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9-β-D-Arabinofuranosyl-2-fluoroadenine 5′-monophosphate pharmacokinetics in plasma and tumor cells of patients with relapsed leukemia and lymphoma*

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Summary. The pharmacokinetics of 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A) in plasma and its biologically active 5′-triphosphate (F-ara-ATP) in leukemic cells obtained from the peripheral blood and bone marrow was evaluated in patients with hematologic malignancies subsequent to the first dose of 20–125 mg/m^2 per day for 5 days of F-ara-A 5′-monophosphate (F-ara-AMP) administered as an IV bolus over 30 min. The terminal half-lives of elimination of both F-ara-A (8 h) in plasma and intracellular F-ara-ATP (15 h) were not dependent upon the dose of F-ara-AMP. The area under the concentration × time curves for F-ara-A and F-ara-ATP, on the other hand, were increased in proportion to the prodrug dose. There was a high correlation between F-ara-ATP levels in circulating leukemic cells and those in bone marrow cells aspirated at the same time. DNA-synthetic capacity of leukemic cells was inversely related to the associated F-ara-ATP concentration. A linear trend was noted when F-ara-ATP levels in pretreatment peripheral blood leukemic cells incubated with F-ara-A in vitro were compared with the amount of F-ara-A that was incorporated into nucleic acids. Finally, F-ara-ATP concentrations were three times higher in bone marrow cells from patients with lymphomatous bone marrow involvement than from those without evidence of marrow disease.

Introduction

The striking antileukemic activity of the pyrimidine analogue 1-β-D-arabinofuranosylcytosine (ara-C) prompted the search for purine nucleoside derivatives that might be similarly effective agents. 9-β-D-Arabinofuranosyl-adenine (ara-A) proved to be an active antiviral agent [28], but had limited antitumor activity because of its rapid deamination by adenosine deaminase [2, 7, 12]. The antitumor efficacy of ara-A could be augmented by combining this agent with a nontoxic dose of the adenosine deaminase inhibitor 2′-deoxycoformycin [5, 13, 20]. Such combination chemotherapy, however, which elevates the natural nucleotide dATP, may produce a cellular milieu that is antagonistic to the inhibitory action of the active moiety, ara-ATP, on DNA synthesis [24]. The observation that a fluorine atom substituted for a hydrogen at the 2-position of the purine component of adenosine could render this compound relatively resistant to deamination led to the synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A) [17, 18]. In the absence of adenosine deaminase inhibitors, F-ara-A produced cytotoxicity against cultured human lymphoblastoid cells that was comparable to that of ara-A combined with 2′-deoxycoformycin [22].

Fludarabine phosphate (F-ara-AMP), the soluble 5′-monophosphate derivative of F-ara-A, is currently undergoing Phase I–II clinical trials [9, 10, 16, 31] (L. Danhauser, W. Plunkett, J. Liliemark, V. Gandhi, S. Iacoboni, M. Keating, 1985, submitted for publication). F-ara-AMP is rapidly dephosphorylated to F-ara-A in both animals and man [6, 15, 19] (L. Danhauser et al., 1985,submitted for publication]. F-ara-A is initially phosphorylated by deoxycytidine kinase [4, 8] and then is converted to its biologically active 5′-triphosphate, F-ara-ATP [3, 22] (L. Danhauser et al., 1985, submitted for publication). F-ara-A irreversibly inhibits S-adenosylhomocysteine hydrolase [32], whereas F-ara-ATP inhibits ribonucleotide reductase and the DNA polymerases with subsequent inhibition of DNA synthesis [22, 30, 32]. In addition, F-ara-ATP becomes incorporated into RNA as well as into DNA [20].

The aim of this investigation was to evaluate the pharmacokinetics of F-ara-A in plasma and of F-ara-ATP in leukemic cells obtained from the peripheral blood and bone marrow of patients with hematologic malignancies who had relapsed or failed to respond to prior chemotherapy. We hoped to identify parameters that would prove useful to predict the efficacy of this agent.

