Minimum Dose of Fludarabine for the Maximal Modulation of 1-β-d-Arabinofuranosylcytosine Triphosphate in Human Leukemia Blasts during Therapy

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ABSTRACT
1-β-d-Arabinofuranosylcytosine (ara-C), an effective drug for acute leukemias, must be phosphorylated to its 5'-triphosphate, ara-CTP, for activity. Our previous studies during therapy of acute myelogenous leukemia (AML) patients demonstrated that the accumulation of ara-CTP in circulating leukemia blasts was increased by a median of 2-fold when fludarabine (30 mg/m²/day over 30 min) was infused 4 h prior to intermediate dose ara-C. The augmentation was dependent on the cellular concentration of fludarabine triphosphate (F-ara-ATP). To determine the lowest dose of fludarabine needed for modulation of ara-C metabolism, the present study administered fludarabine at a test dose (15 mg/m² over 30 min) followed by 2 g/m² ara-C infused over 4 h. The next day, the fludarabine/ara-C couplet was repeated but with a standard dose (30 mg/m²) of fludarabine. There was a dose-dependent accumulation of F-ara-ATP in circulating leukemia blasts; the median peak concentrations were 33 and 41 μM with 15 and 30 mg/m² of fludarabine, respectively. These intracellular levels of F-ara-ATP effectively increased ara-CTP accumulation to similar levels. To further titrate the dose of fludarabine, the next cohort of patients (n = 4) initially received fludarabine test doses of 7.5 or 5 mg/m², followed by the 30 mg/m² dose of fludarabine on the next day; each dose was infused 4 h prior to 2 g/m² of ara-C. The peak levels of F-ara-ATP at 7.5 and 5 mg/m² fludarabine were between 3 and 39 μM. The AML blasts that achieved ≥10 μM intracellular F-ara-ATP accumulated ara-CTP similar to the levels achieved after 30 mg/m² of fludarabine. However, <10 μM intracellular F-ara-ATP resulted in less ara-CTP accumulation compared to that observed after the conventional dose of fludarabine. These data suggest that the modulation of the ara-CTP accumulation by fludarabine is dependent on the cellular concentration of F-ara-ATP, and that 15 mg/m² fludarabine infused over 30 min consistently produces cellular F-ara-ATP levels that maximize ara-CTP accumulation in AML blasts. These findings point to the feasibility of intensifying the fludarabine/ara-C regimen by using fludarabine as a 15 mg/m²/dose twice daily with intermediate-dose ara-C.

INTRODUCTION
ara-C, one of the most effective agents for therapy of AML (1), must be phosphorylated to its 5'-triphosphate (ara-CTP) to act as a cytotoxic agent (2, 3). Significant correlations have been observed between the pharmacokinetics of ara-CTP in circulating leukemia blasts and clinical response to single-agent high-dose ara-C therapy given either on an intermittent schedule (4, 5) or by continuous infusion (6). These results illustrate the importance of ara-CTP accumulation and retention to treatment outcome and suggest the utility of therapeutic strategies that augment ara-CTP levels in leukemia blasts.

Our previous studies demonstrated that infusion of fludarabine (F-ara-A monophosphate) prior to intermittent infusion of intermediate-dose ara-C increases ara-CTP accumulation by a median 2-fold in circulating leukemia blasts of patients with AML receiving this combination regimen (7). This biochemical modulation was due to the influence of F-ara-A triphosphate (F-ara-ATP) on the rate of ara-CTP accumulation (7, 8). The standard fludarabine dose (30 mg/m²) administered to patients with AML or CLL resulted in a range of cellular F-ara-ATP concentrations (median, 16 μM; range, 8–58 μM). However, consistent with studies in human leukemia cell lines (8), the fludarabine-induced increase in the rate of ara-CTP accumulation appeared to be dependent upon the cellular concentration of F-ara-ATP in CLL lymphocytes (n = 8) and AML blasts (n = 10) (9). Although it was clear that ara-CTP accumulation was not further augmented by peak cellular F-ara-ATP concentrations greater than 15 μM, only two patients in these groups had peak F-ara-ATP levels less than 10 μM. This distribution precluded determination of the lowest effective cellular F-ara-ATP concentration for enhancing ara-CTP metabolism.

