Phase I trial of continuous infusion 9-aminocamptothecin in patients with advanced solid tumors: 21-day infusion is an active well-tolerated regimen

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This study's objectives were to determine the maximum tolerated dose (MTD) of 9-aminocamptothecin (9-AC), given as a prolonged continuous infusion (CI) for 7–21 days, when formulated in dimethylacetamide/polyethylene glycol 400 (DMA) and then later as a colloidal dispersion (CD), and to determine the steady-state pharmacokinetics of 9-AC. Patients with solid tumors refractory to standard therapy were enrolled on this study. Total dose/cycle of 9-AC/DMA was initially escalated by duration (7–21 days), while keeping the dose rate constant at 6.2 μg/m^2/h (1.04–3.12 mg/m^2/4-week cycle). Then, the dose rate was escalated from 6.2 to 21.1 μg/m^2/h (3.12–10.6 mg/m^2/4-week cycle) while keeping the infusion duration constant at 21 days. CD formulation was escalated from 14.1 to 25 μg/m^2/h (7.11–12.60 mg/m^2/4-week cycle) while keeping the infusion duration constant at 21 days and then escalated from 28.1 to 37.5 μg/m^2/h (9.44–12.60 mg/m^2/3-week cycle) while keeping the infusion duration constant at 14 days. Sixty-two patients were evaluable for toxicity; 61 received prior chemotherapy (median 3 regimens/patient). No consistent dose-limiting toxicity (DLT) was encountered with the DMA formulation until dose level 10.60 mg/m^2/cycle, when two patients experienced DLTs. With the 21-day CD formulation, the MTD was 12.60 mg/m^2/cycle with three DLTs out of five patients. When 9-AC was given on the 14-day schedule, DLT was seen at 9.44, 11.20 and 12.60 mg/m^2/cycle, with consistent DLT at the two highest dose levels. All DLTs for both formulations were grade 4 hematologic toxicities (neutropenia and/or thrombocytopenia), while non-hematologic toxicities were relatively mild (including gastrointestinal toxicities and fatigue). One patient with ovarian cancer had a complete response and three had partial responses (PRs). One patient each with non-Hodgkin's lymphoma and cancer of unknown primary had a PR. Pharmacokinetic studies of both formulations of 9-AC revealed a linear relationship between increasing plasma 9-AC lactone concentration and dose. The median plasma 9-AC lactone concentration for 9-AC/CD was approximately twice that achieved by 9-AC/DMA for the same dose level. Both 9-AC formulations, given as a 21-day CI, were well tolerated with dose-limiting myelosupression at the MTD. This dose intensity exceeds that of other 9-AC phase I/II schedules. The recommended phase II dose (RPTD) is 9.42 mg/m^2/4-week cycle, given as a 21-day infusion. The 14-day schedule of 9-AC/CD was equally myelosuppressive with the RPTD of 9.44 mg/m^2/3-week cycle, although two heavily pre-treated patients (one with pelvic radiotherapy) could not tolerate this dose. Objective responses were observed in six out of 57 heavily pre-treated patients, most of which had ovarian cancer. *Anti-Cancer Drugs* 17:571–579 © 2006 Lippincott Williams & Wilkins.

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Introduction

20(*S*)-Camptothecin is a plant alkaloid isolated from the oriental tree *Camptotheca acuminata* [1]. Camptothecins interact in a unique way with DNA topoisomerase I (Topo I), a cellular enzyme essential for various DNA functions such as replication, transcription and recombination [2]. The Topo I:DNA interaction involves the formation of a covalent DNA:Topo I complex and a transient breakage of one of the DNA strands to relieve torsional strain on the DNA molecule. Camptothecins change these complexes into a cellular 'poison', but do not deplete Topo I. The drug interaction with the complex triggers a cascade of events that result in apoptosis [2].

9-Aminocamptothecin (9-AC) was the first analog amongst several camptothecins, including irinotecan, topotecan and GG-211, to be synthesized for clinical application [3,4]. Unlike topotecan or irinotecan, 9-AC is poorly water soluble and requires a lipophilic formulation. The National Cancer Institute (NCI) initially developed the dimethylacetamide/polyethylene glycol 400 (DMA) formulation, which required the use of glass syringes for

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handling and proved difficult to manufacture in large quantities. Pharmacia & Upjohn (Kalamazoo, Michigan, USA) later developed a 9-AC colloidal dispersion (CD) formulation that was water soluble and could be produced in commercial volume. This formulation became available after our protocol had commenced accrual and resulted in the sequential testing of the two 9-AC formulations. Preclinical studies of the CD formulation had shown the same toxicity profile as the 9-AC DMA formulation [5,6].

