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Phase I and Pharmacokinetic Study of Arabinofuranosyl-5-azacytosine (Fazarabine, NSC 281272)

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ABSTRACT

A Phase I clinical trial of 1-β-D-arabinofuranosyl-5-azacytosine (ara-AC or fazarabine) given as a 72-h continuous infusion on a 21-day cycle was conducted in 27 adult patients with refractory cancer. The major toxicity was reversible granulocytopenia and thrombocytopenia. Dose-limiting toxicity was observed at a dose rate of 1.96 mg/m²/h in which Grade IV leukopenia (WBC <1,000/mm³) occurred in 4 of 11 patients and Grade IV thrombocytopenia (platelets <25,000/mm³) occurred in 3 of 11 patients. Plasma steady-state levels ranged from 0.13 to 0.6 μM for doses of 1.25 to 5.94 mg/m²/h. Mean total body clearance was 647 ml/min/m². Minor clinical responses were seen in one patient with testicular cancer, one patient with colon cancer, one patient with breast cancer, and one patient with acute nonlymphocytic leukemia. Another patient with adenocarcinoma of unknown primary had stable disease during 13 cycles of therapy. Based on the results of this study, the recommended dose for Phase II studies of 1-β-D-arabinofuranosyl-5-azacytosine administered as a 72-h continuous infusion is 2.0 mg/m²/h (48 mg/m²/day).

INTRODUCTION

ara-AC[2] (fazarabine; NSC 281272) is a synthetic pyrimidine nucleoside containing the structural features of two effective antineoplastic agents, ara-C and 5-AC (Fig. 1). ara-AC combines the arabinose sugar of ara-C (with its inverted hydroxyl group at the 2′-position) with the triazine base of 5-AC, in which nitrogen is substituted for the C-5 of cytidine. This triazine ring is highly susceptible to reduction and nucleophilic reactions. Thus, ara-AC, like 5-AC, undergoes decomposition in aqueous solution to inactive products through the addition of water to its 5-6 double bond with concomitant ring opening. In contrast, when ara-AC is dissolved in organic solvents such as DMSO, its stability is markedly enhanced and there is little hydrolysis of the triazine ring (3, 4).

ara-AC, like ara-C, apparently enters cells via a nucleoside transport mechanism and is subsequently activated through phosphorylation by deoxycytidine kinase (5, 6). Degradative pathways for both ara-AC and ara-C involve phosphatases which can cleave phosphate groups, from the arabinofuranosyl moiety. While ara-C can be converted to an inactive metabolite, 1-β-D-arabinofuranosyluridine, by cytidine deaminase (7), ara-AC is a poor substitute for this enzyme and is relatively refractory to inactivation by deamination (5).

The similarity in activation via deoxycytidine kinase suggests that cross-resistance between the two nucleoside analogues, ara-C and ara-AC, can occur (8, 9). Indeed, P388 murine leukemia cells that are resistant to ara-C because of a decrease in deoxycytidine kinase activity are also cross-resistant to ara-AC (9). In contrast, HL-60 cells deficient in uridine/cytidine kinase are resistant to 5-AC (which undergoes intracellular phosphorylation by this enzyme) but sensitive to both ara-C and ara-AC (10).

Although the mechanism of ara-AC cytotoxicity is not well understood, this analogue is incorporated into cellular DNA in a dose- and time-dependent manner (5, 6, 11). Furthermore, ara-AC inhibition of DNA synthesis in L1210 cells occurs at drug doses that do not affect RNA or protein synthesis. Thus, the mechanism of ara-AC cytotoxicity, like that of ara-C, is apparently related to its ability to inhibit DNA synthesis.

One of the most interesting features of ara-AC is its relatively broad spectrum of activity in both *in vivo* and *in vitro* preclinical screens (8, 12–15). In contrast to both parent compounds, ara-C and 5-AC, which show little if any activity against solid tumors, ara-AC demonstrated significant activity in a National Cancer Institute panel of human tumor xenografts. In addition to the above-mentioned activity against human and mouse leukemia cell lines, ara-AC displayed activity against human colon, lung, breast, and ovarian cancer xenografts.

