

Assessment of Ifosfamide Pharmacokinetics, Toxicity, and Relation to CYP3A4 Activity as Measured by the Erythromycin Breath Test in Patients With Sarcoma

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BACKGROUND. Ifosfamide is a chemotherapeutic agent that requires cytochrome P450 3A (CYP3A) for bioactivation and metabolism. To the authors' knowledge, the correlation between dose, pharmacokinetics, CYP3A, and toxicity has not been fully evaluated. A randomized Phase II trial was performed on 22 soft tissue sarcoma patients treated with doxorubicin (60 mg/m²/cycle) and either high-dose ifosfamide (12 g/m²/cycle) or standard-dose ifosfamide (6 g/m²/cycle). The pharmacokinetics of ifosfamide and CYP3A measurements observed are reported.

METHODS. Pharmacokinetic parameters for ifosfamide, 2-dichloroethylifosfamide (2-DCE), and 3-dichloroethylifosfamide (3-DCE) were collected after the first ifosfamide infusion in 13 patients. Bayesian designed limited pharmacokinetic data were collected from an additional 41 patients. The erythromycin breath test (ERMBT) was performed on 81 patients as an in vivo phenotypic assessment of CYP3A activity.

RESULTS. Fourteen-hour (peak) plasma levels of ifosfamide, 2-DCE, and 3-DCE were found to correlate strongly with the respective area under the curve (AUC) 0–24 values ($r = 0.97, 0.94, \text{ and } 0.95; P < .0001$). Patients who experienced a grade 3–4 absolute neutrophil count (ANC), platelet, or creatinine toxicity (using the National Cancer Institute Common Toxicity Criteria [version 2]) were found to have statistically significantly higher median 14-hour plasma levels of ifosfamide, 2-DCE, and 3-DCE compared with patients with grade 0–2 toxicity. ERMBT was not found to correlate with pharmacokinetic parameters of ifosfamide and metabolites or toxicity.

CONCLUSIONS. The 14-hour plasma level of ifosfamide, 2-DCE, and 3-DCE is a simple and appropriate substitute for describing the AUC of ifosfamide after 1 day of a 1-hour to 2-hour infusion of drug. Fourteen-hour plasma levels of ifosfamide and metabolites are useful predictors of neutropenia, thrombocytopenia, and creatinine toxicity. ERMBT was not found to accurately correlate with ifosfamide pharmacokinetics or clinical toxicity. *Cancer* 2007;109:2315–22. © 2007 American Cancer Society.

KEYWORDS: ifosfamide, cytochrome P450 3A, erythromycin breath test, sarcoma, pharmacokinetics.

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Ifosfamide is a cancer chemotherapeutic drug that belongs to the oxazaphosphorine class of alkylating agents. Significant clinical toxicities involve the central nervous system and the genitourinary system, in addition to myelosuppression, nausea, emesis, alopecia, and elevated liver enzymes.¹⁻³ Ifosfamide has complex pharmacokinetics (Fig. 1), which have been extensively reviewed. The cytochrome P450 3A (CYP3A) family, mainly CYP3A4 and CYP3A5, are the major enzymes involved in bioactivation of this pro-drug.^{4,5} Cytochrome 2B6 has also been implicated to a minor degree.^{6,7} This process involves 4-hydroxylation and ultimately yields phosphoramidate mustard and acrolein.^{8,9} Phosphoramidate mustard is the therapeutically active metabolite that possesses DNA alkylating activity.^{9,10} Acrolein is an inactive agent that is responsible for bladder toxicity.

A second metabolic pathway of ifosfamide involves detoxification of the drug by side-chain N-dechloroethylation (Fig. 1). CYP3A is the main enzyme responsible for the formation of therapeutically inactive metabolites 2-dechloroethylifosfamide (2-DCE) and 3-dechloroethylifosfamide (3-DCE).^{5,6} The creation of 2-DCE and 3-DCE yields equal amounts of chloroacetaldehyde, the metabolite responsible for neurotoxicity from ifosfamide.^{9,11,12} Chloroacetaldehyde is rapidly degraded,¹³ and 2-DCE and 3-DCE measurements are often preferred assessments of this pathway.