As part of a PO-1-funded research project, the activity of 9-AC was tested in a tumor xenograft model using NCI-1 mice. The rationale behind these experiments was based on an observation that elevated Topo I levels can be detected relative to normal tissues, in surgical specimens of colon cancer [7], lymphoma [8] as well as in xenografts of malignant melanoma, non-small cell lung cancer (NSCLC), and cancer of the breast and stomach [9]. In xenograft studies, 9-AC induced complete remissions in all treated animals when injected s.c. or i.m. on a twice-weekly schedule over a period of 4–6 weeks [7,10]. This dramatic activity was observed in tumors resistant to cytotoxic drugs such as doxorubicin, 5-flurouracil and methotrexate [9]. Furthermore, the KB-V1 and CEM-V cell lines, which express the multidrug-resistant phenotype, were found to be sensitive to 9-AC, while topotecan showed reduced cytotoxicity against these cell lines [10].

Pharmacologic studies have shown that the plasma level of the biologically active lactone form of 9-AC formulated in intra-lipid remains detectable for up to 72 h following s.c. or i.m. injection, indicating a low rate of drug elimination. This differs profoundly from 9-AC solubilized in DMA, injected i.v. or s.c. as a bolus: the 9-AC lactone peak levels are followed by rapid elimination with a half-life of several hours. Repeated treatment of murine tumors with solubilized 9-AC showed only limited effectiveness and substantial toxicity, probably due to the drug's short half-life [9,11].

Preclinical studies indicated that plasma 9-AC lactone concentration, elevated above 10 nmol/l for a prolonged time, is needed for optimal therapeutic effects [12,13]. Based on this experimental data, repeated courses of 72-h continuous infusion (CI) were recommended for clinical studies by the NCI.

Two phase I trials (Dana Farber Cancer Institute and NCI-Navy Medical Oncology Branch) established the recommended phase II dose (RPTD) for 9-AC delivered as a 72-h CI, at 45 μg/m^2/h every 3 weeks (3.24 mg/m^2/course), 35 μg/m^2/h every 2 weeks (2.51 mg/m^2/course) and 47 μg/m^2/h every week (3.38 mg/m^2/course) with granulocyte colony-stimulating factor (G-CSF) support [11,14]. Leukopenia was the dose-limiting toxicity (DLT) in all three studies. Grade 3 thrombocytopenia and minor toxicities such as nausea, vomiting, alopecia and diarrhea grade 2 or less also occurred. Minor responses were noted in patients with colon cancer, NSCLC and gastric cancer.

Two phase II studies conducted in patients with metastatic colorectal carcinoma have found the 72-h CI to be ineffective. The dose levels explored were 2.51 and 3.6–4.25 mg/m^2/course with G-CSF, both of which yielded no objective response [15,16]. A preliminary report in patients with relapsed non-Hodgkin's lymphoma showed objective responses in 10 out of 40 patients (25%) [17]. Partial responses were also observed in 33% of previously untreated patients with small cell lung cancer and in 19% of patients with ovarian carcinoma refractory to platinum-based therapy [18,19]. Although these studies demonstrated some activity with the 72-h CI, the overall response to this schedule has been judged as poor [18]. The rationale for administering 9-AC on a more prolonged schedule is to improve efficacy by exposing tumor cells to a constant low dose of drug for a prolonged period of time, thus allowing the delivery of a higher total dose.

We adopted a schema that we previously used in a phase I trial of low-dose CI topotecan. This schema involves an initial dose escalation by duration (from 7 to 21 days) and later by dose rate [20]. The rationale for prolonged administration of both 9-AC and topotecan is that both drugs have a narrow therapeutic window for dose escalation, and hence this limits their utility for monotherapy or in combination therapy [21–23].

We report here the results of a phase I study of 9-AC CI in patients with advanced refractory solid tumors. The 9-AC dose was initially escalated by duration of infusion (from 7 to 21 days) and this was then followed by a dose rate escalation where the duration of the infusion was kept constant at 21 days. The CD formulation of 9-AC was then given as a 21-day CI, with dose escalation beginning one level below the RPTD of the DMA formulation. Later, the same schema was followed on a 14-day infusion schedule.

Patients and methods

Study design

This phase I study was conducted under the auspices of the NCI/Cancer Therapy Evaluation Program and was performed at the Kaplan Comprehensive Cancer Center (KCCC) of the New York University (NYU) School of Medicine. The study accrued patients between April 1995 and February 1998. The protocol was reviewed by the Clinical Executive Management Committee (Scientific Review Committee) of the KCCC, by the Institutional Board of Research Associates (IRB) of the NYU School of Medicine and by the Review Board of the NCI

(CTEP T92-0163). Informed consent was obtained in accordance with IRB and federal guidelines.

Eligibility criteria

In order to be eligible for this protocol, patients were required to have a biopsy-proven malignant solid tumor, have failed conventional therapy or have a tumor type for which no standard therapy exists. Furthermore, patients had to be at least 18 years of age, with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2 and have measurable or assessable disease. Subjects were required to have adequate organ function, with a leukocyte count $\geq 4 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, creatinine and bilirubin ≤ 1.5mg/dl, and SGOT and alkaline phosphatase ≤ 3 times the upper limit of normal. At least 4 weeks had to have elapsed since the completion of chemotherapy and/or radiotherapy, with the resolution of all prior toxicities. Patients previously treated with nitrosourea compounds or mitomycin C were eligible if there had been no prior grade 4 hematological toxicity or delay in treatment due to prolonged myelosuppression. Pregnancy and breast-feeding was not allowed, and patients had to have an estimated life expectancy in excess of 12 weeks.