Preclinical pharmacokinetic and toxicological studies have been performed on both mice and dogs (12, 15) and limiting toxicities appeared to be myelosuppression and gastrointestinal distress, with the occurrence of bloody diarrhea and emesis (15). Other toxicities that were not dose limiting included lethargy, tremors, and ataxia. Histological evidence of drug-related toxicity included bone marrow depletion, lymphoid organ depletion, necrosis of gastrointestinal epithelium, and maturation arrest with necrosis of the seminiferous tubules of the testis (15).

Because of its broad spectrum of preclinical activity, we initiated a Phase I clinical study of this agent. Preclinical studies indicated that the activity of this agent is schedule dependent and that continuous infusion and frequent intermittent administration are apparently more effective than single-dose schedules (12–15). Thus, in this Phase I trial ara-AC was administered as a 72-h infusion to patients with clinically refractory cancer.

MATERIALS AND METHODS

Patient Selection. Patient characteristics are shown in Table 1. Twenty-seven (26 evaluable for toxicity) patients, ranging in age from 25 to 72 years were entered into the study. All patients had a histologically proved diagnosis of malignant disease with definitive evidence of metastatic spread and/or of inoperable local recurrence. One patient with treatment-induced acute nonlymphoblastic leukemia was given ara-AC under a special exemption; this patient was included in the pharmacokinetic evaluation but not in the toxicity evaluation since he was neutropenic and thrombocytopenic before entering this study. The patients in this study were, in general, heavily pretreated. All but four patients had previously received chemotherapy. In addition to chemotherapy, 13 patients (48%) were treated previously with radiation therapy.

Prior to beginning treatment with ara-AC, each patient underwent a comprehensive evaluation including complete history, physical examination, and evaluation of measurable disease by appropriate radio-

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[2] The abbreviations used are: ara-AC, 1-β-D-arabinofuranosyl-5-azacytosine; ara-C, 1-β-D-arabinofuranosylcytosine; 5-AC, 5-azacytidine; HPLC, high-performance liquid chromatography; C_{ss}, plasma steady-state concentration; DMSO, dimethyl sulfoxide.

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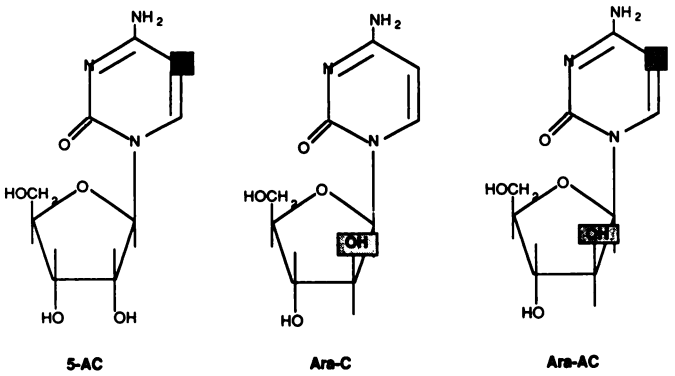

Fig. 1. Chemical structures of 5-AC, Ara-C, Ara-AC. □, changes in structure compared to cytidine.

Table 1 *Patient characteristics*

No. of patients entered	27
No. evaluable	26
Total no. of cycles (evaluable)	82 (79)
Age, yr	
Mean	52
Range	25–74
Male/female	17/10
Mean performance status (Karnofsky)	82
No. of patients with prior therapy	24
Chemotherapy only	10
Radiation therapy only	1
Chemotherapy and radiation therapy	12
Diagnosis	
Acute nonlymphocytic leukemia	1
Adenocarcinoma liver, unknown primary	1
Adrenal cancer	1
Breast cancer	3
Colon cancer	4
Diffuse lymphoma	4
Embryonal cell carcinoma	1
Fibrohistiocytoma	1
Glioma	1
Nodular lymphoma	2
Non-small cell lung cancer	1
Osteogenic cancer	1
Ovarian cancer	1
Pancreatic carcinoma	1
Renal cell carcinoma	1
Small cell lung cancer	1
Squamous cell cancer, anus	1
Uterine sarcoma	1

