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Clinical and pharmacokinetic overview of parenteral etoposide phosphate

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Abstract. Etoposide phosphate (Etopophos, BMY-40481) is a water-soluble derivative of the widely used podophyllotoxin etoposide (VP-16). The phosphate ester renders the compound water-soluble, eliminating the need for formulation in polysorbate (Tween) 80, ethanol, and polyethylene glycol. As a result the compound can be given at high concentrations and as a bolus. In animals and in vitro, etoposide phosphate (EP) is rapidly and completely converted to VP-16. Clinical development of the i.v. formulation has focused on the identification of the maximum tolerated dose (MTD) and pharmacokinetic characteristics of the drug using a 5 daily dose schedule and a days 1, 3, and 5 schedule, with the drug being given over 30 or 5 (bolus) min. Myelosuppression was dose-limiting. Data from these trials show the rapid and complete conversion of EP to VP-16, a pharmacokinetic/pharmacodynamic relationship for myelosuppression and exposure to VP-16, and an MTD of 100 and 150 mg/m^2 (molar equivalent to VP-16) when EP is given daily for 5 days and on days 1, 3, and 5, respectively. A formal randomized trial has been conducted to show the pharmacokinetic comparability of EP and VP-16. In this trial, exposure to VP-16 was the same after the parenteral administration of equimolar doses of EP or VP-16. The feasibility of bolus dosing and treatment at high concentrations has been demonstrated, with no effects on the cardiovascular system being noted. Parenteral EP is pharmacokinetically and biologically equivalent to VP-16 and has the advantages of the elimination of potentially toxic excipients; more convenient administration; and ability to be given as a bolus, at high concentrations, and as a continuous infusion.

Key words: Etoposide phosphate – Dose-limiting toxicity – Maximum tolerated dose

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Introduction

The podophyllotoxins are a group of compounds extracted from roots and rhizomes of the plants *Podophyllum peltatum* (May apple or American mandrake) and *Podophyllum emodi*. Extracts (podophyllin) from these plants were used as medicinals (cathartic and anthelminthic) hundreds of years ago by native Americans and natives of the Himalayan regions [4]. The use of podophyllin was taken up by early European settlers in the New World, and it was listed in the first United States pharmacopoeia (USP, 1820) and was deleted in 1942 because of toxicity. Topical use for the treatment of cancer dates to 1862 [1]. By the 1880s, partial purification had been achieved with the isolation of podophyllotoxin [8]. The use of podophyllin to treat warts was described in 1942 [5], and in 1947 its ability to inhibit mitosis was recognized [10].

On the basis of its activity as a "mitotic poison", podophyllotoxin was tested in animal models of cancer and found to be active. Unfortunately, clinical trials in humans were abandoned due to toxicity despite the objective tumor shrinkage obtained in 7 of 18 cases of carcinoma and 20 of 28 leukemia/lymphoma patients, including a complete response observed in a patient with giant follicular lymphoblastoma [2]. An extensive program of chemical modification, led by H. Stähelin at Sandoz Laboratories, was then undertaken. This effort produced two semisynthetic active agents, VP-16–213 (etoposide) and VM-26 (teniposide), by 1971 [4] (see Fig. 1). VP-16 (Vepesid, Bristol-Myers Squibb) has been approved in the United States for use in the treatment of refractory testicular tumors and small-cell lung cancer, whereas VM-26 (Vumon, Bristol-Myers Squibb) has been approved for "the treatment of pediatric patients with refractory and/or relapsed acute lymphocytic leukemia ... in combination with other approved ... agents." In addition, VP-16 is considered by many clinicians to be effective in the treatment of other neoplasms, including lymphoma, leukemia, and non-small-cell lung cancer [3].

Because it is poorly soluble in water, etoposide is formulated with polysorbate 80/Tween 80, polyethylene gly-