3 The abbreviations used are: ara-C, 1-β-d-arabinofuranosylcytosine; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; ara-CTP, 1-β-d-arabinofuranosylcytosine 5'-triphosphate; AUC, area under the concentration time curve; F-ara-A, 9-β-d-arabinofuranosyl-2-fluoroadenine; F-ara-ATP, 9-β-d-arabinofuranosyl-2-fluorojadeline 5'-triphosphate; fludarabine, F-ara-A monophosphate; G-CSF, granulocyte colony stimulating factor; FLG, fludarabine/ara-C/G-CSF; HPLC, high-pressure liquid chromatography.
Nevertheless, these data suggested that a lower dose of fludarabine might result in cellular F-ara-ATP concentrations sufficient to maximize the rate of ara-CTP accumulation. Furthermore, the finding that G-CSF administered with the fludarabine-ara-C couplet on the FLAG protocol (10, 11) was associated with a 40% increase in F-ara-ATP accumulation indicates that a lesser dose of fludarabine might effectively modulate ara-CTP metabolism. Hence, the objective of the present investigation was to determine the minimum dose of fludarabine necessary to augment ara-CTP accumulation to levels achieved by the conventional dose of fludarabine (30 mg/m² over 30 min).

PATIENTS AND METHODS

Patients and Treatment Plans. Nine patients (five males) with relapsed AML, a median age of 57 years (range, 20–78 years), and a median WBC count of 24,000/µl (range, 9,000–39,000/µl) were treated on a modified FLAG protocol (11), which stipulated that the treatment start with a 400 µg/m²/day dose of G-CSF. Infusion of G-CSF continued every day during chemotherapy. On day 2, fludarabine (either 15, 7.5, or 5 mg/m²) was infused over 30 min, followed 4 h later by a dose of 2 g/m² of ara-C given over 4 h. Twenty-four h after infusion of the first fludarabine, patients received another couplet of fludarabine and ara-C, with the fludarabine infused at 30 mg/m² over 30 min (standard dose fludarabine). Subsequently, fludarabine and ara-C were administered daily for 3 days; hence, one course of therapy included five couplets of fludarabine and ara-C. G-CSF was given each day until the recovery of neutrophils (similar to the FLAG protocol; Ref. 11). Two patients (nos. 1 and 6) did not receive G-CSF due to a high WBC count in the peripheral blood.

Drugs. Berlex Laboratories, Inc. (Richmond, CA) provided fludarabine as a sterile, lyophilized powder free of antibacterial preservatives. ara-C was obtained commercially as Cytosar-U from the Upjohn Co. Recombinant human G-CSF (300 µg/vial) was obtained from Amgen, Inc. (Thousand Oaks, CA). The specific activity was 1 × 10⁶ units/mg of protein as assessed by the cell mitogenesis assay. For in vitro investigations, F-ara-A was obtained by alkaline phosphatase treatment of fludarabine. F-ara-ATP was synthesized by Sierra Bioresearch (Tucson, AZ). ara-C and ara-CTP were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade.

Blood Samples for Clinical Pharmacology. To determine the pharmacokinetics of F-ara-ATP and ara-CTP in circulating blasts in patients treated on this protocol, 40-ml blood samples were obtained on days 1 and 2 before therapy; 10-ml samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 9 h after the start of therapy each day. This schedule enabled the investigation of F-ara-ATP pharmacology for 9 h and ara-CTP for 5 h after the start of infusion with the respective drugs. All blood samples were collected in vacutainer tubes containing heparin. The tubes were immediately placed in an ice-water bath and transported promptly to the laboratory for processing. Control studies have demonstrated that under these conditions, leukemia cells are stable for at least 15 h with respect to size, membrane integrity, and cellular nucleotide content (12).

