Cancer Chemother Pharmacol (2007) 60:811–819
DOI 10.1007/s00280-007-0428-5

ORIGINAL ARTICLE

Pharmacokinetic modulation of oral etoposide by ketoconazole in patients with advanced cancer

Wei Peng Yong · Apurva A. Desai · Federico Innocenti · Jacqueline Ramirez · Dale Shepard · Ken Kobayashi · Larry House · Gini F. Fleming · Nicholas J. Vogelzang · Richard L. Schilsky · Mark J. Ratain

Received: 15 October 2006 / Accepted: 15 January 2007 / Published online: 17 February 2007

Abstract
Purpose Etoposide is a widely used cytotoxic drug that is commercially available in both intravenous and oral formulations. High interpatient pharmacokinetic variability has been associated with oral etoposide administration. Various strategies used in the past to reduce such variability have not been successful. Hence, this study was designed to evaluate if pharmacokinetic modulation of oral etoposide with ketoconazole could lead to a favorable alteration of etoposide pharmacokinetics, and to assess the feasibility and safety of this approach.
Methods Thirty-two patients were treated with ketoconazole 200 mg daily with an escalating dose of oral etoposide starting at a dose of 50 mg every other day. Pharmacokinetic samples were obtained during the first treatment cycle after the administration of an oral etoposide and ketoconazole dose. Additional baseline pharmacokinetic studies of etoposide alone were performed 4 days prior to the first treatment cycle.
Results Dose limiting toxicities were neutropenia and fatigue. Ketoconazole increased the area under the plasma concentration–time curve (AUC) of oral etoposide by a median of 20% ($p < 0.005$). Ketoconazole did not reduce the interpatient variability in etoposide pharmacokinetics. Pretreatment bilirubin levels correlated with etoposide clearance (Spearman's $r = -0.48$, $p = 0.008$). The maximum tolerated dose was etoposide administered at 50 mg daily and ketoconazole 200 mg qd for 3 of 5 weeks.
Conclusions Ketoconazole reduces the apparent clearance of oral etoposide, does not alter its toxicity profile and does not reduce interpatient pharmacokinetic variability. Other methods to reduce the pharmacokinetic variability of oral etoposide are needed.

Keywords Pharmacokinetic modulation · Etoposide · Ketoconazole · Drug interaction

W. P. Yong · A. A. Desai · F. Innocenti · D. Shepard · K. Kobayashi · M. J. Ratain (✉)
Committee on Clinical Pharmacology and Pharmacogenomics, University of Chicago, 5841 S Maryland Ave, MC2115, Chicago, IL 60637, USA
e-mail: mratain@medicine.bsd.uchicago.edu

A. A. Desai · F. Innocenti · J. Ramirez · K. Kobayashi · L. House · G. F. Fleming · N. J. Vogelzang · R. L. Schilsky · M. J. Ratain
Department of Medicine, University of Chicago, Chicago, IL 60637, USA

A. A. Desai · F. Innocenti · G. F. Fleming · N. J. Vogelzang · R. L. Schilsky · M. J. Ratain
Cancer Research Center, University of Chicago, Chicago, IL 60637, USA

Introduction

Etoposide [4′-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene)-β-D-glucopyranoside] is used in the treatment of various malignancies such as small cell lung cancer, germ cell tumors, and lymphomas [32, 46]. Although etoposide is most commonly administered intravenously, it can also be given orally as a liquid capsule. The interaction between etoposide and its target, topoisomerase II, is rapidly reversible and the cytotoxic efficacy of etoposide appears to be schedule-dependent, favoring a more prolonged schedule of administration, for which oral etoposide is most suitable [41].

The bioavailability of oral etoposide is unpredictable with high interpatient variability [11, 17, 43, 47]. Hande et al. [16] demonstrated that both interpatient and intrapatient variability in systemic exposure to oral etoposide were more than twice that of intravenous administration. Furthermore, the absorption of oral etoposide is nonlinear, with saturable bioavailability at oral doses above 200–400 mg, which potentially could contribute to interpatient variability [19, 41, 42]. Since variability in systemic exposure leads to unpredictable drug effects, several strategies have been attempted to improve the oral bioavailability of etoposide and reduce the pharmacokinetic variability. However, strategies such as the modification of gastric emptying time and gastric pH have been unsuccessful [23].

Intestinal and hepatic metabolism often plays a major role in determining the bioavailability of agents with high first pass metabolism. The inhibition of drug metabolizing enzymes such as CYP3A4 and dihydropyrimidine dehydrogenase has been shown to improve the bioavailability and, to a lesser degree, reduce interpatient variability of their corresponding substrates such 5-flurouracil, taxanes and protease inhibitors [1, 7, 27, 29]. Oxidative demethylation of etoposide to its corresponding catechol is mediated primarily by CYP3A4 [36, 37]. Glucuronidation by UGT1A1 accounts for the disposition of 15–35% of administered etoposide [9, 15, 50]. CYP3A4 and UGT1A1 are expressed both in the liver and the intestine and thus are potential targets for the modulation of etoposide pharmacokinetics.

Ketoconazole is a commonly used antifungal agent known for its inhibitory effect on CYP3A4 [28]. Recently, ketoconazole has been shown to be a potent inhibitor of UGT1A1 [38, 52]. In this study, we utilized ketoconazole to modulate the pharmacokinetics of oral etoposide in an attempt to: (1) evaluate its effects on systemic exposure of oral etoposide, (2) examine its effect on interpatient variability of etoposide pharmacokinetics and (3) assess the feasibility and safety of this approach.