Drug administration

Patients were required to have a semi-permanent venous access device (e.g. Hickman catheter or mediport). 9-AC was delivered by a CADD ambulatory infusion pump (Pharmacia Deltec, St Paul, Minnesota, USA). The drug and diluent was supplied by NCI, Division of Cancer Treatment, in two formulations. Before 1996, 9-AC in DMA was provided in 1-ml ampules, 5 mg/ml. The dosage of drug sufficient for one course of treatment was adjusted to a total volume of 100 ml by adding an appropriate volume of sterile diluent (50% PEG 400:50% 0.01 phosphoric acid). This formulation (designated as 9-AC/DMA) was delivered to patients at a constant rate of 0.7 ml/h (16.8 ml/day). After May 1996, the trial protocol was amended for use of the improved CD formulation (56 mg dimyristoylphosphatidylcholine, 24 mg dimyristoylphosphatidylglycerol and 100 mg mannitol, USP), which replaced DMA. The new formulation was provided as 1 mg 9-AC with 56 mg CD or 2 mg 9-AC with 112 mg CD. Again, the dose of 9-AC sufficient for one course of treatment was adjusted to a final volume of 100 ml by adding an appropriate volume of a special new diluent (20% dextrose USP, 0.9% NaCl USP and sterile water for injection). With either of the two formulations, a total volume of 100 ml was used to fill the pump cassette. The cassettes were changed after 3 days for 9-AC/DMA or 6 days for 9-AC/CD.

Baseline and follow-up studies

Baseline studies included physical examination, chest X-ray, complete blood counts (CBC), serum chemistries and computerized tomography scans to document the extent of the disease. The CBC was repeated weekly, or twice weekly in patients experiencing grade 3 or 4 myelosuppression. Imaging studies as well as clinical tumor measurements were repeated after 2 cycles of treatment to assess response. Responses were then confirmed whenever possible with repeated imaging and clinical tumor measurements two months later. The WHO response criteria were used [24]

Therapeutic plan

In a prior phase I study, the RPTD for 9-AC (given as a 72-h infusion once every 2 weeks) was 2.51 mg/m^2 without CSF support. We selected the first dose level to be 1.05 mg/m^2/4-week cycle (21% of the above dose rate), because it was assumed that such a relatively low dose would allow dose escalation up to 21 days. The infusion rate was kept constant at 6.2 μg/m^2/h for the first five dose levels, while increasing the infusion duration from 7 to 21 days. From the fifth to the eighth dose level, the duration of the infusion was kept constant at 21 days, but the rate of infusion was increased from 6.2 to 21.1 μg/m^2/h (Table 1). Each dose level increase was 150% that of the previous dose level and all cycles were repeated every 28 days. When we began the studies with the CD formulation, we had already determined the maximum tolerated dose (MTD) for 9-AC/DMA (level 9, 21.1 μg/m^2/h). We therefore began the 21-day CD formulation dosing at one level below the RPTD (i.e. 14.1 μg/m^2/h). Then, in order to improve convenience, we gave the CD formulation as a 14-day infusion and escalated the dose from 28.1 to 37.5, and then reduced it to 33.3 μg/m^2/h (Table 1).

CSFs were not to be given prophylactically in this study, but were allowed at the discretion of the treating physician in the event of neutropenic fever or sepsis, after discontinuing administration of 9-AC.

Clinical pharmacology

The steady-state levels of 9-AC from patients enrolled in this study were monitored using a modification of the

Table 1 Delivered dose levels

Formulation	Dose level	Cycle duration (days)	Dose rate (μg/m^2/h)	Total dose (mg/m^2/cycle)
DMA	1	7	6.2	1.04
DMA	2	10	6.2	1.49
DMA	3	14	6.2	2.08
DMA	4	17	6.2	2.53
DMA	5	21	6.2	3.12
DMA	6	21	9.4	4.74
DMA	7	21	14.1	7.11
DMA	8	21	18.7	9.42
DMA	9	21	21.1	10.60
CD	10	21	14.1	7.11
CD	11	21	18.7	9.42
CD	12	21	25.0	12.60
CD	13	14	28.1	9.44
CD	13a	14	33.3	11.20
CD	14	14	37.5	12.60

solid-phase extraction and HPLC analysis reported by Takimito *et al.* [25]. Briefly, blood was collected in heparinized tubes and kept on wet ice until processed for plasma (centrifugation at 4°C for 10 min at 1000 *g*). The plasma was collected and processed for solid-phase extraction with the addition of camptothecin (from a stock solution of 10 ng/ml) that was used as an internal standard (final concentration 10 ng/ml). The extraction columns (100 mg C_{18}; Varian, Harbor City, California, USA) were pre-treated with 1 ml MeOH followed by 1 ml distilled/deionized water. An application of 1 ml of the patient plasma was added to the extraction column followed by 1 ml water and 1 ml of 25% MeOH/H_2O. After these washes, the extraction columns were subjected to air-drying. The 9-AC was eluted from the extraction columns with the addition of 0.75 ml of 75% MeOH/25 mmol/l KH_2PO_4, pH 2.55, into 12 × 75-mm polypropylene tubes. This eluate was combined with the effluent from an additional amount of 0.25 ml of 25 mmol/l KH_2PO_4 that was added to the extraction columns. The extracts were stored at −70°C for at least 20 min or until processing at a later date for the additional HPLC analysis steps detailed below.

