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Phase I evaluation of 773U82·HCl, a member of a new class of DNA intercalators

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The arylmethylaminopropanediols (AMAPs) are a new class of DNA intercalators. 773U82·HCl is the second of these compounds to enter clinical trial. Significant antitumor activity for 773U82·HCl was documented in a variety of murine and human tumor models. This phase I study examined a 1-, 2- and 6-hour infusion given every 28 days. Thirty-six patients received 58 courses of drug at doses ranging from 15 mg/m^2 to 980 mg/m^2. The dose-limiting toxicity of 773U82·HCl was hemolysis noted at 980 mg/m^2. Change in color of the plasma and decreases in haptoglobin were correlated with drug concentrations of the infusate $\geq$3 mg/ml. Clinically significant changes in hemoglobin levels requiring blood transfusions did not occur. Neurologic toxicity occurred at 720 mg/m^2 with the most severe neurologic toxicity occurring in a patient with the highest peak plasma concentration (4.1 μg/ml). With an increase in duration of the infusion and amount of fluid administered, the neurologic toxicity resolved. Other toxicities included mild nausea and vomiting and a dose-related phlebitis. Pharmacokinetic studies were completed in 22 patients. The mean terminal $t_{1/2}\beta$ was 4.4 h with a mean apparent volume of distribution at steady state (Vd_{ss}) of 314 l/m^2. The mean total body clearance was 72 l/h/m^2. Peak plasma levels ranged from 0.04 to 4.14 μg/ml. Further studies with 773U82·HCl on this schedule at the doses studied are not recommended. Hematologic monitoring for evidence of intravascular hemolysis should be included in future studies with 773U82·HCl.

Key words: Arylmethylaminopropanediols, DNA intercalator, 773U82·HCl, phase I trial.

Introduction

The arylmethylaminopropanediols are a series of DNA intercalators synthesized by investigators at Burroughs Wellcome Company. The antitumor activity of these compounds was noted to be dependent on the type of polycyclic aromatic ring and on the structure and position of the side chain.[1] Evaluation of over 250 analogues resulted in the selection of three compounds for clinical development based on differing biophysical, pharmacologic, and toxicologic properties. Compound 773U82·HCl (Figure 1) was shown to have significant preclinical antitumor activity against P388 and L1210 leukemias, B16 melanoma, M5076 sarcoma and Lewis lung carcinoma. In addition, 773U82·HCl demonstrated significant antitumor activity against breast, colon, kidney, non-small cell lung, melanoma, and ovarian cancers in a human tumor assay at 10 μg/ml (continuous exposure).[2]

Preclinical toxicology studies were conducted in mice, rats, and beagle dogs.[3] In mice and rats, ataxia and tremors were seen. Transient and reversible decreases in total white cell counts and neutrophils

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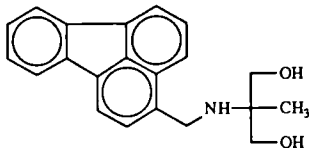

Figure 1. Structure of 773U82·HCl.

were seen in rats at all dose levels. In dogs, myelosuppression, lymphoid atrophy, local vein irritation and GI epithelial damage were observed. At the highest dose (60 mg/kg) CNS toxicity with convulsions occurred. No cardiac, hepatic, or renal effects were noted. Pharmacokinetic studies in the rat and dog (10 mg/kg and 30 mg/kg) revealed substantial first-pass hepatic metabolism with total body clearance values equal to hepatic blood flow. Both the clearance and half-life appeared to be independent of dose, suggesting linear kinetics.[2] The starting dose in this trial was 15 mg/m^2 which represented the human surface equivalent of one-tenth of the LD_{10} (10% lethal dose) in the single-dose study in the mouse.

Materials and methods

Patient selection

All patients entered on this trial had histologic proof of malignant solid tumors refractory to all known forms of effective therapy. Patients with leukemia were not eligible for treatment on this study. All patients were required to have a life expectancy of >12 weeks, a Karnofsky performance status of ≥ 60, and to have recovered from toxic effects of previous chemotherapy, immunotherapy, or radiotherapy. Adequate organ function was required, as evidenced by WBC $\geq 3000/mm^3$, platelets $>100\,000/mm^3$, hemoglobin ≥ 9 g/dl, bilirubin ≤ 2.5 mg/dl, SGOT $\leq 2 \times$ normal, alkaline phosphatase $\leq 2 \times$ normal, creatinine ≤ 2.0 mg%, normal electrolytes, urinalysis, PT, and PTT. Patients were excluded if significant abnormalities, specifically heart block, were present on electrocardiogram or a significant history of heart disease was elicited. Informed consent was obtained from all patients in accordance with federal and institutional guidelines.