Materials and methods

F-ara-A and erythro-9-(2-hydroxy-3-nonyl)adenine were the kind gifts of Ven L. Narayanan, PhD, Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, Md. F-ara-AMP for clinical use was supplied by the National Cancer Institute as a sterile, lyophilized powder (200 mg/vial) free of antibacterial preservatives; it was reconstituted with 10 ml sterile water. [8-^{3}H]F-ara-A (13–18 Ci/mmol), obtained from Moravek Biochemicals (Brea, Calif), was routinely purified by high-pressure liquid chromatography (HPLC) on μBondapak C_{18} (Waters Associates, Inc., Milford, Mass) using previously described methods [1]. The radioactive fractions eluting in

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the region corresponding to F-ara-A were pooled, reduced to dryness, redissolved in water, then rerun isocratically on the HPLC using 20% MeOH as the eluant to desalt the sample. Fractions coeluting with F-ara-A were again pooled, reduced to dryness, and redissolved in 50% ethanol. The resulting [8-^{3}H] F-ara-A was found to be of greater than 99.6% homogeneity. ICN Pharmaceuticals, Inc. (Irvine, Calif) supplied [methyl-^{3}H]thymidine (50 Ci/mmol). All other chemicals were reagent grade.

All patients in the studies reported had been previously treated for leukemia or lymphoma and had persistent or recurrent (refractory) hematologic malignancies. Patients were older than 15 years of age and generally had not received chemotherapy, immunotherapy, or radiotherapy in the 3 weeks prior to being entered in these studies. A total of 19 patients were evaluated, 9 with leukemia [3 with acute myeloblastic leukemia (AML), 1 with acute myelomonocytic leukemia (AMML), 3 with acute lymphocytic leukemia (ALL), 1 with chronic myelogenous leukemia in blastic crisis (CML-BC), 1 with chronic lymphocytic leukemia (CLL)] and 10 with lymphoma [4 with diffuse, large cell lymphoma (DLCL), 2 with nodular, poorly differentiated lymphoma (NPDL), 3 with diffuse, well-differentiated lymphoma (DWDL), 1 with nodular, mixed cell lymphoma (NMCL)]. Twelve patients were male: seven were female. Patients were required to have a performance status of $\geqq 3$ (Zubrod scale) and a life expectancy of at least 6 weeks. Adequate hepatic (bilirubin <2.0 mg/100 ml) and renal (creatinine <1.5 mg/100 ml) functions were necessary for a patient to be eligible for the study. Patients received 20–125 mg/m^2 F-ara-AMP (every day for 5 days) as an IV bolus infused over 30 min. Generally, pharmacological profiles were determined after the first dose of F-ara-AMP.

For pharmacological studies, blood samples (10 ml) or bone marrow aspirates (2–3 ml) were drawn into sterile heparinized tubes and placed in an ice bath. Blood samples were centrifuged at 450 *g* for 5 min, an aliquot of the plasma was removed, and erythro-9-(2-hydroxy-3-nonyl)adenine (1 μ*M*) was added to the plasma prior to storage at −20 °C for later F-ara-A quantitation. F-ara-A-containing plasma was deproteinized by rapid ultrafiltration through Amicon CF-25 conical filters (Amicon Corp., Lexington, Mass). Recoveries of drug added to plasma were in excess of 85%. F-ara-A was separated from natural nucleosides by using a column of μBondapak C_{18} [1].

Mononuclear cells were isolated from whole blood or bone marrow by standard step-gradient density centrifugation procedures [23]. Cells were counted and the mean cell volume determined for each sample with a Coulter counter (Model ZM, Coulter Electronics, Hialeah, Fla) equipped with a cell size analyzer (Model C-1000). The cells were then washed in phosphate-buffered saline (PBS, containing per liter: 8.1 g NaCl, 0.22 g KCl, 1.14 g Na_4PO_4, 0.27 g KH_2PO_4; pH 7.4), and nucleotide pools were extracted with $HClO_4$ [23]. F-ara-ATP was separated from natural nucleotides by HPLC using gradient elution from anion-exchange resin [22]. The identity of F-ara-ATP was confirmed by its coelution with authentic F-ara-ATP, its resistance to periodate oxidation, and the ratio of absorbance at 262 nm:280 nm (3.8). Quantitation of F-ara-ATP was determined by electronic integration of preprogrammed response factors. The intracellular concentration of F-ara-ATP was expressed as the quantity of F-ara-ATP contained in the $HClO_4$-soluble fraction extracted from a given number of cells of determined mean volume. Total intracellular exposure to antimetabolites (area under the concentration × time curve, AUC), and half-life of elimination, $t_{1/2}$, was quantitated as described elsewhere [14] (L. Danhauser et al., 1985, submitted for publication).