Patients and methods

Eligibility criteria

Patients with histologically confirmed solid tumors or lymphomas refractory to standard therapy or for which no known effective therapy existed were eligible for the study. Additional eligibility criteria included: presence of measurable or evaluable disease, age at least 18 years, Karnofsky performance status of $\geq$60%, life expectancy of $\geq$8 weeks; adequate organ function defined as: white blood cell count $\geq 3.5\times10^9$/L, platelet count $\geq 100\times10^9$/L, total bilirubin <1.6 mg/dL, serum albumin $\geq$3.0 g/dL, AST and ALT $\leq$3 times the upper limit of the normal, serum creatinine $\leq$1.5 mg/dL. Eligible patients had to be fully recovered from prior therapy and could not have received any chemotherapy or radiotherapy within 3 weeks (6 weeks for nitrosoureas or mitomycin C) before initiating therapy on this protocol. Patients were excluded if their underlying medical conditions made administration of oral etoposide or ketoconazole hazardous. Other exclusion criteria consisted of: history of small bowel or gastric resections, chronic diarrheal diseases and malabsorption syndromes; patients requiring concomitant administration of antacid, H_1- or H_2-blockers, CYP3A4 substrates, inhibitors and inducers; and pregnant or lactating patients. All patients provided written informed consent before study enrollment in accordance with institutional and Federal guidelines.

Study design

Etoposide, provided by Bristol-Myers Oncology Division, was supplied in 50 mg liquid-filled gelatin capsules. All patients were administered a 50 mg test dose of etoposide on day −3 (see Figs. 1, 2). The actual treatment began on day 1 with a fixed dose of ketoconazole at 200 mg/day until day 22. Patients were administered both ketoconazole and etoposide starting on day 2.

The starting dose of etoposide was selected based on the predicted increase in etoposide exposure observed in other studies that involved modulation of etoposide with the CYP3A4 inhibitor cyclosporine [26, 51]. The study was divided in two parts. In part 1, an initial cohort of 9 patients was used to confirm our hypothesis that pharmacokinetic modulation of etoposide with ketoconazole would increase systemic exposure of oral etoposide. In this cohort, etoposide was initially administered at 50 mg daily one hour after the ketoconazole dose from days 2 to 22 on a 28-day cycle for the first 8 subjects. Due to greater than anticipated toxicities (prolonged neutropenia that delayed treatment), the protocol was amended to increase the cycle to 35 days for a subsequent subject in this cohort.

After establishing that ketoconazole modulation increased the systemic exposure of oral etoposide, the second part of the study was initiated. Part 2 of the study involved dose escalation of etoposide with a fixed dose of ketoconazole. The following etoposide dose levels were studied: 50 mg on alternate days (level I), 50 mg on 2 of 3 days (level II), 50 mg on 3 of 4 days

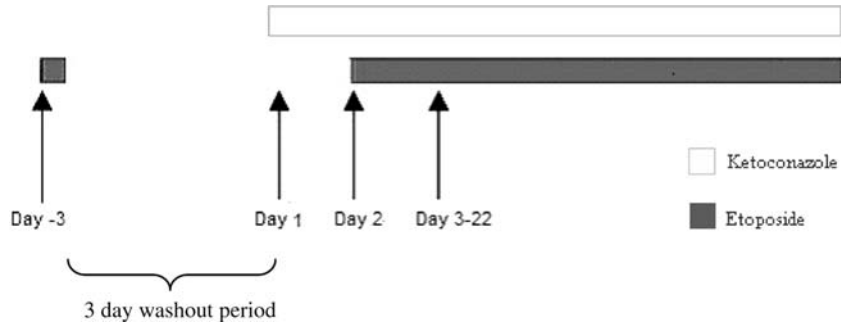

Fig. 1 Cycle 1 treatment schedule for etoposide and ketoconazole administration Cycle 1 treatment schema. Day −3: etoposide alone (50 mg), Day 1: ketoconazole (200 mg), Day 2: etoposide (50 mg) + ketoconazole (200 mg/day), Day 3–22: etoposide dose + ketoconazole (200 mg/day). Pharmacokinetic studies were performed on day −3 and day 2. Twenty eight-day cycle for dose level I–III and 35-day cycle for dose level IV

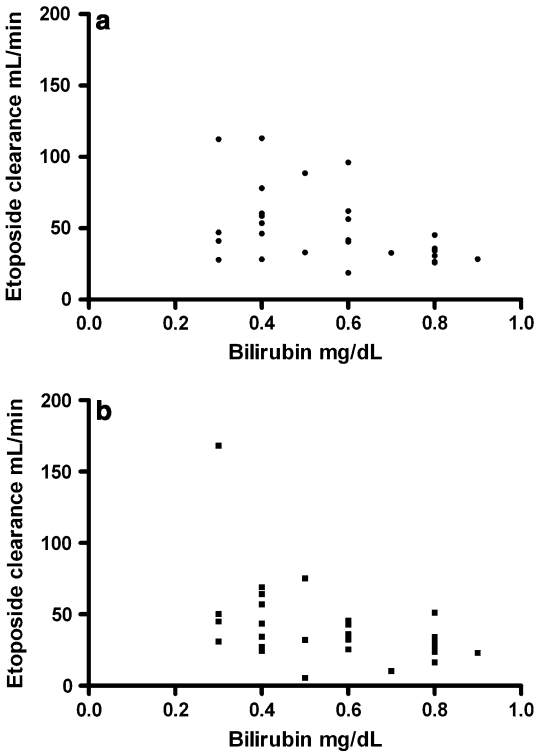

Fig. 2 The relationship between serum bilirubin level versus etoposide clearance in patients receiving **a** etoposide alone (Spearman's $r = -0.45$, $p = 0.016$) and **b** etoposide with ketoconazole modulation (Spearman's $r = -0.48$, $p = 0.008$)

(level III) and 50 mg alternating with 100 mg daily (level IV). Etoposide was administered at the assigned dose one hour after the ketoconazole dose from days 2 to 22, on a 28-day cycle for dose levels I, II and III, and on a 35-day cycle for dose IV. Table 1 summarizes all the dose levels that were studied. All patients were instructed to record the time of administration of each medication in a logbook, to facilitate compliance assessment.