Treatment plan

The compound was supplied by the Burroughs Wellcome Company in sterile 10 ml vials containing 50 mg of 773U82·HCl free base. The drug was formulated as a 5 mg/ml solution in sterile water for injection. The drug was passed through a 0.22 μm filter as it was further diluted with 250 ml D_5W for infusion (D_5W 5% dextrose in water). Stability studies of the diluted product revealed no decomposition with refrigeration at 24 h and stability at room temperature for 8 h. The solution was infused through a free-flowing intravenous line utilizing a controlled infusion pump initially over 1 h. As toxicity occurred, the infusion was lengthened first to 2 h and ultimately to 6 h in 1000 ml D_5W. All patients were hospitalized for treatment and retreated every 28 days in the absence of tumor progression or unacceptable toxicity. Three patients were entered at each dose level with dose escalation at 100% until evidence of a biologic effect then 50% until Grade 1 toxicity followed by escalation at 33% to the maximally tolerated dose (MTD).

Study parameters

Baseline studies obtained on all patients included history and physical examination, complete blood count, serum chemistries, PT, PTT, urinalysis, creatinine clearance, EKG, chest radiograph and appropriate scans or radiographs necessary to evaluate tumor response. Vital signs were monitored frequently during the infusion and for 24 h after the infusion. An EKG was obtained at the end of infusion. All patients were followed on a weekly basis after treatment with toxicity notation, CBC, serum chemistries, PT, PTT, and urinalysis. These studies and tumor evaluation were repeated prior to each course of drug. Standard response criteria and WHO toxicity criteria were used in this study. Patients were free to terminate their participation in the study at any time.

Pharmacokinetic sampling

Blood samples for pharmacokinetic studies were obtained during the first course of therapy from an indwelling intravenous heparin lock in the arm contralateral to the infusion line. Serial 8 ml specimens were collected in EDTA tubes before infusion, 30 min into infusion (1 and 3 h with the 2- and 6-h infusions), and at the end of infusion. Post-infusion samples were collected 10, 20, 40 and 60 min, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h after the infusion. Plasma was separated from whole blood by cold centrifugation, flash frozen and stored at

−20°C in polyethylene tubes. A baseline urine sample was obtained; serial urine samples were collected during and after infusion with the last collection period ending at 48 h. The total volume of each sample was recorded and a 20 ml aliquot removed, labeled and stored at −20°C.

HPLC analysis

A normal phase high-performance liquid chromatographic (HPLC) method was developed for the analysis of 773U83 in plasma and urine samples. A structurally related analogue, 818U83, was added to each plasma/urine sample as an internal standard prior to extraction. Stock solutions and urine/plasma standard curves were prepared on the day of each procedure. A 0.5 ml volume of plasma or urine was alkalinized with 50 μl of 8N KOH and extracted with 2.5 ml of chloroform:methanol (9:1). The mixture was gently rotated on a multipurpose rotator for 10 min and centrifuged for 10 min at 800 × ***g***. The organic layer was transferred to a 12 × 75 mm disposable glass culture tube and evaporated to dryness in a 45°C water bath under a gentle stream of nitrogen. The extraction yield was 80–90% for 773U83 and 87% for the internal standard. The extracted residue was then reconstituted with 250 μl of chloroform and 75 μl injected [Perkin-Elmer (ISS-100) autosampler; Norwalk, CT] onto a 5 μm silica column (4.6 mm × 25 cm; ES Industries, Marlton, NJ). The elution system consisted of 5% methanol in dichloromethane (HPLC grade) with 0.02% perchloric acid pumped (Model 510; Waters, Milford, MA) at a flow rate of 1 ml/min with constant helium purge. UV absorbance (Spectro-Monitor D; Milton/Roy, Rivera Beach, FL) was monitored at 269 nm. Chromatograms and peak height areas were stored and analyzed on a Model DS-802 microcomputer (Digital Specialties, Chapel Hill, NC). The coefficient of variation was less than 5% over the linear range of the assay (5–500 ng/ml), with the lower limit of sensitivity of the assay set at 5 ng/ml.