Prior to the HPLC analysis, the samples were transferred to 1.5-ml Eppendorf micro-centrifuge tubes and centrifuged at 8000 *g* for 7 min. An aliquot of 200 µl of the supernatant was subjected to HPLC analysis. The HPLC analysis system consisted of an M6000 isocratic pump (Waters, Medford, Massachusetts, USA), a WISP 712B autosampler (Waters) and an Applied Biosystems 980 fluorescence detector (Perkin Elmer, Norwalk, Connecticut, USA). The detector was set for an excitation wavelength at 365 nm and fluorescence emission was monitored using a 470-nm cut-off filter. A second Waters M6000 pump was employed as a post-column elution pump. The samples were separated on a Phenomex C_{18}, 3 µm 4.6 × 150-mm Phenosphere Luna Column (Phenomex, Torrance, California, USA) eluted at a flow rate of 1 ml/min with a mobile phase consisting of 25% acetonitrile/25 mmol/l KH_2PO_4 with the outlet connected via a T-fitting (Upchurch Science, Seattle, Washington, USA). The flow rate for the post-column acidification steps was 0.2 ml/min and the post-column acidification buffer consisted of 25% acetonitrile/1% trifluoroacetic acid/distilled water. The above system was managed by a 747 Multi-System Data Controller (Axxiom Scientific, Morepark, California, USA). The five-point standard curve ranged from 0.25 to 4 ng/ml with 10 ng/ml serving as the internal standard. A least-squares analysis of the standard curve was used for calculation of the data. Data are reported as the 9-AC lactone form, which elutes at a retention time of 6 min. Mean values were calculated from at least two samples obtained after steady-state levels had been achieved, generally the 72-h and weekly samples. The pharmacodynamic correlations for absolute neutrophil count (ANC) and white blood cell (WBC) utilized the E_{max} models available in WinNonlin version 1.5 (Pharsight, Mountain View, California, USA).

Toxicity and dose modification

Toxicity was assessed according to NCI Common Toxicity Criteria, version 2.0. DLT was defined as grade 4 neutropenia lasting $\geq$ 4 days, febrile neutropenia, grade 4 thrombocytopenia lasting $\geq$ 4 days or any grade 4 non-hematologic toxicity occurring during the first cycle of treatment, excluding alopecia.

Re-treatment on day 1 of the subsequent cycle required non-hematologic toxicities to be either grade 0 or 1 (except for alopecia), and neutrophils $> 1.5 \times 10^9$/l and platelets $> 75 \times 10^9$/l. If these criteria were not met, then treatment was postponed until such time as these criteria were met (up to a maximum of 4 weeks, after which the patient was removed from the study). Patients experiencing grade 3 neutropenia or thrombocytopenia on day 1 of the subsequent cycle had a dose reduction of one dose level, while patients with grade 4 neutropenia or thrombocytopenia were taken off the study, except by special permission of the Principal Investigator, in which case the dose was reduced by two dose levels. Treatment was interrupted during a cycle if neutrophil=levels fell to $< 0.5 \times 10^9$/l or if platelet=levels fell to $< 50 \times 10^9$/l, until recovery to these levels occurred.

Dose escalation

There was no intra-patient dose escalation. Three evaluable patients were entered at each non-toxic dose level. If one of the three patients experienced DLT at any dose level, then three additional patients were accrued. If none of these patients experienced DLT, then the dose was escalated to the next level. If one or more of the three additional patients had DLT, then the patient entry at that dose level was halted. Up to three further patients were then treated at the next lower level (to a maximum of six patients). The MTD was defined as the lowest dose level at which two or more patients experienced DLT. The RPTD was defined as the prior dose level.

The criteria for removal from the study included declining PS, disease progression, patient's wishes, any grade 4 toxicity or non-compliance with therapy.

Results

Patient characteristics

Sixty-two patients were enrolled on this protocol. All were evaluable for toxicity and 57 for efficacy. All demographic data, unless otherwise stated, refers to the 62 evaluable patients (Table 2). The sex distribution was 23% male and 77% female. The median age of this population was 62 years, with a median ECOG PS of 1. The site of the primary tumor was as follows: ovarian cancer ($n = 19$), colorectal cancer ($n = 13$), breast cancer

($n = 8$), NSCLC ($n = 3$) and cholangiocarcinoma ($n = 3$). There were one or two patients per tumor type for all other cancers. Ninety-eight percent (61 patients) had received prior chemotherapy regimens, with a median of 3 per patient (range 0–11). Sixteen patients (26%) had received prior radiotherapy, while 45 patients (73%) had undergone prior surgery for their tumor (Table 2).