graphic studies. Patients in this study had to have a performance status of greater than 50% on a Karnofsky scale. Pretreatment evaluation also included complete blood cell count, with WBC differential, serum chemistries, coagulation studies, serum creatinine, and creatinine clearance. All patients had adequate liver (bilirubin <1.5 mg/100 ml and serum transaminases less than twice the normal range) and renal (creatinine clearance of 45 ml/min or a serum creatinine <1.5 mg/100 ml) function, as well as normal serum electrolytes and normal coagulation studies. All patients evaluated for toxicity had adequate peripheral blood cell counts (WBC > 3,000/mm^3, platelets > 100,000/mm^3) prior to initiation of study. Complete blood cell counts and liver function tests were done weekly while the patients received therapy and they were seen at least once every 3 weeks prior to a new cycle at which time all laboratory tests were repeated. At each visit, patients were asked to report and subjectively rate every possible drug-related side effect. All patients gave written informed consent prior to therapy in a manner consistent with institutional guidelines. Patients who developed progressive disease after two cycles were removed from study.

Drug Formulation and Dosage. ara-AC was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), as a sterile lyophilized powder in 250-mg vials. Because of limited aqueous stability, the vials were reconstituted with 9.9 ml of 70% (v/v) DMSO in water (Tera Pharmaceuticals) resulting in a final ara-AC concentration of 25 mg/ml. A 24-h supply of drug was diluted to a final volume of 12 ml with 70% DMSO and placed in a 12-ml Luer-lock syringe (Monoject, Model 512936). The syringe was replaced every 24 h with one containing freshly reconstituted drug solution. The drug was delivered by an Autosyringe infusion pump (Travenol Laboratories; Model AS2F) as a "piggyback" into an i.v. solution of 5% dextrose in water and infused at 20 ml/h. A polyolefin-lined extension set (Pacesetter Infusion, Ltd.; Model 126) was used to connect the syringe to the i.v. set. This diluted solution of ara-AC was administered through a peripheral vein or a central line.

Treatment Plan. The starting dose was 0.2 mg/m^2/h administered as a continuous infusion over 72 h. This starting dose was determined on the basis of the tolerated dose in mice during 72-h continuous infusion (10 mg/m^2/h) with an additional consideration given to the potential increased metabolic activation of this drug in humans. Some studies suggest that human cells may contain higher levels of activity of deoxycytidine kinase, the enzyme that converts ara-AC to its toxic metabolites (15). Indeed, previous clinical experience had shown that fludarabine, an agent that is also converted to toxic species by deoxycytidine kinase, was considerably more toxic in humans that it was in mice and other animals (15). Based on this, the starting dose of ara-AC in this Phase I clinical study was chosen to be 0.2 mg/m^2/h for 72 h. Dose escalation was allowed in individual patients. A minimum of three patients were treated at each dose level before escalation to the subsequent dose level was permitted in new patients. The ara-AC dose was initially escalated to 0.4 mg/m^2/h and then to 0.8 mg/m^2/h for 72 h with subsequent doses being escalated by 25% of the previous dose level (as indicated in Table 3). ara-AC was administered every 21 days if the patients had recovered from toxicity of the previous cycle; otherwise, administration of the next dose was delayed for 1 week. The objective of the study was to determine the maximally tolerated dose of ara-AC when given as a 72-h continuous infusion and to determine the pharmacokinetics of this agent in humans. The maximally tolerated dose was defined as the dose at which greater than 50% of the patients treated at a given dose experienced Grade III or greater toxicity. Toxicity was graded according to the guidelines recommended by the Cancer Therapy Evaluation Program, National Cancer Institute.

***In Vitro* Stability.** An appropriate volume of 1.0×10^{-3} M ara-AC in DMSO was added to fresh heparinized human plasma (pH 7.6) from a normal volunteer and to 0.1 M phosphate-buffered saline solution (pH 7.2) at 37°C to give a final ara-AC concentration of 1 μg/ml (4.1 μM). A 0.5-ml aliquot was taken from each sample before incubation ($t = 0$), and the remainder of the sample was then incubated at 37 ± 0.2°C. At predetermined intervals 0.5-ml aliquots were removed from each sample, and ara-AC concentrations were measured by the method described below. Sampling was continued for a minimum of three half-lives.