The effect of F-ara-A treatment on DNA synthesis in malignant cells was determined using reported methods [25]. The incorporation of F-ara-A into nucleic acids was determined by incubation (in duplicate) at 37 °C for 1 h of $1-2\times10^7$ mononuclear cells (obtained prior to F-ara-AMP therapy) in RPMI 1640 medium supplemented with 5% fetal bovine serum (Grand Island Biologicals, Grand Island, NY) and F-ara-A (1 μ*M*) containing [8-^{3}H]F-ara-A (final specific activity of 3×10^9 dpm/μmol). Cells were then washed with 10 volumes of ice-cold PBS, and the F-ara-ATP was extracted with $HClO_4$ [22]. The cell pellet was washed twice with 0.8 *N* $HClO_4$ before addition of 1 ml PBS and 3 drops of 1 *N* KOH to solubilize the pellet. F-ara-ATP was assayed by HPLC [22] and radioactive fractions eluting in the region of F-ara-ATP were collected. The radioactivity in the $HClO_4$-soluble and $HClO_4$-insoluble fractions was measured by liquid scintillation counting. The number of molecules of F-ara-A in nucleic acids per cell was quantitated as:

$$\frac{\dfrac{\text{dpm in HClO}_4\text{-insoluble fraction}}{\text{Specific activity of F-ara-A (dpm/mol)}} \times \text{(6.02} \times 10^{11} \text{ Molecules/pmol)}}{\text{Number of cells}}$$

Results

The plasma pharmacokinetics of F-ara-A in patients receiving 50–125 mg/m^2 of F-ara-AMP are summarized in Table 1 and Fig. 1. F-ara-AMP was undetectable in the plasma at the times when the first samples were obtained. Of patients receiving 20–25 mg/m^2 F-ara-AMP, only two had detectable peak F-ara-A levels (of 1.4 and 2.2 μ*M*). F-ara-A levels were undetectable in this group of patients 3 h after the F-ara-AMP infusion was completed. The disappearance of F-ara-A in plasma was biphasic and independent of dose, with an initial rapid rate of elimination (median of 1.41 h) and a slow terminal rate of elimination

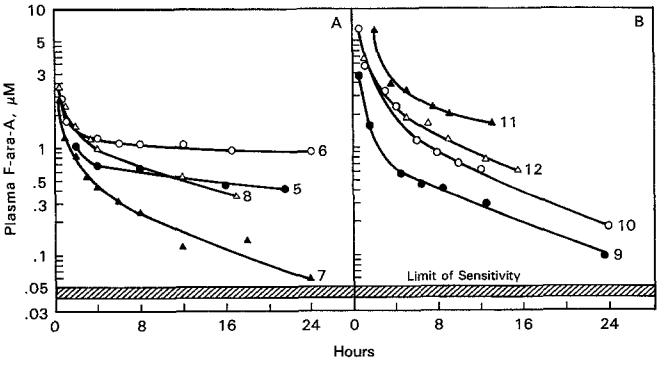

Fig. 1 A, B. Plasma profiles of F-ara-A levels following an IV bolus dose of F-ara-AMP 50 mg/m^2 **(A)** or 100–125 mg/m^2 **(B)**. Plasma samples were deproteinized by rapid ultrafiltration through Amicon CF-25 conical filters. F-ara-A was separated from natural nucleosides by HPLC using gradient elution on a reverse-phase μBondapak C_{18} column (see *Materials and methods)*

Table 1. Pharmacological characteristics for F-ara-A in the plasma of patients with relapsed leukemia

Patient	F-ara-AMP Dose (mg/m²)	F-ara-A Parameters		
		$t_{1/2}\alpha$[a] (h)	$t_{1/2}\beta$[b] (h)	AUC[c] (μM-h)
5	50	3.30[d]	23.90	14
6	50	0.49	>24.00	28
7	50	1.42	7.77	10
8	50	1.25	7.76	16
Median	50	1.34	7.76[e]	15
9	100	1.40	8.90	15
10	100	1.87	6.88	37
11	125	0.93[d]	13.00	94
12	125	2.20	6.22	37
Median	112.5	1.64	7.89	37

Patient numbers as in Table 2

[a] Initial rate of elimination
[b] Terminal rate of elimination
[c] Area under the concentration x time curve calculated to 24 h
[d] As the 2-h sample was the earliest obtained, this value is based on extrapolation of the line to 30 min
[e] The median value excluding patients 5 and 6 whose elevated creatinine levels may signal impaired renal function and thus a longer $t_{1/2}\beta$

(approximately 8 h). Patients 5 and 6 had an excessively long terminal $t_{1/2}\beta$. These patients demonstrated elevations of 43%–62% in their serum creatinine levels during F-ara-AMP therapy. Median F-ara-A AUC values also showed a dose-dependent relationship.

