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ORIGINAL ARTICLE

**Rebecca R. Thomas · William Dahut · Nancy Harold
Jean L. Grem · Brian P. Monahan · Michael Liang
Roger A. Band · Jeff Cottrell · Victor Llorens
Judith A. Smith · William Corse · Susan G. Arbuck
John Wright · Alice P. Chen · Jeremy D. Shapiro
J. Michael Hamilton · Carmen J. Allegra
Chris H. Takimoto**

A phase I and pharmacologic study of 9-aminocamptothecin administered as a 120-h infusion weekly to adult cancer patients

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Abstract *Purpose*: To define the toxicity profile and the recommended phase II doses of 9-aminocamptothecin (9-AC) administered as a weekly 120-h infusion. *Methods*: 9-AC was administered over 120 h weekly to 55 adult cancer patients with solid tumors over doses ranging from 0.41 to 0.77 mg/m^2 per day in a phase I and pharmacologic study. 9-AC formulated in dimethylacetamide/polyethylene glycol (DMA) was administered on a 3 of 4-week schedule, and the newer colloidal dispersion (CD) formulation was given on a 2 of 3-week schedule. *Results*: Overall, 193 courses of therapy were administered over 122 dose levels. On the 3 of 4-week schedule, 9-AC DMA infused at ≥0.6 mg/m^2 per day for 120 h weekly produced dose-limiting neutropenia, thrombocytopenia, and diarrhea, or resulted in 1–2-week treatment delays. Shortening treatments to 2 of 3 weeks resulted in dose-limiting neutropenia and fatigue at infusion rates >0.72 mg/m^2 per day. The ratio of 9-AC lactone to total (carboxylate + lactone) drug plasma concentrations at steady-state was 0.15 ± 0.07. Clinical toxicities and drug pharmacokinetics were not substantially different between the DMA and CD formulations. One objective response was observed in a patient with bladder cancer and minor responses were observed in patients with lung and colon cancers. Plasma area under the concentration versus time curve for 9-AC lactone modestly correlated with the degree of thrombocytopenia ($r = 0.51$) using a sigmoid E_{max} pharmacodynamic model. *Conclusion*: The recommended phase II dose for the 9-AC DMA formulation is 0.48 mg/m^2 per h over 120 h for 3 of 4 weeks and for the 9-AC CD formulation is 0.6 mg/m^2 per day over 120 h for 2 of 3 weeks. Both regimens were well tolerated and feasible to administer.

Keywords Camptothecin · Topoisomerase I poison · Chemotherapy

R.R. Thomas · W. Dahut · N. Harold · J.L. Grem (✉)
B.P. Monahan · M. Liang · R.A. Band · J. Cottrell
V. Llorens · J.A. Smith · A.P. Chen · J.D. Shapiro
J.M. Hamilton · C.J. Allegra · C.H. Takimoto
Developmental Therapeutics Department, Medicine Branch,
Division of Clinical Sciences, National Cancer Institute,
Bethesda, MD 20889, USA

W. Corse
Department of Radiology, National Naval Medical Center,
Bethesda, MD 20889, USA

S.G. Arbuck · J. Wright
Investigational Drug Branch,
Cancer Treatment Evaluation Program,
Division of Cancer Treatment and Diagnosis,
National Cancer Institute,
Bethesda, MD 20892, USA

Correspondence address: J.L. Grem
NCI-Medicine Branch,
National Naval Medical Center, Building 8,
Room 5101, 8901 Wisconsin Avenue, Bethesda,
MD 20892, USA
e-mail: gremj@mail.nih.gov
Tel.: +1-301-4355382, Fax: +1-301-4801683

Introduction

9-Amino-20(S)-camptothecin (9-AC) is a poorly soluble camptothecin derivative and topoisomerase-I-targeting agent with broad spectrum single-agent activity in animal tumor models [1, 2]. It forms a drug-stabilized cleavable complex with the nuclear enzyme, topoisomerase I, in which the protein is covalently bound to DNA at the site of a single strand break. In the presence of ongoing DNA synthesis, these drug-stabilized cleavable complexes lead to cytotoxic DNA damage [3, 4, 5]. 9-AC has a highly labile terminal camptothecin lactone ring that undergoes nonenzymatic hydrolysis in aqueous solutions to form the less-active open-ring carboxylate [6].

9-AC has demonstrated preclinical antitumor activity against a broad range of human tumors including

malignant melanoma, acute leukemias, and colorectal, prostate, ovarian, and bladder cancers [1, 2, 7, 8, 9, 10, 11]. Because of poor aqueous solubility, the initial clinical testing of 9-AC lactone was delayed until a suitable intravenous formulation in dimethylacetamide (DMA)/polyethylene glycol and phosphoric acid could be developed. The first human trials utilized a 72-h infusion of the 9-AC DMA formulation administered every 2 weeks [12] or 3 weeks [13]. The principal dose-limiting toxicity (DLT) was myelosuppression, especially neutropenia, but these treatments were otherwise well tolerated. More recently a more easily administered colloidal dispersion (CD) formulation of 9-AC has been developed [14].