Pharmacokinetic analysis

The plasma concentration–time data were computer-fitted to a two-compartment open model with zero-order infusion using NONLIN[4] with a weighting of $1/y$. The values for A, B, α and β were used to obtain values for the pharmacokinetic parameters $t_{1/2}\beta$ (terminal half-life), V_c (volume of distribution of central compartment), Vd_{ss} (volume of distribution at steady state), and CL (clearance). The area under the plasma concentration–time curve was estimated by the linear trapezoidal method up to the last measurable data point and extrapolated to infinity.

Results

Thirty-six patients with refractory solid tumors were entered on this trial. Thirty-two patients are evaluable for toxicity. Four patients died as a result of disease complications prior to completion of a 4-week course. Patient characteristics are outlined in Table 1. Fifty-eight courses (each course was 28 days in length) of 773U82·HCl were administered at doses ranging from 15 mg/m^2 to 980 mg/m^2 (Table 2). One patient underwent dose escalation twice. Neurologic toxicity with blurred vision, nystagmus, dizziness, and ataxia was seen at 720 mg/m^2 with a 1-h infusion. With prolongation of the infusion to 2 and then 6 h, intravascular hemolysis was noted.

Toxicity—hemolysis

At 840 mg/m^2 given over 2 h, gross hemolysis of the serum of patients was noted during preparation

Table 1. Patient characteristics

Characteristics	Number of patients
Total patients	36
Sex (M:F)	32:4
Median age (range)	62 (38–79)
Performance status (SWOG)	
0–1	20
2	15
3	1
Previous treatment	
None	5
Radiation only	5
Chemotherapy only	9
Radiation and chemotherapy	13
Immunotherapy	4
Type of tumor	
Lung	14
Colon	9
Renal	3
Adeno-unknown primary	3
Prostate	2
Pancreas	2
Melanoma	1
Breast	1
Head and neck	1

Table 2. Dose escalation scheme

Dose (mg/m²)	Length of infusion (h)	Number of patients	Number of courses
15	1	3	8
30	1	6	9
60	1	4	8
120	1	3	4
240	1	3	3
480	1	3	6
720	1 (3 patients) 2 (3 patients)	6	8
840	2 (6 patients) 6 (3 patients)	9	14
980	6	1	1

Table 3. Laboratory parameters

PT	Dose (mg/m²)	Time (h)	HAPTO (mg/dl)	LDH (μ/l)	BILI (mg/dl)	HGB/HCT ((g/dl)/%)
28	840	Pre[a]	—	277	0.3	13.4/42.1
		EOI[b]	—	452	1.1	13.4/40.2
		8	—	423	0.9	12.6/37.8
		24	—	446	0.3	12.9/39.1
29	840	Pre	187	146	0.6	14.1/45.6
		EOI	157	299	1.8	15/43.8
		8	35	207	1.1	13.7/39.1
		39	123	274	0.8	14.1/44.2
35	840	Pre	361	176	0.3	10.7/31.6
		EOI	160	—	0.6	10.0/31
		8	124	—	—	—
		24	140	—	0.7	10.7/32.0
36	980	Pre	362	374	0.2	13.9/42.5
		EOI	106	—	0.9	13.9/39.5
		8	<35	—	0.8	14.0/40.4
		24	68	—	0.4	13.0/37.7

[a] Pretreatment value.
[b] End of infusion.

of the samples for pharmacokinetic studies (see Figure 2). After this initial observation, serial laboratory tests of intravascular hemolysis were obtained on subsequent patients and included haptoglobin, LDH, total bilirubin, hemoglobin and hematocrit (Table 3). In three of the four patients in which values were obtained, a consistent decrease in haptoglobin was seen. The hemolysis was not of clinical importance given the lack of significant change in patients' hemoglobin levels. Hemolysis was positively correlated with the drug concentration of the infusate and the infusion rate as shown in Table 4. Drug concentrations in the infusate ≥ 3 mg/ml were correlated ($p < 0.002$) with gross

