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ORIGINAL ARTICLE

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Pharmacokinetics of ecteinascidin 743 administered as a 24-h continuous intravenous infusion to adult patients with soft tissue sarcomas: associations with clinical characteristics, pathophysiological variables and toxicity

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Abstract *Purpose:* Ecteinascidin 743 (ET-743) is a potent cytotoxic alkaloid of marine origin that has shown promising evidence of antitumor activity during phase I clinical trials. In the study reported here, the influence of clinical characteristics and pretreatment pathophysiological variables on the pharmacokinetics of ET-743 and their associations with drug-related toxicity was examined in sarcoma patients treated in three phase II clinical trials. *Methods:* Adult patients with various histological subtypes of soft tissue sarcoma received 1.5 mg/m^2 of ET-743 by 24-h continuous i.v. infusion once every 3 weeks. Eligibility criteria were similar for each study, except for the histological subtype of the tumor or the extent of prior treatment with other anticancer agents, and all patients had normal or near-normal liver and renal function. The maximum plasma concentration (C_{max}) and area under the plasma profile from time zero to infinity (AUC) of the drug were determined during the first cycle of therapy. Patients were evaluated for toxicity every week. *Results:* Geometric mean ± SD values of the pharmacokinetic parameters in 69 patients were: C_{max} 1.14 ± 0.52 ng/ml, AUC 39.9 ± 16.6 ng·h/ml, and total body clearance (CL) 36.7 ± 16.4 l/h per m^2. The only significant correlation involving physical characteristics of the patients or pretreatment pathophysiological variables was a very weak relationship between alkaline phosphatase and AUC ($r = 0.39$, $P < 0.01$). The 15 patients with any baseline liver function test exceeding the upper limit of the normal ranges had a significantly greater ($P = 0.02$) incidence of severe toxicity (80% vs 44%). Although the mean AUC of ET-743 in patients with elevated serum levels of hepatic enzymes was 17% greater than that in patients with normal pretreatment liver function tests, the difference was not significant ($P = 0.22$). In addition, there was no distinct relationship between the grade of the most severe drug-related toxicity that occurred during the first cycle of therapy and the AUC for the entire cohort. The CL of ET-743 was found to be 27% greater in patients concurrently receiving dexamethasone as a preventative antiemetic than in those who were not, but the difference did not achieve statistical significance ($P = 0.08$). There were no significant associations between CL (liters per hour) and body surface area or any other variable related to body size. *Conclusions:* The risk of developing severe toxicity was substantially enhanced in patients with relatively moderate indications of hepatic dysfunction without a coincident effect on the CL of ET-743. Dexamethasone cotreatment appeared to decrease the incidence of severe toxicity as well as the AUC of the drug. Delivering a fixed amount of drug without

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adjustment for the height or weight of the patient may be more appropriate than dose normalization due to the absence of an association between CL and body surface area. Optimizing dosing strategies to further enhance the therapeutic index of ET-743 may depend upon obtaining a better understanding of the metabolic fate of the drug in humans.

Keywords Cancer · Chemotherapy · Clinical trials · Human · Phase II

Introduction

Ecteinascidin 743 (ET-743) is a potent cytotoxic tetrahydroisoquinoline alkaloid isolated from a marine organism [7, 19]. It covalently binds to DNA by reacting with the *N*-2 amino group of guanine in the minor groove, in a sequence-specific manner, which bends the double helix toward the major groove [15, 17]. An increasing body of evidence suggests that the ensuing molecular events leading to cell death may involve interference with transcription complexes [8, 13]. Recently, the transcription-coupled nucleotide excision repair system has been convincingly implicated in the recognition of these drug-associated lesions and consequent generation of lethal single strand breaks in DNA [26].

ET-743 exhibits broad-spectrum growth-inhibitory effects against in vivo tumor models and has shown promising evidence of activity during phase I clinical trials [1, 6, 20, 26, 29]. Activity of the compound against a variety of tumor types, including sarcoma, breast cancer, hepatoma and melanoma, is currently being evaluated in phase II studies [31]. The most commonly encountered toxicities of the agent are transaminitis, myelosuppression, nausea/vomiting and fatigue [1, 20, 29, 31]. Transaminitis is dose-related with grade 3–4 toxicity occurring in 68% of the patients treated with a dose of 1.5 mg/m^2 as a 24-h continuous i.v. infusion [25]. However, in most cases, it is fully reversible and non-cumulative with serum levels of liver enzymes returning to baseline values within 15 days after treatment [25]. Furthermore, approximately 1% of the total number of patients treated with various schedules of ET-743 during phase I trials developed multiorgan toxicity, including prolonged myelosuppression, rhabdomyolysis, and liver and renal failure, culminating in death [4, 20]. In at least one of these patients, systemic exposure to the drug was markedly greater than observed in other similarly treated patients [20].

There has been considerable interest in further characterizing the pharmacokinetic behavior of ET-743 in the more homogeneous patient populations encountered in phase II efficacy studies. Establishing associations between pharmacokinetic variables, clinical characteristics, baseline pathophysiological variables and the severity of drug-related toxicity has the potential to enhance the overall safety of the drug by identifying patients in advance who may be more susceptible to an adverse event. In this report is presented a collective analysis of the pharmacokinetic data from three concurrently accruing phase II trials of ET-743 in adult patients with soft tissue sarcomas in order to enhance the ability to establish the existence of any such relationship. Other than the histological subtype of the primary tumor or extent of prior treatment with antineoplastic agents, the eligibility criteria of each study were the same and the drug was administered at a dose of 1.5 mg/m^2 by 24-h continuous i.v. infusion once every 3 weeks. Results pertaining to the therapeutic effectiveness of the drug will be reported separately for each study.

