokinetics

The non-compartmental pharmacokinetic parameters of midazolam are shown in Table 3. Compared to intravenous midazolam at 2.5 mg without ketoconazole, the mean midazolam CL was reduced by 6-fold and dose-normalized AUC by 4-fold. The variability of midazolam exposure measured as the % CV in AUC was also reduced from 157 to 67% (F-test, $P < 0.001$). Metabolite ratios (MR) of 1′hydroxymidazolam/midazolam with ketoconazole at 15 min, 1, 2, and 5 h were 0.016 ± 0.009, 0.027 ± 0.012, 0.034 ± 0.015, and 0.039 ± 0.02, respectively, from 11- to 13-fold lower than the ratios 0.21 ± 0.12, 0.32 ± 0.17, 0.4 ± 0.19, and 0.44 ± 0.24 without ketoconazole, confirming the reduction in CL was attributable to CYP3A inhibition.

Table 3 Midazolam noncompartmental pharmacokinetic estimates

PK parameter	Without ketoconazole[a]	With ketoconazole
Dose (mg)	2.5	1
AUC (mg/L h)	0.2 ± 0.3	0.3 ± 0.2
Dose-normalized AUC (h/L)	0.1 ± 0.11	–
CL (L/h)	26.4 ± 12.3	4.3 ± 1.8
CL (L/h/m^2)	17.0 ± 7.9	2.7 ± 1.1
Vss (L)	70.3 ± 35.5	24 ± 4.7
$t_{1/2}$ (h)	2.1 ± 0.8	26.1 ± 13.0

[a] Goh et al. [14]

Docetaxel pharmacokinetics and pharmacodynamics

Forty patients had full pharmacokinetic sampling. First-order, 2- and 3-compartmental models were fitted to the docetaxel data (Table 4). The 2-compartment model best fit the data based on Akaike's information criterion [35]. Non-compartmental pharmacokinetic parameters are reported in Table 5. The overall mean docetaxel CL of all patients with ketoconazole was 9.1 ± 4.2 L/h/m^2 (range 3.8–21.5 L/h/m^2) which was 1.7 times less than the CL without ketoconazole; however, CL variability was not different between the two groups (F-test, $P = 0.179$).

M4/docetaxel and M1 + M3/docetaxel metabolite ratios were 2.4 and 1.7 times higher at 1 h time point ($P < 0.001$ and $P = 0.005$, respectively), 2.7 and 1.5 times higher at 2 h time point ($P = 0.015$ and $P = 0.684$, respectively) in the absence of ketoconazole, indicating significant CYP3A inhibition with ketoconazole modulation. Metabolite concentrations at sampling times after 2 h were generally below limits of quantification by mass spectrometry, hence AUC ratios were not determined.

Using univariate linear analysis, docetaxel CL was significantly correlated with CrCL (Fig. 1), age and C_{max} of ketoconazole but not with midazolam CL (Fig. 2), serum ALT, AST, albumin, or A1AG (Table 6). Multiple linear regression analysis selected CrCL and ketoconazole C_{max} in the final clearance model: docetaxel CL = 6.82 + 0.14 × CrCL − 0.51 × ketoconazole C_{max} (overall $r = 0.61$, $P < 0.001$).

The mean docetaxel clearances and coefficients of variation for the BSA-dosed groups and the groups with fixed dosing were similar (14.5 ± 7.8 vs 14.1 ± 5.7 L/h, respectively; F-test, $P = 0.18$). Mean AUC of 70 mg with

Table 4 Docetaxel pharmacokinetic parameters using 2-compartmental model

Pharmacokinetic parameter	Mean ± SD
CL (L/h)	14.22 ± 6.87
CLcp (L/h)	8.74 ± 6.33
Vc (L)	8.19 ± 9.19
Vp (L)	58.04 ± 47.62

CL central compartment CL, *CLcp* intercompartmental CL, *Vc* central compartment volume of distribution, *Vp* peripheral compartment volume of distribution