The pharmacokinetic characteristics of F-ara-ATP in circulating leukemic cells following an IV bolus infusion over 30 min of 20–125 mg/m² F-ara-AMP are illustrated in Fig. 2 and listed in Table 2. There was a wide range of variation in F-ara-ATP pharmacological parameters among patients receiving the same F-ara-AMP dose. When the median peak F-ara-ATP concentrations and 24-h AUC values were compared at each dosage increment (20–25 mg/m², 50 mg/m², and 100–125 mg/m²), however, a clear dose-dependence emerged. A doubling of the median F-ara-AMP dose resulted in a proportional increase in the peak F-ara-ATP level and the 24-h cellular F-ara-ATP exposure (AUC). The rate of drug elimination ($t_{1/2}$), on the other hand, was not dependent upon the dose of drug administered and was approximately 15 h at all dose levels.

In two patients studied, the accumulation and retention of F-ara-ATP by circulating leukemic cells were virtually unaffected by subsequent doses of F-ara-AMP, as shown in Fig. 3. Patient 9 had peak F-ara-ATP levels of 112 and 175 μM and AUCs of 2560 and 3510 μM-h, respectively, after the first and second doses of F-ara-AMP. Patient 7 was followed for all five doses and similarly showed no significant alterations in pharmacokinetics. The $t_{1/2}$ values after the first and fifth doses were, respectively, 14.1 and 16.6 h, while the AUC values were 2060 and 2130 μM-h. The mean trough F-ara-ATP concentration measured 24 h after each successive dose was 51 ± 7.4 μM (SD), a coefficient of variation of only 15%.

The concentration of F-ara-ATP in circulating leukemic cells at 12–24 h after the first dose of F-ara-AMP was compared to the F-ara-ATP concentration in the bone marrow cells obtained at the same time point. The results are shown in Figure 4. There was a strong correlation between the F-ara-ATP levels in leukemic cells obtained from the peripheral blood and those found in bone marrow ($r=0.84$, $p=0.01$, 95% confidence interval = 0.33–0.97). Such a correlation suggests that no differential pharmacologic barriers exist in the marrow.

Intracellular F-ara-ATP levels in circulating leukemic

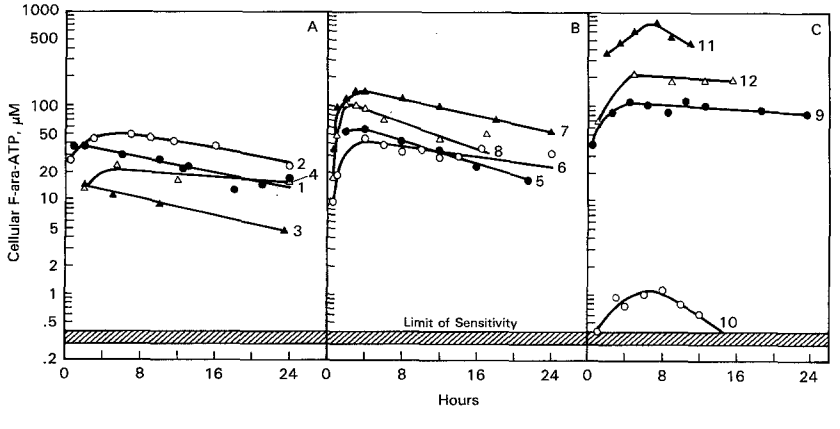

Fig. 2A–C. Concentrations of F-ara-ATP in circulating leukemic cells following an IV bolus dose of F-ara-AMP over 30 min 20–25 mg/m² **(A)** 50 mg/m² **(B)**, or 100–125 mg/m² **(C)**. Patient numbers are as in Table 2. Mononuclear cells were isolated from whole blood by using standard step-gradient density centrifugation procedures on Ficoll-Hypaque. The cells were then washed and the nucleotide pools were extracted with $HC10_4$. F-ara-ATP was separated from natural nucleotides by HPLC using gradient elution from anion exchange resin.

