Phase I Trial of the Colloidal Dispersion Formulation of 9-Amino-20(S)-camptothecin Administered as a 72-Hour Continuous Intravenous Infusion

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ABSTRACT

The camptothecins are a class of potent cytotoxic anticancer agents that interact with the nuclear enzyme topoisomerase I to produce lethal DNA strand cleavages. 9-Amino-20(S)-camptothecin (9AC) was introduced into Phase I clinical trials in dimethylacetamide and polyethylene glycol 400 in a 10 mM phosphoric acid vehicle for i.v. solubility. A lyophilized colloidal dispersion (CD) of 9AC for reconstitution with 20% dextrose in normal saline was developed as an alternative formulation. Patients (ages 25–75 years) with normal liver and kidney function, Eastern Cooperative Oncology Group performance status ≤ 2, and up to two prior chemotherapy regimens were treated. The initial infusion rate was 37.5 μg/m²/h as a 72-h continuous infusion (2.7 mg/m² total dose). Patient cohorts were treated with escalating infusion rates until grade 4 hematological or other grade 3 toxicity developed. Pharmacokinetic sampling was performed on all patients, and 9AC lactone concentrations in plasma were determined by a high-performance liquid chromatographic assay. Twenty-five patients received a total of 65 courses of 9AC CD at doses from 2.70 to 4.65 mg/m². The dose-limiting toxicity was neutropenia, with little nonhematological toxicity. Nonlinear regression analysis of pooled patient data yielded a total plasma clearance of 30.3 ± 4.5 liters/h/m², a half-life of 22.5 ± 8.5 h, a mean residence time of 9.7 ± 3.5 h, and a steady-state volume of distribution of 325 ± 145 liters/m². Although no objective responses were seen, 9 of 25 patients exhibited stable disease for 2–6 months. The plasma pharmacokinetics of 9AC lactone in cancer patients were comparable between the 9AC CD and soluble formulations. The dosing regimen recommended for Phase II trials of the 9AC CD formulation is 54.2 μg/m²/h, given as a 72-h continuous i.v. infusion every 3 weeks.

INTRODUCTION

CAM2 and several of the A ring-substituted derivatives have impressive in vivo activity against a variety of murine leukemia and solid tumor lines. Several of these compounds demonstrate significant activity in a variety of human tumor xenografts, including lines that are resistant to other clinically available chemotherapeutic agents (1–4). Detailed investigation of the cytotoxic and antineoplastic effects of CAM have identified topoisomerase I as its molecular target (5–9).

The structural features of CAM that are essential for topoisomerase I interaction and antineoplastic effect have been conclusively determined (1, 10, 11). The α-hydroxy-δ-lactone moiety constituting the E ring is the principal determinant of the pharmacological, physicochemical, and cytotoxic properties of the CAMs. In addition to an intact E ring, the tertiary alcohol and the chemical structures surrounding the chiral center at position 20 in the S configuration are absolute requirements for antitumor activity. The hydroxyl group renders the lactone highly reactive by enhancing the electrophilicity of the adjacent carbonyl group (1). The CAMs exist as an equilibrium mixture of intact lactone and open ring structures in aqueous solutions and biological fluids (12–15). Acidic conditions favor the intact lactone, whereas the opened ring form predominates at neutral and alkaline pH. The rate of equilibration is slower in solutions at or near neutrality compared to strongly acidic or alkaline solutions. These properties are an important consideration in the design and interpretation of the pharmacokinetic properties of CAM and its analogues in clinical trials (13, 14).

The intact lactone form of CAM is poorly soluble in vehicles suitable for parenteral administration due to the weak basicity effect of the quinoline nitrogen (1). To facilitate clinical development, the initial formulation of CAM was as a water-soluble sodium salt of the carboxylic acid of the opened lactone ring structure (2). The initial trials were disappointing because of a poor response rate and dose-limiting myelosuppression, diarrhea, and hemorrhagic cystitis (16–18). The investigations performed subsequent to these trials showed the sodium salt of CAM is inactive when administered i.v. because conversion to the active form with an intact E ring lactone is negligible (19).