Plasma Pharmacology. Plasma samples prior to therapy and at the end of fludarabine infusion were analyzed for F-ara-A levels at different doses of fludarabine. The plasma samples were deproteinized by rapid filtering through Centrifree micropartition filters (Amicon, Inc., Beverly, MA) as described by the manufacturer. F-ara-A was separated from natural nucleosides using µBondapak C₁₈ columns (Waters Associates, Milford, MA) using a linear gradient of ammonium acetate and methanol. The F-ara-A peaks were quantitated using authentic standard F-ara-A, which was prepared in pooled plasma and filtered similarly. The lower limit of detection was 0.01 nmol, and detection was linear to 1 nmol.

Cellular Pharmacology. After removal of plasma, the cell pellet was resuspended in PBS (8.1 g of NaCl, 0.22 g of KCl, 1.14 g of Na₂HPO₄, and 0.27 g of KH₂PO₄ per liter of H₂O, pH 7.4), and mononuclear cells were isolated by Ficoll-Hypaque density-gradient centrifugation procedures (12). After enumeration and cell size determinations with a Coulter counter (Coulter Electronics, Hialeah, FL), natural and arabinosyl nucleotides were extracted from blasts by HClO₄ and ara-CTP and F-ara-ATP were separated from ribonucleoside triphosphates by HPLC on an anion-exchange Partisil-10 SAX column (13). Ara-CTP and F-ara-ATP were quantified at 262 nm by electronic integration with reference to external standards. The intracellular concentrations of nucleotides were calculated and expressed as the quantity of nucleotides contained in the extract from a given number of cells of a determined mean volume. This calculation assumes that nucleotides are uniformly distributed in total cell water (13).

Calculations and Statistical Analysis. The levels of ara-CTP and F-ara-ATP obtained by HPLC analysis were normalized based on the concentrations of endogenous nucleotides during sampling times in each individual (14). The AUC for the accumulation of ara-CTP or F-ara-ATP in leukemia cells was estimated by gravimetric procedures. The AUC of F-ara-ATP represented the total AUC of accumulation and elimination, whereas the AUC of ara-CTP was the accumulation AUC until 4 h. Pharmacological data obtained during the first and second doses of ara-C or fludarabine were compared using the two-tailed, paired t test.

RESULTS

Fludarabine at 15 mg/m² versus 30 mg/m² Dose

F-ara-ATP Pharmacokinetics. For illustrative purposes, the pharmacokinetics of F-ara-ATP in circulating leukemia blasts of a patient who received fludarabine doses of 15 mg/m² and then 30 mg/m² are presented in Fig. 1. At both infusion doses, F-ara-ATP accumulation was linear for nearly 2 h; thereafter, the rate diminished until a peak was reached 3 or 4 h after the start of the infusion. The elimination of F-ara-ATP, studied for 5–6 h after peak levels, was linear when evaluated by semilogarithmic plots (data not shown) and was not altered at the higher dose infusion of fludarabine. The accumulation of F-ara-ATP was dose dependent, and peak levels of F-ara-ATP were higher with 30 mg/m²/day infusion compared to 15 mg/m²/day dose of fludarabine (Fig. 1A).

