Pharmacology of Fludarabine Phosphate after a Phase I/II Trial by a Loading Bolus and Continuous Infusion in Pediatric Patients

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INTRODUCTION

Fludarabine phosphate is a nucleotide analogue of adenine arabinoside with antitumor activity in murine and human lymphoid malignancies; it has occasional, unpredictable neurotoxicity after high dose bolus injections in adults. To avoid this toxicity, we studied a loading dose plus 5-day continuous infusion in 47 evaluable pediatric patients. Dose limiting myelosuppression was seen in children with solid tumors after a loading dose of 8 mg/m² followed by 23.5 mg/m²/day for 5 days. In children with leukemia, no dose limiting toxicity was seen at dose level 6, consisting of a loading dose of 10 mg/m² and an infusion of 30.5 mg/m²/day for 5 days. One complete and 3 partial remissions were seen in 26 evaluable children with acute lymphoblastic leukemia. 9-B-D-arabinofuranosyl-2-fluoroadenine plasma concentrations and the area under the moment curve in children with leukemia, no dose limiting toxicity was seen at dose level 6, consisting of a loading dose of 10 mg/m² and an infusion of 30.5 mg/m²/day for 5 days. One complete and 3 partial remissions were seen in 26 evaluable children with acute lymphoblastic leukemia. 9-B-D-arabinofuranosyl-2-fluoroadenine plasma concentrations and the area under the moment curve increased linearly with dose. The terminal half-life was similar, while the total body clearance was shorter than that reported for adults receiving bolus or continuous doses. Lymphoblasts isolated from 2 patients during fludarabine phosphate (9-B-D-arabinofuranosyl-2-fluoroadenine) treatment increased their ability to convert 1-B-D-arabinofuranosylcytosine to 1-B-D-arabinofuranosylcytosine 5’-triphosphate by more than 10-fold. The antileukemic activity of 9-B-D-arabinofuranosyl-2-fluoroadenine 5’-phosphate and its ability to alter the metabolism of 1-B-D-arabinofuranosylcytosine indicate that timed combinations of these 2 agents should be tested.

MATERIALS AND METHODS

Patient Selection and Evaluation. This study was open to patients <21 years old from selected institutions of the Childrens Cancer Study Group (Los Angeles, CA) and the Pediatric Branch of the National Cancer Institute (Bethesda, MD). All patients had histological proof of a malignancy for which conventional therapy had proven ineffective or for which no other therapy with established efficacy was available. Patients had recovered from toxic effects of prior therapy, had a life expectancy of at least 8 weeks, and had signed consent for this investigation. At the time of study entry, all patients had adequate liver function (bilirubin, <1.5 mg/dl; aspartate aminotransferase, <100 units/liter) and adequate renal function (serum creatinine <2.0 mg/dl). All patients with solid tumors had adequate bone marrow function (granulocyte count, 1500/mm³; platelet count, 100,000/mm³). Patients with CNS malignancy, CNS metastases, or neurological symptomatology were
excluded because of prior reports of neurotoxicity. Blood counts, liver enzyme values, and creatinine levels were obtained on days 3, 5, 14, and 21 of each 21-day treatment cycle. Plasma pharmacokinetic studies were incorporated as part of the protocol for each patient enrolled during the dose escalation phase of the study. The drug was supplied by the National Cancer Institute to each participating institution.

Treatment Protocol. Patients received a loading bolus followed by a 5-day continuous infusion starting at 50% of the adult maximum tolerated dose. The first dose level was a 5-mg/m² bolus followed by a 13-mg/m²/day continuous infusion. Subsequently, the dose was escalated at 18.5 mg/m² (Table 1). Escalation occurred when at least 3 patients (2 without bone marrow disease) were evaluable at the current dose level. Despite no dose limiting non-bone marrow toxicity, dose escalation was stopped at level 6 due to concern about the reports of irreversible CNS toxicity seen at higher bolus doses in adults. Subsequently, the study was continued for children with acute lymphoblastic leukemia to evaluate its efficacy at level 6. A three-stage multiple testing procedure was used (25). Only 2 partial responses were seen after 16 patients were entered at level 6, so the study was terminated after 2 stages.

Criteria for Response and Toxicity. A complete remission was defined as the absence of symptoms and signs due to the malignancy and radiographic studies showing absence of tumor or a bone marrow with <5% blasts and normal peripheral blood counts. Partial remission was defined as ≥50% shrinkage of the tumor or a bone marrow with >5% and ≤25% blasts. Toxicity was graded by the Cancer Clinical Trials Common Toxicity Scale, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. This is a I-IV scale with IV defined as life-threatening; specific limits for each toxicity grade depended on the organ system.