Given its complex pharmacokinetics and the potential for severe toxicities, we wished to better understand the relation between metabolite levels and ifosfamide toxicity and to explore the use of a predictive test of hepatic CYP3A activity when using ifosfamide. The erythromycin breath test (ERMBT) is performed by intravenous injection of a trace dose of radiolabeled erythromycin. CYP3A is the major enzyme catalyzing the subsequent N-demethylation of erythromycin and conversion to carbon dioxide.¹⁴ Exhaled radiolabeled carbon dioxide is then measured to quantify this reaction.¹⁵

This test has been validated as a measure of CYP3A activity. We have previously shown that the ERMBT correlates with lower CYP3A activity levels and clinical toxicity¹⁶ in patients receiving docetaxel, a drug metabolized by the CYP3A system. To our knowledge, there has been no prior report of using predictive tests to assay CYP3A activity to assess the pharmacokinetics and toxicity of ifosfamide.²

Recently, we reported on a Phase II evaluation of high-dose ifosfamide (12 g/m²/cycle) versus standard dose ifosfamide (6 g/m²/cycle) in conjunction with doxorubicin (60 mg/m²/cycle) in patients with soft tissue sarcoma.¹⁷ To better understand and predict

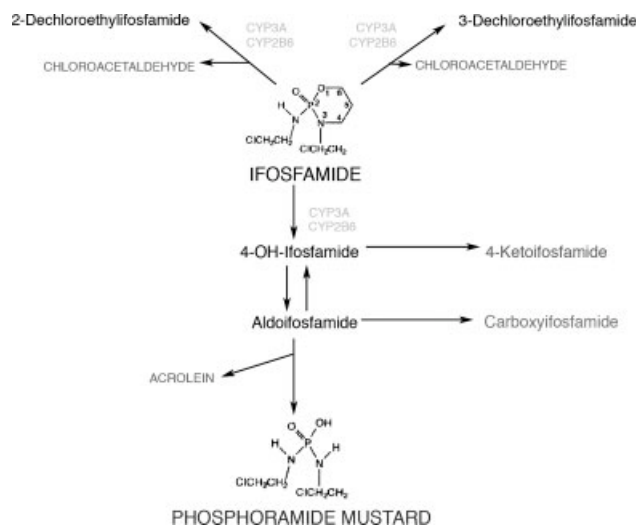


FIGURE 1. Activation and metabolism of ifosfamide.

the pharmacokinetics and toxicity of these treatments, we performed serum and urine measurements of ifosfamide and metabolites (2-DCE, 3-DCE) and ERMBT to assess CYP3A activity. Herein, we examine the correlation between dose, drug and metabolite levels, ERMBT, and toxicities.

MATERIALS AND METHODS

Trial Design

Eligible patients were enrolled in a single-institution randomized Phase II trial. Eligibility criteria included patients with documented soft tissue sarcoma, American Joint Committee on Cancer (AJCC) Stage IIIB disease (grade G3, >5 cm in greatest dimension); AJCC Stage IIB disease (grade G2, >5 cm in greatest dimension and deep to initial fascia); or patients with measurable, metastatic, high-grade (\geq G2 lesions) disease who were previously untreated with chemotherapy. Patients who had undergone primary surgical resection received adjuvant chemotherapy, whereas patients with unresected primary disease received neoadjuvant chemotherapy. Patients with metastases received palliative treatment. Participants were required to be at least 16 years old with a Zubrod performance status of \leq 2. Further eligibility requirements included an absolute neutrophil count (ANC) of \geq 1500 cells/mm³, a platelet count of \geq 100,000 cells/mm³, total bilirubin \leq 1.5 times the upper limit of normal (ULN), and a serum creatinine \leq 1.2 times the ULN. Patients were required to have 2 functioning kidneys and an estimated creatinine clearance of \geq 50 cc/min. The study was approved by the local Institutional Review Board and written informed consent was obtained from each patient.