Patients and methods

Patient selection

Pharmacokinetic studies were performed in adult patients with unresectable advanced or metastatic soft tissue sarcoma who were enrolled into three phase II trials designed to evaluate the activity of the drug against different histological subtypes of the malignancy and the number of prior therapies permitted. One trial was restricted to patients with gastrointestinal stromal tumors (GIST) without any limitation on prior chemotherapy (99-129). The second study included patients with advanced soft tissue sarcomas who had not received any prior chemotherapy other than in a neoadjuvant or adjuvant setting terminating at least 12 months before entry into this study (99-130). The third study included patients with this same tumor who progressed after treatment with at least one, but not more than two, prior chemotherapy regimens (99-143).

Physical examinations of prospective patients were performed within 2 weeks of treatment. Baseline hematology and serum biochemistry tests were determined within 7 days of initiating therapy. Patients were eligible for the studies if the following criteria were satisfied: histologically documented soft tissue sarcoma measurable by computed tomography; ECOG performance status ≤ 1; age $\geq$18 years; life expectancy $\geq$3 months; and full recovery from toxicity associated with any prior therapies. Adequate bone marrow reserve, renal function and liver function, as indicated by the following laboratory parameters, were required: absolute neutrophil count (ANC) $\geq$1500/μl; platelet count $\geq$100,000/μl; creatinine $\leq$ 1.5 mg/dl; serum total bilirubin and alkaline phosphatase not more than the upper limit of normal (ULN); serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) less than 2.5 times the ULN; albumin $>$25 mg/ml. Patients with any other serious or uncontrolled medical conditions, history of another neoplastic disease, or evidence of involvement of the tumor within the central nervous system were excluded. The study protocols were approved by the Institutional Review Boards of both participating institutions. All patients signed an informed consent document that satisfied all federal and institutional requirements as a condition of registration into the studies.

Drug administration and toxicity assessments

ET-743 was supplied by Pharma Mar (Madrid, Spain) as a sterile lyophilized powder in glass vials containing 250 μg of the drug, 0.25 mmol of sodium phosphate, and 250 mg of mannitol. Reconstitution by adding 5 ml of Sterile Water for Injection, USP, afforded a clear solution buffered to pH 4. CADD programmable ambulatory infusion pumps (SIMS Deltec, St. Paul, Minn.) were used to deliver the drug through a central venous catheter. The required volume of reconstituted drug solution (50 μg/ml) was loaded into a 100-ml medication cassette reservoir and further

diluted with Normal Saline for Injection, USP, such that the desired daily dose was delivered in a volume of 96 ml. ET-743 was administered on an outpatient basis as a 24-h continuous i.v. infusion at a dose of 1.5 mg/m^2 repeated once every 3 weeks.

All patients received a serotonin receptor antagonist prior to dosing and on an as-needed basis thereafter to control nausea and vomiting. Shortly after the studies were initiated, dexamethasone was added to the antiemetic regimen, typically as an i.v. injection of a 10-mg dose shortly before beginning the ET-743 infusion and, in most cases, discontinued after giving a second dose on the following day. Prophylactic use of hematopoietic colony-stimulating factors (i.e., G-CSF and GM-CSF) was not permitted. No other medications were specifically excluded from use. However, patients receiving known inducers or inhibitors of hepatic cytochrome P450 3A4 were carefully monitored.

Toxicities were graded according to the National Cancer Institute's Comprehensive Toxicity Criteria version 2.0 (http://www.ctep.nci.nih.gov). Physical examinations, complete blood counts, and serum biochemistry tests were performed on a weekly basis. Treatment with the second cycle of therapy could be delayed for up to 2 weeks to allow grade 3-4 elevations in serum transaminase levels to return to baseline values, in which case the dose was reduced to 1.2 mg/m^2. The dose was also reduced to 1.2 mg/m^2 in the event of grade 4 neutropenia (ANC $< 500/\mu l$) associated with fever or persisting for more than 5 days, as well as for grade 4 thrombocytopenia (nadir platelet count $< 25{,}000/\mu l$), which fully recovered to baseline values within 3 weeks after dosing.

Pharmacokinetic sample collection and analysis

The definition of the plasma concentration-time profile of ET-743 during the first cycle of therapy was based upon specimens obtained before dosing and in addition at 2, 23.5, 24.5, 25, 48, and 72–96 h relative to the start of the 24-h infusion. Blood samples (7 ml) were drawn from an arm vein into a Vacutainer tube with freeze-dried sodium heparin anticoagulant (Becton Dickinson, Franklin Lakes, N.J.). Sample tubes were mixed by inversion, placed on wet ice until centrifuged (1800 *g*, 10 min, 4°C) within 15 min, upon which the plasma was removed and stored at –70°C until assayed. The beginning and ending times of the drug infusion and sample collection intervals were monitored with a digital timer.

A validated analytical method involving preliminary isolation of the drug and an added internal standard from plasma by solid-phase extraction followed by isocratic reversed-phase high-performance liquid chromatography with electrospray ionization mass spectrometric detection was used, as previously described, to measure the concentration of ET-743 in plasma [20]. In the present study, the between-day accuracy and precision of the assay were assessed by analyzing the interpolated drug concentrations from 36 standard curves run over a 60-week period. The grand mean ± SD values of the between-day accuracy and precision for all points in the standard curve, which ranged from 50 to 1000 pg/ml, were 101.1 ± 7.2% (range 91.8–110.9%) and 11.2 ± 3.6% (range 5.3–15.2%), respectively. At the lowest drug concentration included in the standard curve, 50 pg/ml, the accuracy was 110.9% and the precision was 13.3%.

Pharmacokinetic data analysis

Actual sample times were calculated from the beginning of the drug infusion to the midpoint of each sample collection interval. Individual patient plasma concentration-time data were analyzed by noncompartmental methods using routines supplied in the WinNonlin Version 1.1 software package (Scientific Consulting, Apex, N.C.). The areas under the plasma profiles from time zero to infinity (AUC) were estimated by the logarithmic-linear trapezoidal algorithm to the last data point, with extrapolation to time infinity using the slope of the terminal log concentration versus time data ($-\lambda_z$). Total plasma clearance (CL) was calculated as the dose divided by the AUC. Mean values of the pharmacokinetic variables were calculated as the geometric mean of the individual patient values [14]. Standard deviations for the geometric mean values were estimated by the jackknife method [12].

