Phase I Trial and Pharmacokinetic Evaluation of Fazarabine in Children

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ABSTRACT

A phase I trial of fazarabine (1-beta-D-arabinofuranosyl-5-azacytosine, NSC 281272) administered as a 24-h continuous infusion was performed in 16 children with refractory malignancies. Dose-limiting toxicity consisting of reversible granulocytopenia and thrombocytopenia was observed in 4 of 4 solid tumor patients treated at the starting dose of 20 mg/m²/h. Subsequent patients were treated at a dose of 15 mg/m²/h which was determined to be the maximum tolerated dose. Moderate nausea and vomiting were the only other toxicities observed. Plasma steady-state concentrations of fazarabine were attained by 2-4 h in all patients and were 1.8 and 2.5 μM at the 15- and 20-mg/m²/h doses, respectively. The total body clearance of fazarabine was 571 and 550 ml/min/m² at the 15- and 20-mg/m²/h doses, respectively. In three of four patients evaluated, fazarabine was detectable in the cerebrospinal fluid (CSF). Steady-state CSF concentrations ranged from 0.29 to 0.74 μM in these three individuals and the steady-state CSF/plasma ratios ranged from 0.22-0.25. Both the plasma and CSF steady-state concentrations were within the 0.1 to 1 μM range reported to be cytotoxic in vitro against the Molt-4 human T-lymphoblastic leukemia cell line. Based on the above, the optimal dose for phase II trials of fazarabine administered as a 24-h infusion is 15 mg/m²/h (360 mg/m²/day).

INTRODUCTION

AAC (fazarabine, NSC 281272) is a new hybrid nucleoside which possesses the arabinoside sugar of ara-C and the pyrimidine base of 5-AC (Fig. 1). AAC resembles 5-AC in that both compounds are resistant to deamination by cytidine-deoxycytidine deaminase, and both undergo spontaneous degradation in aqueous solutions (1-3). Like ara-C, AAC requires intracellular activation to the nucleotide triphosphate by deoxycytidine kinase before interfering with DNA synthesis both by direct internucleotide incorporation as well as by inhibition of DNA polymerase (2, 4).

AAC has demonstrated activity against a variety of tumors in the National Cancer Institute preclinical tumor screening panel. Like 5-AC and ara-C, it was active against both systemic and intracranially implanted L1210 and P388 murine leukemias (1, 3-5), and it has demonstrated in vitro activity against the Molt-4 T-lymphoblastic and other human leukemia cell lines (2). However, in contrast to its parent compounds, ara-C and 5-AC, AAC demonstrated greater activity against both murine and human solid tumors, including the Lewis lung carcinoma, B-16 melanoma, and the MX-1 mammary, TE-671 medulloblastoma, and LX-1 lung human tumor xenografts (1, 3, 4).

Like most other nucleosides, the antitumor activity of AAC is schedule dependent. Administered on an every-3-h, day 1, 5, and 9 schedule, AAC was significantly more active against L1210 leukemia in vivo than when given on once daily schedules of up to 9 days (3). Schedule dependency has also been noted in canine preclinical toxicology studies, as well as in in vitro cell cytotoxicity studies (4).

This report details the results of a Phase I trial and pharmacokinetic study of AAC administered as a 24-h continuous infusion to children. This schedule was chosen to exploit the known schedule dependency of this agent.

MATERIALS AND METHODS

Patient Eligibility. Patients less than 21 years old with malignancies refractory to conventional therapy were eligible for this trial. All patients were required to have prior histological documentation of malignancy, an Eastern Cooperative Oncology Group performance level of 3 or less (6), and a life expectancy of at least 8 weeks.

Prior to entry, patients were required to have recovered from the toxic effects of previous antineoplastic therapy, and to have adequate hepatic (bilirubin less than 2 mg/100 ml and normal transaminases), and renal function (serum creatinine less than 1.5 mg/100 ml or creatinine clearance greater than 60 ml/min/1.73 m²), as well as a normal coagulation profile, serum electrolytes, and uric acid. Patients with solid tumors (without bone marrow involvement) were also required to have adequate peripheral blood counts (granulocytes greater than 1500/mm³, and platelets greater than 100,000/mm³) prior to treatment.