Response rates were calculated as the number of patients who exhibited either a partial or complete response divided by the number of evaluable patients. The 95% confidence interval (26) for the particular response rate was also calculated. The response rate and associated confidence interval were calculated for each dose level and for the aggregate patient population.

Pharmacokinetics. Fludarabine phosphate was provided by the National Cancer Institute. HPLC solvents and other chemicals were of HPLC or analytical grade.

Venous blood samples were obtained 2 h after completion of the loading bolus and every 24 h thereafter for 5 days. Specimens were obtained at frequent time intervals immediately following the completion of the 5-day F-ara-AMP regimen to determine the elimination kinetics of F-ara-A in plasma. The lumen of the central venous catheter was flushed with normal saline prior to withdrawal of each sample. The catheter lumen used for sampling was different from the one through which F-ara-AMP was infused. Blood samples were collected in heparinized syringes, placed in an ice bath, and centrifuged to separate the plasma. The plasma was immediately separated from the samples and was either assayed immediately or frozen at −20°C until assay (22). Measurements of F-ara-A were performed on HPLC reverse phase column as previously described (4, 15, 22). The results are presented as the arithmetic mean plasma F-ara-A concentrations ± SD. The pharmacokinetic parameters were calculated according to the method of least squares. AUC and AUMC were estimated by the linear trapezoidal rule for the duration of the infusion and up to the final determined plasma concentration and extrapolated to infinity by addition of the terms:

\[
\text{AUC}_{0-\infty} = \frac{C_{\text{max}}}{\beta} \\
\text{AUMC}_{0-\infty} = \frac{C_{\text{max}}}{\beta} + \frac{C_{\text{max}}}{\beta^2}
\]

where \(C_{\text{max}}\) is the initial determined plasma F-ara-A concentration and \(\beta\) is the terminal elimination rate constant. The proportion of the extrapolated \(\text{AUC}_{0-\infty}\) was on average <10% of the total \(\text{AUC}_{0-\infty}\). Model-independent pharmacokinetic methods were used to determine TBC and \(V_d\) by the formulas:

\[
\text{TBC} = \frac{k_0}{C_{\infty}} \quad \text{and} \quad \text{TBC} = \frac{\text{dose}}{AUC_{0-\infty}} \\
V_d = \frac{k_0}{C_{\infty} \times \beta^2}
\]

where \(k_0\) and \(C_{\infty}\) are the infusion rate and steady state plasma F-ara-A concentrations, respectively.

Determination of F-ara-AMP Cellular Concentrations in Peripheral Blast Cells. PBC were isolated from whole blood from 4 patients who had a high peripheral blast count by standard bilayer centrifugation procedures (27). The PBC were counted and the mean cell volume was estimated for each sample with a Coulter Counter (model ZBI) coupled with a cell size analyzer (Coulter Channelizer, Coulter Electronics, Hialeah, FL). Pretreatment PBC, 1 × 10⁷ cells, were incubated in vitro with 400 µM purified [3H]F-ara-A for 1 h at 37°C. The cells were washed once with phosphate buffered saline and extracted with PCA for nucleotides and nucleotide analogues as described earlier (4, 15, 27). F-ara-AMP was separated from the other nucleotide triphosphates in the neutralized PCA extract by HPLC analysis, using a strong anion exchange column (SAX-10) and a linear gradient elution with phosphate buffers over 40 min (15). F-ara-AMP was identified in a similar manner as described earlier (4, 15).

Nucleoside triphosphate standards were purchased from Sigma Chemical Co. (St. Louis, MO) and standard calibration curves have been conducted for each of them. The quantitation of F-ara-ATP was conducted by integration of the analogue signal by a Digital Equipment Co. computer (model PC-350) and preprogramed response factors. The amounts of F-ara-AMP were converted to cellular concentrations with conversion factors accounting for the variability in the cell size of the samples.

Measurement of ara-CTP Levels in Lymphoblasts from Patient during F-ara-AMP Treatment. Lymphoblasts, 1 × 10⁷ cells, were isolated from the blood of 3 patients before and during treatment and incubated with 1 mM ara-C for 1 h at 37°C. The cells from 2 patients were extracted with PCA as described earlier (27) and the cellular concentrations of the phosphorylated anabolite, ara-CTP, were determined. In an earlier study in pediatric patients with ALL, results of cellular concentrations of ara-CTP from the ara-C incubations (ara-C sensitivity test) were linearly correlated with the ara-CTP concentrations achieved during treatment with high doses of ara-C (27).