Statistical methods

Ideal body weight (IBW) in kilograms was calculated as 50 + (2.3×height in inches over 5 feet) for males, and as 45.5 + (2.3×height in inches over 5 feet) for females, body mass index (BMI) was calculated as (weight, kg)/(height, m)^2, and body surface area (BSA) was calculated according to the following equation:

$$\mathrm{BSA}\ (\mathrm{m}^2) = \sqrt{\frac{\mathrm{height\ (cm)} \times \mathrm{weight\ (kg)}}{3{,}600}}$$

The impact of therapy on hematological and serum biochemistry parameters was quantified by calculating the maximum percent change observed during the first course of treatment:

$$\Delta\%_{\max} = \frac{|\mathit{pretreatment\ value} - \mathit{observed\ peak\ or\ nadir\ value}|}{\mathit{pretreatment\ value}} \times 100$$

Pearson correlation coefficients (r) were calculated to identify relationships between the AUC or C_{max} of ET-743 and continuous patient characteristics, pretreatment serum biochemistry tests, and $\Delta\%_{max}$ values. Comparisons of discontinuous variables between subgroups of patients were assessed by the two-tailed Student's t-test. Parametric statistical tests of pharmacokinetic variables were performed after logarithmic transformation of the data. Independent associations between two variables in each population were evaluated using the Chi-squared test or Fisher's Exact test, as appropriate. All tests were two-sided and values of $|r| \geq 0.4$ and/or $P < 0.05$ were used as the criteria for significance. All statistical analyses were performed using the SPSS/PC program (SPSS, Chicago, Ill.).

Results

Patient population

The three phase II clinical trials were concurrently opened for enrollment at the Dana Farber/Harvard Cancer Center and the Memorial Sloan Kettering Cancer Center. A total of 90 patients were treated with ET-743 administered as a 24-h continuous i.v. infusion at a dose of 1.5 mg/m^2 between August 1999 and May 2000. Pharmacokinetic data were obtained from 69 of these patients during the first cycle of therapy. The large majority (90%) of the pharmacokinetic studies were performed at the Dana Farber/Harvard Cancer Center. As shown in Table 1, the clinical and baseline pathophysiological characteristics of the patients evaluated in each trial were very similar. The overall patient population was relatively young, with a median age of 50 years (range 22–78 years), and comparably distributed between the genders. The percentages of patients with ECOG performance statuses of 0 and 1 were 57% and 43%, respectively. The median number of prior chemotherapy regimens received was one (range none to three). The predominant histological subtypes of the malignancies were leiomyosarcoma (25%), liposarcoma (20%) and GIST (28%). All of the

Table 1. Clinical and pathophysiological characteristics of the patients. All values represent the number of patients unless specified otherwise (*99–129* GIST and not more than two prior chemotherapy regimens, *99–130* first-line therapy for soft tissue sarcoma, *99–143* advanced chemorefractory soft tissue sarcoma, *GIST* gastrointestinal stromal tumor, *PNST* peripheral nerve sheath tumor. *ULN* upper limit of normal)

Characteristic	Phase II trial			Combined population
	99–129	99–130	99–143	
No. of patients	19	22	28	69
Age (years)				
Median	44	53	50	50
Range	22–78	30–77	22–69	22–78
Sex				
Male	13	10	10	33 (48%)
Female	6	12	18	36 (52%)
ECOG performance status				
0	11	11	17	39 (57%)
1	8	11	11	30 (43%)
Primary tumor type				
GIST	19	0	0	19 (28%)
Leiomyosarcoma	0	8	9	17 (25%)
Liposarcoma	0	6	8	14 (20%)
PNST	0	3	1	4 (6%)
Synovial sarcoma	0	1	6	7 (10%)
Other	0	4	4	8 (12%)
No. of prior chemotherapy regimens				
0	9	22	0	31 (45%)
1	4	0	12	16 (23%)
2	4	0	16	20 (29%)
3	2	0		2 (3%)
Liver function tests				
≤ ULN	14	18	22	54 (78%)
1–1.5×ULN	3	2	4	9 (13%)
>1.5×ULN	2	2	2	6 (9%)
Dexamethasone pretreatment				
+	13	18	25	56 (81%)
–	6	4	3	13 (19%)

patients had normal or near-normal liver and renal function (Table 1).

Pharmacokinetic parameter estimates

The plasma pharmacokinetics of ET-743 were characterized during the first cycle of therapy using an empirically based limited sampling schedule that included six samples collected at times ranging from 2 h after starting the infusion to 48–72 h after the end of the 24-h infusion. Thus, the time that the patients remained in the outpatient clinic for pharmacokinetic sampling did not exceed 2 h after starting or ending the drug infusion and they were required to return to provide single specimens on only two subsequent days. Considerations that entered into selecting the sample collection times included the following: (a) delivering the infusion on an outpatient basis; (b) maximizing the number of days on which treatment could be initiated; (c) minimizing inconvenience to the patients; and (d) estimating the AUC with acceptable accuracy by the trapezoidal method. The ability of the limited sampling schedule to estimate the AUC was assessed by analyzing data sets generated at these six time points from simulations of a 1.5-mg/m^2 dose of ET-743 given as a 24-h continuous i.v. infusion using values of the pharmacokinetic parameters that were previously determined by nonlinear regression analysis in a group of 12 patients during a phase I trial with intensive sampling [20]. The AUC values obtained from numerical integration of these six concentration-time points by the log-trapezoidal method were then compared to the theoretical AUC values calculated from the coefficients and rate constants for the equations of the simulated plasma profiles.