Table 5 Summary of noncompartmental pharmacokinetic estimates of docetaxel

PK parameter	75–100 (mg/m^2) single agent[a] (n = 31)	Docetaxel dosing levels (with ketoconazole)							Docetaxel overall with ketoconazole (n=40)
		10 (mg/m^2) (n = 1)	20 (mg/m^2) (n = 6)	25 (mg/m^2) (n = 6)	30 (mg/m^2) (n = 5)	50 (mg) (n = 6)	60 (mg) (n = 6)	70 (mg) (n = 10)	
AUC (mg/*L h*)	6.0 ± 1.7	1.4	2.8 ± 1.2	2.8 ± 1.2	3.3 ± 0.8	4.9 ± 1.0	4.0 ± 1.2	5.7 ± 2.9	-
Dose-normalized AUC (h/L)	0.21 ± 0.07	0.1	0.1 ± 0.05	0.07 ± 0.03	0.07 ± 0.01	0.1 ± 0.02	0.07 ± 0.02	0.07 ± 0.03	0.08 ± 0.03
CL (L/h)	-	11.5	14.0 ± 12.3	16.1 ± 6.6	13.9 ± 2.3	10.5 ± 1.9	16.8 ± 7.3	14.6 ± 5.4	14.3 ± 6.6
CL (L/h/m^2)	15.3 ± 4.0	6.2	8.4 ± 6.8	10.4 ± 4.3	9.4 ± 2.3	6.3 ± 0.9	10.5 ± 5.1	10.3 ± 3.5	9.1 ± 4.2
*V*ss (L)	155.1 ± 150.9	115.2	70.1 ± 67.1	116.1 ± 65.5	70.6 ± 15.8	31.3 ± 11.5	79.5 ± 31.8	83.1 ± 38.4	77.0 ± 4.5
$t_{1/2}$ (h)	15.1 ± 9.6	8.5	8.6 ± 6.6	11.9 ± 1.8	10.8 ± 1.6	9.2 ± 3.7	9.1 ± 3.7	11.7 ± 3.5	10.3 ± 3.8

[a] Goh et al. [14]

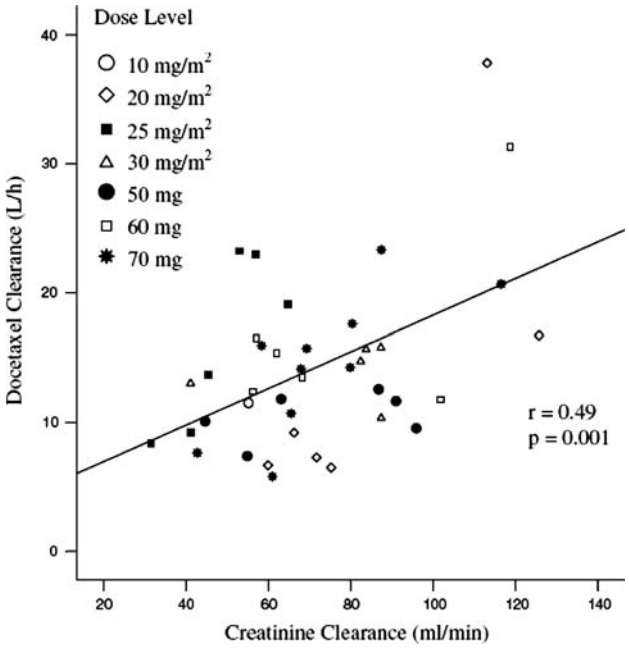

Fig. 1 Correlation between docetaxel and creatinine clearances (n = 40). Points at each dose level are represented by symbols as shown in the figure. *Solid line* represents the line of best fit. Correlation coefficient (r) and its significance level (P) are shown