Patients were randomized to receive either high-dose ifosfamide (3.0 g/m²/day given over 1–2 hours on Days 1–4) and mesna (1800 mg/m²/day on Days 1–4) or standard dose ifosfamide (1.5 g/m²/day given over 1–2 hours on Days 1–4) and mesna (1800 mg/m²/day on Days 1–4). All patients received doxorubicin (60 mg/m² given as a 72-hour continuous infusion starting Day 1) and filgrastim (5 mcg/kg/dose) until the ANC was >1500 cells/mm³. Details of the clinical design and primary clinical results have been previously published.¹⁷

Laboratory Monitoring

Before enrollment, patients had a history and physical examination. Pretherapy laboratory studies included a complete blood cell count with differential and platelet counts (CDP) and serum chemistries. Chemistries were monitored weekly and before each cycle. A CDP was obtained on Days 1, 8, 10, 12, 15, 17, and 19 of each cycle.

Blood Sampling for Pharmacokinetic Evaluation

In 13 patients, blood samples were collected at Hours 0, 2, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 24.0, 26.0, and 28.0 during the first ifosfamide infusion of the first cycle. Additional patients had blood samples taken at Hours 0, 14, and 26 of ifosfamide infusion. With each sample, a total of 6 mL of blood was collected, centrifuged, separated into plasma, and frozen at –20°C. Ifosfamide, 2-DCE, and 3-DCE were measured via a gas chromatograph/mass spectrometer (GC/MS)-stable isotope method. The accuracy and precision of ifosfamide quantitation is 107.5% and 4.7%, respectively, at 1.5 µmol/L; 95.4% and 1.37%, respectively, at 20.0 µmol/L; and 95.4% and 1.37%, respectively, at 100 µmol/L.

Urine was collected for 24 hours after the start of the first ifosfamide infusion in a random subset of patients treated on trial. Plasma and urine samples were spiked with an internal standard solution containing the oxazaphosphorine derivative trofosfamide and were subsequently extracted twice with dichloromethane. Simultaneous determination of ifosfamide, its dechloroethyl metabolites, and trofosfamide was performed by means of nitrogen/phosphorus (N/P) flame ionization gas chromatography/nitrogen phosphorus flame ionization (GC/NPFI) using a fused-silica capillary column (HP5 [5% PhMe Silicone]) under isothermal conditions, with N₂ serving as the carrier gas. The temperature of the oven, injector, and detector were set at 195°C, 300°C, and 300°C, respectively.¹⁸

Erythromycin Breath Test

Four µCi of [¹⁴C N-methyl] erythromycin dissolved in 2 mL of normal saline was injected intravenously

into patients before the initiation of any therapy.¹⁴ A single sample of exhaled carbon dioxide was taken 20 minutes later into vials that contained 4 mL of benzethonium hydroxide and ethanol (1:1) and a trace amount of thymophthalein. A total of 12 mL of Aquasol was added to each sample, which was then sealed in darkness for 24 hours. A scintillation counter measured the amount of radioactivity present. The percentage of administered carbon exhaled per minute was calculated assuming an endogenous carbon dioxide production of 5 mmol CO₂/m²/min.

Toxicity Measurements

National Cancer Institute (NCI) Common Toxicity Criteria (version 2.0) were used to classify adverse events.

Statistical Analysis

The area under the curve (AUC) was calculated for the time period 0 to 24 hours using the standard trapezoidal method. For ifosfamide only, Kel, the elimination rate constant, was calculated using a mixed effects model for the log₁₀ of ifosfamide concentration. Each patient was allowed to have a random intercept (3-hour plasma measurement). The Kel values were used to complete the exponential decay of ifosfamide to calculate the AUC(0 to infinity). Metabolite AUC and 14-hour levels, and ERMBT and 14-hour drug levels were compared using Spearman correlation coefficient. The occurrence of toxicity was dichotomized by grade into serious (grades 3 and 4) and none to mild/moderate (grades 0 to 2) using the NCI Common Toxicity Criteria. Comparisons between continuous pharmacokinetic parameters and dichotomous toxicity outcomes were conducted using the Wilcoxon rank-sum test statistic, which tests whether the median values between toxicity groups were similar.