A minimum of three patients was evaluated at each dose level. No more than one new patient was treated per week. If none of the 3 evaluable patients developed dose limiting toxicity (DLT), subsequent patients were treated at the next dose level. If one patient developed DLT, then the dose level was expanded to at least six patients, and further dose escalation occurred only if no more than one of six patients had DLT (see below for definition). Dose escalation was continued until a maximum tolerated dose (MTD) was identified. MTD was defined as the dose level at which half of the subjects experienced DLT. For the purposes of determining the MTD, only toxicities occurring during the first cycle of the therapy were considered.

DLT was defined as any grade 3 or greater non-hematological toxicity, grade 4 hematological toxicity or grade 3 myelosuppression that was not resolved to grade 1 or less by the first day of the next scheduled treatment cycle. Elevation of liver function tests to grade 3 was considered dose limiting if it was not resolved to grade 2 or less by the next scheduled treatment day. Anemia, nausea, vomiting and alopecia were excluded as dose limiting criteria. Patients who experienced a DLT were allowed to continue on protocol with a dose modification at the discretion of the treating physician.

Patient evaluation

Patients were evaluated for toxicity by history, physical examination and laboratory evaluation once a week during the first cycle of treatment, and every 3 weeks during subsequent cycles. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0. Tumor response assessments were performed after the first two cycles of therapy and every two cycles thereafter. Complete

Table 1 Dose levels studied and corresponding dose limiting toxicities

Drug	Dose level	Dose (mg/day)	No. of patients treated (no. of patients eligible for DLT assessment)	No. of cycles	Dose limiting toxicities
Etoposide	Initial cohort	50 mg daily (50)	9	16	3[a]
	I	50 mg every other day (25)	5	31	0
	II	50 mg 2 of 3 days (33)	11 (9)	17	1[b]
	III	50 mg 3 of 4 days (37)	3	5	0
	IV	50/100 mg alternate day (75)	4	9	1[c]

[a] (1) Grade 3 febrile neutropenia; (2) grade 4 neutropenia

[b] Grade 4 neutropenia with grade 3 fatigue

[c] Grade 4 neutropenia

response was defined as the disappearance of all clinical and radiological evidence of the tumor for a minimum of 4 weeks without the appearance of new lesions. Partial response was defined as ≥50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions and no appearance of new lesions. Progressive disease was defined as >25% increase in the size of any measurable lesion or the appearance of new lesions. Stable disease was attributed when these criteria were not met. Patients with partial and complete response or stable disease were allowed to continue the therapy.

Pharmacokinetic evaluation

Plasma samples for pharmacokinetic analyses were obtained on days −3 (etoposide alone) and 2 (etoposide with ketoconazole) at the following time points: baseline, 10, 20, 30, 40, 50, 60, 75, 90 and 105 min and 2, 3, 4, 6, 8 and 24 h after the ingestion of etoposide. Blood samples were collected in heparinized tubes and centrifuged to separate the plasma, which was then stored at −80°C until analysis. Etoposide was administered under direct observation by the study team on the days when pharmacokinetic samplings were required.

The etoposide assay was based on the published method by Sinkule and Evans [40]. Briefly, a liquid–liquid extraction was carried out by adding 100 μL of saturated ammonium sulfate and 4 mL ethyl acetate to 0.5 mL plasma. The organic layer was dried under nitrogen and reconstituted using 1 mL 50% methanol/50% deionized water. One hundred μL of teniposide (VM-26) at a concentration of 40 μg/mL was used as an internal standard. After reconstitution, the sample was injected onto the HPLC system. A μBondapak phenyl reverse phase column (3.9 × 300 mm, 10 μm; 125 Å, Waters Corp., Milford, MA) was used with fluorescence detection at an excitation wavelength of 215 nm and an emission wavelength of 328 nm. The mobile phase consisted of methanol (A) and deionized water (B) with the following gradients: from 0 to 15 min, 40% A and 60% B, from 16 to 40 min, 55% A and 45% B, from 40.1 to 50 min, 40% A and 60% B. At the elution rate of 1.0 mL/min the retention times for etoposide and teniposide were 18 and 27 min, respectively. The assay was validated over a range of 50.8−1868.4 ng/mL, with an interday accuracy from 97.5 to 103.5% and precision of ≤6.4% and intraday accuracy ranging from 95.2 to 107.3% and precision of ≤14.1%, respectively. Samples with concentrations that fell outside of the range of the standard curve were reanalyzed by diluting with blank plasma prior to extraction and the final concentration values were determined after applying the appropriate dilution factor.