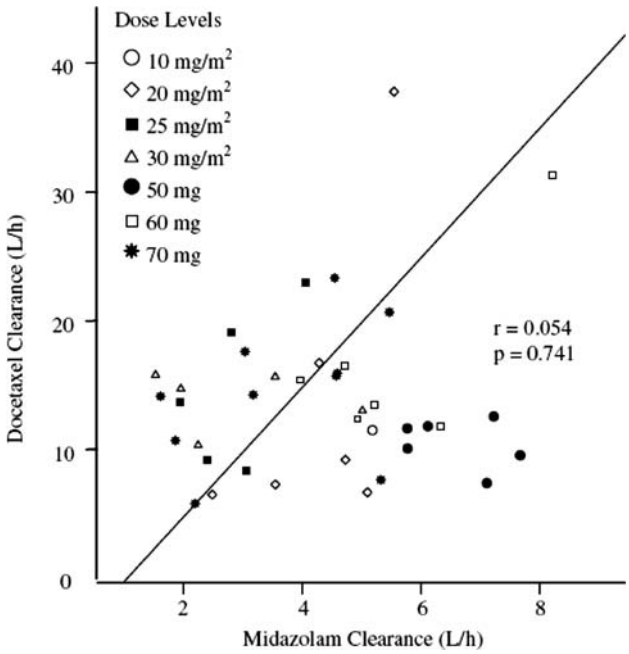

Fig. 2 Correlation between docetaxel and midazolam clearances (n = 40). Points at each dose level are represented by symbols as shown in the figure. *Solid line* represents the line of best fit. Correlation coefficient (r) and its significance level (P) are shown

ketoconazole and 75 mg/m^2 without ketoconazole were similar (5.7 ± 2.9 vs 5.1 ± 1.6 mg/L h; P = 0.36 and F test, P = 0.32) as was % change in ANC (84.6 ± 11.1 vs 84.7 ± 12%; P = 1.0 and F-test, P = 0.42, respectively).

Tumor responses

At 70 mg of docetaxel with ketoconazole, confirmed partial responses were observed in three patients (30%), two with metastatic breast cancer, and one with adenocarcinoma of unknown primary. Stable disease was observed in one patient with nasopharygeal carcinoma and one patient with breast cancer.

Discussion

There are several theoretical reasons to combine docetaxel with ketoconazole in the treatment of cancer. Firstly, from a pharmacological point of view, inhibition of CYP3A may reduce the pharmacokinetic variability of docetaxel by abrogation of factors that contribute to interindividual variability of CYP3A activity. Secondly, CYP3A may be overexpressed in some tumors and this expression is associated with response to taxanes treatment in breast cancer cell lines and colorectal [21, 23, 24]. Lastly, reduced docetaxel

Table 6 Univariate correlations of docetaxel clearance

Predictors	Pearson's correlation coefficient (*r*)	*P* value
CrCL	0.48	0.002
Ketoconazole C_{max}	−0.36	0.024
Age	−0.31	0.026
BSA	0.03	0.875
KPS	0.2	0.200
Midazolam CL	0.18	0.263
Albumin	0.09	0.566
AAG	−0.21	0.203
AST	−0.15	0.358
ALT	−0.14	0.401
ALP	−0.07	0.661
Bilirubin	−0.16	0.304

CrCL creatinine clearance, *BSA* body surface area, *AAG* α_1-acid glycoprotein, *AST* aspartate aminotrasnferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase

dose may lead to improved cost savings. Therefore, we conducted this phase I study to determine the safe dose of docetaxel that could be administered concurrently with ketoconazole, and to predict docetaxel clearance using midazolam as a probe for CYP3A activity.

In this study, adequate CYP3A inhibition was achieved as shown by plasma ketoconazole levels that were relevant for CYP3A inhibition and markedly reduced midazolam clearance. The safe dose of docetaxel that could be administered with ketoconazole was a fixed dose of 70 mg, once in every 3 weeks. The toxicity profile of docetaxel with ketoconazole was similar to docetaxel alone, with neutropenia, fatigue, and diarrhea predominate. At the 70 mg dose, only two out of ten patients treated experienced dose-limiting neutropenia. Further substantiating this clinical endpoint was the mean docetaxel AUC at this dose, which reached levels close to that of single agent docetaxel at 75 mg/m^2 [14], and the clinical responses observed. Fixed dosing of docetaxel was studied because BSA correlated poorly with docetaxel clearance in the initial cohorts of patients studied. There was also no increase in variability of clearance between the fixed dosing group compared to the BSA-based dosing group. Although previous studies of single agent docetaxel showed that BSA, to a certain extent, correlated with docetaxel clearance [5, 6], CYP3A inhibition with ketoconazole may abrogate this relationship.