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2 The abbreviations used are: CAM, camptothecin; 9AC, 9-amino-20(S)-camptothecin; DMA, dimethylacetamide; CIV, continuous i.v.; CD, colloidal dispersion; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; AUC, area under the concentration-time curve; CL, clearance; CV, coefficient of variation.
There have been continued efforts to identify analogues with greater therapeutic efficacy based on understanding the chemistry of CAM, the recognition of topoisomerase I as the target, and the finding of elevated levels of topoisomerase I in cancer cells (20). Substitutions in the A ring at the 9, 10, and 11 positions confer better solubility characteristics and enhance antitumor activity (4). 9AC is one such analogue. 9AC has exceptional efficacy in several resistant human colon cancer cell lines in athymic mice, producing cures in resistant human colon cancer cell lines in athymic mice, producing

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There have been continued efforts to identify analogues with advanced tumors without substantial toxicity (21). 9AC is a neutral compound at physiological pH and, in contrast to topotecan, is not a substrate for the multidrug-resistant efflux pump (22, 23). These observations supported the further preclinical and eventual clinical development of 9AC.

Under the direction of the National Cancer Institute, a parenteral vehicle of DMA/polyethylene glycol 400 in 10 mm phosphoric acid (2:49:49, v/v/v; soluble DMA) was developed to solubilize the drug. This soluble formulation was used for the initial Phase I clinical trials of 9AC administered as a 72-h CIV infusion, which began in 1992 (24, 25). A lyophilized CD for reconstitution with 20% dextrose in normal saline was subsequently developed as an alternative parenteral formulation. Comparative preclinical trials concluded that the toxicity profiles of the soluble and CD formulations of 9AC in the rat and dog are similar. This study represents the first report of the CD formulation of 9AC administered as a 72-h CIV in cancer patients. The results demonstrate that this formulation is well tolerated in cancer patients and that the pharmacokinetics of the CD and soluble DMA formulations are comparable.

PATIENTS AND METHODS

Patient Selection. Eligibility criteria for this study included: age of >18 years, a histologically confirmed malignancy, no potentially curative therapy, and a life expectancy of >2 months. The study was limited to patients with solid tumor malignancies who had received up to two previous chemotherapy regimens and who had completed any prior chemotherapy, surgery, or radiation >3 weeks prior to entry. Other requirements were: an Eastern Cooperative Oncology Group performance status of 0–2, a serum creatinine level of <1.5 mg/dl, serum aspartate aminotransferase and bilirubin levels of <1.5 times the upper limit of normal, WBCs at >3,000/μl, and platelets at >100,000/μl. A central venous access device was placed if not already present. All patients signed an informed consent, which met federal and institutional requirements.

Dosage and Drug Administration. A lyophilized CD of 9AC for injection was supplied as 1- or 2-mg vials, together with 20% dextrose-0.9% sodium chloride in 50- or 100-ml glass vials by the National Cancer Institute (Bethesda, MD). Each 1-mg vial of 9AC also contained 56 mg of dimyristoylphosphatidylcholine, 24 mg of dimyristoylphosphatidylglycerol, and 100 mg of mannitol USP. Shortly before administration, the drug was dispersed to a concentration of 100 μg/ml by the addition of 20% dextrose-0.9% sodium chloride and gentle shaking. The dispersion was loaded into a 50-ml medication cassette reservoir (SIMS Deltec, St. Paul, MN) and further diluted with the same vehicle to concentrations as close to 20 μg/ml 9AC as practical, depending upon the intended dose. CADD-1 Model 5100 or CADD-Plus Model 5400 programmable ambulatory infusion pumps (SIMS Deltec) were used to deliver the drug through a central venous catheter, without an in-line filter, at the desired infusion rate. New medication cassettes containing freshly reconstituted 9AC dispersions were placed in the pump every 24 h due to stability considerations of the formulation.

Treatment Plan. All patients had complete laboratory studies and disease assessments performed within 14 days of the start of treatment. Patient histories and physical examinations were performed weekly, and a complete blood count was performed twice weekly. Three patients were entered at each dose level, with three additional patients added upon occurrence of DLT, defined as grade 4 hematological toxicity for ≥7 days or grade 3 nonhematological toxicity (Common Toxicity Criteria; Ref. 26). With occurrence of a DLT in two patients in any cohort of three to six, the preceding dose was established as the MTD, and 13 additional patients were enrolled at that level.

Response Criteria. Complete response was defined as the disappearance of all measurable disease, signs, symptoms, and biochemical changes related to the tumor for 4 weeks, during which time no new lesions appeared. Partial responses were defined as >50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions for ≥4 weeks, with the appearance of no new lesions. Stable disease was defined as <50% decrease or <25% increase in the sum of the products of the perpendicular diameters of all measurable lesions and the appearance of no new lesions for 4 weeks. Progressive disease was defined as a >25% increase in the sum of the products of the perpendicular diameters of any measurable lesion or the appearance of any new lesion.