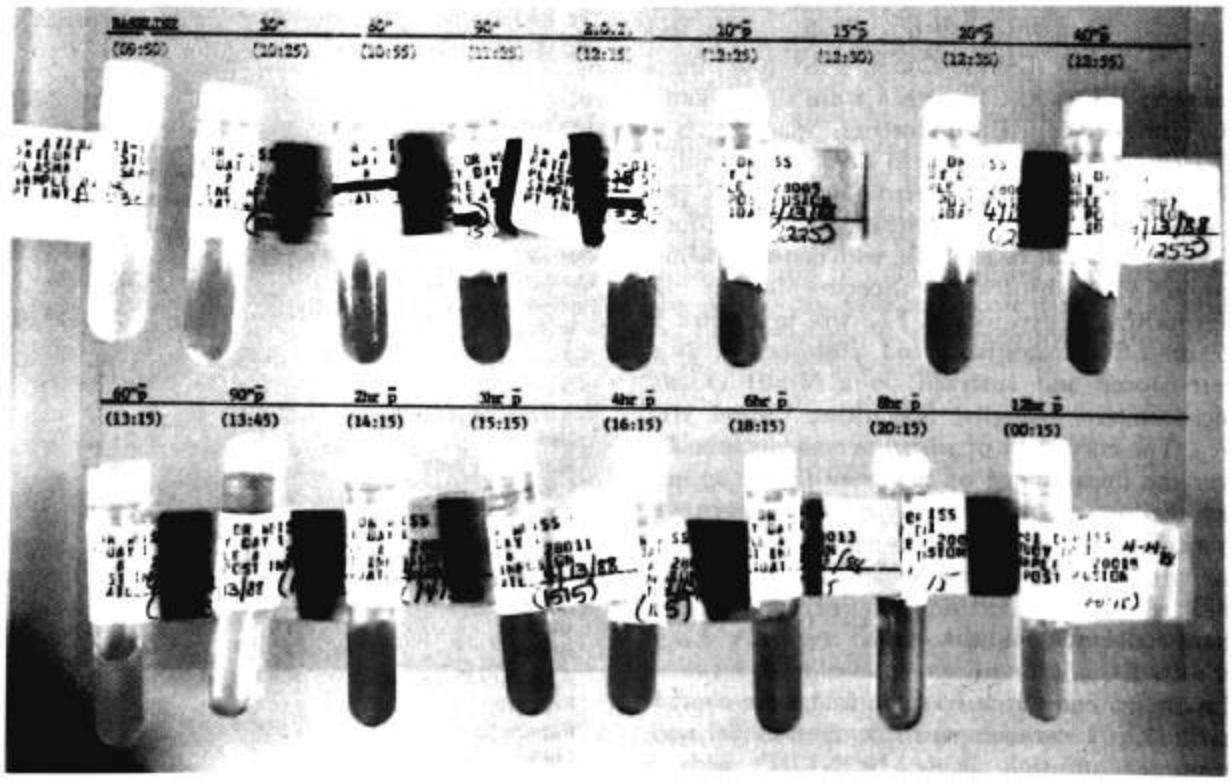

Figure 2. Photograph of plasma samples from patient no. 28 treated at 840 mg/m² in 500 ml over 2 h.

Table 4. Drug concentration vs hemolysis

Patient no.	Total dose (mg)	Infusion volume (ml)	Infusion rate (mg/h)	Concentration (mg/ml)	Hemolysis
27	1680	500	737	3.36	+
28	1528	500	656	3.06	+
29	1537	500	635	3.07	+
33	1537	1000	243	1.50	−
34	1150	1000	166	1.15	−
35	1600	1000	268	1.60	±
36	1960	1000	305	1.96	+

Spearman's correlation between drug concentrations vs hemolysis: $r = 0.9$; $p = <0.002$.

hemolysis in the plasma and a decrease in haptoglobin levels (Figure 3). Patients with drug concentrations of 1.5 mg/ml and less did not have gross hemolysis or a change in laboratory parameters consistent with intravascular hemolysis. There was no relationship to peak plasma concentrations or other pharmacokinetic parameters.

Other toxicity

Neurologic toxicity was noted at 720 mg/m^2 in two of the three patients treated at this dose with a 1-h infusion. Symptoms included blurred vision, dizziness, ataxia and nystagmus. In one patient, these symptoms were severe enough to warrant discontinuation of the drug at approximately 45 min into the infusion. With prolongation of the infusion to 2 h, mild blurred vision and dizziness which did not interrupt treatment were reported at 720 mg/m^2 and 840 mg/m^2. At 840 mg/m^2 over 6 h, the only neurologic symptom reported was mild dizziness in one patient.