Drug delivery

In total, 206 completed cycles of chemotherapy were delivered to 59 patients, with a median of 3 cycles per patient and ranging up to 11 cycles per patient. Five patients were not re-evaluated for response for the following reasons: one patient was lost to follow-up after 1 cycle, one patient died of gastrointestinal hemorrhage secondary to grade 4 thrombocytopenia after having received 15 days of therapy during cycle 1, two patients withdrew consent after receiving, respectively, 5 and 2 days of therapy during the first cycle (grade 3 nausea and lethargy and grade 3 vomiting and constipation), and one patient was removed from the study after developing febrile neutropenia and Gram-negative sepsis during the first cycle.

Table 2 Patient characteristics at study entry

Characteristic	No. of patients
No. of patients	
total	62
evaluable for toxicity	62
evaluable for response	57
Sex	
male	14 (23%)
female	48 (77%)
Median age [years (range)]	62 (35–86)
ECOG PS	
0	16
1	45
2	1
Primary tumor	
ovarian	19
colorectal	13
breast	8
cholangiocarcinoma	3
NSCLC	3
Pancreatic	2
Renal	2
non-Hodgkin's lymphoma	2
cancer of unknown primary	2
miscellaneous (one patient/tumor type)	8
Prior therapy	
chemotherapy	61 (98%)
median (range) no. of chemotherapy regimens	3 (0–11) per patient
radiotherapy	16 (26%)
surgery	45 (73%)

Toxicity

With the DMA formulation of 9-AC there was no DLT for dose levels 1–7 (1.04–7.11 mg/m^2/cycle). One patient, however, did experience grade 3 nausea at dose level 3 without vomiting, although this was not clearly drug related. A fourth patient was added to this dose level without any DLT. At dose level 8 (9.42 mg/m^2/cycle), one out six patients experienced DLT (grade 4 neutropenia and thrombocytopenia). At dose level 9 (10.60 mg/m^2/cycle), two patients experienced DLT (grade 4 neutropenia and thrombocytopenia) and, hence, dose level 8 was the RPTD. One patient each experienced grade 3 anemia at dose levels 7–9. One patient had grade 3 diarrhea at dose level 9 (Table 3).

With the CD formulation of 9-AC infused over 21 days, there was an episode of DLT (grade 4 neutropenia) in one out of three patients at dose level 10 (7.11 mg/m^2/cycle). However, this myelosupression was out of proportion to that seen in the other two patients on this dose level and was felt to be due to his extensive prior treatment (three prior chemotherapy regimens and 1 year of methotrexate for psoriatic arthritis). In light of this fact, the next dose level was commenced without adding a further three patients at this dose level. At dose level 11 (9.42 mg/m^2/cycle), one patient out of six experienced DLT (grade 4 neutropenia and thrombocytopenia). At dose level 12 (12.60 mg/m^2/cycle), three out of five patients experienced DLT (three with grade 4 neutropenia and two with grade 4 thrombocytopenia). The

Table 3 Toxicity summary for the first cycle

Formulation and dose level	Total dose (mg/m^2/cycle) (days of CI)	No. of patients evaluable for toxicity	Grade 4 neutropenia	Grade 4 thrombocytopenia	Grade 3 anemia	Grade 3 non-hematologic toxicities
DMA 1	1.04 (7)	3	0	0	0	0
DMA 2	1.49 (10)	3	0	0	0	0
DMA 3	2.08 (14)	4	0	0	0	1(nausea, fatigue)
DMA 4	2.53 (17)	3	0	0	0	0
DMA 5	3.12 (21)	3	0	0	0	0
DMA 6	4.74 (21)	3	0	0	0	0
DMA 7	7.11 (21)	3	0	0	1	0
DMA 8	9.42 (21)	6	1	1	1	0
DMA 9	10.60 (21)	5	2	2	1	1 (diarrhea)
CD 10	7.11 (21)	3	1	0	2	1 (diarrhea)
CD 11	9.42 (21)	6	1	1	1	1 (vomiting)
CD 12	12.60 (21)	5	3	2	2	3 (1 vomiting, 2 diarrhea)
CD 13	9.44 (14)	5	2	2	1	0
CD 13a	11.20 (14)	7	2	1	1 (grade4)	3 (1 vomiting and fatigue, 1 diarrhea, 1 constipation)
CD 14	12.60 (14)	3	3	2	2	0