Previously, we have demonstrated the feasibility of administering 9-AC as a continuous infusion for up to 72 h per week [12]. In this study, our goal was to prolong the duration of the weekly 9-AC infusion up to 120 h. There are several theoretical reasons why prolonging the duration of 9-AC infusion may increase antitumor efficacy. First, because 9-AC is an S-phase cell cycle-specific cytotoxic agent with a short half-life [15], longer drug infusion times could increase the number of tumor cells exposed to drug during the sensitive phase of the cell cycle [16]. Second, in preclinical studies of nude mice bearing human tumor xenografts, repeated administration of low doses of camptothecins has been shown to decrease systemic toxicity, yet to preserve antitumor activity [17]. Furthermore, clinical administration of prolonged continuous infusions of other camptothecin analogues has been shown to be tolerable by Hochster et al. and others [18, 19, 20]. Finally, at the molecular level, Danks et al. have found that intermittent rather than continuous exposures to topotecan allows greater cleavable complex formation and produces maximal cytotoxicity [21].

Consequently, we postulated that a prolonged continuous exposure to 9-AC with periodic ''breaks'' during the infusion might maximize clinical efficacy and decrease toxicity. Therefore, we evaluated a prolonged 120-h infusion of the DMA formulation of 9-AC given weekly for 3 of 4 weeks. Once the recommended phase II dose on this schedule was established, the duration of treatment was shortened and the recommended phase II dose of the CD formulation of 9-AC on a weekly for 2 of 3 weeks schedule was also determined.

Materials and methods

Patient selection

In order to be eligible for this study, patients had to be 18 years or older, and diagnosed with a refractory solid tumor. The Eastern Cooperative Oncology Group (ECOG) performances status had to be less than or equal to 2, and patients had to have an absolute granulocyte count greater than 2000 μl, a platelet count greater than 100,000/μl, a total bilirubin less than 1.8 mg/ml, an aspartate aminotransferase (AST) level less than or equal to four times the upper limit of normal, and a serum creatinine concentration less than or equal to 1.6 mg/dl. Measurable disease was not required. At least 4 weeks had to have elapsed since the completion of any prior chemotherapy, radiation therapy, or immunotherapy. If prior mitomycin C, nitrosoureas, or suramin treatments had been given, at least 6 weeks must have elapsed. Patients were ineligible if they had any active infections requiring antibiotic therapy, central nervous system metastases, human immunodeficiency virus antibody positivity, or other serious concurrent medical illness that would preclude receiving experimental chemotherapy. The institutional review boards of the National Cancer Institute (NCI) and the National Naval Medical Center approved the protocol, and all patients gave written informed consent. Patients were enrolled from 11 October 1994 through 27 January 1998.

Dose escalation

The initial seven patients treated at the starting dose level of 0.41 mg/m^2 per day infused over 120 h every 2 weeks were enrolled as part of a preliminary pilot study to demonstrate the feasibility of infusing the 9-AC DMA formulation for longer than 72 h. Once the logistics and safety of administering a 120-h infusion were established, a standard three patients per dose level phase I escalation scheme was instituted, with the initial dose levels defined by the duration of drug infusion (Table 1). In the subsequent dose levels, the duration of drug infusion was increased to 1.5, 2, 2.5, and

Table 1 Dose levels

Dose level	Dose rate (mg/m^2/day)	Duration (weeks)	New patients entered	Total patients treated at this dose level	Evaluated courses
1-DMA	0.41	1 every 2[a]	7	7	46
2-DMA	0.41	1.5 every 3[b]	3	3	10
3-DMA	0.41	2.0 every 3[c]	4	7	17
4-DMA	0.41	2.5 every 4[d]	3	5	7
5-DMA	0.41	3.0 every 4[e]	4	4	8
6-DMA	0.48	3.0 every 4[e]	5	7	28
7-DMA	0.60	3.0 every 4[e]	9	10	28
8-DMA	0.77	3.0 every 4[e]	5	5	7
1-CD	0.48	2.0 every 3[c]	4	5	7
2-CD	0.60	2.0 every 3[c]	8	9	16
3-CD	0.72	2.0 every 3[c]	3	4	6
4-CD	0.84	2.0 every 3[c]	0	1	4
Total	–	–	55	–	184

[a]Week 1 120 h, week 2 no treatment
[b]Week 1 120 h, week 2 48 h, week 3 no treatment
[c]Week 1 120 h, week 2 120 h, week 3 no treatment
[d]Week 1 120 h, week 2 120 h, week 3 48 h, week 4 no treatment
[e]Week 1 120 h, week 2 120 h, week 3 120 h, week 4 no treatment

finally 3 weeks while maintaining the infusion rate at 0.41 mg/m^2 per day. Once the targeted treatment schedule of a 120-h infusion weekly for 3 of every 4 weeks had been achieved, the rate of drug infusion was escalated to 0.48, 0.60, and 0.77 mg/m^2 per day (Table 1). A shorter, more convenient schedule administering 9-AC as the CD formulation weekly for 2 of 3 weeks was also examined over a dose range of 0.48 to 0.84 mg/m^2 per day (Table 1). This regimen matched the 2 of 3-week treatment schedule utilized in preclinical animal models [17].