Figure 1A shows a simulated plasma profile for a 1.5-mg/m^2 dose of the drug given as a 24-h infusion together with the six time points comprising the sampling schedule used in this study. When the last plasma sample was obtained 48 h after the end of the infusion, as was the case for 67 of the 69 patients in this study, the mean error between the true and estimated AUC values was less than 2%, as illustrated in Fig. 1B. However, since only two samples were obtained after the terminal disposition phase was achieved, pharmacokinetic parameters that depend upon accurate definition of the terminal phase, which include the biological half-life, apparent volume of distribution at steady-state, and mean residence time, were not reliably estimated. It should be noted that simulated data were used for this analysis, which yielded a measure of the intrinsic ability of the limited sampling schedule to estimate the true AUC, but did not provide an indication of the accuracy

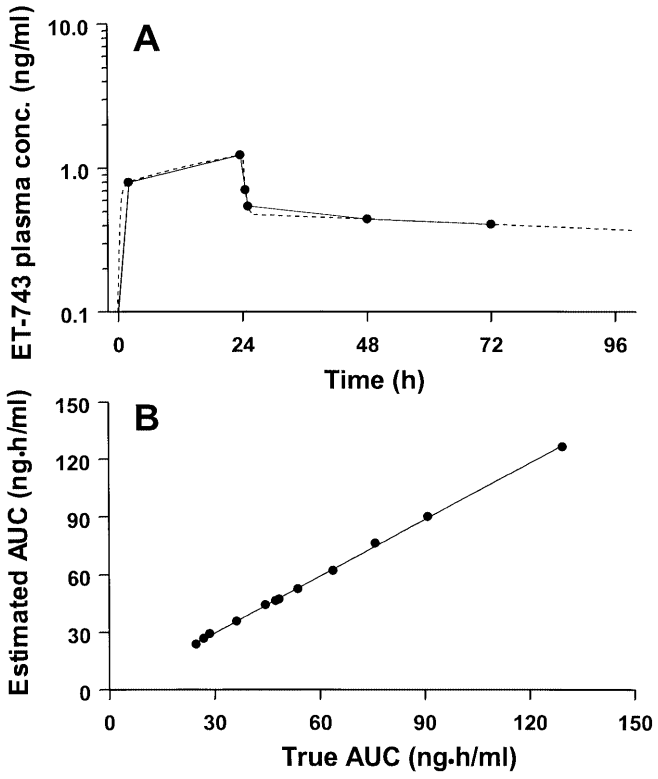

Fig. 1. **A** Simulated plasma concentration-time profile for a 1.5-mg/m^2 dose of ET-743 given as a 24-h continuous i.v. infusion (*dashed line*) showing the six time points comprising the limited sampling schedule used in this study, which are also connected by *line segments*. **B** Scatter plot depicting the relationship between the estimated AUC determined by application of the log-linear trapezoidal method to the ET-743 concentrations at the six time points in the limited sampling schedule and the true AUC of the simulated plasma profile for a set of 12 patients. The *solid line* was generated from linear regression analysis of the data

and precision of the method as applied to experimental data.

The mean ± SD peak plasma concentration (C_{max}) of ET-743 achieved at the end of the infusion was 1.14 ± 0.51 ng/ml (range 0.45–4.00 ng/ml) for the entire cohort of 69 patients. The grand mean ± SD AUC was 39.9 ± 16.6 ng·h/ml (range 16.4–114.3 ng·h/ml) and the CL was 36.7 ± 16.4 l/h per m^2. Interpatient variability as indicated by the percent coefficient of variation (CV) was 45.1% for C_{max} and 41.5% for AUC. There was a strong linear relationship ($r = 0.812$) between the AUC and C_{max} in individual patients.

Associations between baseline patient characteristics and AUC

There were no significant differences in the mean AUC of patients categorized according to gender, age or performance status (Table 2). In addition, regression analysis did not reveal any evidence of an association between age and AUC ($r = 0.15$). Subgroup analysis of patients categorized according to the histological subtype of their primary tumor revealed that the 19 patients with GIST had a mean AUC (34.2 ± 12.5 ng·h/ml) that was significantly lower ($P = 0.04$) than all other patients grouped together (42.3 ± 17.8 ng·h/ml, $n = 50$). Furthermore, the CV of the AUC was considerably lower in GIST patients (34.2% vs 42.1%). An additional finding of this analysis was that the eight patients with a primary tumor located in the retroperitoneum had a significantly greater ($P = 0.04$) mean AUC (54.8 ± 22.0 ng·h/ml) than patients with a primary tumor occurring in all other sites (38.3 ± 15.4 ng·h/ml, $n = 61$). The only significant relationship between the AUC and baseline serum biochemistry tests, considered both as the observed value and the value expressed relative to the upper limit of the normal range for liver function tests, was a weak association with alkaline phosphatase ($r = 0.39$, $P < 0.01$). Although the mean AUC of ET-743 in the group of patients with slightly elevated serum levels of liver enzymes before treatment was 17% greater than in those with normal liver function tests, the difference was not significant ($P = 0.22$; Table 2).

Toxicity

Severe toxicities experienced by the patients during the first cycle of therapy with ET-743 are summarized in Table 3. Among the 69 patients in whom the pharmacokinetics of ET-743 were characterized, the most commonly encountered severe toxicity was grade 3 or 4 elevations in serum transaminase levels in 23 patients (33%). However, in all but five patients, this was readily reversible with transaminase levels returning to baseline by day 21. Clinically significant changes were not observed in any other serum biochemical liver function test including total bilirubin and alkaline phosphatase. The only other severe nonhematological toxicities were nausea and vomiting in four patients (6%), two of whom did not receive dexamethasone pretreatment, fatigue in three patients (4%) and dyspnea in one patient (1%). The most common severe hematological toxicities were neutropenia in 15 patients (22%) and leukopenia in 14 patients (20%). In addition, four patients (6%) developed anemia and thrombocytopenia occurred in only one patient (1%).