Table 2. Pharmacological characteristics for F-ara-ATP in circulating leukemic cells of patients with relapsed leukemia or lymphoma

Patient	Diagnosis	F-ara-AMP Dose (mg/m^2)	F-ara-ATP Parameters		
			Peak (μM)	$t_{1/2}$[a] (h)	AUC[b] (μM-h)
1	CLL[c]	20	42	13.3	600
2	DWDL[d]	20	51	16.8	840
3	DLCL[e]	25	15	13.7	220
4	NMCL[f]	25	24	>24.0	480
	Median	22.5	33	15.3	540
5	AMML[g]	50	58	10.7	780
6	AML[h]	50	47	>24.0	700
7	AML	50	147	14.1	2060
8	ALL[i]	50	105	12.8	1340
	Median	50	82	13.5	1060
9	AML	100	112	>24.0	2560
10	CML-BC[j]	100	1	6.0	10
11	ALL	125	757	5.2	3470
12	ALL	125	226	>24.0	6050
	Median	112.5	169	15.0	3015

[a] Half-life of elimination
[b] Area under the concentration x time curves calculated to 24 h
[c] Chronic lymphocytic leukemia
[d] Diffuse, well-differentiated lymphoma
[e] Diffuse, large cell lymphoma
[f] Nodular mixed cell lymphoma
[g] Acute myelomonocytic leukemia
[h] Acute myeloblastic leukemia
[i] Acute lymphoblastic leukemia
[j] Chronic myelogenous leukemia in blast crisis

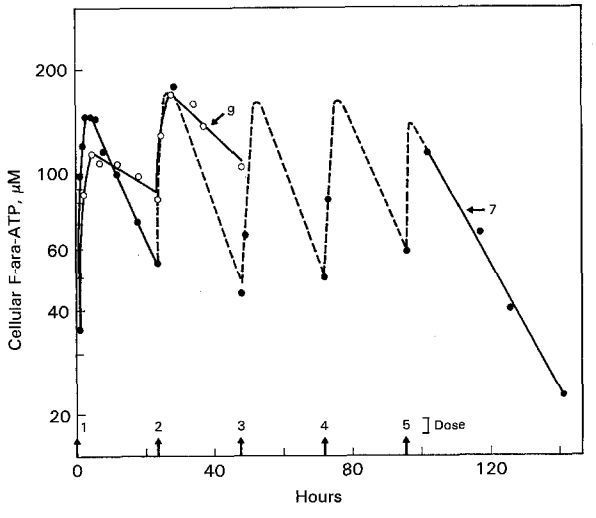

Fig. 3. Accumulation and retention of F-ara-ATP by circulating leukemic cells following two (○, patient 9) or five (●, patient 7) bolus doses of F-ara-AMP (50 mg/m^2, patient 7; 100 mg/m^2, patient 9). *Solid lines* represent the data from which the $t_{1/2}$ and AUC values were obtained. *Dashed lines* represent estimated F-ara-ATP profiles for patient 7, based on limited trough and peak values. F-ara-ATP concentrations were determined as described in the legend to Fig. 2

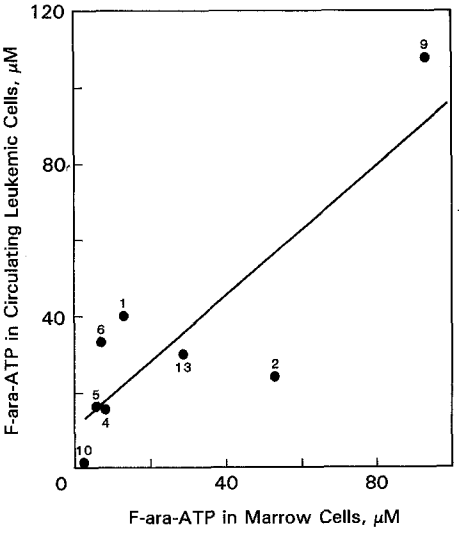

Fig. 4. Relationship between the concentration of F-ara-ATP in circulating leukemic cells and the level of F-ara-ATP in bone marrow cells. Patients' numbers correspond to those listed in Table 2. Patient 13, diagnosed with diffuse well-differentiated lymphoma, received 25 mg/m^2 F-ara-AMP. Bone marrow and peripheral blood F-ara-ATP levels were measured in samples obtained at the same time (12–24 h after the start of the F-ara-AMP bolus). F-ara-ATP was quantitated as described in the legend to Fig. 2.