All patients or their legal guardians signed a document of informed consent consistent with federal and local institutional guidelines, stating that they were aware of the investigational nature of this trial.

Study Design. This pediatric trial began simultaneously with those in adults. Thus, an adult MTD was not available, and our starting dose was instead based on preclinical toxicology data. It had been demonstrated that a dose of 108 mg/m²/h for 24 h was tolerable in canine tumor toxicology studies (4). However, because of the reportedly higher levels of the drug-activating enzyme (deoxycytidine kinase) in humans (7), and the concern that greater toxicity might occur as a result of enhanced concentrations of AAC triphosphate, the canine MTD was reduced by 80% to give a starting dose of 20 mg/m²/h.

AAC was administered as a 24-h continuous i.v. infusion once every 21 days, provided that patients had recovered from the toxic effects of the previous course. One dose constituted one course of therapy. At least 3 patients evaluable for toxicity were entered at each dose level studied. If dose-limiting toxicity occurred in 2 of 3 patients, 3 additional patients were entered at that dose level. Dose seeking was terminated when such toxicity was identified in 4 of 6 evaluable patients at any dose level. Patients were monitored weekly with complete blood counts, serum creatinine, blood urea nitrogen, serum glutamic-oxaloacetic transaminase, bilirubin, electrolytes, uric acid, as well as physical examinations and measurement of any palpable lesions. Radiographic studies, computed tomography or magnetic resonance imaging scans and bone marrow examinations (where appropriate) were obtained prior to treatment and repeated at the end of each course of therapy to determine response to treatment. Patients who experienced objective disease progression were removed from study.

Drug Preparation and Administration. AAC was obtained from the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute, Building 10, Room 13N 240, Bethesda, MD 20892. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
was then quantitated the same day by using a previously described high-concentration of the delivery vehicle. Dilution of this DMSO concentration as close to the patient as possible to minimize any hydrolytic drug decomposition prior to intravenous delivery.

The polyolefin-lined tubing is compatible with the high DMSO concentration of the delivery vehicle. Dilution of this DMSO concentration by the running i.v. is necessary to prevent leaching of plasticizer from standard, commercially available polyvinyl chloride infusion tubing.

Drug Assay and Sampling Times. During the AAC infusion, 3-ml heparinized blood samples were obtained at 15 and 30 min, and at 1, 1.5, 2, 4, 8, 12, and 24 h. Lumbar CSF samples (1 ml) were obtained in 4 patients at 9 h (1 patient) and at 12 h (3 patients) into the drug infusion.

Blood samples were immediately chilled on ice, and the plasma was separated by centrifugation at 400 × g at 5°C. AAC in plasma and CSF was then quantitated the same day by using a previously described high-pressure liquid chromatography assay. Standard curves encompassing an appropriate concentration range were prepared in the pretreatment plasma of each individual studied. The limit of quantitation was 50 ng/ml (0.2 µM).

Pharmacokinetics. The steady-state plasma concentrations of AAC at each dose level were determined from the geometric mean (concentration × time) data. Clearance was calculated by dividing the infusion rate by the steady-state plasma concentration. The steady-state CSF:plasma concentration ratio for AAC was calculated by dividing the CSF steady-state concentration by the plasma steady-state concentration.

RESULTS

Phase I Trial. Table 1 lists the characteristics of the patients entered on this trial. Sixteen heavily pretreated patients between the ages of 2 and 19 years were accrued. Ten patients with solid tumors were fully evaluable for toxicity, and 5 patients with acute lymphoblastic leukemia were evaluable for nonhematological toxicity only. One patient with nonlymphoblastic leukemia, who died of disease-related complications 10 days after AAC, was not evaluable.

The dose-limiting toxicity in this study was myelosuppression, characterized primarily by granulocytopenia and to a lesser extent by thrombocytopenia. Table 2 defines the grades of hematological toxicity, and shows its relationship with the dose of AAC. At the 20-mg/m²/h starting dose of AAC, 4 of 4 patients with solid tumors demonstrated dose-limiting granulocytopenia, and 2 of 4 also had dose-limiting thrombocytopenia. Average granulocyte and platelet nadirs occurred on days 19 and 13, respectively. The average time to recovery for both an absolute granulocyte count of 1,500/mm³ and platelet count of 100,000/mm³ was day 22.