Individual patient dose escalations were permitted every third cycle provided that no worse than grade 1 toxicity was experienced in the previous two cycles (excluding nausea, vomiting and alopecia). However, individually escalated patients were not included in the determination of the maximally tolerated dose (MTD). DLT was defined as any of the following occurring during cycle 1: absolute neutrophil count (ANC) less than 500/μl for longer than 5 days; platelet count less than 25,000/μl; grade 3 or 4 nonhematologic NCI Common Toxicity Criteria [22]; or greater than 2-week delay due to drug-related toxicity. If a DLT was observed in any new patient during cycle 1, the number of new patients treated at that dose level was expanded to six. The MTD was defined as the dose level that induced a DLT in fewer than 33% of patients during cycle 1. Routine use of colony-stimulating factors was not permitted and high-dose loperamide was not used in this study. Treatment was resumed when the ANC had recovered to greater than 1500/μl, the platelet count was greater than 100,000/μl, and all clinically significant nonhematologic toxicities had resolved. Dose reductions were made by decreasing the rate of drug infusion and not by shortening the duration of drug administration because preservation of the prolonged infusion schedule was a primary study objective.

Drug administration

9-AC was supplied by the Cancer Treatment Evaluation Program, Division of Cancer Treatment and Diagnosis, NCI, in vials that contained 9-AC concentrate in DMA (5 mg/ml). A volume of 1 ml of this concentrate was added to a second vial that contained 49 ml 50% polyethylene glycol (400) (PEG) and 50% 0.01 mol/l phosphoric acid. This solution was diluted in 0.9% sodium chloride and the concentration was kept at less than 1 μg/ml to prevent precipitation. 9-AC was administered over 120 h in a single cassette via a central venous access device using CADD I or CADD-PLUS (Pharmacia Deltec, St Paul, Minn.) pumps. Subsequently, 9-AC was also provided as a lyophilized powder prepared with dimyristoylphosphatidylcholine, dimyristoylphophatidylglycerol and mannitol, which could be added to 20% dextrose in normal saline to yield a 100 μg/ml CD formulation for parenteral administration. Premedications were not routinely administered with 9-AC.

Pretreatment and follow-up studies

Complete blood cell counts, laboratory chemistries, urinalysis and a history and physical examination were performed every cycle. In addition, complete blood cell counts were obtained twice weekly, and if the ANC decreased to less than 1000/μl, they were obtained at least three times per week. Formal tumor measurements were performed after every two cycles of therapy. Treatment was continued indefinitely in the absence of disease progression. Standard response criteria as employed in previous NCI studies were used [23].

Pharmacokinetic and pharmacodynamic analyses

Plasma samples were obtained from 47 of 55 patients during cycle 1 of treatment at baseline and every 24 h until the end of the infusion. Clinical sample processing and quantitation of the 9-AC lactone and total drug concentrations were performed using our previously reported assay which uses immediate solid-phase extraction of the sample to separate the lactone from the open-ring carboxylate [15]. The lower limit of quantitation of 9-AC lactone was 0.25 nmol/l (0.09 ng/ml) and for 9-AC total drug was 2.5 nmol/l (0.9 ng/ml) for both the DMA and CD drug formulations.

Plasma concentrations were measured at 24, 48, 72, 96, and 120 h. Total body clearance (CL) was calculated by dividing the dose rate infused by the steady-state plasma concentrations (Css) [24]. The area under the concentration versus time curve (AUC) was estimated during cycle 1 for each patient by multiplying the measured Css by the duration of infusion for subsequent pharmacodynamic analyses.