Pharmacokinetic Sampling and Quantitation. Blood specimens were acquired to characterize the plasma pharmacokinetics of 9AC lactone in all patients during the first course of treatment. Blood was drawn directly into Vacutainer Brand plasma tubes with freeze-dried sodium heparin (Becton Dickinson, Franklin Lakes, NJ) from a peripheral venous catheter placed in an arm of the patient. Samples were collected shortly before treatment and at 4, 24, 48, 72, 74, 75, 80, 96, 104, and 120 h after the start of infusion. Sample tubes were immediately placed on ice and transported to the analytical laboratory. All blood specimens were centrifuged (800 × g, 10 min, 10°C) within 1 h after collection, whereupon duplicate aliquots of the separated plasma were immediately prepared for the analysis of 9AC lactone using a reported solid-phase extraction procedure (27). The extracts were quantified by reversed-phase high-performance liquid chromatography with in-line postcolumn acidification prior to fluorescence detection, as described previously (14).

Quantitation was performed by similarly assaying, in duplicate, a series of six plasma standards with added concentrations of 9AC lactone ranging from 0.25 to 10.0 nm and a drug-free sample, together with each set of patient samples. The ratio of the chromatographic peak area for 9AC to that of the internal standard (CAM) was calculated. Standard curves were constructed by plotting the average peak area ratios against the corresponding known drug concentration. The drug concentration in patient samples was calculated using the slope and intercept of the line fit to the standard curve by unweighted linear regression. Specimens with concentrations exceeding the upper limit of the standard curve were reassayed upon dilution with pretreatment plasma.

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Pharmacokinetic Data Analysis. Individual patient plasma concentration-time data were analyzed by noncompartmental methods using the WinNonlin Version 1.1 software package (Scientific Consulting, Apex, NC; Ref. 28). Area under the plasma profiles from time zero to infinity (AUC) was estimated using the logarithmic-linear trapezoidal algorithm to the last data point with extrapolation to time infinity using the estimated value of the slope of the terminal logarithmic-linear disposition phase (−λ2). Total plasma CL was calculated as the dose divided by AUC. The apparent steady-state plasma concentration (CSS) of 9AC was calculated as the geometric mean of the observed values at 24, 48, and 72 h during the infusion.

Geometric mean plasma concentrations of 9AC at each time point were calculated from the individual patient data in groups that received the same dose. Equations having the general form:

\[ C = \sum_{i=1}^{n} C_i \left( e^{-\lambda_i \tau} - e^{-\lambda'_i} \right) \]

were fit to the geometric mean plasma concentration-time profiles by weighted nonlinear least squares regression using WinNonlin. The equation was derived according to the principles developed by Benet (29) and simplified to eliminate compartmental attributes. The value of \( t' \) is zero until the infusion of duration \( \tau \) has terminated, upon which it becomes defined as \( t' = t - \tau \), where \( t \) denotes time relative to the beginning of the infusion. The coefficients \( C_i \) are intercept values, corresponding to iv bolus administration of the dose, of each logarithmic-linear phase with a slope \( \lambda_i \), such that \( \lambda_1 > \lambda_2 > ... > \lambda_n \). Parameters corresponding to the terminal disposition phase are designated with a subscript \( \tau \) by convention. Initial estimates of the iterated parameters (\( C_i \) and \( \lambda_i \)) were determined automatically by the program and then refined using the Levenberg-Hartley modification of the Gauss-Newton algorithm, with upper and lower boundaries on the parameters specified. For each plasma profile, the number of parameters in the fitted equation and influence of the weighting factor, \( y_{obs}^{-n} \) (\( n = 0, 1, \) or 2), were evaluated to ascertain the best fit of the experimental data. This was assessed by a variety of considerations, which included visual inspection of the predicted profile, residual analysis, values of the Akaike and Schwartz Information Criterion, sum of weighted squared residuals, mean SD, degree of correlation between parameters, and WCs of the parameter estimates (30). In each case, weighting according to \( y_{obs}^{-2} \) proved optimal. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic terms according to standard equations (28).

Mean values of pharmacokinetic parameters and related terms are reported as the arithmetic mean ± SD, with the following exceptions: harmonic mean half-lives ± jackknife estimate of the SD (31); and geometric mean apparent steady-state or maximum plasma concentrations ± jackknife estimate of the SD.