Pharmacokinetic analysis

Non-compartmental methods were used to calculate the pharmacokinetic parameters of etoposide using WinNonlin Version 4.0 (Pharsight Corporation, Mountain View, CA). The peak concentration (C_{max}) was the maximum plasma concentration measured. The apparent elimination rate constant (λ_z) was estimated by performing a linear regression of the terminal log-linear portion of the concentration–time plot. The AUC, which was extrapolated to infinity, was determined by the linear trapezoidal method. Calculated parameters included elimination half-life ($t_{1/2} = \ln 2/\lambda_z$), clearance ($CL/F$ = Dose/AUC) and volume of distribution ($Vd/F = CL/\lambda_z$). All pharmacokinetic parameters were calculated using actual collection times.

Statistical considerations

Data were expressed as median with range for non-normally distributed data. A paired non-parametric test (Wilcoxon signed-rank test) was used for 2-group comparisons. Although the original statistical plan called for 1-sided testing, we chose the more conservative

2-sided test for final analysis. Spearman's r analysis was used for comparing etoposide clearance with various clinical parameters. Pitman's T test was used for analysis of variance for correlated samples. A p value of <0.05 was considered to be statistically significant. The significant level cut-off was not adjusted for multiple comparisons because these analyses were considered exploratory. The statistical analyses of the data were conducted using GraphPad Prism 4 (San Diego, CA).

Results

Patient characteristics

Thirty-two patients were enrolled on the trial. The detailed characteristics of the patients enrolled are listed in Table 2 The most common tumor types include ovarian cancer, colorectal cancer, prostate cancer and breast cancer. More than two-thirds of the patients had been treated with at least two prior treatment regimens. All patients who received etoposide were assessed for toxicity; two patients were not evaluable for DLT because they did not complete the first cycle of therapy due to rapid disease progression.

Table 2 Patient characteristics

	Number of patients
Total enrolled	32
Gender	
Male	12
Female	20
Age (years)	
Median	60
Range	25–80
Karnofsky performance status (%)	
100	8
90	8
80	12
70	4
Disease	
Ovarian	7
Colorectal	6
Prostate	4
Breast	4
Gastric	2
Lung	2
Sarcoma	2
Other	5
Prior treatment regimens[a]	
≤1	10
2	7
3	9
≥4	5

[a] Data on prior treatment regimen was not available for one patient

Twenty-eight patients were eligible for response evaluation. Four patients were not evaluable for response because they were discontinued from the study before the first scheduled tumor response assessment (three had unacceptable treatment related toxicity and one withdrew consent).

Adverse events and response evaluation

Table 1 provides a summary of the DLTs at each dose level and Table 3 illustrates the toxicities for all dose levels in the first cycle. Myelosuppression was the most common toxicity and neutropenia was the most common DLT. One-third of the patients in the initial cohort (etoposide 50 mg daily) developed DLT, and all DLTs from this cohort were due to febrile neutropenia. Hence, the dose escalation phase of the study started at a lower dose of 50 mg every other day (level I). None of the patients treated at this level developed a DLT. At dose level II, one grade 4 febrile neutropenia (this patient also developed grade 3 thrombocytopenia) and one grade 3 neutropenia were observed. No hematological toxicity was seen in the three patients treated at dose level III. At dose level IV, 2 of 4 patients developed grade 3 or 4 neutropenia; the patient with grade 4 neutropenia also developed grade 3 thrombocytopenia. Mild anemia (≤grade 2) was common and was attributed to both the advanced disease of patients and the study drug. Only one patient developed grade 4 anemia (at dose level I). Six patients had mild (≤grade 2) thrombocytopenia and 3 patients had grade 3 thrombocytopenia. Further accrual to the study was suspended, due to concerns of the overall hematological toxicity observed at dose level IV.

Fatigue, nausea and vomiting were the most commonly reported non-hematological toxicities. About 25% of all patients treated developed grade 2 or 3 fatigue; it was essentially observed at all doses and did not correlate with the dose of etoposide. Two patients at dose level IV had grade 3 events unrelated to etoposide therapy. One had a transient ischemic attack with dysarthria, while the other patient with ovarian cancer and bulky intra-abdominal disease developed deep vein thrombosis and required anticoagulation. Two deaths occurred on the study. Both patients developed rapid symptomatic progression of lung metastases from breast and lung cancer; one occurred in cycle 1 and the other in cycle 2 of the treatment.

A total of 78 cycles of treatment were administered with a median of 2 cycles per patient (1–11 cycles). No partial or complete responses were observed. Twelve patients (38%) were noted to have stable disease for up to 11 months (range 4–11 months, median 4 months).

Table 3 Toxicity for all dose levels

Dose level	No. of patients																			
	Initial cohort 50 mg/day				I 50 mg every other day				II 50 mg 2 of 3 days				III 50 mg 3 0f 4 days				IV 50/100 mg alternate day			
	(n = 9)				(n = 5)				(n = 11)				(n = 3)				(n = 4)			
Toxicity/grade	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Gastrointestinal																				
Nausea/emesis	3	2	–	–	2	–	–	–	2	–	–	–	1	–	–	–	1	–	–	–
Mucositis	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Constitutional																				
Anorexia	2	2	–	–	2	–	–	–	1	1	–	–	–	–	–	–	1	–	–	–
Fatigue	2	1	1	–	–	2	–	–	2	1	1	–	2	–	–	–	2	2	–	–
CNS																				
Peripheral neuropathy	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–
Hematologic																				
Neutropenia	–	1	1	3	1	1	–	–	2	1	1	1	–	–	–	–	–	1	1	1
Thrombocytopenia	1	–	1	–	2	–	–	–	2	1	1	–	–	–	–	–	–	–	1	–
Anemia	1	5	1	1	1	2	–	–	3	5	1	–	1	1	–	–	–	3	–	–
Other																				
Metabolic	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Infection	–	–	2	–	–	1	–	–	–	–	1	–	–	–	1	–	1	–	–	–