Pharmacodynamic relationships were examined by plotting the percent change in the ANC or platelet count versus the cycle 1 AUC. Because the time of the lowest observed blood counts on this prolonged infusion schedule frequently occurred during the first week of cycle 2 (i.e. 3 or 4 weeks after the start of the drug infusion), the absolute degree of myelosuppression resulting from the initial drug exposure during cycle 1 was defined as the nadir blood count occurring during either cycle 1 or cycle 2 of therapy. The percent change in blood counts was determined by subtracting the nadir blood count observed at any time during the first two cycles of therapy from the pretreatment baseline count and expressing this value as a percent of the baseline level. Data were fitted to either a simple maximum-effect (E_{max}) model or a sigmoidal maximum-effect model (sigmoidal E_{max}) using WinNonLin, version 2.1 (Pharsight Corporation, Mountain View, Calif.). Goodness of fit and model selection were based upon analysis of the Akaike's Informational Criteria [25], the precision of the pharmacodynamic parameter estimates, and visual inspection of the weighted residual plots. In all cases, the best model for these data was the simple E_{max} model, which is defined by the formula for drug effect $E = (E_{max} \times AUC)/(AUC + EC_{50})$, where E is the drug effect, E_{max} is the maximal drug effect, and EC_{50} is the AUC associated with one-half of the maximal drug effect.

Results

Patient demographics

A total of 55 patients were enrolled in this study of whom 40 received 151 courses of 9-AC using the DMA formulation over eight different dose levels and 15 received 33 courses of 9-AC using the CD formulation over four different dose levels (Table 1). The median number of courses administered per patient was two and ranged from 1 to 19 cycles. Of the 55 patients, 49 (89%) had an ECOG performance status (PS) of 0 to 1. Five patients were completely chemotherapy-naive, 34 had received prior chemotherapy, and 15 had received prior chemotherapy and radiation therapy. The median number of prior chemotherapy regimens was one with a range of one to five (Table 2). Of 53 patients evaluated for toxicity, 39 were on the 3 of 4-week escalation schedule, and 14 were on the 2 of 3-week schedule. One nonevaluated patient with pancreatic cancer was enrolled on dose level 4-DMA (0.41 mg/m^2 per day over 120 h weekly for 2.5 weeks repeated every 4 weeks), but developed a new cerebral vascular event early in cycle 1. A second patient on dose level 1-CD (0.48 mg/m^2 per day over 120 h for 2 of 3 weeks) was taken off study during cycle 1 for an inferior vena cava thrombosis secondary to tumor compression.

Dose limiting toxicities

Once the feasibility of administering 0.41 mg/m^2 per day of 9-AC DMA as a 120-h infusion every other week had been established in a pilot group of seven patients, further dose escalations were implemented adding three new patients per dose level (Table 1). The targeted schedule of administering 9-AC as a weekly 120-h infusion for 3 of 4 weeks was reached at dose level 5-DMA, after which the rate of infusion was increased in subsequent dose levels from 6-DMA to 8-DMA (Table 1). At dose level 8-DMA (0.77 mg/m^2 per day of 9-AC DMA over 120 h weekly for 3 of 4 weeks), dose-limiting grade 4 diarrhea, thrombocytopenia and febrile neutropenia were observed during cycle 1 in two of five patients. Therefore, additional patients were treated at dose level 7-DMA (0.60 mg/m^2 per day over 120 h weekly for 3 of 4 weeks) to define the MTD. At this level, only one patient of nine experienced dose-limiting febrile neutropenia and diarrhea during cycle one, meeting our criteria for the MTD.

However, the time of the nadir ANC was often delayed and frequently occurred after 21 to 28 days of therapy. Consequently, four of nine patients treated at dose level 7-DMA experienced delays in starting cycles 2 or 3, or required early termination of cycles 2 or 3 due to neutropenia or thrombocytopenia. Thus, maintaining the planned dose intensity at dose level 7-DMA was not feasible. At the next lowest dose level 6-DMA (0.48 mg/m^2 per day over 120 h weekly for 3 of 4 weeks), five patients received 18 courses of therapy, and no clinically significant neutropenia, thrombocytopenia, or diarrhea were observed (Tables 3 and 4). None of these patients required dose escalations or reductions. Therefore, our recommended phase II dose for the DMA formulation of 9-AC is 0.48 mg/m^2 per day over 120 h weekly for 3 of 4 weeks (dose level 6-DMA).

For the 2 of 3-week schedule using the 9-AC CD formulation, dose-limiting grade 4 neutropenia and grade 3 fatigue were observed at dose level 3-CD (0.78 mg/m^2 per day over 120 h weekly for 2 of 3 weeks) in two of three patients (Tables 3 and 4). Therefore, additional new patients were accrued until a total of eight new patients were treated with 16 courses at the next lower dose level 2-CD (0.60 mg/m^2 per day over 120 h weekly for 2 of 3 weeks). One patient at this dose level was diagnosed with CMV esophagitis during cycle 1 and required treatment with ganciclovir. She ultimately developed grade 4 neutropenia and *Clostridium difficile* colitis with grade 3 diarrhea that required stopping protocol therapy. Another patient developed febrile neutropenia after two cycles of therapy. However six additional patients tolerated dose level 2-CD well, including one patient who was successively dose escalated twice and finally tolerated four courses at 0.84 mg/m^2 per day without problems. Nonetheless, because only two of eight patients (< 33%) experienced DLT during cycle 1 to 2, this dose level met our definition of MTD. Overall, for the majority of patients treated on the 2 of 3-week schedule, neutropenia and fatigue were common,