Stability of 9AC in Human Blood. The stability of 9AC was determined in fresh whole blood maintained ice-cold and at ambient temperature. Heparinized blood obtained from a healthy male volunteer was pipetted into glass test tubes and allowed to equilibrate at room temperature or within an ice bath. Kinetic runs were initiated by adding a solution of 9AC in DMSO (10 μM) to the temperature-equilibrated blood to provide an initial concentration of 20 nm. After mixing by repeated inversion, an aliquot of blood (2 ml) was withdrawn and immediately processed to determine the 9AC lactone concentration in plasma (0.5 ml), as described above. Additional aliquots of blood were periodically withdrawn and similarly analyzed for 4–6 h. The contents of the tube were frequently mixed by gentle inversion during the course of the experiment to ensure that the erythrocytes remained uniformly suspended. The plasma derived from each blood sample was assayed in duplicate. Apparent first-order rate constants for the loss of 9AC lactone (kobs) were determined by linear regression analysis of a semilogarithmic plot of the observed 9AC lactone concentrations in plasma as a function of time. The time for 5% degradation of 9AC lactone in whole blood was calculated as \( -\ln(0.95)k_{obs} \).

RESULTS

Patient Characteristics. Table 1 lists the distribution of age, sex, performance status, cancer type, and prior therapy characteristics for the 25 patients who received a total of 65 courses of 9AC CD. The patient population was similar to that in the previous 9AC trial of the soluble formulation performed at the Dana-Farber Cancer Center (24). There were no previously untreated patients in this group. The median number of courses was three, with a maximum of eight.

Toxicity. 9AC CD administered as a 72-h CIV infusion was reasonably well tolerated. No allergic or other acute problems related to the agent itself occurred. Myelosuppression was the major toxicity encountered. Neutropenia was the DLT observed with this schedule. The MTD was 3.9 mg/m² (54.2 μg/m²/h over 72 h), repeated every 3 weeks. As listed in Table 2, severe neutropenia occurred at every dose level except for the first (2.7 mg/m²). Because the definition of neutropenic DLT in
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Table 2  Hematological toxicity

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>n</th>
<th>Hematocrit (%)</th>
<th>Platelets 10³/µl</th>
<th>Leukocytes 10³/µl</th>
<th>Neutrophils 10³/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Course 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.7</td>
<td>3</td>
<td>31.8 (26.5–35.8)*</td>
<td>234 (131–305)</td>
<td>6.76 (3.80–9.10)</td>
<td>4.20 (2.80–4.15)</td>
</tr>
<tr>
<td>3.3</td>
<td>3</td>
<td>28.1 (27.6–28.8)</td>
<td>99 (190–106)</td>
<td>1.63 (1.10–2.00)</td>
<td>0.73 (0.04–1.20)</td>
</tr>
<tr>
<td>3.9</td>
<td>16</td>
<td>30.4 (18.9–39.6)</td>
<td>141 (5–286)</td>
<td>3.46 (0.30–6.80)</td>
<td>2.47 (0.04–4.82)</td>
</tr>
<tr>
<td>4.65</td>
<td>3</td>
<td>27.5 (22.9–31.2)</td>
<td>59 (31–99)</td>
<td>1.27 (0.6–2.31)</td>
<td>0.29 (0.02–0.61)</td>
</tr>
<tr>
<td>All courses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.7</td>
<td>5</td>
<td>28.8 (26.9–32.4)</td>
<td>174 (66–324)</td>
<td>4.23 (2.40–6.90)</td>
<td>2.20 (1.10–4.10)</td>
</tr>
<tr>
<td>3.3</td>
<td>8</td>
<td>27.6 (21.3–31.2)</td>
<td>195 (80–262)</td>
<td>3.46 (2.1–5.00)</td>
<td>2.00 (0.22–3.90)</td>
</tr>
<tr>
<td>3.9</td>
<td>28</td>
<td>30.37 (23.7–39.3)</td>
<td>189 (15–257)</td>
<td>3.63 (0.30–5.20)</td>
<td>2.24 (0.06–8.70)</td>
</tr>
<tr>
<td>4.65</td>
<td>3</td>
<td>27.0 (28.0–31.3)</td>
<td>160 (114–208)</td>
<td>2.53 (1.40–3.60)</td>
<td>1.18 (0.40–1.9)</td>
</tr>
</tbody>
</table>

* Values in parentheses represent ranges.