The pattern of changes in hematological parameters (white blood cell count [WBC], platelets, hemoglobin) over the first 4 cycles was analyzed using a random effects model including fixed effects for dose, cycle, day within each cycle, and interactions. Measurements of serum creatinine were converted into creatinine clearance using the Cockcroft-Gault formula.

RESULTS

Ifosfamide, 2-DCE, 3-DCE Level at 14 Hours Correlates With AUC

Thirteen patients had extensive sampling of ifosfamide, 2-DCE, and 3-DCE levels after the first dose of ifosfamide, which was used to calculate an AUC(0–

TABLE 1
Correlation of Drug AUC to 14-Hour Level in 13 Patients

Metabolite	AUC	Correlation coefficient*	P
Ifosfamide	0-24	0.97	<.0001
Ifosfamide	0-infinity	0.97	<.0001
2-DCE	0-24	0.94	<.0001
3-DCE	0-24	0.95	<.0001

AUC indicates area under the curve; 2-DCE, 2-dichloroethylifosfamide; 3-DCE, 3-dichloroethylifosfamide.

* Spearman correlation coefficient.

24 h). The coefficients of elimination of ifosfamide used to calculate the AUC(0 to infinity) were 0.04398 and 0.03889 for the high- and low-dose groups, respectively.

The AUC (n = 13) values for each metabolite were found to be strongly correlated with their 14-hour (peak) levels (Table 1). Given this robust relation and the difficulty in obtaining extensive sampling of these metabolites in all patients, we collected 14-hour drug levels in 41 additional patients and performed further correlations based on these values. This provides the basis for future evaluations of ERMBT and toxicity using our larger sample size of 14-hour metabolite levels (n = 54).

ERMBT Results Do Not Correlate With Ifosfamide 14-Hour Levels

ERMBT was performed on 81 patients. Thirteen patients had ifosfamide and metabolite AUC data for comparison and 52 patients had 14-hour levels available for comparison. There was no relation observed between ifosfamide, 2-DCE, 3-DCE AUC or 14-hour levels and ERMBT (Table 2).

Urinary Ifosfamide and Metabolites Concentration Correlates With 14-Hour Plasma Levels

Twenty-five patients treated on the high-dose ifosfamide arm had 24-hour urinary metabolite evaluations. The median total amount (in micromoles) excreted for ifosfamide, 2-DCE, and 3-DCE were 2377 (range, 1075-4416), 814 (range, 288-1450), and 893 (range, 455-1703), respectively. Twenty-nine patients treated with standard dose ifosfamide had median total amounts (in micromoles) of ifosfamide, 2-DCE, and 3-DCE excreted in the urine of 3680 (range, 705-8622), 1101 (range, 301-3253), and 1500 (range, 363-2974). The 14-hour plasma measurements were positively and significantly correlated with the 24-hour urinary concentration (Table 3).

TABLE 2
Correlation Between ERMBT and Ifosfamide and Metabolites

Sample size	Metabolite	Measurement	Correlation coefficient*	P
13	Ifosfamide	AUC ₀₋₂₄	0.016	.96
13	Ifosfamide	AUC _{0-infinity}	0.016	.96
13	2-DCE	AUC ₀₋₂₄	0.14	.65
13	3-DCE	AUC ₀₋₂₄	0.17	.59
52	Ifosfamide	14-hour	-0.34	.087
52	2-DCE	14-hour	-0.13	.36
52	3-DCE	14-hour	-0.13	.35

ERMBT indicates erythromycin breath testing; AUC, area under the curve; 2-DCE, 2-dichloroethylifosfamide; 3-DCE, 3-dichloroethylifosfamide.

* Spearman correlation coefficient.

Characterization of Hematologic Toxicity in Patients Treated on High- vs Low-Dose Ifosfamide Arms

WBCs, ANC, hemoglobin, and platelet counts were determined at 7 different time points during each cycle of therapy. This information is plotted in Figure 2 and reveals the differential degree of hematologic toxicity in patients treated on high-dose or low-dose ifosfamide arms in conjunction with granulocyte-colony stimulating factor (G-CSF). Leukopenia, anemia, and thrombocytopenia were found to be more severe in the high-dose ifosfamide arm and worsened with subsequent cycles of chemotherapy.