In the 15 patients with any baseline liver function test above the ULN range, the incidence of severe toxicity was 80%, as compared to 44% in patients with normal hepatic function prior to initiation of therapy, which proved to be statistically significant ($P = 0.02$). Among these 15 patients, three developed grade 3-4 transaminitis, severe hematological toxicity occurred in four, and five experienced both severe hepatotoxicity and severe myelosuppression. In addition, the 25 patients with liver metastases had a substantially, but not significantly ($P = 0.12$), increased incidence of severe toxicity (64% vs 44%). No other clinical or pathophysiological variables among those listed in Table 1 was significantly associated with the occurrence of toxicity of grade 3 or more.

Table 2. Statistical comparisons of mean pharmacokinetic variables between subpopulations stratified according to baseline clinical, pathological, or treatment-related factors. Pharmacokinetic parameter values are geometric means ± SD

Variable	No. of patients	AUC ng·h/ml	*P* value[a]	C_{max} ng/ml	*P* value[a]
Age (years)					
< 50	34	36.7 ± 13.5	0.099	1.10 ± 0.41	0.343
≥50	35	43.3 ± 19.3		1.22 ± 0.61	
Gender					
Male	33	41.3 ± 16.3	0.503	1.18 ± 0.57	0.732
Female	36	38.6 ± 16.8		1.14 ± 0.47	
ECOG performance status					
0	39	40.5 ± 14.7	0.757	1.21 ± 0.50	0.324
1	30	39.2 ± 18.7		1.09 ± 0.52	
Serum liver enzyme levels					
≤ ULN	54	38.5 ± 16.6	0.219	1.13 ± 0.46	0.303
> ULN	15	45.2 ± 27.8		1.26 ± 0.70	
Hepatic metastases[b]					
+	25	39.8 ± 15.6	0.485	1.13 ± 0.38	0.710
–	43	40.7 ± 17.0		1.19 ± 0.58	
Clinical trial					
99-129	19	34.2 ± 12.5		1.10 ± 0.44	
99-130	22	45.5 ± 20.7	0.086	1.34 ± 0.61	0.174
99-143	28	40.0 ± 15.6		1.07 ± 0.47	
Dexamethasone cotreatment					
+	56	38.1 ± 15.4	0.077	1.12 ± 0.47	0.266
–	13	48.5 ± 20.3		1.34 ± 0.69	

[a]Two tailed *t* test or ANOVA, as appropriate, of the log transformed data
[b]Evidence of hepatic disease involvement could not be evaluated in one patient ($n = 68$)

Table 3. Severe toxicity during the first cycle of therapy

	No. of patients	Any severe toxicity[a]	Hematological	Nonhematological	
				Hepatic	Other
All patients	69	36 (52%)	21 (30%)	23 (33%)	7 (10%)
No. of prior chemotherapy regimens					
0	31	20 (65%)	11 (35%)	12 (39%)	4 (13%)
1	16	5 (31%)	2 (13%)	5 (31%)	2 (13%)
2–3	22	11 (50%)	8 (36%)	6 (27%)	1 (5%)
Serum liver enzyme levels					
≤ ULN	54	24 (44%)	12 (22%)	15 (28%)	3 (6%)
> ULN	15	12 (80%)	9 (60%)	8 (53%)	4 (27%)
Hepatic metastases					
+	25	16 (64%)	8 (32%)	12 (48%)	4 (16%)
–	43	19 (44%)	12 (28%)	11 (26%)	3 (7%)
Dexamethasone cotreatment					
+	56	27 (48%)	17 (30%)	16 (29%)	5 (9%)
–	13	9 (69%)	4 (31%)	7 (54%)	2 (15%)

[a]Severe toxicity is defined as a drug-related adverse event of grade 3 or more according to the NCI Common Toxicity Criteria

Relationships between toxicity and AUC

As illustrated in Fig. 2, there was no marked association between the most severe drug-related toxicity that occurred during the first cycle of therapy and the AUC or C_{max} (data not shown) of ET-743. The frequency of grade 3-4 toxicity was 68% in patients with an AUC greater than 50 ng·h/ml and 46% among those with lower AUC values; however, this difference was not statistically significant (Chi-squared test, $P = 0.096$).

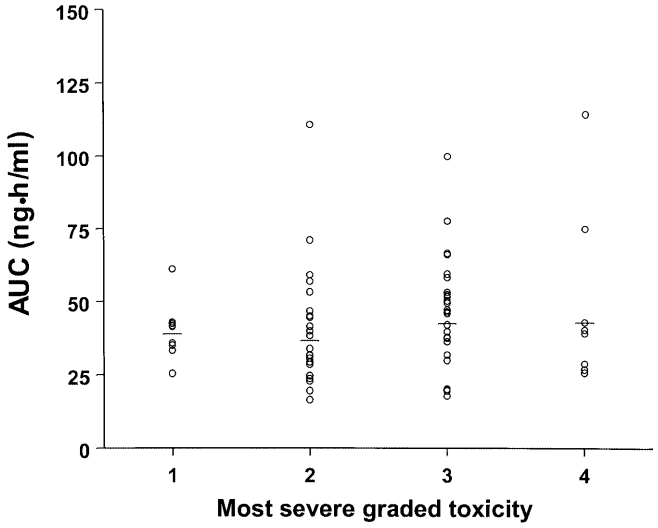

Fig. 2. Plot demonstrating the relationship between the AUC of ET-743 and the most severe grade of any toxicity observed during the first cycle of therapy. The points (*open circles*) are the observed AUC values in individual patients and the *horizontal bars* are the geometric mean values for each grade of toxicity

Furthermore, the incidence of severe toxicity in patients younger than 50 years (53%) was comparable to that in older patients (51%, $P = 0.90$). There were no significant correlations between the $\Delta\%_{max}$ in any hematological or serum chemistry parameter that was monitored (i.e., hemoglobin, WBC, ANC, platelets, creatinine, total bilirubin, albumin, SGOT, SGPT, creatine phosphokinase, alkaline phosphatase) and the AUC or C_{max} of ET-743.