Nausea and vomiting were sporadic and mild throughout the study and were controlled with oral antiemetic therapy. At 980 mg/m^2 a moderately severe phlebitis with local inflammation and swelling was noted in the one patient treated at this dose level. Mild phlebitis with local erythema and tenderness was seen in two of three patients treated with a 6-h infusion at 840 mg/m^2.

Pharmacokinetic results

The pharmacokinetic results are presented in Table 5. A representative plasma concentration versus time profile for one patient at 720 mg/m^2 over 2 h is shown in Figure 4. The plasma concentration declined biexponentially with a mean terminal $t_{1/2}\beta$ of 4.4 h. The mean apparent volume of distribution at steady state was 314 l/m^2 and the mean total body clearance was 72 l/h/m^2. Peak plasma concentrations ranged from 0.04 to 4.14 μg/ml with the highest peak concentration occurring at 720 mg/m^2 over 1 h. The highest peak level was seen in the patient with the most severe degree of neurologic toxicity seen in this trial. As noted, hemolysis was directly related to the concentration of the drug in the infusion without correlation with other pharmacokinetic parameters.

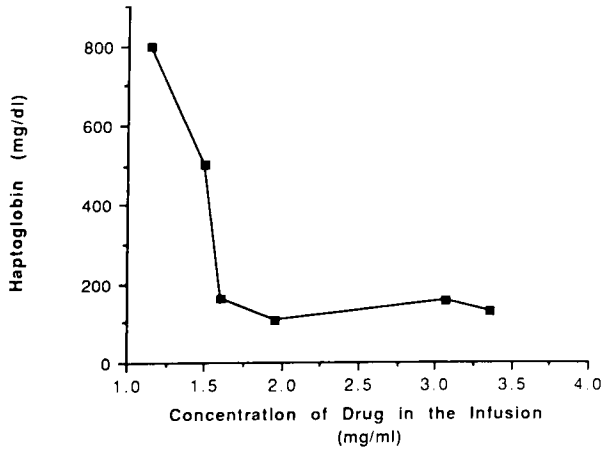

Figure 3. End of infusion haptoglobin levels vs drug concentration in the infusion in five patients at doses 840 mg/m^2 and one patient at 980 mg/m^2 (1.96 mg/ml).

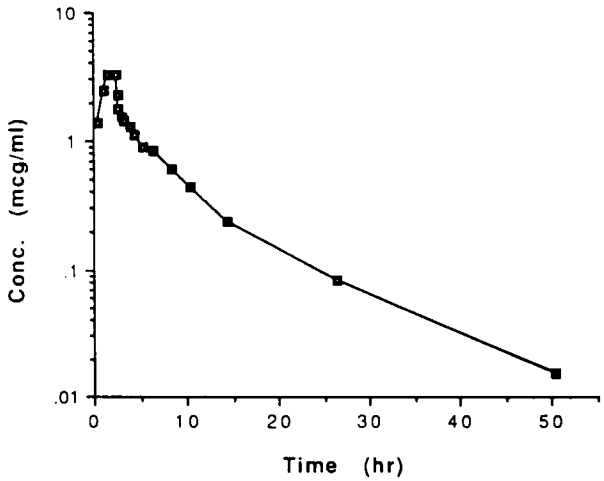

Figure 4. Plasma concentration vs time curve; patient no. 25 (720 mg/m^2 over 2 h).