Hematological toxicity in the patients treated at 20 mg/m²/h necessitated a dose reduction to 15 mg/m²/h in subsequent patients. Six patients were treated at the 15-mg/m²/h dose level; 3 demonstrated dose-limiting granulocytopenia or thrombocytopenia. The average day of granulocyte and platelet nadirs in the 6 patients treated at this dose were 15 and 12 days, respectively. The average time to recovery of an absolute granulocyte count of 1,500/mm³ and platelet count of 100,000/mm³ was 23 and 19 days, respectively. This dose, 15 mg/m²/h, was determined to be the MTD for the 24-h continuous infusion schedule used in this trial.

Clinically significant alterations in hepatic or renal function, neurotoxicity, and mucositis were not observed at either dose of AAC. Nausea and vomiting occurred in 10 patients. It was generally mild to moderate in nature and resolved shortly after the drug infusions stopped.

Fifteen patients were evaluable for response on this study. No objective tumor responses to AAC were noted at either dose level evaluated. Two patients, one with Ewing’s sarcoma and another with an undifferentiated sarcoma, each had stable disease for a 6-week period.

Fig. 1. Structure of AAC and its parental compounds ara-C and 5-AC.

Cancer Institute, Bethesda, MD, as a sterile lyophilized powder in 250-mg vials. The contents of each vial were dissolved in 4 ml of a sterile 70% DMSO, 30% water (v/v) diluent (supplied with the drug). A rubber-stoppered polypropylene syringe affixed to a small volume infusion pump (Autosyringe AS20S, Travenol Laboratories, Inc., Hookset, NH 03104) served as the reservoir for infusion. AAC, in the 70% DMSO delivery vehicle was delivered through a length of polyolefin-lined infusion tubing (supplied with the drug) as a "piggyback" infusion into a 5% dextrose in 0.45% saline i.v. solution infusing at a rate of 1800 ml/m²/24 h through a peripheral i.v. or central venous catheter. The point of entry for the piggyback into the patients i.v. was kept as close to the patient as possible to minimize any hydrolytic drug decomposition prior to intravenous delivery.

Table 1 Patient characteristics

<table>
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<th>Diagnoses</th>
<th>No. fully evaluable</th>
<th>No. of patients entered</th>
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</thead>
<tbody>
<tr>
<td>Acute lymphoblastic leukemia</td>
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<tr>
<td>Osteogenic sarcoma</td>
<td>2</td>
<td>15</td>
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<tr>
<td>Ewing’s sarcoma</td>
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<td>15</td>
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<tr>
<td>Undifferentiated sarcoma</td>
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<td>15</td>
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</tr>
<tr>
<td>Rhabdomyosarcoma</td>
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<td>15</td>
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<td>15</td>
</tr>
<tr>
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<td>15</td>
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<td>Astrocytoma</td>
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Table 2 Hematological toxicity of fazarabine

<table>
<thead>
<tr>
<th>Fazarabine dose (mg/m²/h for 24 h)</th>
<th>No. of evaluable patients</th>
<th>Grade of hematological toxicity*</th>
</tr>
</thead>
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<tr>
<td>15 mg</td>
<td>6</td>
<td>0 I I I I IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (2/2)</td>
</tr>
<tr>
<td>20 mg</td>
<td>4</td>
<td>0 0 0 0 4 0 (4/2)</td>
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</table>

* Grade III toxicity: 1,000-1,999 total leukocytes, 500-999 absolute granulocytes, 25,000-49,000 platelets (all per µl), hemoglobin 5-7 g/dl. Grade IV toxicity: <1,000 total leukocytes, <500 absolute granulocytes, <25,000 platelets (all per µl), hemoglobin <5 g/dl.
* Maximally tolerated dose.
* Numbers in parentheses, number of patients with indicated grade of granulocytopenia/number with thrombocytopenia.
mg/m²/h 24-h continuous infusions. After the start of the drug infusions, there was a rapid rise in plasma concentrations of AAC, and steady-state plasma concentrations were achieved within 2 to 4 h of the start of infusion in all patients. Table 3 shows the mean pharmacokinetic parameters determined for the two AAC dose levels studied. Mean steady-state plasma concentrations were 1.8 and 2.5 μM, and clearances were 571 and 550 ml/min/m² at the 15- and 20-mg/m²/h dose levels, respectively.