ara-CTP Pharmacokinetics. To determine whether the levels of F-ara-ATP accumulated after the 15 mg/m² dose would
be sufficient to augment ara-CTP accumulation similarly to the standard dose fludarabine, ara-CTP accumulation profiles were compared after fludarabine doses of 15 and 30 mg/m²; ara-CTP accumulated at near linear rates during the infusion duration of ara-C. Despite the differences in F-ara-ATP accumulation shown in Fig. 1A, the rates of ara-CTP accumulation in circulating leukemia blasts of the same patient did not differ (Fig. 1B) between doses of fludarabine. The linear rates of ara-CTP accumulation in leukemia cells of this patient were 290 and 230 μM/h after 15 and 30 mg/m² dose of fludarabine, respectively. The pharmacokinetics of F-ara-ATP and ara-CTP were compared in the blasts of four patients after 30-min fludarabine infusions of 15 and 30 mg/m² (Table 1). The accumulation rates and the peak levels of ara-CTP were similar after each dose of fludarabine, suggesting that the cellular F-ara-ATP levels that resulted from 15 mg/m² of fludarabine were sufficient to maximally modulate ara-CTP accumulation. Although there was interpatient heterogeneity in the absolute values, the rate of F-ara-ATP accumulation was about 2-fold higher at the 30-mg/m² dose compared to that achieved with 15 mg/m² dose (data not shown). The median peak levels of F-ara-ATP in AML blasts were 33 μM (range, 20–60 μM) at 15 mg/m²/day (Table 1) of fludarabine. Because the ara-CTP pharmacokinetics did not change significantly (P = 0.187) after each dose of fludarabine (15 or 30 mg/m²), these data suggest that a median 33 μM peak of F-ara-ATP achieved with 15-mg/m² dose of fludarabine is sufficient to maximally modulate ara-CTP accumulation in these cells during the combination therapy.

**Fludarabine at 7.5 mg/m² versus 30 mg/m² Dose**

**F-ara-ATP Pharmacokinetics.** To further titrate the dose of fludarabine needed for ara-CTP modulation, the first fludarabine dose was lowered to 7.5 mg/m² in a cohort of three patients. Again for comparison purposes, the second infusion of fludarabine was set at 30 mg/m². The pharmacokinetic profile of F-ara-ATP in the leukemia blasts of one representative patient is shown in Fig. 2A. The peak levels of F-ara-ATP after infusion of 7.5 mg/m² of fludarabine (median, 10 μM) were proportional.

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*Fig. 1* F-ara-ATP (A) and ara-CTP (B) pharmacokinetics in circulating leukemia blasts of a patient (no. 2) after 15 or 30 mg/m²/day of fludarabine infusion with ara-C. The patient received G-CSF on day 1, 15 mg/m² fludarabine with ara-C on day 2, and 30 mg/m² fludarabine with ara-C on day 3. F-ara-ATP and ara-CTP accumulation were quantitated on day 2 (after 15 mg/m²/day of fludarabine; ○) and on day 3 of therapy (after 30 mg/m²/day of fludarabine; ●) as described in “Patients and Methods.”

**Table 1** Comparison of ara-CTP and F-ara-ATP pharmacokinetics after 15 or 30 mg/m² dose of fludarabine

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Ara-CTP accumulation</th>
<th>F-ara-ATP accumulation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Peak, μM</td>
<td>Rate, μM/h</td>
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<tr>
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<td>15⁰</td>
<td>163</td>
</tr>
<tr>
<td>2</td>
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* Dose of fludarabine in mg/m² over 30 min.
Fig. 2  F-ara-ATP (A) and ara-CTP (B) pharmacokinetics in circulating leukemia blasts of a patient (no. 5) after 7.5 or 30 mg/m²/day of fludarabine infusion with ara-C. The patient received G-CSF on day 1, 15 mg/m² fludarabine with ara-C on day 2, and 30 mg/m² fludarabine with ara-C on day 3. F-ara-ATP and ara-CTP accumulation were quantitated on day 2 (after 7.5 mg/m²/day of fludarabine; ○) and on day 3 of therapy (after 30 mg/m²/day of fludarabine; ◦) as described in “Patients and Methods.”

Table 2  Comparison of ara-CTP and F-ara-ATP pharmacokinetics after 7.5 or 30 mg/m² dose of fludarabine

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Ara-CTP accumulation</th>
<th>F-ara-ATP accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak, µM</td>
<td>Rate, µM/h</td>
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<td></td>
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<td>30°</td>
</tr>
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<td>630</td>
<td>520</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>ND</td>
</tr>
</tbody>
</table>

- Dose of fludarabine in mg/m² over 30 min.
- First dose of fludarabine was 5 mg/m² over 30 min.
- ND, not done.