Patients on the low-dose ifosfamide arm exhibited 2 WBC peaks each cycle. This potentially indicates an early rise in WBC due to G-CSF therapy followed by suppression due to chemotherapy and a second peak due to continued G-CSF effect. The decrement in peak observed in the high-dose arm during the fourth cycle of chemotherapy may reflect depletion of stem cells.

Reduction in hemoglobin was cumulative and worsened with each subsequent cycle in both doses. Platelet counts dropped more dramatically in the high-dose arm and worsened with each cycle.

Correlation of Hematologic Toxicity With 14-Hour Levels of Ifosfamide and Metabolites, ERMBT, and 24-Hour Urinary Concentrations

Fourteen-hour levels of ifosfamide, 2-DCE, and 3-DCE were compared with hematologic toxicity experienced during the first cycle of treatment. There was a statistically significant difference in the 14-hour levels of each metabolite and both neutropenia and thrombocytopenia (Table 4). Each 10-μL/L incremental increase in the 14-hour ifosfamide level increased the likelihood of grade 3/4 neutropenia by more than 2-fold (odds ratio of 2.1; 95% confidence interval, 1.4-3.1). No correlation was observed with

TABLE 3
Correlation Between 14-Hour Plasma Levels and 24-Hour Urine Concentration of Ifosfamide and Metabolites

14-hour metabolite plasma level	24-hour urine concentration ($\mu\text{mol/L}$)		
	Ifosfamide	2-DCE	3-DCE
Ifosfamide: correlation coefficient/sample size	0.62 ($P < .0001$)/51	0.50 ($P = .0003$)/50	0.59 ($P < .0001$)/51
2-DCE: correlation coefficient/sample size	0.60 ($P < .0001$)/51	0.64 ($P < .0001$)/50	0.70 ($P < .0001$)/51
3-DCE: correlation coefficient/sample size	0.49 ($P = .0003$)/51	0.54 ($P < .0001$)/50	0.68 ($P < .0001$)/51

2-DCE indicates 2-dichloroethylifosfamide; 3-DCE, 3-dichloroethylifosfamide.

hemoglobin toxicity, except with 3-DCE, but only 4 patients were found to experience grade 3/4 toxicity after 1 cycle of therapy.

ERMBT results were also compared with hematologic toxicity and controlled for patients treated on the high-dose and standard-dose ifosfamide arm. No correlation was noted with ERMBT results and ANC and hemoglobin toxicity. Patients with an ERMBT ≤ 2.0 were more likely to experience severe thrombocytopenia.

Urinary concentrations of metabolites were found to exhibit a statistically significant correlation with ANC toxicity (Table 5), but there was no significant association noted with either hemoglobin or platelet toxicity.

Correlation of Nonhematologic Toxicity With 14-Hour Levels of Ifosfamide and Metabolites and ERMBT

In all, 52 patients with relevant pharmacokinetic data and creatinine toxicity data were evaluated. Two patients experienced grade 1 toxicity, 2 patients experienced grade 2 toxicity, and 1 patient experienced grade 4 toxicity. Despite the small numbers of patients experiencing renal toxicity, there was a significant difference in 14-hour plasma levels in patients with grade 0/1 toxicity compared with grade 2/3/4 toxicity (Table 6). Median levels of ifosfamide, 2-DCE, and 3-DCE in patients experiencing grade 2/3/4 creatinine toxicity were more than twice that of other patients. No correlation was identified with 24-hour urinary concentrations or ERMBT results after stratifying for both high-dose and low-dose ifosfamide arms (data not shown).

Seven patients with available pharmacokinetic data were found to have any form of neurologic toxicity. No statistically significant difference was noted in the 14-hour plasma levels and ERMBT results when comparing patients with and without neurologic toxicity. However, the trend was similar to that observed with renal toxicity. Patients with grade 0 neurologic toxicity were found to have a statistically significant difference in 24-hour urinary concentra-

tion values compared with patients with grade 1–3 toxicities (498.6 vs 805.6; $P = .019$). No other correlations were observed with the urinary concentrations.