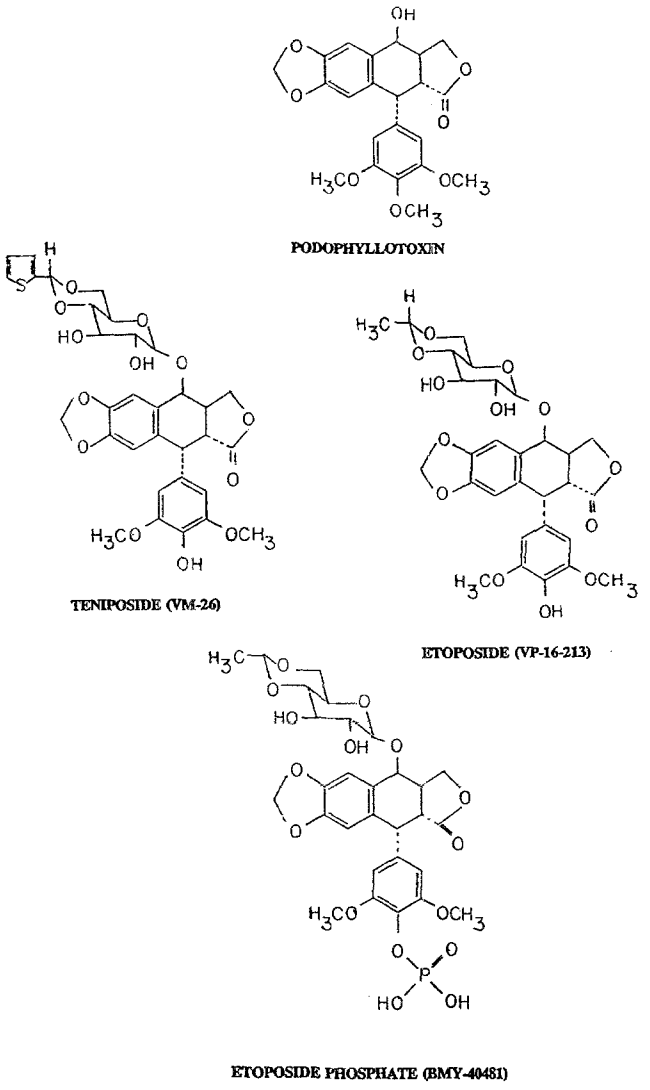

Fig. 1. Structures of the podophyllotoxins; podophyllotoxin, teniposide (VM-26), etoposide (VP-16-213), and etoposide phosphate (BMY-40481)

col, and alcohol. This formulation must be diluted to a concentration of 0.2–0.4 mg/ml of etoposide and must be given by slow infusion (30–60 min). As part of an ongoing program to generate "better" podophyllotoxins, Bristol-Myers Squibb modified etoposide by adding a phosphate group at the 4 position in the "E" ring (Fig. 1). This compound, etoposide phosphate (BMY-40481), proved to be water-soluble and rapidly converted to etoposide in plasma through the cleavage of the phosphate group [9]. The water solubility suggested that etoposide phosphate could be prepared at higher concentrations and given over a relatively short period of time. With rapid conversion of etoposide phosphate to etoposide, patients given etoposide phosphate were expected to be exposed to etoposide and only briefly exposed to the phosphate derivative. The development of etoposide phosphate has been based on this rapid and complete conversion.

Data are available from five recently completed phase I trials of the parenteral formulation as well as from a randomized pharmacokinetic trial comparing etoposide (VP-16) and etoposide phosphate (EP). Because EP (mol. wt., 668.55 Da) has a higher molecular weight than VP-16 (mol. wt., 588.57 Da), doses of EP are calculated and expressed as molar equivalents of VP-16 (113.6 mg of EP is the molar equivalent of 100 mg of VP-16).

Table 1. Phase I trials of parenteral EP

Study number	Schedule	Administration	Investigators
136-002	Days 1–5	30 min	H. Calvert, Newcastle upon Tyne
136-006	Days 1–5	30 min	A. Miller, Memphis F. A. Greco, Nashville
136-009	Days 1–5	5 min	D. Alberts, Tucson
136-005	Days 1, 3, 5	30 min	P. O'Dwyer, Fox Chase S. Fields, SUNY Syracuse
136-008	Days 1, 3, 5	5 min	D. Budman, North Shore

Table 2. Phase II randomized pharmacokinetic comparison of VP-16 and EP: study 136-012 investigators

Site	Investigator, Location
-001	P. O'Dwyer, Fox Chase, Philadelphia
-002	S. Fields, SUNY, Syracuse
-004	G. Goss, Ottawa Regional Cancer Center, Ottawa
-005	R. Bukowski, Cleveland Clinic, Cleveland
-006	N. Levitan, Ireland Cancer Center, Cleveland
-007	C. Belani, University of Maryland, Baltimore
-008	R. DeLap, Georgetown University Hospital, Washington
-009	M. Kosty, Scripps Clinic, La Jolla
-011	D. Gandara, VA Medical Center, Martinez