Relationship between Dose of Fludarabine and Pharmacology of F-ara-A and F-ara-ATP

The pharmacokinetic studies conducted with F-ara-ATP accumulation suggested a dose dependency between the fludarabine dose rate and the intracellular F-ara-ATP level. This was consistent with the fact that the peak plasma level of F-ara-A was dose dependent. The peak plasma F-ara-A concentrations were 0.2, 1.4 ± 0.6, 2.6 ± 0.9, and 3.2 ± 1.4 µM at 5.0 (n = 1), 7.5 (n = 2), 15 (n = 3), and 30 (n = 7) mg/m² of fludarabine, respectively. The accumulation of F-ara-ATP seems to be de-
peak concentrations of ara-CTP remain similar after identical dose fludarabine (Fig. 3). The median levels of peak F-ara-ATP at ATP, peak levels of F-ara-ATP were plotted against doses of between different doses of fludarabine and intracellular F-ara-
dose fludarabine compared to that achieved with lower doses of
higher levels of F-ara-ATP were achieved with the standard pendent on the plasma pharmacokinetics, and significantly higher levels of F-ara-ATP were achieved with the standard dose fludarabine compared to that achieved with lower doses of fludarabine (P = 0.005; n = 8). To determine the relationship between different doses of fludarabine and intracellular F-ara-ATP, peak levels of F-ara-ATP were plotted against doses of fludarabine (Fig. 3). The median levels of peak F-ara-ATP at each dose were 10, 33, and 41 μM at 7.5, 15, and 30 mg/m² fludarabine, respectively. These data demonstrate a strong, direct, and linear relationship between these two parameters (r = 0.75, P = <0.001 when each peak F-ara-ATP value was taken as a separate point; or r = 0.99, P = 0.002 when the mean and SE F-ara-ATP at each dose level was considered as a point).

DIscussion

Our previous studies using the fludarabine and ara-C combination regimen demonstrated that the rate of ara-CTP accumulation could be augmented with conventional dose fludarabine (7), when ara-C is given at a dose that otherwise saturates the rate of ara-CTP synthesis (15, 16). The modulatory effect of fludarabine on ara-CTP metabolism was not limited to AML blasts (7); similar effects were observed in CLL lymphocytes (20) and ALL lymphoblasts (19). During these separate clinical trials, the fludarabine was infused to all patients over 30 min at 30 mg/m² (standard dose of fludarabine). Previously, we have compared the pharmacokinetic profiles of ara-CTP during first and second infusions of ara-C, when the standard dose of fludarabine with intermediate-dose ara-C couples were infused serially. The data from five AML patients who were studied during serial infusions of fludarabine and ara-C demonstrated that the peak concentrations of ara-CTP remain similar after identical infusions on consecutive days (Table 3). This is also illustrated by the observation that the AUC of ara-CTP remained similar during two consecutive doses of single-agent ara-C infusions (median AUC ratio, 1.0; range, 0.7 -1.2; Refs. 17 and 18). These data suggest that comparison of ara-CTP accumulation in serial doses of ara-C is appropriate to test the modulatory influence of effectors such as different doses of fludarabine.