Effect of dexamethasone on ET-743 pharmacokinetics and toxicity

Only 13 of the 69 patients (19%) were not concurrently treated with dexamethasone to control nausea and vomiting before and during the 24-h infusion of ET-743 in the first cycle of therapy. The mean ± SD AUC of ET-743 was 38.1 ± 15.4 ng·h/ml for the group of patients who received dexamethasone and 48.5 ± 20.3 ng·h/ml for those who did not (Table 2). Although the difference between these mean AUC values was 27%, it did not achieve statistical significance ($P = 0.08$). The use of dexamethasone reduced the frequency of grade 3 or 4 nausea and vomiting from 15% to 4% ($P = 0.16$; Fisher's Exact test). Similarly, the incidence of any severe toxicity (i.e., grade 3 or more) was 48% in patients receiving dexamethasone and 69% in those who did not ($P = 0.22$; Fisher's Exact test; Table 3). Thus, while cotreatment with dexamethasone appeared to diminish the incidence of severe toxicity experienced by patients, failure of the effect to achieve statistical significance was probably due to the relatively low number of patients who did not receive dexamethasone in these studies.

Table 4. Correlations between clinical variables of the patients and unnormalized total body clearance

Independent variable	Pearson's correlation coefficient	*P* value
Age	0.15	0.22
Gender	–	0.50[a]
Dose (mg)	0.08	0.49
Height	0.19	0.12
Total body weight	0.03	0.82
Body surface area	0.08	0.49
Ideal body weight	0.17	0.16
Body mass index	0.07	0.55

[a]Two-tailed *t*-test of means using log-transformed data

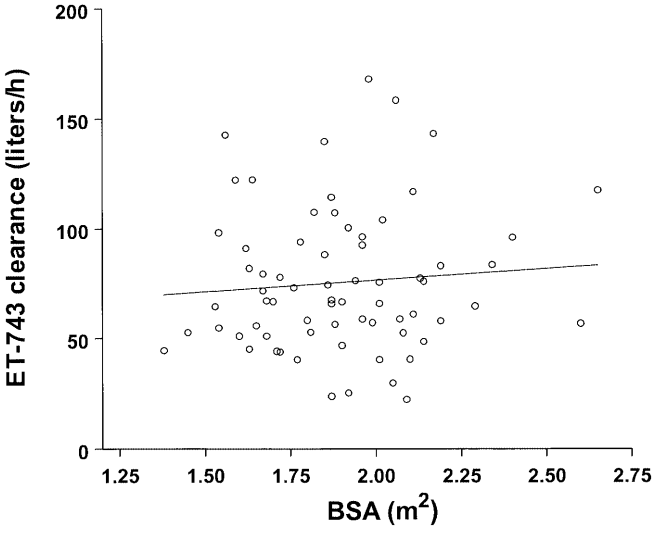

Fig. 3. Scatter plot depicting the relationship between the clearance of ET-743 and body surface area (BSA). Points (*open circles*) represent the observed values in individual patients and the *solid line* was generated from linear regression analysis of the individual patient values. The correlation coefficient of the best-fit line was 0.084 and the slope, 10.7, was not significantly different from zero ($P = 0.49$)

Influence of clinical variables on ET-743 clearance

Relationships between CL and variables related to body size were examined to assess the appropriateness of normalizing the dose of ET-743 to BSA or other measures of body size (Table 4). The patients had a wide range of body weight, from 44 to 147 kg (median 80 kg), with BSA values spanning an approximate twofold range from 1.4 to 2.7 m^2 (median 1.9 m^2). The amount of drug corresponding to a BSA-normalized dose of 1.5 mg/m^2 ranged from 2.07 to 3.98 mg (median 2.82 mg). The mean ± SD CL of ET-743 was 68.9 ± 30.1 l/h when calculated without normalizing to BSA. Interpatient variability in the mean CL for the entire cohort of 69 patients was remarkably similar whether normalized to BSA (45%) or not (44%). As shown in Fig. 3, the relationship between BSA and unnormalized CL was very weak ($r = 0.084$) and there were

no significant correlations between any other parameter related to body size and CL (Table 3).

Discussion

Phase I trials of ET-743 have been performed in adult patients with solid malignancies to evaluate its administration as a single i.v. infusion for periods of 1, 3, 24 and 72 h, as a 1-h infusion on five consecutive days, and a 3-h infusion given once a week for 3 weeks [3, 20, 25, 27, 28, 29, 30]. In consideration of the dose intensity, tolerability, and duration of systemic exposure to potentially effective drug concentrations, the dosing regimen selected for initial single-agent phase II trials was 1.5 mg/m^2 administered as a 24-h continuous i.v. infusion once every 3 weeks. The C_{max} of ET-743 provided by this dosing regimen, 1 to 4 n*M*, is well above the 50% inhibitory concentration against human tumor cell lines that are responsive to the cytotoxic effects of the drug [25, 29]. The pharmacokinetics of ET-743 are linear and independent of the duration of infusion at doses not exceeding those recommended for phase II studies [20, 29]. The biological half-life of the drug is long, being in the range 69–106 h, and it also has a relatively large steady-state apparent volume of distribution (2000–6000 l/m^2), suggestive of extensive tissue binding.

The principal routes by which ET-743 is eliminated from the body have not been quantitatively established in humans or laboratory animals. Phase I trials revealed that less than 2% of the dose is excreted unchanged in the urine [20, 24]. In vitro studies suggest that the drug is extensively metabolized, principally by enzymes in the cytochrome P450 3A superfamily, with CYP3A4 being the predominant isoenzyme mediating the biotransformation of ET-743 in a panel of human liver slices [10, 18]. In addition, the compound has a phenolic acetyl group that is susceptible to hydrolysis in plasma and it has been shown to be a substrate for purified UDP-glucuronyl transferase [24]. Nevertheless, metabolites have not been detected in urine, bile or plasma specimens obtained from patients during treatment with the drug.