Patients and methods

Trials with parenteral EP began in August 1990. Five dose-escalation phase I trials were conducted (Table 1). In each trial, cohorts consisting of three patients were treated at increasing dose levels until toxicity was observed. At dose levels showing toxicity, additional patients were treated for a full assessment of EPs toxic potential. The maximum tolerated dose of EP was defined as the highest dose that, in one-third of the patients, produced predictable and reversible WHO grade III or IV toxicity. Patients treated with bolus EP were intensively monitored for hemodynamic changes (blood pressure and pulse) before, during, and after treatment. Pharmacokinetic samples were collected to assess dose proportionality and pharmacokinetic/pharmacodynamic relationships. Entry criteria were typical for phase I trials with the exception that patients with tumors that might respond to VP-16 at the dose level being tested could be included regardless of prior treatment or a lack thereof. Otherwise, patients were required to have a malignancy not amenable to "conventional" treatment, to have recovered from any prior therapy, and to have a life expectancy of 3 months; a performance status of 0 or 1 [Eastern Cooperative Oncology Group (ECOG) scale]; adequate renal, hepatic, and marrow function; and the ability to give informed consent. Drug was delivered using a calibrated infusion pump over 5 (bolus) or 30 min. In a trial conducted by A. H. Calvert (136-002), as the MTD was approached, patients were randomized to VP-16 or EP for course 1 and to the alternative drug for course 2. In this study the number of patients treated at the MTD was expanded to allow the collection of comparative pharmacokinetic data.

Blood and urine samples for pharmacokinetic evaluation were collected from patients beginning on day 1 of the first course of treatment with EP. Patients receiving a higher dose during subsequent courses of therapy were observed for toxicity, but blood and urine

Table 3. Patients treated in phase I trials of parenteral EP

Duration of administration		Treatment schedule		Total
		Days 1, 3, 5	Days 1–5	
5 min (bolus)	Total	36	27	63
	M	20	13	33
	F	16	14	30
30 min	Total	39	88	127
	M	19	47	66
	F	20	41	61
Totals	Total	75	115	190
	M	39	60	99
	F	36	55	91

samples for pharmacokinetic studies were not collected during therapy at the higher dose. Serial blood samples (7 ml) were to be collected predosing and at 5, 10, 20, 30, 45, and 60 min and 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, and 32 h after dosing.

The randomized comparison (136-012) of parenteral EP and VP-16 pharmacokinetics was based on findings from the phase I trials (Table 2). A days 1, 3, and 5 treatment schedule was chosen to allow adequate drug washout between assessments (8 half-lives of 6 h). Patients had to meet entry criteria similar to those of the phase I trials. Eligible patients were randomly assigned to treatment with EP on day 1 and VP-16 on days 3 and 5 or with VP-16 on day 1 and EP on days 3 and 5. Samples for pharmacokinetic analysis were collected following therapy on days 1 and 3. Therapy after the first course was given at the investigators discretion and could include EP. Drug at 150 mg/m^2 (the MTD on this schedule in phase I) was delivered over 3.5 h to conform with the labeled directions for VP-16 dosing. A minimal sample size of 22 was chosen to ensure that the 90% confidence interval for the difference in the mean area under the curve (AUC) of the two drugs would be contained within $\pm 10\%$ with a 90% power.

The pharmacokinetic parameters of VP-16 and EP were determined by non-compartmental methods. The following pharmacokinetic parameters were determined: maximal plasma concentration, CMAX; time to achieve CMAX, TMAX; area under the plasma concentration versus time curve from time zero to the last measurable plasma concentration time point, AUC(O-T), and to infinity, AUC(INF); terminal elimination half-life, $t_{1/2}$; total body clearance, CLT; renal clearance, CLR; steady-state volume of distribution, VSS; and cumulative percentage of the dose excreted unchanged in urine, UR.

Results

Dose-limiting toxicity/maximum tolerated dose

The dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) were evaluated for two different dosing schedules and two different durations of drug administration. Data were collected from 190 patients (Table 3). Not included are patients being entered in an ongoing extension of one phase I trial in which pharmacokinetic comparison at the MTD is being conducted. Myelosuppression in the form of leukopenia and granulocytopenia was the dose-limiting toxicity. Although thrombocytopenia was observed especially at the higher doses, it was less profound than leukopenia and was not dose-limiting. On the days 1, 3, and 5 schedule, a dose of 150 mg/m^2 produced reversible WHO grade III and IV myelosuppression in about one-third of courses. This was true whether the drug was given over 5 or 30 min. Although some patients have been successfully treated at doses of up to 200 mg/m^2 using this schedule, the 150-mg/m^2 dose seems appropriate in most cases (Table 4).