Pharmacokinetics

Pharmacokinetic data were not available in one subject because the subject refused venous sampling. Thirty-one patients had samples available for etoposide pharmacokinetic data analysis from both day −3 and 2. Of these 31 patients, four were not assessable for clearance and terminal half-life, due to missing sampling points at the terminal log-linear portion of the concentration–time plot. The pharmacokinetics of etoposide with and without ketoconazole are shown in Table 4. Co-administration with ketoconazole resulted in an increase in the AUC and a reduction in the clearance of etoposide by a median of 20% ($p < 0.005$) and 18% ($p = 0.03$), respectively. The addition of ketoconazole did not significantly reduce interpatient variability in peak plasma concentration (61% to 50%, $F = 0.025$, $p = 0.87$). In fact, interpatient variability of AUC and clearance increased from 43% to 89% ($T = 8.12$, $p < 0.001$) and 52% to 71%, ($T = 0.714$, $p = 0.24$), respectively, in the presence of ketoconazole, which was opposite to the hypothesized effect.

Several parameters were assessed for their correlation with etoposide clearance (Table 5). In the absence of ketoconazole, both bilirubin and creatinine correlated with etoposide clearance. With ketoconazole, pretreatment bilirubin was the only factor that correlated significantly with etoposide clearance. A similar trend was noted with multivariate analysis. Etoposide AUC correlated negatively with log nadir absolute neutrophil counts (ANC) (Spearman's $r = -0.37$, $p = 0.05$). Amongst the pretreatment parameters, bilirubin was significantly correlated to nadir log ANC (Spearman's $r = -0.47$, $p = 0.007$).

Discussion

This study demonstrates that ketoconazole decreases the apparent oral clearance of etoposide, although the

Table 4 Pharmacokinetic parameters (non-compartmental analysis) expressed as median (range)

Pharmacokinetic parameter	Etoposide alone	Etoposide + ketoconazole	Ratio (% change)	p value
Dose (mg)	50	50/200		
C_{max} (μg/mL)	2.3 (0.4–6.9)	2.3 (0.4–6.0)	1.0(0)	0.86
T_{max} (h)	1.1 (0.5–8.0)	1.5 (0.5–8.0)	1.0 (0)	0.09
AUC (μg h/mL)	20.3 (7.4–44.3)	25.6 (5.0–148.2)	1.2 (20)	0.003
CL/F (mL/min)	41.0 (18.8–113.0)	32.5 (5.67–168.2)	0.82 (−18)	0.03
Vd/F (L)	25.6 (11.8–92.0)	23.6 (4.3–53.5)	0.78 (−22)	0.09
$T_{1/2}$ (h)	6.4 (2.6–35.6)	6.5 (3.1–110.0)	1.0 (0)	0.55

Table 5 Univariate correlations of etoposide clearance

Predictor	Etoposide alone		Etoposide + ketoconazole	
	Spearman's *r* correlation	*p* value	Spearman's *r* correlation	*p* value
Performance status	−0.13	NS	0.10	NS
Age	−0.22	NS	0.00	NS
Weight	−0.10	NS	0.16	NS
Albumin	0.26	NS	0.04	NS
Bilirubin	−0.45	0.016*	−0.48	0.008**
Creatinine	−0.46	0.015*	−0.07	NS
ALT	0.32	NS	0.14	NS
AST	0.35	NS	0.07	NS
Alkaline phosphatase	0.05	NS	−0.13	NS

NS not significant
* $p < 0.05$
** $p < 0.01$

combination of ketoconazole with reduced dose etoposide was well tolerated. Table 6 compares the AUC following the ingestion of a single 50 mg of oral etoposide with ketoconazole to etoposide alone (50 mg/m^2 and 100 mg) based on previously published data [8, 11, 17, 30, 33, 35, 39, 41, 47, 48]. Also, ketoconazole did not substantially alter the toxicity profile of oral etoposide compared with published studies of equivalent systemic exposure [11, 33]. As expecteded, myelosuppression was the most common dose limiting toxicity. The proportion of patients who developed grade 3 or 4 hematologic toxicity was slightly higher in patients who received 50 mg of etoposide daily with ketoconazole modulation compared to patients receiving 50 mg/m^2 in the studies by Paredes et al. and el-Yazigi et al. (44% vs 23–34%) [11, 33]. This difference may be related to the patient selection and the sample size of the studies. The study subjects in el-Yazigi et al. were chemonaive and two thirds of the patients in Paredes study were exposed to one or fewer previous chemotherapy regimens whereas majority of the study subjects in the current trial were heavily pretreated. Although patients were instructed to keep a log to monitor compliance, the data for most patients were missing. Hence we were unable to compare our patient's compliance data with other studies.