DSC and Elimination of Blast Cells after F-ara-AMP Treatment. The effect of F-ara-AMP concentrations on [3H]thymidine incorporation into DNA of blast cells before and after treatment was determined during the 5-day fludarabine phosphate treatment as described earlier (4, 27). DSC was also determined after in vitro incubation with ara-C in 3 patients. The elimination of circulating blast cells was monitored by daily peripheral blood counts and differentials.

RESULTS

Patients. Sixty-three patients entered the study. Sixteen were not fully evaluable: 8 died of progressive disease before day 21, 5 were started on alternative therapy before day 21, and 3 did not have sufficient data recorded. Of the 47 evaluable patients, 29 had acute lymphoblastic leukemia, six had acute nonlymphoblastic leukemia, and 12 had solid tumors. All children had previously received at least two conventional therapeutic regimens and had received two or more investigational drugs. At least 4 patients, including 2 with solid tumors, were evaluable at each level (Table 1). Because of dose limiting myelosuppression, level 5 was the last level for accrual of patients with solid tumors.

Nonhematological Toxicity. The regimen was well tolerated in this study population. Minimal nausea and vomiting were
TABLE 1  F-ara-AMP doses in pediatric patients

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Loading dose (mg/m²)</th>
<th>Infusion rate (mg/m²/24 h)</th>
<th>Total dose (mg/m²)</th>
<th>Evaluable Patients</th>
<th>Evaluable Courses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>13.0</td>
<td>70.0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>16.5</td>
<td>88.5</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>7.0</td>
<td>20.0</td>
<td>107.0</td>
<td>5</td>
<td>6</td>
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<td>4</td>
<td>8.0</td>
<td>23.5</td>
<td>125.5</td>
<td>10</td>
<td>12</td>
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<tr>
<td>5</td>
<td>9.0</td>
<td>27.0</td>
<td>144.0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>30.5</td>
<td>162.5</td>
<td>19</td>
<td>33</td>
</tr>
</tbody>
</table>

PHARMACOLOGY OF F-ara-AMP IN CHILDREN

 Experienced by only 5 patients. One patient had diarrhea and 1 had a slight elevation of creatinine from 1.3 to 1.8 mg/dl. Three had pruritis and 2 had macular rashes. There were moderate reversible increases in transaminase enzyme levels to grade II in 9 patients at all dose levels and grade III in 1 patient each at dose levels 1 and 4. These toxicities did not appeared to be dose related.

Two patients developed neurological symptoms during the period of evaluation. One patient experienced a single transitory episode of tremors and confusion lasting <24 h on day 3 of an infusion at level 2 of F-ara-AMP. A second patient with non-Hodgkin's lymphoma died of progressive disease, 22 days after the beginning of treatment with level 4. Three days before his death, he developed progressive confusion. These episodes were not thought to be secondary to the treatment. Thus, the maximally tolerated dose for patients with leukemia was not reached.

Hematological Toxicity. Neutropenia and thrombocytopenia were not seen at dose levels 1–3 in patients without bone marrow involvement from their malignancy. At dose level 4, two patients had no toxicity, one had a grade 1 thrombocytopenia, and two patients had grade IV neutropenia and grade III or IV thrombocytopenia. One of 3 patients at dose level 5 had grade IV neutropenia and grade II thrombocytopenia with each of 2 courses of F-ara-AMP. Anemia was observed at dose levels 3–5. Thus, dose level 3 appears to be the maximally tolerated dose for patients with solid tumors.

Efficacy. One complete remission and three partial remissions were seen in children with acute lymphoblastic leukemia. The complete remission lasted for 55 days after the level 2 dose of F-ara-AMP. A partial remission after a level 6 dose persisted for 87 days. The other two partial remissions at levels 2 and 6 lasted for 34 and 38 days. The estimated response rate at dose level 6 was 13% (95% confidence interval, 2–40%). The estimated response rate for the aggregate study population was 15% (95% confidence interval, 4–35%). There were no responses in the other disease categories.