DISCUSSION

In the current study, we described ifosfamide pharmacokinetics in a trial of soft-tissue sarcoma patients treated with 6 g/m² or 12 g/m² of ifosfamide in combination with doxorubicin (60 mg/m²). We demonstrated that the 14-hour level of ifosfamide taken on Day 1 of a 3-day treatment is a useful substitute in describing the pharmacokinetics of ifosfamide, and correlates well with hematologic toxicity in the first cycle of therapy. Clinically, this observation may be useful in treating patients at high risk for complications with myelosuppression (ie, underlying low-grade infections, poor performance status). With further validation, it could potentially allow for decreasing the dose of ifosfamide on subsequent treatment days to decrease the risks associated with hematologic toxicity. Of course, such an intervention would also decrease the tumor's dose of ifosfamide, which may or may not impact patient outcomes.¹⁷

As hematologic toxicities progressively worsen in subsequent cycles (Fig. 2), the value of this observation made only during the first cycle of treatment could be questioned. In addition, ifosfamide has been reported to be subject to autoinduction and thus drug metabolism may increase over time.^{3,19,20} Evaluation of similar pharmacokinetic parameters in subsequent cycles may provide useful information.

Renal and neurologic toxicities were reported to occur relatively infrequently in this limited population, making it difficult to reach conclusions regarding the predictive value of pharmacokinetic parameters. A significant difference was observed for all metabolites and renal toxicity. A significant difference was noted only in 14-hour plasma levels of ifosfamide in patients with and without any neurologic toxicity. Because chloroacetaldehyde is believed to be the metabolite responsible for neurologic toxicity, we