Table 5. 773U82 pharmacokinetic parameters

Patient no.	Dose (mg/m^2)	Total dose (mg)	$t_{1/2}\beta$ (h)	CL ($l/hr/m^2$)	C_{max} ($\mu g/ml$)	V_c (l/m^2)	Vd_{ss} (l/m^2)	AUC ($\mu g/ml \cdot h$)
1	15	31	3.54	118.1	0.04	60.0	500	0.13
2	15	29	1.33	113.2	0.06	41.5	352	0.13
4	30	48	2.33	134.6	0.11	86.6	329	0.23
5	30	52	7.57	49.0	0.13	52.6	473	0.57
6	30	60	8.44	32.3	0.17	21.3	340	0.93
7	30	58	3.74	48.2	0.23	25.9	203	0.62
9	60	112	6.09	53.5	0.28	59.6	403	1.12
10	60	125	4.39	54.1	0.38	22.4	266	1.11
12	120	198	2.74	129.4	0.57	82.7	353	0.93
13	120	221	3.39	85.2	0.40	43.1	330	1.41
17	240	492	2.97	88.6	1.38	31.8	263	2.71
18	480	1056	4.67	63.7	1.96	83.7	308	7.53
19	480	810	3.01	49.5	3.19	18.0	169	9.71
21	720	1252	2.97	67.2	2.85	27.1	231	10.71
23	720	1440	4.63	67.8	4.14	39.2	339	10.62
25	720	1288	4.90	44.3	3.30	50.3	237	16.25
27	840	1680	4.41	55.7	2.83	51.3	291	15.08
28	840	1530	4.62	53.9	3.88	41.3	235	15.58
29	840	1540	6.35	61.6	2.42	60.9	397	13.65
33	840	1537	4.59[a]	36.1[a]	3.90	4.6[a]	73[a]	23.25
34	840	1150	5.63	65.7	0.94	60.9	288	8.94
36	980	1960	4.19	77.6	1.90	50.0	294	12.60
Mean ±SD			4.38 ±1.73	72.0 ±29.1		48.1 ±20.3	314 ±83	

[a] Not used in calculation.

Antitumor activity

No objective tumor responses were documented on this trial.

Discussion

Of the three AMAPs to enter phase I trials, each has been examined at this institution with infusions of 1 to 6 h. Although structurally related in that they contain a methylamino-propanediol side chain attached to different carbocyclic ring structures, the toxicity profiles differ markedly. Reversible neurologic toxicity was dose-limiting with crisnatol (770U82·mesylate), the first and most lipophilic of the AMAPs tested.[5] The neurologic toxicity was correlated with peak plasma levels of crisnatol. The neurologic effects of 773U82 resembled those of crisnatol but were substantially less severe at comparable plasma drug concentrations. Dose-limiting toxicity of the third AMAP, 502U83, when used on the 1-, 2-, or 4-h infusion schedule was different and was manifest as prolongation of PR, QRS, and QT intervals.[6] Significant cardiac effects were not seen with crisnatol or 773U82. Neurologic toxicity was absent from the 502U83 trial.

The maximum dose given in the present study of 773U82·HCl was 980 mg/m^2 over 6 h which was accompanied by evidence of intravascular hemolysis. Although clinically not significant in terms of decreases in hemoglobin or hematocrit, the hemolysis was persistent and reproducible over two dose levels and with prolongation of the infusion. Since a 24-h infusion schedule was undergoing concurrent evaluation, further prolongation of our infusion schedule was not warranted. As presented, the hemolysis and decreases in haptoglobin were directly related to the concentration of drug in the infusion. There are many examples of hemolysis as a result of drugs and various toxins.[7–16] The mechanisms vary from immunologically-mediated damage[10,14,16] to a direct toxic effect on red cell membranes through thermal damage,[17] membrane lysins,[11,12] oxidative damage[18] or increased sensitivity to an offending agent due to an enzyme deficiency such as glucose-6-phosphate dehydrogenase (G6PD) deficiency. Changes in the color of the plasma were seen within 30 min of the start of the infusion in this study and, therefore, imply a

direct effect of 773U82·HCl on red blood cells rather than immunologically-mediated damage. The most likely explanation of this effect is disruption of a membrane component through lysis or oxidative damage that probably occurred where drug concentration was highest, i.e. at the point of initial mixing between blood and infusate. It is unlikely that all patients were G6PD deficient although patients were not tested for this abnormality during this trial.

As for a related compound, crisnatol, neurologic toxicity was seen with 773U82·HCl. Significant neurologic toxicity was noted in the patient with the highest peak plasma concentration of drug which suggests a correlation of neurologic toxicity with plasma levels. This toxicity was ameliorated with prolongation of the infusion from 2 to 6 h and concomitant peak plasma level reduction.

With evaluation of a prolonged infusion schedule continuing, further studies of the shorter infusion at the doses evaluated in this study are not recommended at present. Additional studies involving this agent on different schedules should continue to include hematologic monitoring for intravascular hemolysis.

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