Pharmacokinetic Studies. Blood samples were obtained in 10-ml heparinized tubes both prior to administration of ara-AC and at 24-h intervals after the initiation of therapy. Each sample was maintained on ice to retard hydrolysis of the parent drug, and plasma was separated from cells as soon as possible by centrifugation at 1000 × *g* for 10 min. Because of limited sample stability, an aliquot of plasma was processed and analyzed within 4 h using a modification of a reverse-phase high-performance liquid chromatography assay (16).

Briefly, 2 μl of 2.25×10^{-3} M (1.0 μg) 2′-deoxy-5-azacytidine were added to a 0.5-ml aliquot of plasma as an internal standard. The sample was vortexed for 10 s to mix the internal standard. A 1.0-ml phenylboronic acid solid-phase extraction cartridge (Bond Elut PBA; Analytichem International, Harbor City, CA) was activated by washing with 1 ml of methanol followed by 1 ml of 0.01 M phosphate buffer, pH 8.0. Attached to this by an adaptor was a 3.0-ml disposable cartridge to which the plasma sample was transferred. The sample was then slowly pushed through the cartridge and the eluant was collected. The cartridge was rinsed with 0.5 ml of 0.01 M phosphate buffer, pH 8.0, and this was then ultrafiltered by centrifugation at 1000 × *g* for 30 min at 4°C in a Centrifree partition system (Amicon Corp., Danvers, MA).

A 100-μl aliquot was analyzed using a Model 204W liquid chromatography system (Walters Associate, Milford, MA) consisting of a U6K injector, a Model 6000 A solvent delivery system, and a Model 116

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UV detector (Gilson Medical Electronics, Middleton, WI). The sample was separated on a 4.6- x 250-mm, 5-μm Ultrasphere octadecylsilane analytical column (Altex/Beckman, Berkeley, CA) with a mobile phase of 0.5% CH_3CN in 0.01 M phosphate buffer, pH 6.8, with a flow rate of 1 ml/min. The detector signal was integrated by a dual channel Spectra-Physics 4200 computing and recording integrator, which was interfaced with a ChromStation-AT data system (Compaq 386 version; Spectra-Physics, Santa Clara, CA) for further data reduction and storage.

Standard curves of ara-AC in plasma were prepared for each patient's samples by addition of known amounts of ara-AC to the corresponding pretreatment sample. These spiked standards were processed in the same manner as above. A standard curve was prepared for analysis each day for the range 0 to 400 ng/ml. This curve was the best straight line defined by least squares regression analysis and possessed a correlation coefficient >0.997. Ratios of peak height of ara-AC standard to peak height of internal standard were obtained for a minimum of four concentrations each day. Samples were run concurrently, and concentrations were calculated from the appropriate standard curve. ara-AC peaks below the automatic detection threshold of the integrator were measured using the data system software. UV detection at 240 nm allowed a limit of quantitation (S/N >5) of 70 ng/ml (0.3 μM) and a limit of detection of 30 ng/ml (0.12 μM). ara-AC concentrations between these two limits could also be estimated; but, since these values have a greater error, they are indicated as falling in the range of less-certain quantitation (17) in Table 2.

Kinetic Calculations. For determination of *in vitro* stability, half-lives were calculated from the best straight line obtained by linear regression analysis of the logarithm of ara-AC concentration *versus* time. C_{ss} was the average of measured 24-h ara-AC plasma concentrations, since ara-AC levels were observed to reach a plateau by 8 h in patients given higher doses on a 24-h infusion schedule (16). The apparent total body clearance was then defined as the rate of infusion divided by C_{ss}.