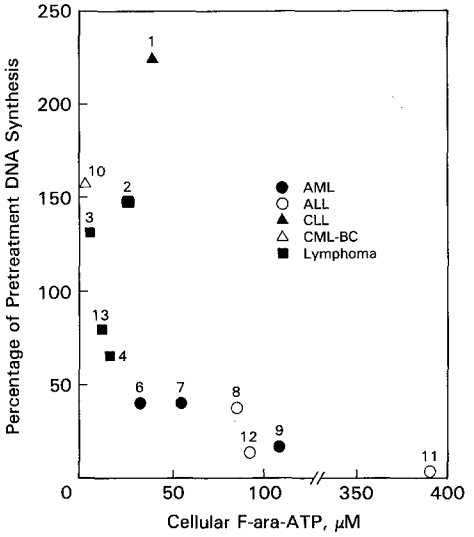

Fig. 5. Relationship between the cellular concentration of F-ara-ATP in circulating leukemic cells 12–24 h after an F-ara-AMP bolus (20–125 mg/m^2) and the DNA synthetic capacity of those cells relative to pretreatment values. F-ara-ATP was determined as described in the legend to Fig. 2. To determine DNA synthetic capacity, $0.5-1.0 \times 10^7$ cells in duplicate were washed with PBS and resuspended in RPMI 1640 medium containing 5% fetal bovine serum. Following the addition of [methyl-^{3}H]thymidine, cells were incubated for 30 min at 37 °C before addition of 10 volumes of ice-cold PBS. Cells were collected on a glass-fiber filter and washed once with PBS, twice with $HC10_4$, and twice with ethanol before the filters were dried and radioactivity was determined by liquid scintillation counting. DNA synthesis in leukemic cells isolated after F-ara-AMP bolus was expressed as a percentage of that determined in cells from the same patient before treatment. Patient numbers are the same as those listed in Table 2. Patient 13 is described in the legend to Fig. 4. Abbreviations for diagnoses are explained in Table 2

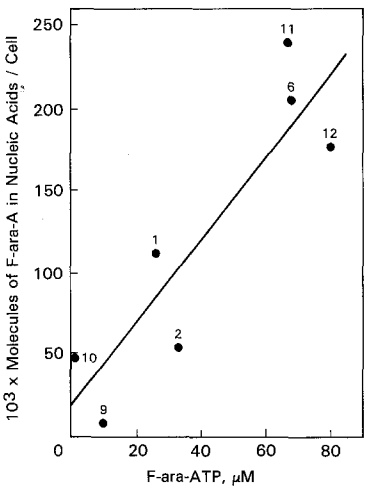

Fig. 6. Relationship between the incorporation of F-ara-A into nucleic acids and the intracellular level of F-ara-ATP in circulating leukemic cells incubated with F-ara-A in vitro. Patient numbers are the same as those listed in Table 2. Blood samples were obtained from patients prior to F-ara-AMP therapy. Mononuclear cells were isolated on Ficoll-Hypaque, washed in PBS and resuspended in RPMI 1640 medium containing 5% fetal bovine serum. F-ara-A (1 μ*M*) containing [8-^{3}H]F-ara-A was added to the medium and cells were incubated at 37 °C for 1 h. Cells were next washed with ice-cold PBS and the F-ara-ATP was extracted with $HC10_4$. The cell pellet was washed twice with $HC10_4$ before it was solubilized in dilute KOH. The radioactivity in the $HC10_4$-soluble (after HPLC) and $HC10_4$-insoluble fractions were measured by liquid scintillation counting

cells at 12–14 h after F-ara-AMP infusion were also related to the DNA synthetic capacity of those cells relative to pretreatment values (Fig. 5). DNA synthesis remained maximally inhibited (>80%) until the cellular concentration of F-ara-ATP fell below approximately 90 μ*M*. There was an inverse relationship between DNA synthesis at 12–24 h and the associated cellular F-ara-ATP concentration. At lower concentrations of F-ara-ATP there was relatively greater DNA synthetic capacity in circulating cells. The patients with CLL and with CML-BC (patients 1 and 10, respectively) showed no inhibition of DNA synthesis following F-ara-AMP. In fact, the cells from these patients showed an increase in DNA synthesis following treatment. Patients with lymphoma fell intermediate on the curve, whereas AML and ALL patients all had greater than 50% inhibition of DNA synthesis following F-ara-AMP.