Values in parentheses represent total numbers of courses administered.

Table 3 Nonhematological toxicity

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>Nausea Gr2/Gr3&amp;4*</th>
<th>Diarrhea Gr2/Gr3&amp;4</th>
<th>Mucositis Gr2/Gr3&amp;4</th>
<th>Bilirubin Gr2/Gr3&amp;4</th>
<th>LFTs Gr2/Gr3&amp;4</th>
<th>Alopecia Gr2/Gr3&amp;4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Course 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.7 (3)*</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>3.3 (3)</td>
<td>1/0</td>
<td>0/0</td>
<td>1/0</td>
<td>0/0</td>
<td>0/0</td>
<td>2/0</td>
</tr>
<tr>
<td>3.9 (16)</td>
<td>2/0</td>
<td>1/0</td>
<td>0/0</td>
<td>0/1</td>
<td>0/1</td>
<td>0/0</td>
</tr>
<tr>
<td>4.65 (3)</td>
<td>1/0</td>
<td>1/0</td>
<td>1/0</td>
<td>0/1</td>
<td>0/1</td>
<td>1/0</td>
</tr>
<tr>
<td>All courses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.7 (6)</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>3/1</td>
<td>1/0</td>
<td>0/0</td>
</tr>
<tr>
<td>3.3 (8)</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>3.9 (27)</td>
<td>4/0</td>
<td>2/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>4.65 (3)</td>
<td>1/0</td>
<td>1/0</td>
<td>1/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
</tbody>
</table>

* Gr. grade: LFT, liver function test.

Responses. There were no complete or partial responses to 9AC in this study. Stabilization of previously progressive disease was noted in 9 of 24 patients presenting with measurable tumors. Three patients had stable disease for over 3 months, and a single patient with refractory gastric adenocarcinoma had stabilization of her malignant disease over a period of eight courses for 6 months. Five of nine patients with colorectal adenocarcinomas had stabilization of their tumors, three had stabilization for five courses over 4 months, and two had stabilization for four courses over 3 months. Two of four patients with non-small cell lung cancer had stable disease for four courses given over 3 months. One patient with a small bowel adenocarcinoma and one with a renal cell carcinoma experienced brief stabilization of disease. All patients with measurable cancers eventually demonstrated tumor progression during treatment.

Pharmacokinetics. An assessment of the stability of 9AC lactone in whole blood, which had not been previously reported, was undertaken to determine whether hydrolysis to the opened lactone ring form of the drug prior to the initial centrifugation of patient specimens would significantly affect the assay results. 9AC lactone was added to ice-cold and ambient-temperature whole blood. The concentration of 9AC lactone in plasma derived from aliquots of blood, removed periodically during a 4–6-h period, decreased in an apparent first-order manner.
manner. The half-life and time for 5% degradation of 9AC lactone in whole blood maintained at ambient temperature were 58.2 min and 4.3 min, respectively. In contrast, the half-life was 11.8 h in ice-cold blood, and the time for 5% degradation was 52 min. These findings affirmed that it is absolutely critical that patient samples be rapidly cooled over ice immediately upon collection and prepared for analysis within 1 h thereafter.

Standard curves of 9AC lactone in plasma at concentrations ranging from 0.25 to 10.0 nm were prepared and assayed together with each set of patient samples. Accuracy and precision of the assay were determined by analyzing the back-calculated drug concentrations and regression parameters for 14 standard curves assayed during a 9-month period. The 9AC lactone-to-internal standard (CAM) peak area ratios were directly proportional to the added drug concentration in plasma throughout this concentration range. The mean correlation coefficient of the standard curves was 0.9993 ± 0.0006. The mean slope was 0.0669 ± 0.0172, and the y-intercept (-0.0139 ± 0.0340) was not significantly different from the origin. The CV for interday quantitation of 9AC lactone in plasma ranged from 0.36% for the 10.0 nm standards to 6.3% at 0.25 nm, the lowest concentration included in the standard curve. The mean predicted concentration of 9AC lactone in the plasma standards ranged from 98.7 to 103.8% of the actual concentration. Accuracy for the quantitation of 9AC lactone did not appear to be dependent upon its concentration.