Table 2 *Pharmacokinetic parameters of ara-AC*

Patient	Cycle	Dose (mg/m²/h)	C_{ss}[a] (ng/ml)	Clearance[b] (ml/min/m²)
W. P.	5	1.25	32	651
	7	1.75	50	583
	8	2.44	57	713
	10	3.83	71	899
	11	4.75	108	733
	13	5.94	137	729
Q. W.	3	1.96	74	441
J. F.	2	1.56	37	703
	3	1.96	72	454
R. D.	3	2.44	47	865
M. T.	1	1.96	61	536
	2	1.96	62	527
K. S.	7	2.96	71	695
	11	2.44	62	656
E. W.	4	1.56	70	371
V. N.	3	2.44	45	904
P. S.	1	1.96	56	583
D. W.	2	1.96	54	605
H. W.	2	2.44	68	598
T. S.	1	2.44	58	701
R. J.	1	2.44	62	656

[a] Measured values of 30–69 ng/ml are in the range of less-certain quantitation.
[b] Mean ± SD, 647 ± 141 (N = 21).

RESULTS

***In Vitro* Stability.** ara-AC was unstable in both phosphate-buffered saline and human plasma at the clinically achievable concentration of 1 μg/ml (4.1 μM) (18). The disappearance of ara-AC in these *in vitro* experiments was apparent first order, with the half-life of 2.5 ± 0.2 h (n = 2) in fresh human plasma incubated at 37°C being much more rapid than the 9.8 ± 0.3 h (n = 3) observed during incubation in phosphate-buffered saline under the same conditions.

Pharmacokinetics. ara-AC was measured in the plasma of 13 patients receiving 21 cycles of therapy at doses above 1.25 mg/m²/h. C_{ss} ranged from 32 ng/ml (0.13 μM) to 137 ng (0.6 μM) (Table 2). For Patient W. P., who received 13 cycles of therapy and had the most extensive blood level study, a good linear relationship of measured C_{ss} with dose was calculated (Fig. 2). A relatively rapid mean clearance of 647 ml/min/m² was observed for all patients (Table 2).

Toxicity. Twenty-six evaluable patients were treated with a total of 79 cycles of ara-AC. The dose-limiting toxicity was reversible leukopenia and thrombocytopenia (Table 3). Grade IV leukopenia was observed in 1 of 7 patients (1 of 7 cycles) given ara-AC at 1.25 mg/m²/h, in 1 of 8 patients (2 of 11 cycles) at 1.56 mg/m²/h, and in 4 of 11 patients (5 of 14 cycles) at 1.96 mg/m²/h. Overall, Grade III or Grade IV leukopenia occurred in 6 of 11 patients treated at 1.96 mg/m²/h and in 4 of 7 treated at 2.44 mg/m²/h. This was not a reflection of cumulative drug toxicity, inasmuch as 2 of 4 new patients who entered treatment with ara-AC at 1.96 mg/m²/h and 4 of 4 new patients who entered at 2.44 mg/m²/h experienced Grade III or IV neutropenia. Thrombocytopenia also occurred but overall was less prominent than leukopenia. Grade IV thrombocytopenia was observed in 1 of 7 patients (1 of 7 cycles) treated at 1.25 mg/m²/h, 1 of 8 patients (2 of 11 cycles) treated with 1.56 mg/m²/h, and 3 of 11 patients (3 of 14 cycles) treated with 1.96 mg/m²/h for 72 h. The median time to WBC nadir was 18 days (range, 7 to 31), while the median time to the corresponding platelet nadir was 13 days (range, 7 to 22).