For the 5-day dosing schedule, myelosuppression in the form of leukopenia and granulocytopenia again proved to be dose-limiting. On this dose schedule the MTD was determined to be 100 mg/m^2, regardless of the duration of

Table 4. MTD of parenteral EP, days 1, 3, and 5 schedule – frequency of WHO grade III and IV toxicity by dose level following the first course

Dose level (mg/m^2)	White cells			Granulocytes			Platelets		
	Infusion time (min)			Infusion time (min)			Infusion time (min)		
	5[a]	30[b]	All (%)	5[a]	30[b]	All (%)	5[a]	30[b]	All (%)
50	0/3	1/4	1/7 (14)	0/3	0/4	0/7 (0)	0/3	0/4	0/7 (0)
75	0/4	0/3	0/7 (0)	0/4	0/3	0/7 (0)	0/4	0/3	0/7 (0)
100	0/3	4/10	4/13 (31)	0/3	3/10	3/13 (23)	0/3	0/10	0/13 (0)
125	3/6	0/6	3/12 (25)	3/6	0/6	3/12 (25)	3/6	0/6	3/12 (25)
150	0/4	5/15	5/19 (26)	2/4	7/15	9/19 (47)	0/4	1/15	1/19 (5)
175	0/3	1/1	1/4 (25)	1/3	1/1	2/4 (50)	0/3	1/1	1/4 (25)
200	2/2	–	2/2 (100)	2/2	–	2/2 (100)	0/2	–	0/2 (0)

Data represent numbers of patients with toxicity/numbers of courses

[a] D. Budman trial (136-008)

[b] P. ODwyer/S. Fields trial (136-005)

Table 5. MTD of parenteral EP, Days 1–5 schedule – frequency of WHO grade III and IV toxicity by dose level following the first course

Dose level (mg/m^2)	White cells			Granulocytes			Platelets		
	Infusion time (min)			Infusion time (min)			Infusion time (min)		
	5[a]	30[b]	All (%)	5[a]	30[b]	All (%)	5[a]	30[b]	All (%)
25	–	0/3	0/3 (0)	0/3	–	0/3 (0)	0/3	–	0/3 (0)
50	1/3	2/11	4/14 (21)	1/3	1/11	2/14 (14)	0/3	3/11	3/14 (21)
75	1/3	3/13	4/16 (25)	2/3	6/13	8/16 (50)	0/3	1/13	1/16 (6)
100	13/21	18/29	31/50 (62)	13/21	20/29	33/50 (66)	5/21	2/29	7/50 (14)
110	–	2/4	2/4 (50)	–	3/4	3/4 (75)	–	0/4	0/4 (0)
125	–	1/1	1/1 (100)	–	1/1	1/1 (100)	–	0/1	0/1 (0)

Data represent numbers of patients with toxicity/numbers of courses
[a] D. Alberts trial (136-009)
[b] A. H. Calvert trial (136-002) and A. Miller/F. A. Greco trial (136-006)

Table 6. Days to nadir white cell and absolute neutrophil counts and duration of nadir absolute neutrophil counts following the first course of treatment at the MTD

Study	Schedule, infusion time	Median days to WBC nadir (range)	Median days to ANC nadir (range)	Mean duration of ANC $<2000/mm^3$ in days (SD)
002	Days 1–5, 30 min	15 (11–19)	15 (8–19)	8 (3.4)
006	Days 1–5, 30 min	11 (4–22)	15 (3–22)	11.1 (5.4)
009	Days 1–5, bolus	13 (4–18)	15 (4–19)	8.1 (4.1)
005	Days 1, 3, 5, 30 min	16 (3–22)	18 (3–22)	8.2 (4.0)
008	Days 1, 3, 5, bolus	17 (12–19)	20 (15–22)	11.5 (9.2)

ANC, Absolute neutrophil count; WBC, white blood cell count

Table 7. Median blood pressure before, during and after bolus dosing of EP at 100 mg/m^2, D. Alberts trial (136-009)