To determine whether or not there was a relationship between intracellular F-ara-ATP levels and the amount of F-ara-A that was detected in nucleic acids, peripheral blood leukemic cells obtained prior to F-ara-AMP therapy were incubated in vitro with 1 μ*M* [8-^{3}H]F-ara-A (comparable to the median plasma concentration of F-ara-A at the time of peak F-ara-ATP accumulation in patients undergoing F-ara-AMP therapy). The results shown in Fig. 6 reflect the linear trend that emerged when the concentration of F-ara-ATP in pretreatment leukemic cells following a 1-h exposure to F-ara-A was compared with the amount of F-ara-A that was incorporated into nucleic acids under the same conditions. There was a highly significant correlation between the two parameters ($r=0.89$, $P<0.01$, 95% confidence interval = 0.42–0.98). The higher the 1-h accumulation of F-ara-ATP, the greater the number of molecules of F-ara-A that were found in the $HC10_4$-insoluble fraction.

Finally, patients with and without lymphomatous bone marrow involvement were compared as to their ability to accumulate and retain F-ara-ATP at 12–24 h after F-ara-AMP. The data are summarized in Table 3. The median F-ara-ATP value for patients with lymphomatous involvement 24 h after F-ara-AMP infusion was 10 μ*M*, whereas that for patients without marrow involvement was <6 μ*M* at 12 h. This is consistent with our previous finding that patients with marrow involvement who received high-dose ara-C therapy retained higher cellular concentrations of ara-C 5′-triphosphate (ara-CTP) than did those without marrow disease [11].

Discussion

There is a significant relationship between leukemic cell retention of ara-CTP in vitro and response to ara-C therapy [26, 27]. Furthermore, a positive correlation between trough (12-h) ara-CTP levels measured during ara-C treatment and clinical response has been demonstrated [25]. Because F-ara-AMP is metabolized and acts similarly to ara-C, several metabolic parameters relevant to the therapeutic outcome of ara-C treatment were examined to assess their potential role in F-ara-AMP therapy. As seen with ara-CTP [14, 25], there was a high degree of heterogeneity in

Table 3. Relationship between bone marrow involvement and F-ara-ATP concentration in bone marrow cells in patients with lymphoma

Patient	Diagnosis	F-ara-AMP Dose (mg/m^2)	Bone marrow involvement	Time of aspirate (h)	Bone marrow F-ara-ATP (μM)
14	DLCL[a]	20	–	12	2.1
15	DLCL	20	–	16.5	11.1
16	NPDL[b]	20	–	12	8.0
17	DWDL[c]	25	–	24	0.9
18	DLCL	30	–	12	3.1
				Median	3.1
2	DWDL	20	+	24	53.3
19	NPDL	25	+	23	12.0
4	NMCL[d]	25	+	24	8.0
13	DWDL	25	+	24	5.7
				Median	10.0

All patients received chemotherapy with or without radiotherapy prior to receiving F-ara-AMP (20–30 mg/m^2 every day $\times 5$ days as an IV, bolus over 30 min. Bone marrow aspirates and biopsy specimens were obtained at 12–24 h after the first dose of F-ara-AMP was started. Mononuclear cells were isolated on Ficoll-Hypaque prior to extraction of F-ara-ATP by $HC10_4$ F-ara-ATP was separated from normal nucleotides by HPLC (see *Materials and methods)*

[a] Diffuse, large cell lymphoma
[b] Nodular, poorly differentiated lymphoma
[c] Diffuse, well-differentiated lymphoma
[d] Nodular, mixed cell lymphoma

F-ara-ATP AUC values and trough (24-h) F-ara-ATP concentrations (Fig. 2, Table 2). Such variability most probably reflects tumor stem cell heterogeneity among patients [29]. Heterogeneity in drug metabolism has also been observed among patients with the same type of leukemia [14, 25, 32]. Similar parameters for F-ara-A showed less heterogeneity among individuals (Fig. 1, Table 1). Furthermore, repeated doses of F-ara-AMP at 24-h intervals did not result in increasingly higher AUC values for F-ara-ATP (Fig. 3). This is most probably a result of the failure to produce a higher plasma F-ara-A level with successive F-ara-AMP doses [10], since at these F-ara-A concentrations the phosphorylation pathway is not saturated.