In the six patients in whom plasma steady-state concentrations of AAC and the degree of granulocytopenia or thrombocytopenia were both evaluated, no correlation between these variables was apparent by linear regression (r = 0.16).

The CSF concentration of AAC 12 h after the start of the 15-mg/m²/h infusion was 0.30 and 0.29 μM in two patients, and undetectable in a third patient. In one patient at the 20-mg/m²/h dose level, the CSF concentration 9 h after the start of the infusion was 0.74 μM. The CSF:plasma steady-state concentration ratios for these 3 patients were 0.24, 0.22, and 0.25, respectively.

**DISCUSSION**

In the current study, we demonstrated that the MTD for AAC administered as a 24-h continuous infusion to children was 15 mg/m²/h. The dose-limiting and only clinically significant toxicity of AAC in this study was myelosuppression (Table 2). Nonhematological toxicities of AAC were limited to moderate nausea and emesis (1–4 episodes of emesis without the need for hydration), which was less than might have been expected based on the structural similarities between AAC and the very emetogenic 5-AC. The odor produced from respiratory elimination of the DMSO necessary to produce a stable AAC formulation was not a barrier to patient acceptance of this drug.

A comparison of the pharmacokinetic parameters obtained for AAC in the current study (Table 3), and those reported for similar infusion rates for ara-C (8, 9), reveal that the steady-state plasma and CSF concentrations of these two biological analogues are similar. The pharmacokinetic similarities between these two compounds has also been demonstrated in the nonhuman primate (10). This is so despite the different mechanisms of drug elimination for AAC (spontaneous hydrolytic degradation) and ara-C (enzymatic deamination). It appears that resistance to deamination, a feature which might have been expected to lead to a longer in vivo half-life for AAC, may be overcome by its aqueous instability. Thus, we were unable to demonstrate a pharmacokinetic advantage for AAC over ara-C when administered by continuous infusion.

The plasma steady-state concentration of fazarabine observed at the 15- and 20-mg/m²/h dose levels studied in this trial closely match those predicted by our pharmacokinetic model developed in the rhesus monkey (10). The mean plasma steady-state levels for the above two dose levels were 1.8 and 2.5 μM versus predicted values of 1.9 and 2.6 μM, respectively. Additionally, the CSF:plasma concentration ratios of fazarabine reported here are close to the 0.15 ratio reported in the monkey. These results confirm the utility of the above model for use in the clinical setting.

Steady-state plasma and CSF concentrations of AAC achieved with the 15-mg/m²/h infusion fall within a range which has demonstrated in vitro activity against the Molt-4 human T-lymphoblastic leukemia cell line (2). Exposure of this cell line to 0.1 and 1 μM AAC for 24 h resulted in 50 and 98% reduction in clonogenic survival, respectively. However, these in vitro experiments failed to correct for the considerable spontaneous degradation of AAC in aqueous media (half-life, 3.9 ± 0.45 h in RPMI 1640 tissue culture media) (10). By 24 h, the concentration of AAC in culture media would be only 1% of the original concentration. Thus, it is possible that the plasma drug concentrations maintained during continuous infusion may be even more cytotoxic than suggested on the basis of the above in vitro data.

Phase II trials of AAC should initially concentrate on defining its activity against solid tumors, the principal activity unique to AAC in the preclinical tumor screening panels. There may also be significant potential for AAC as an antileukemic agent, based upon its in vitro and in vivo antileukemic activity and the ability to achieve and maintain cytotoxic plasma concentrations with the MTD and schedule described here. However, Phase II trials attempting to demonstrate that AAC is active against human leukemia may be difficult in view of the cross-resistance between AAC and ara-C (11), and the fact that most leukemia patients entering such trials will previously have failed ara-C. Biochemical pharmacological study of leukemic cells from potential patients may be useful in identifying those individuals capable of metabolizing AAC to the triphosphorylated nucleotide necessary for cytotoxic activity.

**REFERENCES**

2. Townsend, A., Leclerc, J. M., Dutschman, G., Cooney, D., and Cheng, Y. C. Metabolism of 1β-D-arabinosyl-5-azacytosine and incorporation into
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