	Position		
Time BP determined	Lying	Seated	Standing
Priot to Rx	128/80	122/80	118/80
Patients (*n*)	17	16	17
Measurements (*n*)	66	67	64
During Rx	130/80	132/75	118/70
Patients (*n*)	6	5	5
Measurements (*n*)	7	6	6
Up to 20 min after Rx	130/80	122/80	128/80
Patients (*n*)	13	13	12
Measurements (*n*)	32	32	31

BP, Blood pressure; Rx, treatment

administration (Table 5). At the MTD, regardless of the treatment schedule or duration of infusion, myelosuppression occurred at 2–3 weeks after the 1st day of treatment. Neutropenia (absolute neutrophil count, $<2000/mm^3$) lasted for between 8 and 11 days (Table 6). Dosing could be repeated no more frequently than every 3 weeks, with the interval rarely exceeding 4 weeks.

Responses

Of the 190 patients treated in phase I studies, 3 (2%) had responses meeting protocol criteria (≥50% reduction in the sum of lesion areas sustained for 4 weeks). Two of these patients had ovarian carcinoma, and one had cervical cancer. The patient with cervical cancer had a complete response. Five other patients had unsustained decreases in measurable disease and two others had stable disease.

Toxicity

No effect of EP on renal or hepatic function was observed nor were changes in serum chemistries noted. No neurologic, pulmonary, or cardiac toxicity occurred. Blood pressure changes were monitored before, during, and after bolus administration of EP with patients lying, seated, and standing. No pattern of blood pressure or pulse change was noted (pulse data not shown; Table 7).

The ability to deliver EP at high concentrations was studied as part of the D. Budman trial (136-008). After the MTD had been defined, 11 subjects were treated at the MTD with increasingly concentrated solutions of the drug: 10, 15, and 20 mg/ml. No unusual toxicity was seen when

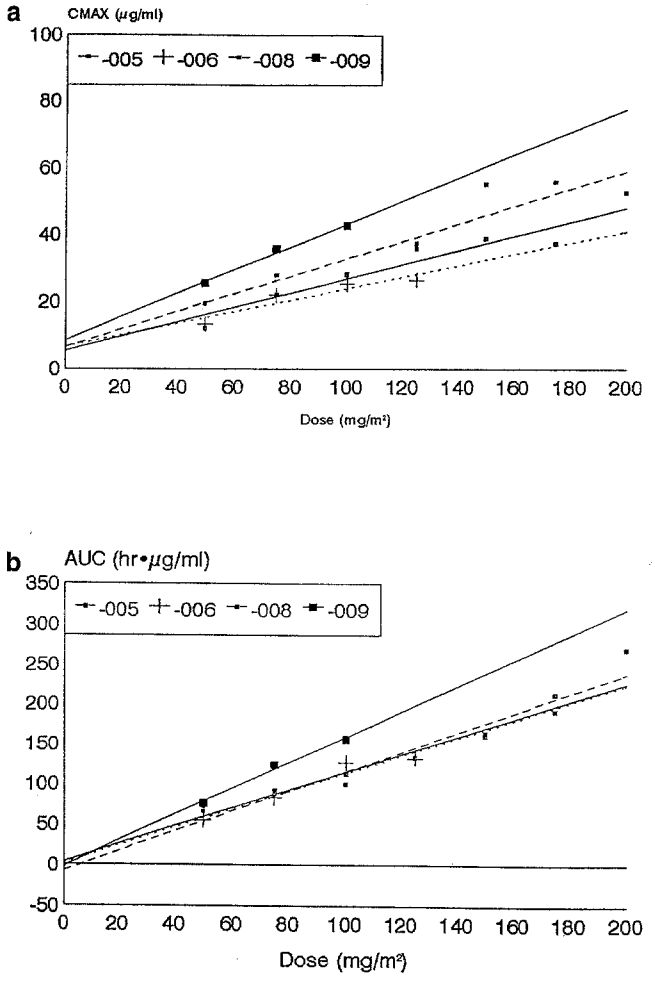

Fig. 2. a Plot of mean CMAX vs dose, studies 136-005, -006, -008, and -009. **b** Plot of mean AUC(INF) vs dose, studies 136-005, -006, -008, and -009

these concentrations were employed. At least 4 of the 190 (2%) patients treated in the phase I trials exhibited classic allergic signs at the time of EP administration. All responded to treatment with antihistamines and steroids and these patients could be and were successfully retreated with appropriate premedication. Six patients developed a rash while on study and another patient developed flushing, which may represent minor allergic reactions to the drug or may not be treatment-related.