Table 6 AUC after single dose of oral etoposide

Dose	AUC (μg h/mL)	$T_{1/2}$ (h)	*n*	Reference
100 mg	22.8	–	17	[48]
100 mg	26	6.6	11	[17]
100mg	30.2	4.8	4	[33]
100 mg	32.5	3.7	15	[30]
100 mg	36.8	6.4	12	[39]
100 mg	56.5	5.6	28	[8]
100 mg	66	8.8	10	[41]
100 mg	87.7	11.5	50	[47]
50 mg/m^2	33.8	9.0	22	[11]
50 mg/m^2	34.3	–	32	[35]
50 mg + ketoconazole	32.5	6.5	33	Current study

Ketoconazole is a known inhibitor of ABCB1. Although modulation by ketoconazole led to an increase in the systemic exposure of etoposide, there was no apparent effect on the peak plasma concentration. In contrast, cyclosporine A, a more potent ABCB1 inhibitor, significantly increased the peak plasma concentration of other ABCB1 substrates (e.g., paclitaxel, docetaxel) [29]. This suggests that the increased systemic exposure to etoposide by ketoconazole modulation is most likely mediated predominantly through the inhibition of etoposide metabolism in the liver rather than the inhibition of transporters in the intestine.

Although deliberate inhibition of metabolizing enzymes or transporters improves the bioavailability of several drugs, this strategy has been less successful in reducing interpatient variability. In this study, we observed marked interindividual variability in etoposide AUC in patients, both with and without ketoconazole modulation. Similar observations have been reported in other studies using oral ketoconazole modulation [10, 12, 49]. In the study reported by Engels et al., the clearance of docetaxel correlated with the AUC of ketoconazole [12]. Engels et al. suggested that the lack of reduction in interpatient variability from ketoconazole modulation might be related to the observed variability in the systemic exposure to ketoconazole. In addition, the increased variability in etoposide clearance may also be a reflection of the differential inhibition of CYP3A isoforms by ketoconazole. As ketoconazole has less of an effect on CYP3A5 (relative to CYP3A4), subjects that express functional CYP3A5, which accounts for about 30% of Caucasians and up to 73% of African Americans, may be less affected by ketoconazole modulation [14, 20, 25, 34].

The lack of reduction in interpatient variability from ketoconazole modulation could also be due to the interpatient variability in UGT1A1 enzyme activity. The *UGT1A1*28* variant has been associated with a reduction in UGT1A1 enzyme activity with resultant pharmacodynamic consequences for drug such as irino-

tecan [3, 21]. The overall contribution of *UGT1A1* polymorphisms to the variability of etoposide kinetics was probably insignificant as CYP3A4 is the predominant metabolic enzyme for etoposide. However, the proportion of etoposide metabolized by UGT1A1 might be increased substantially in the presence of ketoconazole, since ketoconazole is a more potent inhibitor of CYP3A4 than UGT1A1 [22, 38, 52]. Bilirubin is a UGT1A1 substrate and the *UGT1A1*28* polymorphism has been associated with elevated serum bilirubin levels [5, 6, 31]. In this study, the plasma bilirubin was the only parameter that correlated negatively with etoposide clearance and AUC, when modulated with ketoconazole.

Bilirubin can affect etoposide kinetics by competing for albumin binding, resulting in an increase in unbound etoposide. However, such interaction is postulated to occur only at relatively high bilirubin concentrations [13]. Stewart et al. [44] have previously demonstrated that in patients with very high bilirubin levels, the reduction in metabolic clearance of etoposide may be compensated by the increased excretion of unbound etoposide due to the decrease in albumin binding. This provides an explanation as to why studies comparing patients with abnormal bilirubin level, including those with obstructive jaundice, with normal controls failed to detect significant differences in etoposide kinetics [2, 4, 18, 44]. Conversely, studies involving patients with normal bilirubin levels were able to incorporate pretreatment bilirubin for modeling of etoposide kinetics [24, 45]. Thus, the use of bilirubin as a predictor for etoposide toxicity should be further evaluated prospectively in the group of patients with normal bilirubin.

In conclusion, etoposide can be given safely at a lower dose with ketoconazole modulation although this approach does not provide either a clinical or pharmacologic advantage. Modulation by oral ketoconazole did not alter the toxicity profile of oral etoposide, nor did it improve the therapeutic index of oral etoposide.