The present studies conducted in AML blasts isolated during therapy demonstrated that fludarabine infused at 15 mg/m²/d potentiated ara-CTP accumulation in circulating leukemia blasts to the same extent as a dose of 30 mg/m²/day (standard dose; Table 3). Further lowering the dose of fludarabine to 7.5 or 5 mg/m²/day provided mixed results regarding augmentation of ara-CTP. In one of four patients, there was a 30% reduction in ara-CTP accumulation when the 7.5 mg/m² dose of fludarabine was combined with ara-C. In the remaining three patients, the increase in ara-CTP accumulation was similar with the lower dose (5 or 7.5 30 mg/m²/day) and the standard dose (30 mg/m²). Because there was heterogeneity among patients regarding the pharmacokinetics of F-ara-ATP, a relationship was sought between the levels of F-ara-ATP in circulating leukemia blasts and the potentiation effect on ara-CTP accumulation. These studies revealed that seven of seven patients whose circulating leukemia blasts achieved ≥10 μM peak intracellular F-ara-ATP augmented ara-CTP accumulation to similar levels as achieved by conventional dose fludarabine, which produced a median peak of 41 μM (n = 8; Tables 1 and 2) F-ara-ATP. Because the 30 min infusion of 15 mg/m²/day of fludarabine achieved >10 μM F-ara-ATP in circulating leukemia blasts of four of four patients studied, this dose may be recommended to be used with ara-C administration for patients with AML.

Relevant aspects of our previous data obtained in patients with acute lymphoblastic leukemia treated with the fludarabine and ara-C regimen are consistent with the present observation. Leukemic lymphoblasts that accumulated 7 μM peak levels of F-ara-ATP did not augment ara-CTP accumulation during therapy compared to ara-CTP accumulation when ara-C was infused alone (19). Similar results were also obtained in lymphocytes obtained from patients with chronic lymphocytic leukemia whose lymphocytes have a somewhat lesser augmentation of ara-CTP accumulation (median, 1.3-fold) in response to the fludarabine and ara-C combination therapy (20). In these quiescent cells, the influence of F-ara-ATP on the activity of deoxycytidine kinase to modulate ara-CTP metabolism would be a direct effect of F-ara-ATP on the activity of the enzyme (14). More proliferative populations such as AML cells experience, in addition to a direct effect, an indirect effect of F-ara-ATP on the activity of deoxycytidine kinase. This is

**Table 3** Correlation between dose of fludarabine, intracellular F-ara-ATP, and increase in ara-CTP accumulation

<table>
<thead>
<tr>
<th>Test dose of fludarabine</th>
<th>Peak F-ara-ATP, μM</th>
<th>Peak ara-CTP ratio, test dose/standard dose</th>
<th>Median (range)</th>
<th>Median (range)</th>
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<tr>
<td>15</td>
<td>4</td>
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<td>1.1 (1.0–1.4)</td>
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<tr>
<td>7.5 and 5</td>
<td>5</td>
<td>10 (3–39)</td>
<td>1.1 (0.6–1.2)</td>
<td>Present</td>
<td></td>
</tr>
</tbody>
</table>

![Fig. 3 Relationship between intracellular accumulation of F-ara-ATP and fludarabine dose. F-ara-ATP peak levels from all patients studied are plotted against the 5.0 mg/m² (●), 7.5 mg/m² (●), 15 mg/m² (▲), and 30 mg/m² (○) dose of infusion. F-ara-ATP was separated and quantitated by HPLC as described in "Patients and Methods."](Image)
mediated via F-ara-ATP inhibition of ribonucleotide reductase and the subsequent release of feedback regulation of deoxycytidine kinase exerted by dCTP. The IC_{50} of F-ara-ATP for inhibition of ribonucleotide reductase, which range between 1 and 12 μM (21–25), are consistent with this mechanism and greater effect on the rate of ara-CTP synthesis (median, 2-fold).

Hence, it appears that 10 μM intracellular F-ara-ATP is sufficient for both direct and indirect actions to activate ara-C phosphorylation by deoxycytidine kinase.