Pharmacokinetics/pharmacodynamics

In all trials, EP has been rapidly and completely converted to VP-16 following parenteral administration. Exposure to EP as measured by the AUC at all dose levels has been $< 1\%$ of that for VP-16, to which it is converted. This was true for bolus and 30-min dosing. Pharmacokinetic and pharmacodynamic evaluations were therefore based on VP-16 levels in plasma. A linear and proportional relationship between dose and CMAX and AUC was observed in all studies (Fig. 2). The extent of exposure to VP-16 (after administration of EP) as measured by the AUC correlated with the degree of leukopenia and neutropenia in some but not all of the trials.

Comparative pharmacokinetics – EP and VP-16

The critical comparison of EP and VP-16 was performed in a randomized trial of 49 patients (28 men, 21 women). Eight patients could not be included in the evaluation due to inadequate sample collection. Patients were randomly assigned to treatment with EP or VP-16 on day 1 and received the alternative drug on day 3. All were treated at 150 mg/m^2, the MTD for the days 1, 3, and 5 schedule. Drug was diluted to 0.4 mg/ml and given over 3.5 h to conform to the labeling for VP-16. To assure accuracy, samples of the dosing solutions were collected and assayed for drug concentration. The study was carried out at nine institutions with a central randomization (Table 2). All samples were analyzed by Bristol-Myers Squibb.

The mean (SD) CMAX values for VP-16 following administration of EP and VP-16 were 19.9 (3.82) and 20.2 (4.66) μg/ml, respectively; the AUC values were 168 (49.3) and 162 (47.5) h·μg/ml, respectively. EP and VP-16 were pharmacokinetically equivalent (90% confidence interval for the difference in mean within $\pm 10\%$) for both CMAX and AUC(INF), the critical indicators of bioequivalence.

Discussion

EP has been developed as a prodrug of VP-16. Because of its water solubility, it was expected that EP could be delivered rapidly and in high concentrations. In addition, it was hoped that the avoidance of polysorbate 80/Tween 80, polyethylene glycol, and alcohol in the formulation would result in a "kinder and gentler" dosage form, possibly producing reduced side effects. The nature of the modification made to the basic VP-16 molecule led to the expectation that EP would act as a prodrug for VP-16 and would rapidly and completely convert to the parent compound.

The clinical data now available demonstrate that these expectations were correct. EP is rapidly and completely converted to VP-16. When treated with equimolar doses of the two drugs, patients are exposed to equivalent levels of VP-16. Levels of EP are very low and the drug disappears quickly from the circulation. The biological effects of EP proved to be equivalent to those previously observed for VP-16. The drug is myelosuppressive with a primary effect being exerted on white cells and granulocytes with thrombocytopenia being observed at the higher dose levels. Cardiac, renal, hepatic and CNS toxicities were not observed in these studies. Alopecia was common but for many patients antedated treatment with EP.

Classic allergic responses were seen in some of these patients, suggesting that the drug rather than the excipients required in the formulation of VP-16 is responsible for at least some of these reactions in patients treated with VP-16. It was possible, however, to rechallenge some of these patients successfully with appropriate premedication. Of particular interest, it proved to be possible to give EP as a

bolus and at high concentrations. The rapidity of administration appeared to have no impact on the biologic effects (myelosuppression) and was not accompanied by hemodynamic changes. High concentrations (up to 20 mg/ml) of EP could be given without resulting in local or systemic toxicity.

EP would therefore appear to have a number of potential advantages over the parent compound. Excipients known to be toxic [6, 7] have been eliminated from the formulation. The drug can be given safely by bolus administration, thus potentially reducing the costs of preparation and administration, increasing patients convenience, and reducing the time needed to treat. Because the drug can be reconstituted and is stable at and can be given at high concentrations, it is ideal for use in high-dose and continuous-infusion regimens. VP-16, with its limited solubility, requires large volumes of diluent when given in high doses. When used as a continuous infusion, VP-16 dosing solutions must be changed daily to keep the volume manageable and reduce the risk of precipitation. EP, on the other hand, is stable for 4 days at concentrations as high as 20 mg/ml. Trials to evaluate the utility of EP for high-dose and continuous-infusion therapy are currently under way.

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