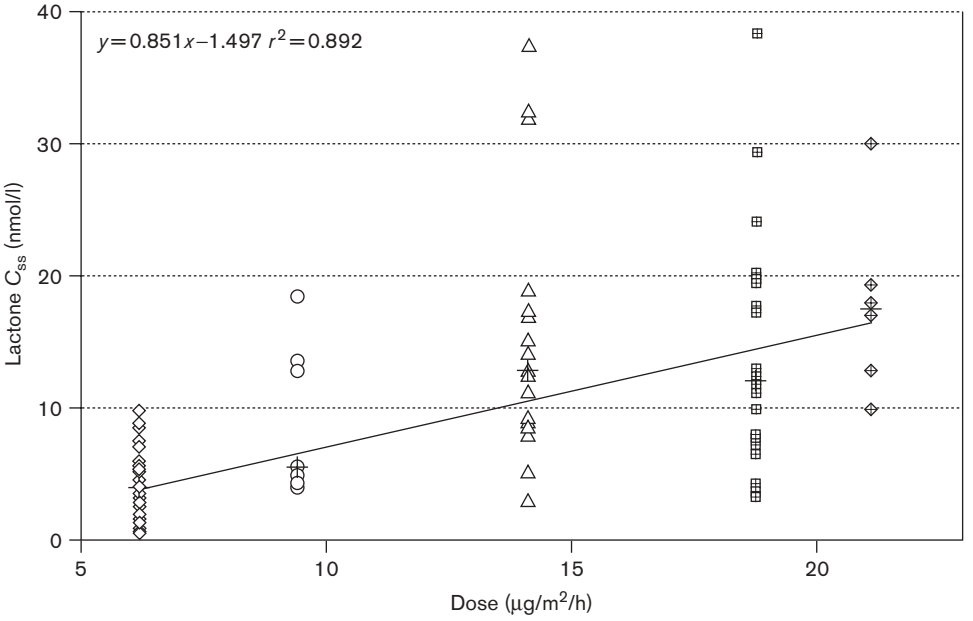
RPTD was therefore 9.42 mg/m^2/cycle (dose level 11), which was the same dose level as determined for the DMA formulation. Grade 3 anemia was experienced by two, one and two patients at dose levels 10, 11, and 12 respectively. As for non-hematologic toxicity, one patient developed grade 3 diarrhea and one patient grade 3 vomiting at dose levels 10 and 11, respectively. At dose level 12, three patients developed non-hematologic toxicities (one patient with grade 3 vomiting and two patients with grade 3 diarrhea).

At the request of the NCI, we then tested 14-day CI 9-AC/CD cycled over 21 days. At dose level 13 (9.44 mg/m^2/cycle), the initial three patients did not experience DLT. At dose level 14 (12.60 mg/m^2/cycle), three patients experienced DLT (three had grade 4 neutropenia and two had grade 4 thrombocytopenia) and two patients developed grade 3 anemia. Due to the severe toxicities encountered, we de-escalated to an intermediate dose level (13a: 11.20 mg/m^2/cycle). Even at this dose level there were two hematologic DLTs (grade 4 neutropenia in two patient and grade 4 anemia in one patient), as well as grade 3 non-hematologic toxicities in three patients (vomiting, diarrhea, constipation and fatigue). We then added two further patients to dose level 13 (9.44 mg/m^2/cycle) and both patients experienced DLT (grade 4 neutropenia and thrombocytopenia). The first patient was heavily pre-treated (six prior regimens as well as radiotherapy to their hemi-pelvis, chest wall and supraclavicular fossa), while the second patient, who was elderly (86 years old), had three prior regimens including mitomycin C. We therefore felt that the MTD for the 14-day schedule was 11.20 mg/m^2/cycle with the RPTD of 9.44 mg/m^2/cycle in patients with moderate prior therapy and without extensive radiotherapy.

Efficacy

Efficacy of treatment was a secondary endpoint in this phase I trial. Amongst the 31 evaluable patients (of 33 treated) with the DMA formulation, there was one complete response (CR), two partial responses (PRs), 15 cases of disease stabilization (SD) and 13 cases of progressive disease (PD). Amongst the cohort of 26 evaluable patients (of 29) receiving the CD formulation, there were three PRs, 10 SDs and 13 PDs. The largest cohort of patients by tumor type was that with ovarian cancer ($n = 18$ evaluable), in which we observed one CR and three PRs (response rate of 22%). All ovarian cancer patients achieving an objective response had received prior platinum, paclitaxel and cyclophosphamide. One patient with non-Hodgkin's lymphoma and one with cancer of unknown primary had a PR. The patient with lymphoma had received four prior regimens, while the cancer of unknown primary patient had received one prior regimen. The overall response rate was six out of 57 patients (11%).

Fig. 1



Steady-state 9-AC lactone levels for the DMA formulation. Each point on the graph represents a mean value per patient from three measurements taken at 24 h, 72 h and 1 week. Median values at each dose level are represented by (+). The dose level is represented by: (◇) 6.2, (○) 9.4, (△) 14.1, (⊞) 18.7 and (⊕) 21.1 µg/m^2/h. Correlation coefficient of least-squares fit of median values $r^2 = 0.89$.