The rate of F-ara-ATP elimination was first-order and was not dependent upon the dose of F-ara-AMP administered or the initial peak cellular concentration of F-ara-ATP. The $t_{1/2}$ was quite prolonged relative to the $t_{1/2}$ for ara-CTP [14]. The median peak time of F-ara-ATP accumulation was 3.5 h (3 h after termination of the F-ara-AMP infusion). Cellular F-ara-ATP declined when the median plasma F-ara-A concentration decreased below 1.2 μM. Maintenance of intracellular F-ara-ATP levels probably depends upon the levels of F-ara-A in the plasma, since its terminal $t_{1/2}$ is approximately 8 h. Thus, F-ara-ATP may continue to be accumulated intracellularly as a result of the sustained source of F-ara-A in the plasma. In contrast, the relatively rapid rate of ara-C elimination from plasma may prevent leukemic cells from maintaining inhibitory ara-CTP concentrations [14].

The cellular concentration of F-ara-ATP present at 24 h after the initial F-ara-AMP dose represents the lowest cellular level of toxic metabolite in tumor cells during the course of therapy. Because of the positive correlation between trough ara-CTP levels and clinical response [25], it may be possible to determine trough F-ara-ATP concentrations that discriminate between responders and nonresponders. Analysis of Phase II studies should permit evaluation of intracellular F-ara-ATP metabolism with respect to clinical response. Initially, however, we have compared the trough F-ara-ATP levels in peripheral blood leukemic cells with trough F-ara-ATP in bone marrow cells (Fig. 4). Because of the significant correlation between the two parameters, measuring the F-ara-ATP levels in peripheral blood samples most likely reflects the F-ara-ATP metabolism that occurs in less accessible bone marrow. In patients with and without lymphomatous involvement, clearly those patients with involved marrows had the highest F-ara-ATP levels (Table 3). Similar results were reported for ara-CTP following high-dose ara-C therapy in non-Hodgkin's lymphoma [11]. Thus, it appears that tumor cells have a greater capacity to accumulate and retain nucleoside analogue triphosphates than do normal cells. This may provide a pharmacological explanation for the higher response rate in ara-C patients with lymphomatous bone marrow involvement [11]. Further studies are needed to evaluate response rate with respect to the cellular pharmacology of F-ara-ATP following F-ara-AMP infusion.

The percentage of inhibition of DNA synthesis in leukemic leukemic cells following high-dose ara-C is directly correlated with trough cellular concentrations of ara-CTP, as is the likelihood of achieving a complete remission [25]. A similar pattern emerged for the relationship between the DNA synthetic capacity and the trough level of F-ara-ATP following F-ara-AMP (Fig. 5). Therefore, it should be possible to define the F-ara-ATP limits that produce >90% inhibition of DNA synthesis and relate this property to clinical response in future studies.

Last, as others have found in vitro drug accumulation and retention studies performed on pretreatment peripheral blood mononuclear cells to be of importance in clinical response [26, 27], we looked for in vitro parameters that might predict sensitivity of tumor cells to F-ara-A. If one mechanism of F-ara-A cytotoxicity is due to its effects on nucleic acids and the resultant inhibition of DNA synthe-

sis, then incubation of blast cells with F-ara-A prior to F-ara-AMP therapy might identify those patients with a high capacity to accumulate F-ara-ATP and, consequently, incorporate the drug into nucleic acids. The results in Fig. 6 indicate the direct correlation between F-ara-ATP accumulation at 1 h and the amount of F-ara-A found in nucleic acids. Patients who retained high trough F-ara-ATP levels during therapy also had the greatest inhibition of DNA synthesis. If these in vitro results are compared with the results (Fig. 5) generated during therapy, several patterns emerge. Patients 11 and 12 showed less than 15% DNA synthesis and high in vitro incorporation of F-ara-A into acid-insoluble material. Patients 1, 2, and 10, on the other hand, had low F-ara-ATP levels in vitro and after F-ara-AMP treatment, as well as fewer molecules of F-ara-A in nucleic acids and no inhibition of DNA synthesis. The results for patients 6 and 9, both with AML, followed no clear pattern. Studies with more patients are necessary to ascertain whether or not these in vitro parameters can be predictive for F-ara-AMP sensitivity.

The results shown in this investigation demonstrate that several F-ara-ATP pharmacokinetic and metabolic parameters are similar to those for ara-CTP. Because of the prolonged $t_{1/2}$ of F-ara-A in the plasma and of F-ara-ATP intracellularly, however, the spectrum of activity of F-ara-AMP may be quite different from that of ara-CTP. A greater number of patients is therefore needed to evaluate the relationship between clinical response and F-ara-ATP accumulation and retention, DNA synthetic capacity, and in vitro F-ara-A sensitivity assays.

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