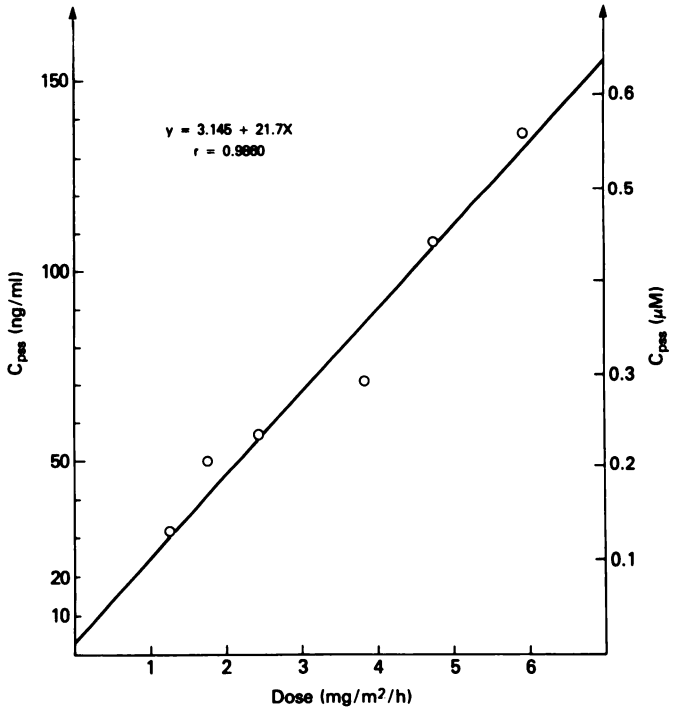

Fig. 2. Correlation between ara-AC plasma concentrations (ng/ml or μM) at steady state and dose (mg/m²/h) in a single patient (W. P.) given multiple cycles of therapy.

There were five episodes of fever and neutropenia requiring

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Table 3 *Hematological toxicity of ara-AC*

Dose (mg/m²/h)	No. of patients (new/total)	No. of cycles	WBC nadir[a]				Platelet nadir[b]			
			I	II	III	IV	I	II	III	IV
0.2	3/3	3								
0.4	3/5	5		1		1				
0.8	3/6	7			2		3			
1.0	1/6	7		1	2		3	1		
1.25	4/7	7	1	2	2	1		2		1
1.56	4/8	11	1	2	3	1	1			1
1.96	4/11	14	2	2	2	4	1		1	3
2.44	4/7	17		1	3	1	3			
3.05	0/3	4				1			1	
3.83	0/1	1							3	
4.75	0/1	1								
5.94	0/1	2	1						1	

[a] Total leukocytes/mm³: Grade I 3,000–3,999; Grade II, 2,000–2,999; Grade III, 1,000–1,999; Grade IV, <1,000.

[b] Platelets/mm³: Grade I, 75,000–90,000; Grade II, 50,000–74,000; Grade III, 25,000–49,000; Grade IV, <25,000.

hospitalization for i.v. antibiotic treatment during this study. In three cases no source of infection was detected. One patient developed *Candida* and herpes esophagitis during the period of fever and neutropenia. One patient with adrenocortical cancer developed *Listeria* meningitis at which time the WBC was 1400/mm³. She was treated successfully and recovered without sequelae. Another patient became neutropenic during his 12th cycle of ara-AC therapy. He subsequently developed staphylococcal septicemia and *Candida* endocarditis on a prosthetic mitral valve (he had a previous history of *Candida* endocarditis). Although his WBC returned to normal, he subsequently died of cardiac complications. This was the only treatment-related death on the study.

Increases in liver function tests were observed in 14 patients treated with ara-AC. This was predominantly manifested by increases in alkaline phosphatase although there were also some minor increases in transaminases. Overall, there was no apparent relationship between ara-AC dose and liver toxicity. Grade I liver toxicity (increased alkaline phosphatase or transaminases) was seen in 19 cycles (occurring in doses ranging from 0.4 to 2.44 mg/m²), Grade II in 13 cycles (in doses ranging from 0.8 to 5.94 mg/m²), Grade III in one cycle (at 1.56 mg/m²), and Grade IV toxicity in 2 cycles (at 1.96 mg/m²). In each case, the hepatic abnormality was transient and returned to baseline prior to the next cycle. One-half (7 of 14) of the patients who developed elevated liver function tests in this study had evidence of liver involvement with tumor, thus complicating the evaluation for hepatotoxicity.

There were few other side effects reported in this trial. Since ara-AC was reconstituted in DMSO in order to enhance its stability during prolonged infusions, the odor of DMSO was noticeable to everyone who entered the patients' rooms. However, the patients themselves did not complain of the odor, nor did they complain of any garlic-like taste. Nausea and vomiting were uncommonly (7 of 27 patients) reported in this trial. Overall, there were only 5 episodes of Grade I and 2 Grade II nausea and vomiting. One patient received a total of 13 cycles with an escalation from 0.2 mg/m²/h to 5.94 mg/m²/h for 72 h. This 44-year-old patient complained of sleep disturbances (nightmares and excessive tiredness) beginning after Cycle 4 and of impotence after Cycle 6. These toxicities were not observed in any other patient.