The predominant dose-limiting toxicities of ET-743 when given as a 24-h i.v. infusion once every 3 weeks were neutropenia and thrombocytopenia [25, 29]. In addition, grade 3 or 4 elevations in serum transaminase levels occurred in 68% of the 25 patients treated with a 1.5-mg/m^2 dose during the phase I trial of this schedule. These events were not considered to be dose-limiting because hepatotoxicity proved to be both reversible and noncumulative. Typically, maximum serum transaminase elevations occurred during the first week after dosing and returned to pretreatment levels near the end of the second week. Delaying treatment for longer than 7 days to permit complete recovery of liver function tests was required for only 3 of the 73 cycles of therapy administered to 25 patients [25]. The severities of the hematological and nonhematological toxicities were found to be correlated with the AUC and C_{max} of ET-743 [25, 29]. The AUC in patients experiencing a dose-limiting toxicity was 94% greater on average than in all other patients [25]. In addition, the frequency of dose-limiting toxicity was 3.8% in patients with an AUC less than 40 ng·h/ml, 14.3% for AUC values ranging from 40 to 70 ng·h/ml, and 40.0% when the AUC exceeded 70 ng·h/ml. Patients with grade 3 or 4 elevations in serum transaminase levels had a significantly higher ($P < 0.005$) mean AUC than those with grade 2 or lower changes [29].

Multivariate regression analysis has revealed that pretreatment serum alkaline phosphatase activity is the only statistically significant pathophysiological factor associated with dose-limiting toxicities [25]. The severity of transaminitis also tends to be greater in patients with higher baseline alkaline phosphatase levels. Accordingly, the eligibility criteria for liver function tests were made considerably more stringent for the phase II trials of ET-743, as compared to the phase I studies, which permitted serum transaminase and alkaline phosphatase levels up to three times the ULN in the absence of hepatic disease involvement and up to five times the ULN in patients with liver metastases [29].

In this study, the pharmacokinetic behavior of ET-743 was characterized in a much larger, more homogeneous population of patients than previously encountered during the phase I clinical evaluation of the drug. Identifying patient-related factors that were potentially predictive for susceptibility to a severe adverse effect, which could significantly enhance the overall safety of the drug, was the primary motivation for undertaking this investigation. The AUC and C_{max} of ET-743 were determined in a group of 69 adult patients with several histological subtypes of soft tissue sarcoma during the course of three phase II clinical trials. The characteristics of the patients in each of these clinical trials were very similar: they were relatively young, had a good or better performance status, and a large majority showed normal liver function.

The most prevalent severe toxicities (i.e., grade 3 or more) encountered during the first cycle of therapy in this population were neutropenia and transaminitis, which occurred in 22% and 33% of the patients, respectively. The only other severe drug-related toxicities were thrombocytopenia in a single patient, anemia, and nausea/vomiting, with an incidence of 6% for both. The number of patients in whom administration of the second cycle of therapy was either delayed or the dose reduced due to drug-related toxicity was 24. The overall frequency of severe toxicity in this group of patients was 52% following the first infusion of the drug. Similar findings have been described in a preliminary report of phase II trials undertaken in Europe to evaluate the activity of ET-743 against sarcomas using this same schedule [31].

Patients with even minor elevations in any pretreatment liver function test experienced a significantly increased incidence of grade 3-4 toxicity. In contrast to patients with normal hepatic function, 44% of whom

showed severe toxicity, the frequency of grade 3 or 4 toxicity was almost twofold greater in patients with pretreatment serum levels of liver enzymes that were 1- to 2.5-times the ULN ranges. These findings are comparable to the experience encountered with the 1.5-mg/m^2 dose in the phase I trial of the 24-h infusion schedule, where none of ten patients who developed dose-limiting toxicity had baseline serum enzyme elevations suggestive of some degree of hepatic dysfunction [25]. However, the grand mean values of the AUC and C_{max} of ET-743 were 27% and 39%, respectively, lower than reported for the group evaluated at this dose level during the phase I trial [29]. Furthermore, there did not appear to be a significant difference in the CL of ET-743 between patients with normal and those with moderate elevations in liver function tests.

Thus, while a 20% lower dose of ET-743 would be better tolerated by patients with indications of relatively mild hepatic dysfunction, as demonstrated in the phase I trial of this administration schedule, systemic exposure to the drug would be commensurately diminished. Thus, the potential consequences of lower plasma levels of the drug on its therapeutic effectiveness should be thoroughly evaluated before advocating any specific guidelines for modifying the dose. In the absence of such information, it may not be advisable to treat patients with any evidence of compromised hepatic function with ET-743.

This study failed to identify any significant relationships between the most severe grade of toxicity experienced by patients and either the AUC or C_{max} of ET-743. Furthermore, the 24 patients for whom the second cycle of therapy was delayed to permit full recovery from drug-related toxicity, or otherwise reduced, and those who received the full dose as scheduled had comparable mean AUC values. Although associations between AUC and toxicity have been demonstrated for many cytotoxic chemotherapeutic agents, including ET-743 during phase I studies [20, 25, 29], it remains possible that the toxic manifestations of ET-743 are not directly mediated by the parent drug, but rather a metabolite, the pharmacological effects of which are not closely associated with the AUC of ET-743. Alternately, toxicity may be more closely associated with another pharmacokinetic parameter, such as the biological half-life or time above a threshold concentration, that was not determined during this study.

In concordance with findings of the phase I trials of ET-743 [25, 29], the AUC and C_{max} were independent of the age, gender, performance status, and presence of hepatic metastases in sarcoma patients. The only significant correlation involving a pretreatment serum biochemistry test was a very weak relationship between alkaline phosphatase and AUC ($r = 0.39$, $P < 0.05$). However, a rather interesting difference was found upon examining the mean AUC values of ET-743 in patients categorized according to the histological subtype of their disease. In comparison to all other patients, the 19 patients with GIST had a 19% lower AUC, that was statistically significant ($P = 0.04$), with markedly less interpatient variability. This difference did not appear to be related to the use of dexamethasone for antiemetic prophylaxis, since the mean AUC in those patients pretreated with dexamethasone (33.0 ± 12.7 ng·h/ml, $n = 13$) and those who were not (36.9 ± 13.2 ng·h/ml, $n = 6$) were similar ($P = 0.53$).