The pharmacokinetics of F-ara-ATP showed a strong, direct, and linear relationship between infusion dose of fludarabine and accumulation levels of F-ara-ATP in circulating leukemia blasts. This was expected because the peak plasma concentration of F-ara-A achieved after a 30-min infusion of a 30-mg dose of fludarabine was about 3 μM (26). At 15 and 7.5 mg/m² infusion doses, proportionally lower F-ara-A concentrations in plasma would be expected. This is based on the fact that a linear relationship was observed between increasing doses of fludarabine and plasma concentrations of F-ara-A (27). In the cells, F-ara-A is phosphorylated to its monophosphate, which is then converted to di- and triphosphates (28). Cellular deoxycytidine kinase catalyzes the conversion of F-ara-A to its monophosphate, which is the rate-limiting step in the formation of its triphosphate (29, 30). Because the K_{m} for F-ara-A phosphorylation by deoxycytidine kinase is between 100 and 600 μM (30–32), the plasma F-ara-A concentration achieved after a conventional dose of fludarabine is far below optimal levels, but it could be predicted that the accumulation of F-ara-ATP would be linear. Our data (Fig. 3) are consistent with this prediction and the fact that higher doses of fludarabine (100–125 mg/m²/ day over 30 min) resulted in a median 7 μM (n = 4) peak plasma levels of F-ara-A and greater concentrations of intracellular F-ara-ATP (median, 170 μM) in leukemia blasts than that obtained through a conventional dose of fludarabine (33). Consistent with these results, bolus (2–5 min) administration of different doses of fludarabine demonstrated dose-dependent peak levels of F-ara-A; 30–40 μM at 80–120 mg, 60 μM at 160 mg, and 100 μM at 260 mg dose (34).

The present investigation has clear implications for therapeutic applications involving the combination of fludarabine and ara-C for patients with AML. When ara-C is given on an intermittent schedule, it generally is administered twice daily (every 12 h; Refs. 35 and 36). This is done to intensify the therapy by maintaining a trough level of ara-CTP in circulating leukemia blasts that is inhibitory to DNA synthesis. Daily doses of fludarabine (80–150 mg/m²) have resulted in grade 3–4 and irreversible neurotoxicity (37, 38), making it unsafe to administer, twice a day, the standard dose of fludarabine with ara-C. The standard daily dose of fludarabine (30 mg/m²), administered on a 5-day schedule, however, is established to be safe and effective. It is, therefore, not unreasonable to propose that fludarabine could be infused twice each day at a dose of 15 mg/m² before intermediate-dose ara-C infusions. This would result in sufficient intracellular F-ara-ATP to modulate metabolism of ara-C infused 4 h after fludarabine administration.

A second application of lower dose of fludarabine with ara-C could be for patients with suboptimal renal function. Previous studies combining fludarabine with ara-C demonstrated that this regimen is well tolerated (10, 39, 40). AML or myelodysplastic syndrome patients with normal renal function did not experience any central nervous system-related toxicity when treated with regimens that include 30 mg/m²/day of fludarabine (10, 39). However, patients with impaired renal function and/or in an age group of >70 years had a trend toward increased toxicity caused by fludarabine (41), and reduction in fludarabine dose is recommended for these patients. Because coadministration of 15 mg/m²/day of fludarabine with ara-C would maximally potentiate ara-CTP accumulation, this lower dose of fludarabine will result in less risk of toxicity in patients with suboptimal renal function without sacrificing the beneficial effect of biochemical modulation of ara-CTP accumulation.

In conclusion, the present study illustrates that a lower dose of fludarabine (such as 15 mg/m²) infused over 30 min is sufficient to produce 10 μM or higher intracellular levels of F-ara-ATP to biochemically modulate ara-CTP accumulation to the same level as standard dose fludarabine. Hence, the 15 mg/m² dose of fludarabine may be combined with ara-C to provide twice-a-day infusions of fludarabine and ara-C couplet in patients with suboptimal renal function. A Phase I protocol for treatment of patients with relapsed acute leukemia has been activated recently; the treatment intensity is being increased by escalating the number of fludarabine/ara-C couplet administrations in subsequent patients.

REFERENCES


Minimum dose of fludarabine for the maximal modulation of 1-beta-D-arabinofuranosylcytosine triphosphate in human leukemia blasts during therapy.