The plasma pharmacokinetics of 9AC lactone were studied in all 25 patients during the first cycle of treatment, with the CD formulation given as a 72-h i.v. infusion at doses ranging from 2.70 to 4.65 mg/m². As shown in Fig. 1, the plasma concentration of 9AC lactone increased rapidly after the infusion was started. Plasma levels of the active form of the drug were essentially constant from the time that the first sample was acquired 4 h after dosing until the infusion was terminated. The apparent Cₘₙₐₜ of 9AC lactone, calculated from the geometric mean of the observed concentrations at 24, 48, and 72 h in individual patients, increased from a mean value of 3.49 ± 0.54 nm (n = 3) in the group treated with the starting dose of 2.70 mg/m² to 6.16 ± 2.64 nm in the patients receiving a total dose of 4.65 mg/m². Mean values of the pharmacokinetic parameters estimated by noncompartmental analysis of the individual patient data were presented in Table 4. Because 16 patients were entered at the MTD of 3.9 mg/m² (54.2 μg/m²·h) and only three patients were entered at each of the other three dose levels, the MTD cohort dominates the cumulative mean values of pharmacokinetic parameters.

The pharmacokinetic behavior of 9AC lactone appeared to be linear, as demonstrated by the tendency toward proportionate increases in the AUC, as well as the apparent Cₘₐₓ, with escalation of the total dose administered (Fig. 2). The mean total plasma CL was 39.7 ± 21.0 liters/h/m² in 24 evaluable patients, and the mean residence time of the drug in the body was 7.0 ± 4.8 h (mean ± SD; n = 20). The terminal disposition phase of 9AC lactone was reliably estimated in only a few patients. The apparent volume of distribution at steady state was relatively large, with a mean value of 305.5 ± 142.3 liters/m² (n = 20). The pharmacokinetic parameters of 9AC lactone exhibited a moderate degree of interpatient variability, as indicated by the CVs for the total plasma CL, mean residence time, and steady state-apparent volume of distribution for the entire cohort of patients, which ranged from 46.6 to 69.3%.

Although the loss of drug from plasma after the infusion had ended was not well characterized in individual patients, a marked improvement in the data were afforded by calculating the geometric mean of the observed 9AC lactone concentrations at each time point for groups of patients that received the same dose. The decrease in 9AC lactone plasma levels was distinctly biexponential in each of the geometric mean plasma profiles, with the exception of the 3.30 mg/m² dose group, for which the postinfusion data remained confounded (Fig. 1). Nonlinear regression afforded excellent fits of the entire geometric mean plasma concentration-time profiles for the 2.70, 3.90, and 4.65 mg/m² dose groups. Values of the pharmacokinetic parameters for 9AC lactone estimated by nonlinear regression analysis of
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Table 4  Mean 9AC lactone pharmacokinetic parameters estimated by noncompartmental analysis of individual patient plasma concentration-time data

<table>
<thead>
<tr>
<th>Total dose (mg/m²)</th>
<th>No. of patients</th>
<th>Apparent Cₘ₀ (nm)</th>
<th>AUC (nMh)</th>
<th>CL (liter/h/m²)</th>
<th>MRT* (h)</th>
<th>Vₘ₀ (liter/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.70</td>
<td>3</td>
<td>3.49 (0.54)</td>
<td>251.8 (54.8)</td>
<td>30.4 (6.3)</td>
<td>7.8 (1.4)</td>
<td>247.5 (85.4)</td>
</tr>
<tr>
<td>3.30</td>
<td>3</td>
<td>4.39 (0.9)</td>
<td>353.8 (70.0)</td>
<td>26.3 (5.1)</td>
<td>11.5 (2.0)</td>
<td>300.0 (95.3)</td>
</tr>
<tr>
<td>3.90</td>
<td>16</td>
<td>4.13 (2.46)</td>
<td>317.8 (223.1)</td>
<td>46.2 (3.6)</td>
<td>5.7 (3.8)</td>
<td>309.2 (169.1)</td>
</tr>
<tr>
<td>4.65</td>
<td>3</td>
<td>6.16 (2.64)</td>
<td>515.8 (228.4)</td>
<td>28.0 (10.9)</td>
<td>16.2 (1.8)</td>
<td>378.2 (186.4)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td>39.7 (21.0)</td>
<td>7.0 (4.8)</td>
<td>305.5 (142.3)</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td></td>
<td></td>
<td>52.8</td>
<td>69.3</td>
<td>46.6</td>
</tr>
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* MRT, mean residence time; Vₘ₀, apparent volume of distribution at steady state.