References

1. Ahmed FY, Johnston SJ, Cassidy J, O'Kelly T, Binnie N, Murray GI, van Gennip AH, Abeling NG, Knight S, McLeod HL (1999) Eniluracil treatment completely inactivates dihydropyrimidine dehydrogenase in colorectal tumors. J Clin Oncol 17:2439–2445
2. Aita P, Robieux I, Sorio R, Tumolo S, Corona G, Cannizzaro R, Colussi AM, Boiocchi M, Toffoli G (1999) Pharmacokinetics of oral etoposide in patients with hepatocellular carcinoma. Cancer Chemother Pharmacol 43:287–294
3. Ando Y, Saka H, Asai G, Sugiura S, Shimokata K, Kamataki T (1998) UGT1A1 genotypes and glucuronidation of SN-38, the active metabolite of irinotecan. Ann Oncol 9:845–847
4. Arbuck SG, Douglass HO, Crom WR, Goodwin P, Silk Y, Cooper C, Evans WE (1986) Etoposide pharmacokinetics in patients with normal and abnormal organ function. J Clin Oncol 4:1690–1695
5. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP, Chowdhury NR (1995) The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 333:1171–1175
6. Bosma PJ, Seppen J, Goldhoorn B, Bakker C, Oude Elferink RP, Chowdhury JR, Chowdhury NR, Jansen PL (1994) Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. J Biol Chem 269:17960–17964
7. Buss N, Snell P, Bock J, Hsu A, Jorga K (2001) Saquinavir and ritonavir pharmacokinetics following combined ritonavir and saquinavir (soft gelatin capsules) administration. Br J Clin Pharmacol 52:255–264
8. Chen GL, Yang L, Rowe TC, Halligan BD, Tewey KM, Liu LF (1984) Nonintercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J Biol Chem 259:13560–13566
9. D'Incalci M, Rossi C, Zucchetti M, Urso R, Cavalli F, Mangioni C, Willems Y, Sessa C (1986) Pharmacokinetics of etoposide in patients with abnormal renal and hepatic function. Cancer Res 46:2566–2571
10. Desai AA, Innocenti F, Janisch L, DeMario M, Shepard D, Ramirez J, Fleming G, Ratain MJ (2004) A phase I trial of pharmacokinetic modulation of carboxyamidotriazole (CAI) with ketoconazole in patients with advanced cancer. Cancer Chemother Pharmacol 54:377–384
11. el-Yazigi A, Ezzat A, Berry J, Raines DA, Yusuf A, al-Rawithi S, Legayada ES (2000) Optimization of oral etoposide dosage in elderly patients with non-Hodgkin's lymphoma using the fraction of dose absorbed measured for each patient. J Clin Pharmacol 40:153–160
12. Engels FK, Ten Tije AJ, Baker SD, Lee CK, Loos WJ, Vulto AG, Verweij J, Sparreboom A (2004) Effect of cytochrome P450 3A4 inhibition on the pharmacokinetics of docetaxel. Clin Pharmacol Ther 75:448–454
13. Fleming RA, Evans WE, Arbuck SG, Stewart CF (1992) Factors affecting in vitro protein binding of etoposide in humans. J Pharm Sci 81:259–264
14. Gibbs MA, Kunze KL, Howald WN, Thummel KE (1999) Effect of inhibitor depletion on inhibitory potency: tight binding inhibition of CYP3A by clotrimazole. Drug Metab Dispos 27:596–599
15. Hande K, Anthony L, Hamilton R, Bennett R, Sweetman B, Branch R (1988) Identification of etoposide glucuronide as a major metabolite of etoposide in the rat and rabbit. Cancer Res 48:1829–1834
16. Hande K, Messenger M, Wagner J, Krozely M, Kaul S (1999) Inter- and intrapatient variability in etoposide kinetics with oral and intravenous drug administration. Clin Cancer Res 5:2742–2747
17. Hande KR, Krozely MG, Greco FA, Hainsworth JD, Johnson DH (1993) Bioavailability of low-dose oral etoposide. J Clin Oncol 11:374–377
18. Hande KR, Wolff SN, Greco FA, Hainsworth JD, Reed G, Johnson DH (1990) Etoposide kinetics in patients with obstructive jaundice. J Clin Oncol 8:1101–1107
19. Harvey VJ, Slevin ML, Joel SP, Johnston A, Wrigley PF (1986) The effect of dose on the bioavailability of oral etoposide. Cancer Chemother Pharmacol 16:178–181
20. Hustert E, Zibat A, Presecan-Siedel E, Eiselt R, Mueller R, Fuss C, Brehm I, Brinkmann U, Eichelbaum M, Wojnowski