Midazolam pharmacokinetic variability, assessed by coefficient of variability of plasma AUC, was reduced in patients with ketoconazole modulation compared to those without, supporting our strategy of using CYP3A inhibition to reduce variability in CYP3A substrate clearance. However, no reduction in interpatient variability of docetaxel clearance was seen with the addition of ketoconazole. This observation was recently reported in patients treated with high dose ketoconazole and docetaxel at 15 mg/m^2 [11] The less potent effect of ketoconazole on docetaxel metabolism may be attributable to the activation of other alternative pathways of clearance, such as renal clearance, which are in turn subject to significant interpatient variability. This could explain the difference in effect of ketoconazole on pharmacokinetic variability of both drugs.

Although previous studies of single agent docetaxel showed that the BSA, to a certain extent, correlated with docetaxel clearance [5, 6], CYP3A inhibition with ketoconazole may abrogate this relationship. CYP3A inhibition also appeared to abrogate the influence of hepatic covariables previously determined to be important predictors of docetaxel clearance, such as α_1 acid glycoprotein, albumin and serum transaminases [5, 6]. This could be explained by the correlation between CYP3A activity with $\alpha_1$acid glycoprotein, serum alanine, aspartate transaminase, and bilirubin levels [1]. Renal function emerged as the most significant predictor of docetaxel clearance, strongly suggesting that with potent inhibition of hepatic CYP3A clearance, renal elimination assumes an important route of clearance. This is supported by recent evidence showing that ketoconazole modulation reduced the recovered fecal and urinary docetaxel metabolites to total [^{3}H]-docetaxel ratio from 35.1 to 19.9%; whereas, urinary excretion of parent drug remained unchanged [10].

A study in a Western population had shown the feasibility of administering 55 mg/m^2 of docetaxel q3 weekly with oral ketoconazole 400 mg [32]. Reported docetaxel AUC for five patients treated at this dose level was 6.2 ± 3.5 mg/L h, which is comparable with the AUC of the 70 mg dose in our study. In addition, docetaxel pharmacokinetics in this study supports studies where a cross-over design was used to show that docetaxel clearance was reduced by half with ketoconazole [11, 12]. However, using a cross-over design does not permit determination of a safe dose or description of the toxicity profile of varying doses of docetaxel with ketoconazole. Compared to our previous data of docetaxel without ketoconazole, our study showed that docetaxel clearance was consistently reduced at all dose levels. In vitro studies suggest that probe drugs may be used to predict CYP3A inhibition [19]. However, in vivo, midazolam clearance did not correlate with docetaxel clearance. Differential activation of alternative pathways of elimination, like renal clearance, during CYP3A inhibition may be a possible explanation. Percentage inhibition of CYP3A metabolism by a competitive drug like ketoconazole is dependent on the hepatic extraction ratios of the substrate in the presence and absence of inhibitor; therefore, differences in effect of ketoconazole on the hepatic extraction ratios of both drugs may account for this lack of correlation in vivo [3, 4]. Furthermore, both drugs may be metabolized by

CYP3A4 and CYP3A5 to different extents, so interindividual differences in CYP3A4 and CYP3A5 content may lead to variable inhibitory effect of ketoconazole on the overall CYP3A activity [15].

In conclusion, reduction of pharmacokinetic variability by CYP3A inhibition using ketoconazole is substrate dependent. Fixed dose administration of docetaxel (70 mg), once in every 3 weeks with ketoconazole is the recommended dose for further evaluation in Asian patients, and together with theoretical advantage of reducing tumor resistance due to overexpression of CYP3A, should be considered for further evaluation in efficacy studies in cancers like breast cancer and colorectal cancer.

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Conflict of interest All authors express no conflicts of interest in this work.

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