Table 2 Patient demographics

Age (years)	
Median	53
Range	25–77
Gender	
Female	25
Male	30
Performance status	
0–1	49
2	6
Primary tumor	
Colorectal	32
Other gastrointestinal	6
Lung	6
Head and neck	3
Other	8
Prior therapy	
Chemotherapy only	34
Chemotherapy + radiation	15
Radiation only	1
No prior chemotherapy or radiation therapy	5
Prior chemotherapy regimens	
Median	1
Range	1–5

Table 3 Hematologic toxicity: worst toxicity grade in any cycle (*ANC* absolute neutrophil count, *WBC* white blood count, *PLT* platelet count)

Dose level	Dose rate (mg/m^2/day)	Duration (weeks)	Evaluated patients treated[a]	ANC (grade 3/4)	WBC (grade 3/4)	PLT (grade 3/4)	Hemoglobin (grade 3/4)
1-DMA	0.41	1 every 2	7	0/0	0/0	0/0	0/0
2-DMA	0.41	1.5 every 3	3	0/0	0/0	0/0	1/0
3-DMA	0.41	2.0 every 3	7	0/0	0/0	0/0	1/0
4-DMA	0.41	2.5 every 4	5	0/0	0/0	0/0	0/0
5-DMA	0.41	3.0 every 4	3	0/0	1/0	0/0	0/0
6-DMA	0.48	3.0 every 4	7	1/0	0/0	0/0	2/1
7-DMA	0.60	3.0 every 4	10	2/1	1/1	1/0	2/1
8-DMA	0.77	3.0 every 4	5	1/2	0/2	0/2	0/0
1-CD	0.48	2.0 every 3	4	0/0	0/0	1/0	1/0
2-CD	0.60	2.0 every 3	9	1/2	2/1	3/0	3/0
3-CD	0.72	2.0 every 3	4	1/1	2/0	0/0	1/0
4-CD	0.84	2.0 every 3	1	0/0	0/0	0/0	0/0

[a]All patients treated at that specific dose, including patients undergoing dose modification

Table 4 Nonhematologic toxicity: worst toxicity grade in any cycle

Dose level	Dose rate (mg/m^2/day)	Duration (weeks)	Evaluated patients treated[a]	Diarrhea (grade 1/2/3/4)	Fatigue (grade 1/2/3/4)	Nausea (grade 1/2/3/4)	Vomiting (grade 1/2/3/4)
1-DMA	0.41	1 every 2	7	1/2/0/0	2/2/0/0	4/3/0/0	4/2/0/0
2-DMA	0.41	1.5 every 3	3	0/2/0/0	1/2/0/0	0/2/0/0	1/0/0/0
3-DMA	0.41	2.0 every 3	7	0/1/0/0	0/4/1/0	3/2/0/0	3/1/0/0
4-DMA	0.41	2.5 every 4	5	1/0/0/0	1/1/0/0	1/1/0/0	1/0/0/0
5-DMA	0.41	3.0 every 4	3	2/0/0/0	1/1/0/0	2/0/0/0	0/0/0/0
6-DMA	0.48	3.0 every 4	7	3/1/0/0	4/2/0/0	2/2/0/0	1/1/0/0
7-DMA	0.60	3.0 every 4	10	2/1/0/1	5/1/0/0	1/3/1/0	1/3/1/0
8-DMA	0.77	3.0 every 4	5	0/0/1/2	2/0/1/0	1/1/0/0	0/1/0/0
1-CD	0.48	2.0 every 3	4	1/0/0/0	1/2/0/0	2/0/0/0	0/0/0/0
2-CD	0.60	2.0 every 3	9	2/1/1/0	5/3/0/0	6/2/0/0	2/2/0/0
3-CD	0.72	2.0 every 3	4	1/1/0/0	0/1/0/0	3/0/1/0	0/1/0/0
4-CD	0.84	2.0 every 3	1	0/0/0/0	1/0/0/0	0/0/0/0	0/0/0/0

[a]All patients treated at that specific dose, including patients undergoing dose modification

but generally mild (Tables 3 and 4). Thrombocytopenia and diarrhea were also less pronounced on this shorter schedule. Our recommended phase II dose for the CD formulation of 9-AC is 0.60 mg/m^2 per day infused over 120 h weekly for 2 of 3 weeks (dose level 2-CD).

Other toxicities

Mild anemia was commonly observed on both schedules. For the 3 of 4-week treatment, 6 of 39 patients required blood transfusions, while on the 2 of 3-week schedule, 3 of 14 patients required transfusional support. Two patients treated with the DMA formulation developed grade 1 eosinophilia in the absence of signs of allergic reaction or infection.