L, Burk O (2001) Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. Drug Metab Dispos 29:1454–1459
21. Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramirez J, Rudin CM, Vokes EE, Ratain MJ (2004) Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 22:1382–1388
22. Ito K, Brown HS, Houston JB (2004) Database analyses for the prediction of in vivo drug-drug interactions from in vitro data. Br J Clin Pharmacol 57:473–486
23. Joel SP, Clark PI, Heap L, Webster L, Robbins S, Craft H, Slevin ML (1995) Pharmacological attempts to improve the bioavailability of oral etoposide. Cancer Chemother Pharmacol 37:125–133
24. Kaul S, Srinivas NR, Igwemezie LN, Barbhaiya RH (1996) A pharmacodynamic evaluation of hematologic toxicity observed with etoposide phosphate in the treatment of cancer patients. Semin Oncol 23:15–22
25. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 27:383–391
26. Lum BL, Kaubisch S, Yahanda AM, Adler KM, Jew L, Ehsan MN, Brophy NA, Halsey J, Gosland MP, Sikic BI (1992) Alteration of etoposide pharmacokinetics and pharmacodynamics by cyclosporine in a phase I trial to modulate multidrug resistance. J Clin Oncol 10:1635–1642
27. Malingre MM, Richel DJ, Beijnen JH, Rosing H, Koopman FJ, Ten Bokkel Huinink WW, Schot ME, Schellens JH (2001) Coadministration of cyclosporine strongly enhances the oral bioavailability of docetaxel. J Clin Oncol 19:1160–1166
28. Maurice M, Pichard L, Daujat M, Fabre I, Joyeux H, Domergue J, Maurel P (1992) Effects of imidazole derivatives on cytochromes P450 from human hepatocytes in primary culture. Faseb J 6:752–758
29. Meerum Terwogt JM, Malingre MM, Beijnen JH, ten Bokkel Huinink WW, Rosing H, Koopman FJ, van Tellingen O, Swart M, Schellens JH (1999) Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. Clin Cancer Res 5:3379–3384
30. Millward MJ, Newell DR, Yuen K, Matthews JP, Balmanno K, Charlton CJ, Gumbrell L, Lind MJ, Chapman F, Proctor M, et al (1995) Pharmacokinetics and pharmacodynamics of prolonged oral etoposide in women with metastatic breast cancer. Cancer Chemother Pharmacol 37:161–167
31. Monaghan G, Ryan M, Seddon R, Hume R, Burchell B (1996) Genetic variation in bilirubin UPD-glucuronosyltransferase gene promoter and Gilbert's syndrome. Lancet 347:578–581
32. O'Dwyer PJ, Leyland-Jones B, Alonso MT, Marsoni S, Wittes RE (1985) Etoposide (VP-16–213). Current status of an active anticancer drug. N Engl J Med 312:692–700
33. Paredes J, Kahn JO, Tong WP, Feldstein ML, Lin S, Bennett JM, Metroka CE, Ratner L, Krown SE (1995) Weekly oral etoposide in patients with Kaposi's sarcoma associated with human immunodeficiency virus infection: a phase I multicenter trial of the AIDS Clinical Trials Group. J Acquir Immune Defic Syndr Hum Retrovirol 9:138–144
34. Patki KC, Von Moltke LL, Greenblatt DJ (2003) In vitro metabolism of midazolam, triazolam, nifedipine, and testosterone by human liver microsomes and recombinant cytochromes p450: role of cyp3a4 and cyp3a5. Drug Metab Dispos 31:938–944
35. Perdaems N, Bachaud JM, Rouzaud P, Murris-Espin M, Hermant C, Mihura J, Lochon I, Houin G, Canal P, Chatelut E (1998) Relation between unbound plasma concentrations and toxicity in a prolonged oral etoposide schedule. Eur J Clin Pharmacol 54:677–683
36. Relling MV, Evans R, Dass C, Desiderio DM, Nemec J (1992) Human cytochrome P450 metabolism of teniposide and etoposide. J Pharmacol Exp Ther 261:491–496
37. Relling MV, Nemec J, Schuetz EG, Schuetz JD, Gonzalez FJ, Korzekwa KR (1994) O-demethylation of epipodophyllotoxins is catalyzed by human cytochrome P450 3A4. Mol Pharmacol 45:352–358
38. Satoh T, Tomikawa Y, Takanashi K, Itoh S, Yoshizawa I (2004) Studies on the interactions between drugs and estrogen. III. Inhibitory effects of 29 drugs reported to induce gynecomastia on the glucuronidation of estradiol. Biol Pharm Bull 27:1844–1849
39. Sessa C, Zucchetti M, Torri V, Pagani O, D'Incalci M, Gentili D, Martinelli G, deJong J, Alerci M, Cavalli F (1993) Chronic oral etoposide in small-cell lung cancer: clinical and pharmacokinetic results. Ann Oncol 4:553–558
40. Sinkule JA, Hutson P, Hayes FA, Etcubanas E, Evans W (1984) Pharmacokinetics of etoposide (VP16) in children and adolescents with refractory solid tumors. Cancer Res 44:3109–3113
41. Slevin ML, Clark PI, Joel SP, Malik S, Osborne RJ, Gregory WM, Lowe DG, Reznek RH, Wrigley PF (1989) A randomized trial to evaluate the effect of schedule on the activity of etoposide in small-cell lung cancer. J Clin Oncol 7:1333–1340
42. Slevin ML, Joel SP (1993) Prolonged oral etoposide in small cell lung cancer. Ann Oncol 4:529–532
43. Smyth RD, Pfeffer M, Scalzo A, Comis RL (1985) Bioavailability and pharmacokinetics of etoposide (VP-16). Semin Oncol 12:48–51
44. Stewart CF, Arbuck SG, Fleming RA, Evans WE (1990) Changes in the clearance of total and unbound etoposide in patients with liver dysfunction. J Clin Oncol 8:1874–1879
45. Stewart CF, Fleming RA, Arbuck SG, Evans WE (1990) Prospective evaluation of a model for predicting etoposide plasma protein binding in cancer patients. Cancer Res 50:6854–6856
46. Toffoli G, Corona G, Basso B, Boiocchi M (2004) Pharmacokinetic optimisation of treatment with oral etoposide. Clin Pharmacokinet 43:441–466
47. Toffoli G, Corona G, Sorio R, Robieux I, Basso B, Colussi AM, Boiocchi M (2001) Population pharmacokinetics and pharmacodynamics of oral etoposide. Br J Clin Pharmacol 52:511–519
48. van der Gaast A, Vlastuin M, Kok TC, Splinter TA (1992) What is the optimal dose and duration of treatment with etoposide? II. Comparative pharmacokinetic study of three schedules: 1 × 100 mg, 2 × 50 mg, and 4 × 25 mg of oral etoposide daily for 21 days. Semin Oncol 19:8–12
49. Van Veldhuizen PJ, Reed G, Aggarwal A, Baranda J, Zulfiqar M, Williamson S (2003) Docetaxel and ketoconazole in advanced hormone-refractory prostate carcinoma: a phase I and pharmacokinetic study. Cancer 98:1855–1862
50. Watanabe Y, Nakajima M, Ohashi N, Kume T, Yokoi T (2003) Glucuronidation of etoposide in human liver microsomes is specifically catalyzed by UDP-glucuronosyltransferase 1A1. Drug Metab Dispos 31:589–595
51. Yahanda AM, Alder KM, Fisher GA, Brophy NA, Halsey J, Hardy RI, Gosland MP, Lum BL, Sikic BI (1992) Phase I trial of etoposide with cyclosporine as a modulator of multidrug resistance. J Clin Oncol 10:1624–1634
52. Yong WP, Ramirez J, Innocenti F, Ratain MJ (2005) Effects of ketoconazole on glucuronidation by UDP-glucuronosyltransferase enzymes. Clin Cancer Res 11:6699–6704