Pharmacokinetics of F-ara-A. A typical pharmacokinetic profile of F-ara-A from dose level 3 is depicted in Fig. 1. F-ara-A plasma concentrations were at steady state 2 h after the start of the loading bolus and continuous infusion of F-ara-AMP (Table 2). The plasma steady state concentration of F-ara-A increased linearly from 2.7 ± 1.5 to 7.2 ± 1.6 μM/m² with the dose levels 1–6 of F-ara-AMP (Fig. 1, Table 2). The elimination of F-ara-A after the end of the 5-day infusion was biexponential with a mean half-life of distribution of 1.2 ± 0.5 h (range, 0.59–3.7 h) and a mean half-life of elimination of 12.4 ± 3.4 h (range, 5.0–38.5 h). There was no appreciable variation of these parameters in the 6 dose levels of the drug. The AUC and the AUMC also increased linearly with the dose level. The MRT was 63.0 ± 11.2 h⁻¹ (n = 26) and the MRT postinfusion averaged 12.9 ± 5.1 h⁻¹ (n = 20). The mean of means of MRT, in patients from each dose level of F-ara-AMP, postinfusion was 15.4 ± 3.6 h⁻¹ (n = 6).
Table 2  Pharmacokinetic parameters of F-ara-A in plasma of children with cancer

<table>
<thead>
<tr>
<th>Dose level</th>
<th>n</th>
<th>Dose F-ara-AMP/F-ara-A</th>
<th>F-ara-A at 2 h (µM)</th>
<th>Cw (µM)</th>
<th>t1/2 (h)</th>
<th>AUC post-treatment (µM·h/L)</th>
<th>Total AUMC (liters·h/L)</th>
<th>Vd∞ (liters/m²)</th>
<th>TBC* (liters/h/m²)</th>
<th>TBC* (liters/h/m²)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>81.4 ± 4.42/50.1 ± 31.5</td>
<td>3.8 ± 0.6</td>
<td>2.7 ± 0.5</td>
<td>1.6</td>
<td>18.6 ± 13.4</td>
<td>342 ± 185</td>
<td>31.5 ± 20.6</td>
<td>21,100 ± 6,890</td>
<td>17.1 ± 12.7</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>105.1 ± 28.0/74.9 ± 20.0</td>
<td>7.9 ± 2.5</td>
<td>3.8 ± 2.6</td>
<td>3.8 ± 1.0</td>
<td>12.2 ± 3.2</td>
<td>457 ± 326</td>
<td>36.7 ± 19.6</td>
<td>25,900 ± 19,400</td>
<td>13.6 ± 7.3</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>101.2 ± 10.4/81.8 ± 22.0</td>
<td>3.5 ± 2.3</td>
<td>3.3 ± 1.1</td>
<td>0.85</td>
<td>8.9 ± 7.5</td>
<td>408 ± 126</td>
<td>28.4 ± 16.9</td>
<td>27,000 ± 12,200</td>
<td>13.0 ± 14.2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>124.1 ± 7.1/88.5 ± 5.17</td>
<td>6.5 ± 4.2</td>
<td>6.5 ± 4.1</td>
<td>0.7 ± 0.1</td>
<td>9.9 ± 2.1</td>
<td>840 ± 555</td>
<td>51.6 ± 27.8</td>
<td>54,100 ± 34,200</td>
<td>7.3 ± 5.1</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>140.3 ± 16.4/100.1 ± 11.4</td>
<td>8.1 ± 5.6</td>
<td>6.8 ± 3.6</td>
<td>1.1 ± 0.2</td>
<td>13.6 ± 2.0</td>
<td>840 ± 473</td>
<td>53.2 ± 56.3</td>
<td>54,600 ± 3,400</td>
<td>6.6 ± 2.4</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>241.0 ± 56.1/171.9 ± 40.0</td>
<td>7.2 ± 5.2</td>
<td>7.2 ± 1.6</td>
<td>1.9 ± 1.7</td>
<td>11.9 ± 5.0</td>
<td>781 ± 213</td>
<td>51.0 ± 19.6</td>
<td>50,300 ± 13,100</td>
<td>9.9 ± 6.8</td>
</tr>
</tbody>
</table>

Mean ± SD

* TBC* = dose/AUC∞.
† Mean ± SD.
‡ One was treated 3 times. Results from this patient were averaged.
§ One was treated 2 times. Results from this patient were averaged.
¶ One was treated 2 times. Results from this patient were averaged.