Mild fatigue was also frequent, occurring in 79% of the 39 patients entering on the 3 of 4-week schedule and in 86% of the 14 patients treated on the 2 of 3-week regimen. Diarrhea of any severity was seen in 41% of patients on the 3 of 4-week schedule and in 35% of those on the 2 of 3-week schedule. Overall, 19 out of 53 patients showed mild elevations in liver transaminases. In two patients, the abnormalities were associated with biliary obstruction secondary to tumor progression. Mild alopecia, headaches, anorexia, and mucositis were also observed on both schedules. All patients were required to have central venous access while on study, and the majority of these were port devices. Six patients had presumed central line infections. Three had documented gram-positive cocci in blood cultures and three patient required line removal; however, none of these patients was neutropenic. Line removal was also required in one patient with skin breakdown at the Port-a-Cath site in the absence of any infection or neutropenia.

Response

Among 47 patients evaluated for response, 36% had stable disease and 57% experienced disease progression at the initial restaging. A 66-year-old patient with bladder cancer failing prior chemotherapy with methotrexate, doxorubicin, vinblastine, cisplatin and radiation was noted to have an impressive partial response to the 9-AC DMA formulation starting dose level 5-DMA (0.41 mg/m^2 per day over 120 h weekly for 3 of 4 weeks). At the time of entry, the patient had bilateral pulmonary nodules, a suprapubic mass, posterior pelvic mass and bone metastases. After four cycles the pulmonary nodules had decreased by 90% and after nine cycles, the pulmonary nodules and suprapubic mass had disappeared. The only remaining evidence of disease was a previously irradiated site of bony metastases. After three cycles, the patient's dose was escalated to 0.48 mg/m^2 per day, which was well tolerated. The patient received a total of 13 cycles and discontinued the study at his own request. At the time of this report he had remained in a partial response for over 53 months.

A 32-year-old patient with colon cancer who had received one prior chemotherapy regimen showed a 26% decrease in disease after two cycles. The patient was entered at dose level 7-DMA (0.6 mg/m^2 per day over 120 h weekly for 3 of 4 weeks) and did not receive any dose escalations or reductions, but he progressed after ten cycles. Another 40-year-old patient with non-small-cell lung cancer entered at dose level 6-DMA (0.48 mg/m^2 per day over 120 h weekly for 3 of 4 weeks) showed an 82% decrease after four cycles. However, during cycle 5 he was found to have cerebral metastases and was removed from the study. It is unclear whether the patient had prior CNS disease at the start of treatment since brain imaging is not a part of the initial evaluation.

Pharmacokinetics and pharmacodynamics

Pharmacokinetic data were collected from 47 patients and are summarized in Table 5. As in previous studies of 9-AC, the overall interpatient variability was high, with the coefficient of variation of the total lactone clearance being 64% and for total 9-AC clearance 57%. No appreciable differences between the DMA and CD forms of 9-AC were observed when lactone clearance (*t*-test,

Table 5 Pharmacokinetic data. Abbreviations: DMA, dimethylacetamide; CD, colloidal dispersion; Css, steady-state concentration; AUC, area under the concentration versus time curve; CL, clearance; NA, data not available. The mean ± standard deviation are shown

Dose level	Dose rate (mg/m^2/day)	Duration (weeks)	No. of patients	Lactone Css (n*M*)	Lactone AUC (nmol·h/l)	Lactone CL (l/m^2/h)	Total Css (n*M*)	Total AUC (nmol·h/l)	Total CL (l/m^2/h)
1-DMA	0.41	1 every 2	7	2.93 ± 0.83	351 ± 100	17.2 ± 5.0	24.7 ± 11.0	2959 ± 1320	2.43 ± 1.45
2-DMA	0.41	1.5 every 3	3	2.67 ± 0.89	448 ± 149	18.9 ± 6.3	23.4 ± 12.8	3931 ± 2152	2.55 ± 1.57
3-DMA	0.41	2.0 every 3	4	2.47 ± 1.36	592 ± 326	26.2 ± 20.0	15.9 ± 4.9	3810 ± 1164	3.17 ± 0.96
4-DMA	0.41	2.5 every 4	1	5.78	1665	8.10	31.2	8986	1.50
5-DMA	0.41	3.0 every 4	4	2.85 ± 1.64[a]	1027 ± 590[a]	21.9 ± 15.1[b]	30.8 ± 16.6	11,070 ± 5,964	1.93 ± 1.11
6-DMA	0.48	3.0 every 4	5	5.84 ± 3.12	2104 ± 1125	12.3 ± 7.1	42.8 ± 25.0	15,394 ± 8,982	1.65 ± 0.87
7-DMA	0.60	3.0 every 4	9	9.50 ± 5.71	3418 ± 2051	9.63 ± 5.13	69.0 ± 36.7	24,856 ± 1,3227	1.29 ± 0.74
8-DMA	0.77	3.0 every 4	5	11.2 ± 3.7	4022 ± 1319	8.76 ± 3.45	62.6 ± 22.3	22,536 ± 8,037	1.73 ± 1.13
All DMA patients			38			14.49 ± 9.38			1.97 ± 1.16
1-CD	0.48	2.0 every 3	3	5.02 ± 0.83	1204 ± 198	11.2 ± 2.0	47.0 ± 19.1[a]	11,274 ± 4,585	1.35 ± 0.61[a]
2-CD	0.60	2.0 every 3	3	NA	NA	NA	51.0 ± 14.2	12,238 ± 3,403	1.42 ± 0.36
3-CD	0.72	2.0 every 3	3	8.12 ± 0.9	1948 ± 219	10.3 ± 1.2	53.6 ± 8.1	12,872 ± 1,935	1.63 ± 0.18
All CD patients			9			10.82 ± 1.6*			1.45 ± 0.47**
Total	–	–	47	–	–	14.0 ± 8.9	–	–	1.87 ± 1.07