Clinical pharmacology

The steady-state pharmacokinetics was investigated in all patients entered on the trial. Plasma steady-state levels were determined using the 24-h, 72-h and weekly plasma samples. Figure 1 shows a scatter plot of the mean (for individual patients) and median steady-state concentrations for 9-AC lactone, at each dose level, when given as the DMA formulation. The graph demonstrates the linearity of concentration increments with the increasing dose rate level ($r^2 = 0.89$). Similarly, Fig. 2 shows the mean (for individual patients) and median steady-state 9-AC lactone concentrations, when given as the CD formulation, at each dose level. Dose levels 9.4 and 21.1 μg/m^2/h arose as a consequence of dose reductions. The graph again demonstrates a linear relationship between increasing dose levels and increasing plasma 9-AC lactone levels ($r^2 = 0.91$).

Pharmacokinetic studies revealed that total 9-AC plasma concentration, at steady-state, was similar for both formulations of 9-AC. The median lactone concentration for the CD formulation, however, was approximately 2–fold higher than the concentration achieved with same dose of 9-AC/DMA. The gradient of the line connecting the median values is greater for the CD formulation compared to the DMA formulation. If one examines two dose levels (14.1 and 18.7 μg/m^2/h), there is significant scatter between the two groups, but median values are higher for 9-AC/CD. This difference is not statistically significant.

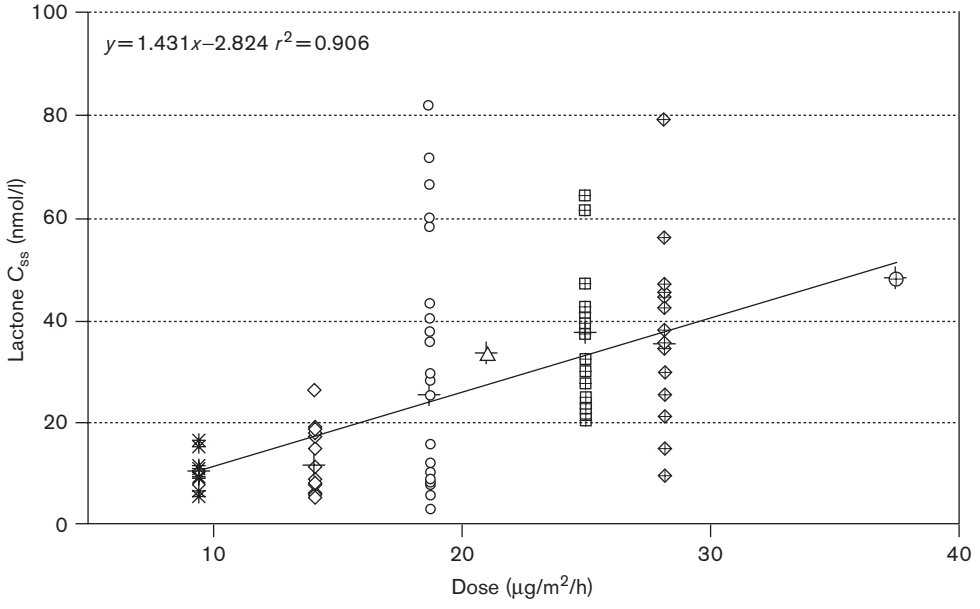
In general, for a given dose level, Fig. 3 shows the fit of the percentage change in neutrophil nadir from baseline to the 9-AC lactone steady-state level, which is fit well by an E_{max} inhibitory model (correlation of observed to predicted values = 0.75). A similar correlation was seen with white blood cell nadirs (correlation of observed to predicted values = 0.70; data not shown).

Discussion

This phase I study used 21- and 14-day continuous ambulatory infusion of 9-AC in two different formulations. As with other studies of this drug, the DLTs for both formulations were hematologic (neutropenia, thrombocytopenia and one case of anemia). Other grade 3 toxicities included anemia, fatigue and gastrointestinal toxicities (especially diarrhea).

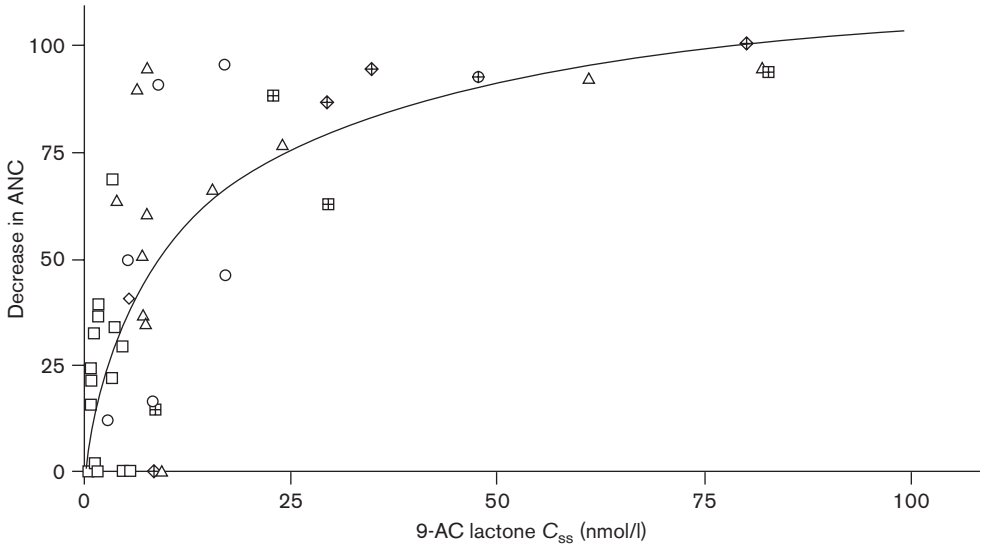
We demonstrated a linear relationship between increasing plasma 9-AC lactone concentration and increasing dose levels for both drug formulations. Interestingly, median 9-AC lactone levels for 9-AC/CD were approximately twice that achieved by 9-AC/DMA for the same dose level. This was not statistically significant for the 14.1 μg/m^2/h dose level, but approached statistical significance for the 18.7 μg/m^2/h dose level ($P = 0.0504$, Mann–Whitney U-test). This phenomenon may be due to improved stability of 9-AC/CD in plasma compared to 9-AC/DMA, thus leading to a higher 9-AC lactone concentration at steady-state. This finding may partially explain the increased toxicity observed with the 21-day