The limiting toxicities were neutropenia and thrombocytopenia. In our study, myelosuppression was observed following 1 mg/m² daily with solid tumors at comparable doses. With a loading dose of 8 mg/m² followed by 23.5 mg/m²/day for 5 days, two of five children had grade IV neutropenia and grade II or IV thrombocytopenia. One patient received 2 courses of 9 mg/m² followed by 27 mg/m²/day for 5 days and grade IV neutropenia.
obtained from the pretreatment (control) PBC. No other dose limiting toxicity was seen in this study. In patients with leukemia this regimen was well tolerated at level 6, a 10-mg/m² bolus followed by 30.5 mg/m²/day for 5 days.

Fludarabine phosphate was rapidly dephosphorylated to F-ara-A in children. This facilitates the pharmacokinetic analysis of F-ara-A in plasma because the pharmacokinetic equations can be applied for F-ara-A as if it was the injected form of the drug and not its plasma metabolite. A linear relationship was seen between plasma F-ara-A concentrations and the dose levels of F-ara-AMP. After the end of the infusion, F-ara-A declined in a biexponential fashion with a half-life of elimination of 12.4 h. The plasma elimination of F-ara-A has been described as bi- and triexponential in two adult studies after single i.v. bolus administration regimens (17, 18). The average terminal half-life of F-ara-A of 10–12 h in those studies is similar to our data in pediatric patients (Table 2).

Since F-ara-A has a terminal half-life of about 12 h, a steady state may not be reached for 1–2 days after the start of a continuous infusion. Our study documents that a steady state concentration is rapidly achieved with a loading bolus (Table 2). A plasma steady state concentration was maintained with the continuous infusion (Fig. 1). In addition, this regimen avoided the high peak plasma concentrations which have been reported for up to 24 h (0.5–1.1 μM) in one of the studies (17).

The plasma F-ara-A concentrations that were achieved in pediatric patients with this regimen are lower than the peak F-ara-A reported in adult patients (17, 18). The steady state concentrations, however, are higher (2–7 μM) than those reported for up to 24 h (0.5–1.1 μM) in one of the studies (17). The area under the curve from the end of the infusion to infinity compares favorably with the AUC in adult patients receiving a bolus injection (Table 2) (17). The total AUC from the beginning of treatment to infinity is much higher after this regimen than after the regimen of daily bolus injections for 5 days (17). Despite this fact, no CNS toxicity was seen in our patients. The average TBC of F-ara-A is 6-fold lower in pediatric patients than in adults (18). Also, the volume of distribution at steady state is lower when compared with that of adult patients (18). Since we used the adult data to estimate the loading bolus and constant rate of infusion of F-ara-AMP (dose levels 1–6), the lower TBC and volume of distribution at steady state may have contributed to the higher than expected Cₘ of F-ara-A in pediatric patients.

Cellular concentrations of F-ara-ATP gradually increased in leukemic blast cells over the 5-day infusion and reached peaks of 100–250 μM in four patients. In one patient, the half-life of elimination of cellular F-ara-ATP concentrations was determined at 13 h. This value is similar to the ones reported by Danhauser et al. (17) after 50- or 100- to 125-mg/m² i.v. bolus injections of F-ara-AMP administered over 30 min in adult patients with leukemia (17). In two patients, DNA synthesis was gradually inhibited throughout the 5-day infusion. The DSC decline in these patients and the elimination of the blast cells from peripheral blood in one patient obeyed first order kinetics after F-ara-AMP administration. Similar observations in the kinetics of these parameters have been made after high dose ara-C administration in pediatric patients with leukemia (27).

Studies of combinations of F-ara-A and ara-C have shown synergism that is both sequence selective and concentration dependent (28, 29). At least 5 μM F-ara-A and 10 μM ara-C are necessary to achieve significant synergism as measured by either increased cellular ara-CTP or decreases in DSC. Our regimen of bolus and continuous infusion of F-ara-AMP achieved this concentration of F-ara-A. F-ara-A must precede the ara-C administration. Lymphoblasts preloaded with F-ara-ATP during treatment of 2 children showed a >10-fold increase in cellular ara-CTP concentrations. Lymphoblasts from a third patient showed a marked decrease in the DSC. Maximal effect was seen after 2–3 days of the F-ara-AMP infusion.

Moderate clinical activity was seen in this highly pretreated population of children with acute lymphoblastic leukemia. On the basis of this activity and the potential interaction between F-ara-A and ara-C, the Childrens Cancer Study Group is further pursuing F-ara-AMP in a phase I study of a timed sequential combination of loading dose and 2-day infusion of F-ara-AMP followed by a 3-day infusion of ara-C.

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