Numbers in parentheses represent SD.

Fig. 2  Plots of the steady Cₘ₀ (A) and AUCs (B) of 9-AC lactone behavior of the drug when administered as a 72-h CIV infusion. Data points, means of the individual patient apparent Cₘ₀ and AUC values in groups treated with 2.70 (n = 3), 3.30 (n = 3), 3.90 (n = 16), and 4.65 (n = 3) mg/m² doses of 9-AC formulated as a CD. bars, SD. ——, generated by linear regression, for which the correlation coefficients were 0.981 for the apparent Cₘ₀ and 0.969 for the AUC relationships.

During the continuous infusion of the drug. This seemingly anomalous behavior can be attributed to the predominant influence of the initial disposition phase on 9AC pharmacokinetics, which not only accounts for 73.7 ± 4.2% of the AUC but also shows a half-life that is rapid (0.90 ± 0.10 h) in comparison to the terminal phase.

Pharmacodynamics. Individual patient total nadir WBC count as a function of total drug exposure (AUC) for all 25 patients during the first course of treatment showed a significant relationship (multiple r² = 0.2011, P = 0.0246) between the total AUC and the nadir WBC count. At the 3.9 mg/m² dose level, a median AUC of ~300 nM-h produced a significant difference between the nadir leucocyte count of those patients above or below this level (Fig. 2).

DISCUSSION

After the initial disenchantment of the early 1970s, a resurgence of interest in CAM followed the elucidation of its pharmacological properties and mechanism of action. There has been a proliferation of analogues developed over the last decade. In 1996, topotecan (for refractory ovarian cancer) and irinotecan (for refractory colorectal cancer) received Food and Drug Administration approval. 9AC showed significant preclinical activity (cures) in human tumor xenografts in athymic mice that are resistant to all clinically available chemotherapeutic agents. 9AC was effective not only against small tumors (0.2–0.25 cm³ in size) but also bulky tumors (2.5–8.0 cm³). Compared to CAM, responses occurred at lower doses and more rapidly with 9AC, and no treatment failures due to resistance were observed during treatment (reviewed in Ref. 32).

Previous investigations have established myelosuppression, principally neutropenia, as the DLT of 9AC. In a Phase I trial of the soluble DMA formulation of 9AC as a 72-h CIV infusion in nonhematological malignancies, the DLT consisted of neutropenia. Thrombocytopenia was also prominent. Nonhematological toxicities were mild and consisted of nausea/vomiting, diarrhea, mucositis, fatigue, and alopecia (24). Similar toxicities were observed in a Phase I trial of the soluble DMA formulation 9AC at the National Cancer Institute (25). As in the Phase I trials of the soluble DMA formulation, here we demonstrated that neutropenia was dose limiting. Severe neutropenia was infrequent (11% of patients and 14% of courses) and brief, so that only four patients (6%) required therapy for febrile neutropenia.

In a Phase I study using the soluble DMA formulation of
9AC, given as a 72-h CIV infusion to patients with characteristics that were generally similar to those in our study, the mean apparent $C_{\text{app}}$ of 9AC lactone was found to increase proportionately from 0.9 to 8.5 nm as the infusion rate was escalated from 5 to 59 $\mu$g/m$^2$/h (24, 31). We observed that values of the $C_{\text{app}}$, derived from nonlinear regression analysis of pooled 9AC lactone plasma profiles at each dose, also increased in a linear manner from 3.4 nm at the initial infusion rate of 37.5 $\mu$g/m$^2$/h to 6.4 nm at 64.6 $\mu$g/m$^2$/h. Accordingly, there was no statistical difference between the mean $C_{\text{app}}$ of 9AC lactone calculated from the averaged CL values at each dose level of the drug administered as the CD (30.3 ± 4.5 liters/h/m$^2$, $n = 4$) and soluble formulation (26.1 ± 6.1 liters/h/m$^2$, $n = 7$; two-tailed $t$ test, $p = 0.05$). However, the degree of interpatient variability of the 9AC lactone apparent $C_{\text{app}}$ tended to be somewhat greater with the CD preparation (median CV, 31.7%; range, 15.5–59.6%) than was reported for the soluble formulation (median CV, 25.4%; range, 15.4–70.8%). This difference was particularly evident at the MTD, for which the CV of apparent $C_{\text{app}}$ values in 16 patients treated with 3.90 mg/m$^2$ of 9AC CD was 59.6%, whereas 15 patients receiving 4.25 mg/m$^2$ of the soluble formulation exhibited a CV of only 20.8%.