Fig. 2



Steady-state 9-AC lactone levels for CD formulation. Each point on the graph represents a mean value per patient from three measurements taken at 24 h, 72 h and 1 week. Median values at each dose level are represented by (+). The dose level is represented by: (*) 9.4, (◇) 14.1, (○) 18.7, (△) 21.1, (⊞) 25.0, (⊕) 28.1 and (⊕) 37.5 μg/m^2/h. Correlation coefficient of least-squares fit of median values $r^2 = 0.91$.

Fig. 3



Hyperbolic E_{max} model fit of the pharmacodynamic correlation between 9-AC lactone concentration at steady-state and decrease in ANC from baseline. The level is represented by: (□) 6.2, (◇) 9.4, (○) 14.1, (△) 18.7, (⊞) 25.0, (⊕) 28.1 and (o ×) 37.5 μg/m^2/h. Correlation of observed to predicted values = 0.75.

CI CD formulation at the 7.11 mg/m^2/cycle dose level, where one heavily pre-treated patient out of three experienced hematologic DLT. The 14-day CD formulation showed increased hematologic toxicity at even the lowest dose level (9.44 mg/m^2/cycle). While prior therapy with radiation, several lines of chemotherapy and advanced age undoubtedly contributed to this phenomenon, increased plasma concentration of the active lactone form may also have caused the severe myelosuppression.

The RPTD for both DMA and CD formulations of 9-AC was 9.42 mg/m^2/cycle, given as a 21-day CI, cycled every 28 days. In addition, a 14-day schedule was studied with the CD formulation to make the regimen more practical to administer. The first dose level explored (9.44 mg/m^2/3-week cycle) was tolerable for moderately pre-treated patients, whereas higher dose levels proved too toxic.

The dose intensity achieved at the RPTD of 9.42 mg/m^2/4-week cycle exceeded that of some other phase I schedules, including the NCI regimen of 2.51 mg/m^2/2-week cycle given as a 72-h infusion, the NCI phase I protocol of 2.4 mg/m^2/week, given as a 120-h infusion 3 weeks out of 4 weeks (total dose of 7.2 mg/m^2/4-week cycle), as well as the Pediatric Oncology Group (POG) RPTD of 52 μg/m^2/h, given as a 72-h CI (3.7 mg/m^2/3-week cycle) [14,18,26]. There was one PR (5%) out of 19 evaluable patients in the POG trial [26]. Another study employed a 30-min infusion of 9-AC 1.1 mg/m^2/day for 5 consecutive days, every 3 weeks (total dose 5.5 mg/m^2/3-week cycle). In this study, only one of 31 patients (3%) had a PR [27]. In our study, 11% (six out of 57) heavily pre-treated patients achieved an objective response, suggesting that the longer CI regimens may have a therapeutic advantage over shorter infusions. This finding is consistent with preclinical data from human xenografts experiments that revealed greater anti-tumor effect with prolonged exposure to Topo inhibitors [28–31]. This is particularly notable in the ovarian cancer cohort, all of whom had received three to five prior regimens, and all had failed platinum, paclitaxel and cyclophosphamide, but still achieved an objective response in 22% (four of 18) of patients. Inferior response rates have previously been reported in second-line treatment of platinum-resistant ovarian cancer patients with topotecan. Gordan *et al.* reported a response rate of 16.8%, while ten Bokkel Huinink *et al.* reported a response rate of 13.3% in such a cohort [32,33].

Future directions with 9-AC include an oral formulation (such as PEG-1000) which may mimic a CI without the inconvenience of a central venous catheter or infusion pump [34]. A phase I trial has been reported with the oral 9-AC formulation, which noted substantial inter-patient variability in the area under the curve [34]. To overcome this difficulty, the authors have used a limited pharmacological sampling model to guide individual patient doses. We are pursuing i.p. 9-AC in a phase I trial of tumors confined mainly to the peritoneal cavity [35]. Other possibilities for 9-AC include combination therapy

(e.g. with cisplatin) and further development in sensitive tumor types, particularly ovarian cancer.

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