*$P = 0.39$, **$P = 0.17$, *t*-test comparison with DMA clearance
[a] $n = 4$ patients
[b] $n = 3$ patients

$P = 0.39$) and total drug clearance ($P = 0.17$) were compared (Table 5). However, when just the DMA patients were examined, there did appear to be a dose-dependent decrease in total 9-AC clearance. Patients treated with 9-AC DMA at 0.48 mg/m^2 per day or higher ($n = 19$) compared to those treated at 0.41 mg/m^2 per day ($n = 19$) had a lower total clearance rate (1.50 ± 0.86 l/m^2 per h vs 2.45 ± 1.25 l/m^2 per h, respectively; *t*-test, $P = 0.01$). Nonetheless, because of high interpatient variation, there was still substantial overlap in the range of total clearance at different dose levels. The overall steady-state lactone to total 9-AC (lactone + carboxylate) AUC ratio was 0.15 ± 0.07, and the total body clearance of total 9-AC was 1.87 ± 1.07 l/h per m^2.

Pharmacodynamic relationships between myelosuppressive toxicities and the 9-AC lactone AUC and total drug AUC were best described using a simple E_{max} model. When all patients were analyzed together, modest correlations were observed between the decrease in platelet count and the 9-AC lactone AUC ($r^2 = 0.51$; Fig. 1) and the 9-AC total AUC ($r^2 = 0.44$). Weaker correlations were observed between the fall in ANCs and the 9-AC lactone AUC ($r^2 = 0.27$) and the total AUC ($r^2 = 0.23$).

Discussion

Our study was designed to mimic preclinical animal models that used frequent administration to increase drug activity [17]. Overall, myelosuppression was common, with the principal DLTs being neutropenia, thrombocytopenia, and diarrhea on the 3 of 4-week regimen, and neutropenia and fatigue on the 2 of 3-week schedule. The recommended phase II dose for the 9-AC DMA formulation is 0.48 mg/m^2 per h over 120 h for 3 of 4 weeks and for the 9-AC CD formulation 0.6 mg/m^2 per day over 120 h for 2 of 3 weeks. Other toxicities were generally mild and both regimens were well tolerated. The toxicity profile observed in this study was not substantially different than that observed in our earlier trial of a 72-h infusion of 9-AC DMA with two exceptions. First, dose-limiting diarrhea was observed on the 3 of 4-week schedule. Second, central line infections were much less common in this present study, occurring in only 11% of 55 total patients instead of the 37.5% reported in our earlier phase I study of 72-h infusions of 9-AC [12].

The poor solubility of 9-AC required its initial formulation in DMA. However, a newer CD formulation was made clinically available while this study was in progress. Advantages of the CD formulation include higher stability and increased ease of administration in standard intravenous fluids. Animal toxicology and preliminary clinical studies suggested that the CD formulation of 9-AC may be equivalent or slightly better tolerated than the DMA formulation [14]. In the present study, no substantial differences were seen in the toxicity profiles of the two formulations. However, a direct