Due to the potential for a relatively high degree of variability in systemic exposure to the active form of the drug, individualizing the 9AC infusion rate in patients expected to receive multiple courses of therapy may be a worthwhile consideration for future Phase II studies using the CD formulation. Because apparent steady-state conditions are rapidly approached, this could be facilitated by measuring 9AC lactone plasma levels in a limited number of specimens acquired during the first infusion of drug to each new patient, such as at 48 and 72 h (25). The benefits of establishing individualized doses include maximizing the possibility for a therapeutic response, limiting toxicity, and obtaining a greater understanding of the population pharmacokinetics of this investigational drug.

Neither the absolute neutrophil count nor the total WBC count were significantly correlated with the dose administered. There was a trend toward grade 3 or 4 leukopenia in patients when the AUC exceeded 400 nm$h$. There are outliers of $>2$ SDs on both ends. The variability in plasma levels of 9AC has a correlate in the variability of response to the drug. There are undoubtedly unknown variables that figure significantly in translating plasma drug exposure into a cytotoxic event, such as distribution of topoisomerase I within populations of various cell types and proliferative states.

There were no objective responses to 9AC in this trial. However, disease stabilization for periods of 2–6 months was evident in 9 patients, including 7 of 16 treated at the MTD of 3.9 mg/m$^2$. Evidence of activity has also been observed in a Phase II study of 9AC in patients with previously untreated advanced stage non-small cell lung cancer. There were three partial responses in 28 patients treated with total doses of 3.3 or 4.2 mg/m$^2$, given as a 72-h CIV infusion every 2 weeks with granulocyte colony-stimulating factor support (32).

Clear indications of antitumor activity have been observed during the early clinical evaluation of 9AC. However, whether the impressive activity of the drug demonstrated against preclinical tumor models (33) will ultimately be realized in the clinical setting remains a matter of some concern. Levels of topoisomerase I, the molecular target of the CAMs, remain relatively constant throughout the cell cycle (34). In contrast, the interaction resulting in eventual cytotoxicity, collision of the DNA replication fork with a stabilized ternary complex consisting of topoisomerase I, single-stranded DNA, and drug, occurs only during the S-phase. Therefore, continuous exposure to the active intact lactone form of the drug should result in optimal therapeutic effects. Consistent with this inference, maintaining the plasma concentration of 9AC lactone above 10 nm for at least 48 h was required to effect complete regressions against a human tumor xenograft model (35). On the basis of these findings, a 3–5-day CIV infusion schedule was recommended for the initial Phase I clinical trials of 9AC. However, the 9AC lactone $C_{\text{app}}$ achieved in patients with solid malignancies during treatment with the MTD of the drug when given as a 72-h CIV infusion are considerably lower than 10 nm. Specifically, the MTD identified here, 3.90 mg/m$^2$, afforded a 9AC lactone geometric mean $C_{\text{app}}$ of only 4.1 ± 2.5 nm ($n = 16$). The mean $C_{\text{app}}$ in patients similarly treated with a total dose of 2.52 mg/m$^2$, using the soluble formulation of 9AC, was 2.6 ± 0.7 nm ($n = 3$; Ref. 25). The MTD established for the soluble formulation of the drug as a 72-h infusion with granulocyte colony-stimulating factor support, 3.48 mg/m$^2$, provided a mean $C_{\text{app}}$ of 5.2 ± 1.1 nm ($n = 15$). In contrast to topotecan, the soluble CAM analogue, which has shown promising clinical activity against refractory ovarian cancer (36), 9AC lactone is essentially completely bound to plasma proteins at the concentrations achieved in patients, further diminishing the effective concentration of the active form of the drug.

In summary, the plasma pharmacokinetics of 9AC lactone in cancer patients treated with the CD were comparable to those of the
soluble DNA formulation. There was an inverse correlation between 9AC lactone AUC and total WBC count. Aside from neutropenia, little toxicity was encountered. No cumulative toxicity was evident. 9AC did not produce any complete or partial neutropenia, little toxicity was encountered. No cumulative toxicity between 9AC lactone AUC and total WBC count. Aside from neutrosoluble DMA formulation. There was an inverse correlation between replication forks and topoisomerase I-DNA cleavage complexes: studies in a cell-free SV-40 DNA replication system. Cancer Res., 53: 5908–5914, 1993.

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