Clinical Activity. In this Phase I study, both prolonged disease stabilization in one patient and minor clinical responses in four other patients were observed. One patient (W. P.) with an adenocarcinoma of the liver of unknown primary had stable disease without any evidence of progression by abdominal computerized axial tomographic scan during 13 cycles of therapy with ara-AC. Of the four patients who were noted to have minor clinical responses, one (E. W.) entered the study with rapidly progressing liver metastases from colon carcinoma. Following two cycles of ara-AC the size of the liver as noted by physical examination diminished from 28 cm to 19 cm. This patient developed disease progression in the retroperitoneum after four cycles of therapy. One patient (V. L.) with metastatic breast cancer had some shrinkage in the size and character of extensive chest wall lesions after three cycles of ara-AC but developed progressive disease after the fourth cycle. One patient (T. R.) previously treated with chemotherapy and radiation therapy for Hodgkin's disease had developed acute nonlymphocytic leukemia. Following treatment with ara-AC, there was a diminution in the number of circulating blasts, a decrease in lactate dehydrogenase, a decrease in fever, and a feeling of subjective improvement. After two cycles of therapy, there was a complete disappearance of biopsy-proved leukemic skin lesions. However, bone marrow biopsies failed to show a decrease in the proportion of blasts, and the patient's therapy was stopped after three cycles.

Another patient (K. S.) in this study had embryonal cell carcinoma and had been previously treated with two different salvage combination chemotherapy regimens. At the time of entering this trial, this patient had bilateral adrenal masses, a testicular mass, pleural effusions, and ascites which required twice-weekly paracentesis. After the first cycle of ara-AC, he no longer required paracentesis, and after the fourth cycle, there was no evidence of ascites by abdominal computerized axial tomographic scan. He was subsequently treated with an additional 9 cycles of ara-AC. Despite clinical improvement there was no apparent change in the size of his adrenal or testicular masses and his α-fetoprotein increased during this time from 600 to >11,000. An orchiectomy was performed which showed an embryonal cell carcinoma with yolk sac elements, which had not been a prominent feature of his previous biopsies. It is possible that ara-AC promoted the differentiation of the tumor toward a yolk sac tumor resulting in enhanced α-fetoprotein production. Indeed, ara-AC does induce differentiation of HL-60 cells (8), a property presumed to be related to its ability to inhibit DNA methylation (11). It is also possible that the testes represented a sanctuary site. Following orchiectomy, this patient's α-fetoprotein returned to normal. The patient developed neutropenia during a subsequent cycle of ara-AC which was complicated by staphylococcal sepsis and *Candida* endocarditis on a prosthetic mitral valve (the patient had developed *Candida* endocarditis during earlier treatments for testicular cancer). The patient subsequently died of cardiac complications. At autopsy, there were only necrotic masses in the abdomen and adrenals without evidence of tumor. He did, however, have two small sites of residual embryonal cancer in his lung.

DISCUSSION

In this Phase I trial, ara-AC was given as a 72-h infusion to adult patients with refractory cancer. The dose-limiting toxicity, which was reversible leukopenia, was reached at 1.96 mg/m²/h for 72 h in this heavily pretreated patient population. Neutropenia was more prominent than thrombocytopenia. Other toxicities included mild elevations in liver function abnormalities. Hepatotoxicity was difficult to evaluate in this patient population since many of the patients had metastatic disease in the liver. Nausea and vomiting were not prominent side effects of this drug, despite the obvious odor produced by the DMSO

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which was required for the effective formulation of the clinical dose.