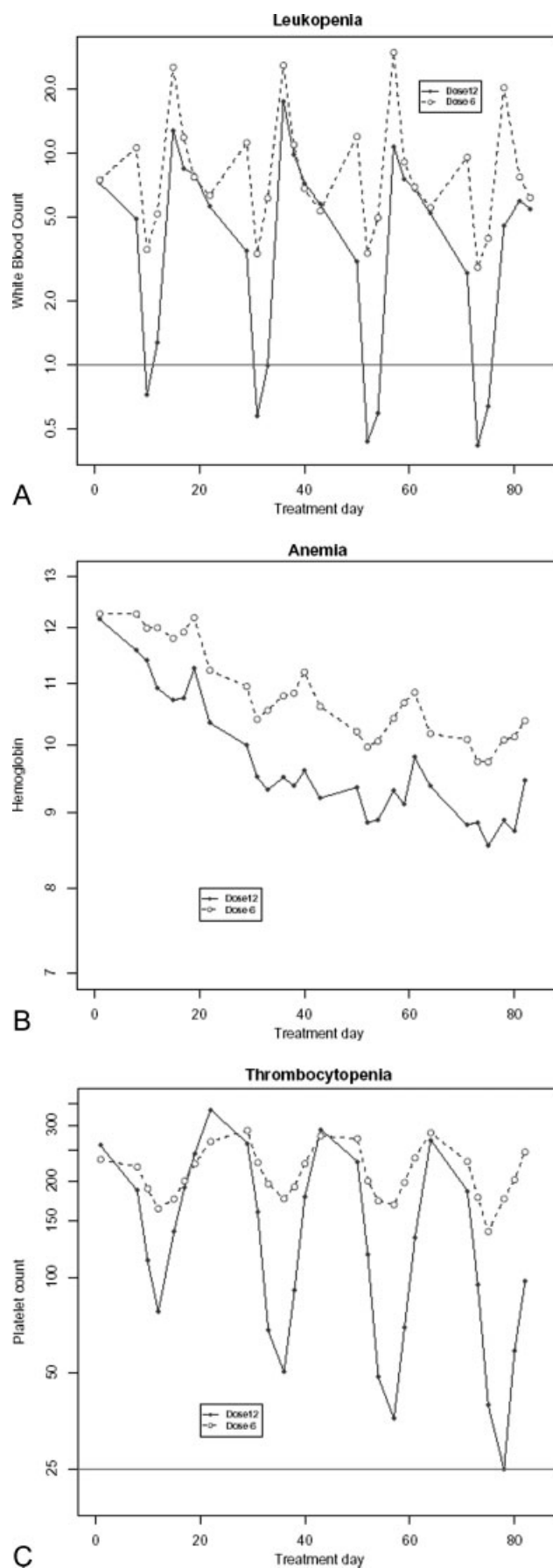


TABLE 4
Hematologic Toxicity and Corresponding Median Plasma Levels of Ifosfamide and Metabolites and ERMBT Values

Toxicity/Grade*	14-hour plasma levels (μmol/L)			No. of patients (%) with ERMBT	
	Ifosfamide	2-DCE	3-DCE	≤ 2.0	>2.0
ANC					
0-2 (n = 24)	64.6	12.0	13.9	8 (47.1)	16 (45.7)
3-4 (n = 28)	123.5	19.7	26.8	9 (52.9)	19 (54.3)
P†	<.0001	.0015	.0016	P‡	.9999
Platelets					
0-2 (n = 45)	80.8	16.0	17.9	12 (70.6)	33 (94.3)
3-4 (n = 7)	155.0	30.9	39.6	5 (29.4)	2 (5.7)
P	.0016	.014	.0086	P‡	.0309
Hemoglobin					
0-2 (n = 48)	91.5	16.1	21.7	15 (88.2)	33 (94.3)
3-4 (n = 4)	105.2	30.2	54.5	2 (11.8)	2 (5.7)
P	.43	.064	.030	P‡	.5890

ERMBT indicates erythromycin breath testing; 2-DCE, 2-dichloroethylifosfamide; 3-DCE, 3-dichloroethylifosfamide; ANC, absolute neutrophil count.

* Toxicity was graded according to the National Cancer Institute's Common Toxicity Criteria (version 2.0).

† Wilcoxon rank-sum test.

‡ Fisher exact test.

TABLE 5
Hematologic Toxicity and Corresponding Median 24-Hour Urinary Concentrations

Toxicity/Grade*	24-hour urinary concentration (μmol/L)		
	Ifosfamide	2-DCE	3-DCE
ANC			
0-2 (n = 23)	409.9	148.9	151.0
3-4 (n = 28)	627.7	227.1	283.6
P†	.0051	.0246	.0020
Platelets			
0-2 (n = 44)	498.6	174.5	214.3
3-4 (n = 7)	610.8	192.2	272.9
P	.13	.53	.23
Hemoglobin			
0-2 (n = 47)	530.3	187.7	235.4
3-4 (n = 4)	555.9	168.4	273.3
P	.65	.97	.55

2-DCE indicates 2-dichloroethylifosfamide; 3-DCE, 3-dichloroethylifosfamide; ANC, absolute neutrophil count.

* Toxicity was graded according to the National Cancer Institute's Common Toxicity Criteria (version 2.0).

† The Wilcoxon rank-sum test was used to test the equality of the median between toxicity groups.

FIGURE 2. Hematologic toxicity. This figure demonstrates the predicted average values of leukopenia, anemia, and thrombocytopenia for the first 4 treatment cycles. Results are derived from fitting a random effects model to all data.

TABLE 6
Nonhematologic Toxicity and Corresponding Plasma Levels of
Ifosfamide and Metabolites

Toxicity/Grade*	14-hour plasma levels		
	Ifosfamide	2-DCE	3-DCE
Creatinine			
0-1 (n = 49)	83.1	16.1	21.5
2-4 (n = 3)	185.7	39.2	51.7
P†	.010	.0081	.0091
Neurologic			
0 (n = 45)	83.1	16.1	21.9
1-3 (n = 7)	141.0	29.9	34.9
P	.063	.23	.28

2-DCE indicates 2-dichloroethylifosfamide; 3-DCE, 3-dichloroethylifosfamide.

* Toxicity was graded according to the National Cancer Institute's Common Toxicity Criteria (version 2.0).