The greater CL of ET-743 in GIST patients is consistent with clinical evidence that they appeared to tolerate the drug better than patients with other histological subtypes of soft tissue sarcoma who were similarly treated. Whereas there were no grade 4 toxicities in the cohort of 19 GIST patients, grade 4 toxicity occurred in 8 of the 50 patients (16%) in the other two phase II trials during the first cycle of therapy, 7 of which were hematological and the other hepatic. Significant differences between these groups were not evident when classifying grade 3 and 4 adverse events together as severe toxicity. In another study involving similarly treated patients with soft tissue sarcomas, the frequency of grade 4 neutropenia was 10–18% and grade 4 thrombocytopenia occurred in 1.7–3.8% of the total number of administered cycles of therapy [1]. Factors that could potentially account for the enhanced CL of ET-743 in GIST patients are not obvious.

Nausea and vomiting of widely varying severity was a frequently encountered side effect of ET-743. Pretreatment with a serotonin receptor antagonist alone did not provide many patients with adequate relief from these complications. The use of dexamethasone as an antiemetic was precluded during phase I trials due to concerns about inducing hepatic metabolism of the drug. However, in consideration of the unexpected severity of nausea and vomiting experienced by the initial patients entered into the phase II studies, dexamethasone was soon incorporated as a regular component of the antiemetic regimen. Dexamethasone can significantly induce the activity of hepatic CYP3A isoenzymes by activating genes which encode their transcription. The magnitude of CYP3A induction is dependent upon the dose, frequency of administration, and duration of treatment with dexamethasone, and the effect gradually reverses upon its discontinuation [16, 22, 23].

The use of dexamethasone as preventative antiemetic therapy in 56 of the 69 patients involved in this study provided an opportunity to perform a preliminary assessment of the effects that it had on the pharmacokinetics of ET-743 and treatment-related toxicities. Information in the literature [9, 11] indicates that administering a 10-mg dose of dexamethasone for only 2 days coincident with the chemotherapy infusion should not markedly alter the elimination of ET-743. However, the CL of ET-743 was found to be 27% greater in patients receiving dexamethasone than in those who did not. Although the difference did not achieve statistical significance ($P = 0.08$), this effect could very well be clinically relevant, particularly if dexamethasone is given at higher doses or over a prolonged period of time.

With regard to the clinical benefit derived from dexamethasone, the incidence of grade 3 nausea and vomiting during the first cycle of therapy was reduced from 15% in patients receiving a serotonin receptor antagonist alone to only 4% in patients receiving the combination with dexamethasone. The overall frequency of any graded symptoms of nausea and vomiting was 69% in patients who did not receive dexamethasone in comparison to 48% in those who did. Furthermore, the percentage of patients with any grade 3 or 4 toxicity was 21% lower in the cohort that received dexamethasone and the incidence of severe transaminitis was 25% lower (54% vs 29%). The latter finding is consistent with a preliminary report of nonclinical studies that the concurrent administration of dexamethasone results in a dramatic decrease in the hepatotoxicity of ET-743 together with an apparent enhancement of its efficacy in tumor-bearing rats [2]. Despite the magnitude of the relative differences, none of these effects proved to be statistically significant, which is most likely attributable to the relatively small number of patients who did not receive dexamethasone in this study. A randomized prospective trial involving two groups of 67 patients would be needed to examine the effects of dexamethasone pretreatment on the pharmacokinetics and pharmacodynamics of ET-743, based upon the ability to detect a 25% difference between the groups, assuming a variance equivalent to that of the grand mean CL of ET-743 observed in this study and a 5% significance level. Unfortunately, undertaking such a study to more definitively address these questions is probably not feasible at this point in the clinical development of the drug.

Individualizing doses of anticancer drugs by normalization to BSA has become a generally accepted clinical practice and is based upon the presumed existence of a relationship between CL and BSA [5, 21]. In this study, the CL of ET-743 was found to be independent of BSA or any other physiological characteristic related to body size. Thus, ET-743 represents another example of the expanding number of chemotherapeutic agents that are predominantly eliminated by the liver for which CL is independent of BSA [21]. Normalizing the dose of such an agent to BSA could actually contribute to a greater degree of variability in systemic exposure to the drug between patients than fixed dosing. Substantiating this by prospectively determining the magnitude of interpatient variability in the AUC of ET-743 in a group of patients treated with a fixed amount of the drug would be an informative and worthwhile endeavor.

In summary, with the exception of baseline hepatic function, there were no distinguishing associations between any pharmacokinetic, clinical, or pathophysiological factors and the incidence of severe drug-related toxicity in a group of 69 adult sarcoma patients, with predominantly normal hepatic function, who were treated with a 1.5-mg/m^2 dose of ET-743 given as a 24-h continuous i.v. infusion. However, relatively minor abnormalities in serum levels of hepatic enzymes seem to markedly predispose patients to severe toxicity without a coincident effect on the CL of ET-743. Under these circumstances, the implications of dose modifications to improving the tolerability of the drug or its potential therapeutic effectiveness requires evaluation. Apparent reductions in the AUC and C_{max} of ET-743, as well as the incidence of severe nausea and vomiting, in patients concurrently receiving dexamethasone as an antiemetic did not achieve statistical significance. Due to the absence of an association between CL and BSA, administering a fixed amount of drug without adjustment for the height or weight of a patient may be more appropriate than BSA dose-normalization. Further optimization of dosing strategies directed by a better understanding of the metabolic fate of the drug in humans could potentially enhance the therapeutic index of this promising anticancer agent.

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