The mean clearance for the 21 cycles of therapy for which ara-AC could be measured was 647 ± 141 ml/min/m^2, which is not statistically different from the mean clearance (571 ml/min/m^2) observed in the Phase I pediatric trial of this agent for a dose of 15 mg/m^2/h over 24 h (18). These clearances also fell into the 480- to 1157-ml/min/m^2 range observed in nonhuman primates for ara-AC doses of 200 mg/kg given over either 15 min or 1 h (19). This rapid clearance of ara-AC is about one-half that of ara-C (20), the nucleoside that ara-AC most closely resembles biochemically. While ara-C is rapidly metabolized by cytidine deaminase, araAC is refractory to catabolism by this enzyme (5). Thus, any clearance of ara-AC through deamination, even considering the high levels of this enzyme in human liver and kidney (21), is expected to be minimal as is the case for 2′,3′-dideoxycytidine, another cytosine nucleoside which is a poor substrate for mammalian cytidine deaminase (22). Based on *in vitro* stability, however, ara-AC probably undergoes substantial hydrolytic degradation *in vivo*. If one assumes a steady-state volume of distribution equivalent to total body water (42 liters for a 70-kg person), which is what has been observed for ara-AC in monkeys (19), a maximum hydrolytic contribution to plasma clearance of 110 ml/min/m^2 can be calculated (23). This indicates that other as yet to be defined processes such as distribution into other compartments (*e.g.*, intracellular transport), renal excretion, and anabolic metabolism may account for the majority of plasma clearance.

Steady plasma levels of ara-AC ranged from 32 to 137 ng/ml (0.13 to 0.6 μM) for patients receiving drug at rates between 1.25 and 5.94 mg/m^2/h (Table 2; Fig. 2). Studies with ara-AC in several *in vitro* systems suggest that these observed concentrations may be sufficient for biological activity, especially if they are maintained for the better part of 72 h. Incubation of Molt-4 human T-lymphoblastic leukemia cells with 1.0 μM ara-AC for 24 h resulted in 98% inhibition in clonogenic assays (5). Although a direct comparison is not possible because drug concentrations with respect to time are not known for the *in vitro* study, drug exposure was probably similar in both cases. If one takes into account the half-life of ara-AC in RPMI 1640 (19), then the average drug concentration for the 24 h of incubation is 0.2 μM, a level that should be achievable at the recommended dose of 2.0 mg/m^2/h for Phase II studies. In addition, the inhibitory concentration for 50% control growth for a 24-h exposure of ara-AC to P388 murine leukemia *in vitro* was found to be 1.9 μM, and exposure of these same cells to 2 μM ara-AC for 3 days resulted in 1 log of cell kill (8). Although these concentrations are approximately an order of magnitude higher than those achieved in this clinical trial, the aforementioned hydrolytic decomposition *in vitro* would substantially reduce effective drug concentrations rapidly. Thus, the average ara-AC concentration *in vitro* over a 24-h period would not be substantially higher than what is observed in patients treated with a 72-h continuous infusion. Since ara-AC induces differentiation at low micromolar concentrations *in vitro*, this process may also be a component of the observed antitumor activity (see above). Some (10 to 44% of viable cells) differentiation of HL-60 cells was observed after 4 days with 2.5 to 10 μM ara-AC (8), while the concentration for 50% inhibition of DNA methylation of DNA in CEM/O cells was 0.25 μM during 24-h drug treatment (24).

Although there were no complete or partial responses observed in this Phase I trial, there was encouraging evidence of clinical activity. Prolonged disease stabilization (13 cycles over 10 months) was seen in one patient with adenocarcinoma of unknown origin involving the liver. Minor clinical responses were observed in one patient with acute nonlymphocytic leukemia, in one patient with colon cancer metastatic to liver, in one patient with breast cancer, and in one patient with embryonal testicular cancer. This wide range of possible clinical activity mirrors the breadth of observed preclinical activity.

ara-AC is thus a new antineoplastic agent which appears to combine the chemical and clinical features of ara-C and 5-AC. The 70% DMSO formulation of ara-AC is well tolerated by patients when it is piggy-backed into a rapidly dripping i.v. solution and given continuously for 72 h. Although all of our patients have been treated in the hospital, it should be possible to deliver ara-AC as an outpatient regimen. The only limiting feature to outpatient therapy may be the noxious odor produced by the DMSO. The recommended dose for Phase II studies in heavily pretreated patients is 2.0 mg/m^2/h for 72 h. Higher dose levels should be tolerated by individuals who have received little or no prior chemotherapy. Preclinical studies and this Phase I study suggest that ara-AC may have a broad spectrum of activity against human solid tumors.

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