† The Wilcoxon rank-sum test was used to test the equality of the median between toxicity groups.

expected to see an increase in both 2-DCE and 3-DCE metabolites. Given the low incidence of these toxicities in this trial, it is not surprising that only a trend and not a more definitive association was observed.

Although drug and metabolite urine concentrations and total amount excreted were found to be significantly correlated with pharmacokinetic parameters, the correlation was not robust. Collecting 24-hour urine samples even in a controlled setting is problematic, and the practical value of urine sampling clinically for drug toxicity prediction is likely limited.

ERMBT was not found to be of value in predicting ifosfamide pharmacokinetics or toxicity in this study of combination ifosfamide and doxorubicin therapy. Although the correlation between lower ERMBT value and more severe thrombocytopenia was considered significant, there were relatively few numbers of affected patients.

ERMBT has been shown to be a reproducible assessor of hepatic CYP3A4 activity in previous studies and has shown to predict the clearance of CYP3A4 substrates, including docetaxel and cyclosporine.^{15,21} Although CYP3A4 is identified as the main enzyme involved in ifosfamide activation and metabolism, CYP3A5 and CYP2B6 have also been shown to be involved to some extent, and are not measured by the ERMBT (Fig. 1).^{5-7,15,22}

In addition, although ERMBT has been shown to correlate with CYP3A activity, the correlation between ifosfamide and CYP3A levels is complex. CYP3A is mainly responsible for the transformation of ifosfamide to active product and the metabolism to inactive product. Additional work to separate out these 2 processes may be necessary to arrive at a

predictive pharmacokinetic test. The concomitant use of dexamethasone and other CYP3A4 inducers and inhibitors by study patients may or may not serve as additional confounding factors.^{16,22,23} The CYP3A family may also play a role in doxorubicin metabolism, which was coadministered with ifosfamide in this trial.²⁴

The ERMBT has been the subject of much pharmacologic criticism. Full discussion has been reviewed elsewhere.²⁵ To summarize, ERMBT has to date been shown to be a reasonable predictor of CYP3A activity of some, but not all, substrates. Many additional probes exist for evaluating CYP3A activity including midazolam, dextromethorphan, verapamil, and cortisol.²⁶ The midazolam clearance test is currently the subject of investigation and offers the theoretical advantage of assessing both hepatic and intestinal CYP3A4 and CYP3A5.²⁷ It is interesting to note that in some studies the ERMBT has been found to have a significantly positive correlation with midazolam clearance, whereas in others no correlation was observed.^{27,28} The midazolam clearance test has been shown to be more predictive than the ERMBT in a study examining the pharmacokinetics of the topoisomerase I inhibitor irinotecan, another antineoplastic agent with complex metabolism involving both CYP3A4 and uridine diphosphate glucuronosyltransferase 1A1.²⁹ Therefore, because the ERMBT did not appear to be useful in this setting, testing using other probes in future studies may prove to be more fruitful.

There may be additional factors that may be useful in predicting ifosfamide toxicity that were not evaluated in the current study. Ifosfamide is administered as a racemic mixture, and some studies suggest that (R)ifosfamide may have a more favorable efficacy and toxicity profile.³⁰ There also may be enantioselective differences in ifosfamide metabolism. Specifically, in vitro studies have shown that CYP2B6 may be the key enzyme in creating (S)-2-DCE and (S)-3-DCE.⁷ We did not investigate the difference in stereoisomers of ifosfamide in this clinical investigation or any in vivo probes of CYP2B6 activity.

Although the metabolism of ifosfamide has been studied extensively, it remains poorly understood. In the current study, we demonstrated that pharmacokinetic data correlate with some forms of ifosfamide toxicity and therefore have the potential to affect clinical practice. Further validation of the use of pharmacokinetic parameters in a prospective setting may prove valuable. Although ERMBT was not found to be a useful in vivo probe for our patients treated with ifosfamide and doxorubicin, further investigation of in vivo CYP3A4 activity using the midazolam

clearance test or other in vivo probes may prove to be more beneficial.

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