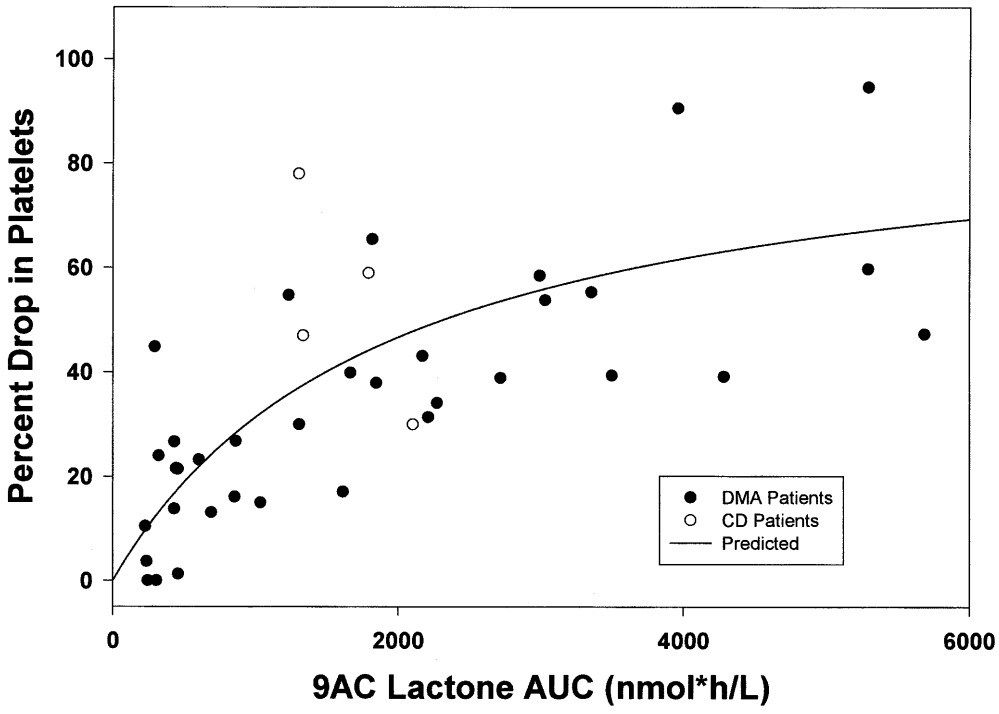

Fig. 1 Pharmacodynamic correlation between 9-AC lactone AUC and percent decrease in platelets. The *solid line* was determined by fitting data to a simple E_{max} model (*closed circles* patients treated with the DMA formulation, *open circles* patients treated with the CD formulation)

clinical comparison cannot be made because the DMA formulation was given every 3 of 4 weeks and the CD formulation was given every 2 of 3 weeks.

No consistent differences in the pharmacokinetics of the DMA and CD formulations were observed; however, both showed substantial interpatient variability. The overall total 9-AC systemic clearance in this study (1.87 ± 1.07 l/h per m^2) was in general agreement with that previously observed during an earlier phase I study of the 9-AC DMA formulation infused over 72 h (2.39 ± 0.94 l/h per m^2) [15]. When just 9-AC DMA patients were analyzed, the total plasma 9-AC clearance decreased as the dose rate increased, although there was still substantial overlap in the range of clearance values over the different dose levels (Table 5). The reasons for this dose dependency are unclear, especially given our earlier observation that total 9-AC clearance tends to increase slightly at higher doses during a 72-h infusion [15]. Pharmacodynamic correlations were identified between drug AUC and myelosuppressive toxicities such as thrombocytopenia (Fig. 1) and neutropenia, but these correlations were less strong than those previously found in our phase I trial of a 72-h infusion of 9-AC [12, 15]. This may have been partially due to the use of a variable duration of infusion, resulting in changes in drug concentrations and durations of exposure in different patients. Nonetheless, the weakness of these correlations suggest that therapeutic drug monitoring of 9-AC plasma levels are unlikely to be of benefit in predicting clinical toxicity in patients receiving 120-h weekly drug infusions.

The full clinical efficacy profile of 9-AC has yet to be reported. In our study, one patient with bladder cancer had a durable response for 53 months with bone metastases as the only site of residual disease. This patient continued to do well at the time of this report although he had been off treatment for 48 months. Another patient with lung cancer had a marked decrease in his primary tumor but he was taken off study for newly recognized brain metastases. The observation that 9-AC dose not readily penetrate the blood-brain barrier [26] may explain why the patient developed brain lesions while extra-CNS sites appeared to respond to 9-AC. It is of interest that these tumor responses occurred despite the relatively low 9-AC steady-state plasma lactone concentrations (< 10 n*M*) achieved in this study. A single phase II trial of a 120-h infusion of 9-AC in untreated colorectal cancer has failed to show antitumor activity [27]. However, further phase II testing in other tumor types must still be reported.

In conclusion, our recommended phase II doses for the 9-AC DMA formulation is 0.48 mg/m^2 per h over 120 h for 3 of 4 weeks and for the 9-AC CD formulation 0.6 mg/m^2 per day over 120 h for 2 of 3 weeks. Although dose-limiting myelosuppression occurred on both schedules, it is unlikely that granulocyte colony-stimulating factor would be of substantial benefit because the nadirs tended to occur during week 1 of cycle 2, and concomitant chemotherapy and growth factor support is generally not advisable. The clinical toxicities and the pharmacokinetic profiles are not substantially different between the DMA and CD formulations of 9-AC.

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