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INTERACTION OF ARABINOSYL NUCLEOTIDES IN K562 HUMAN LEUKEMIA CELLS*

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Abstract—The objective of this investigation was to evaluate the ability of arabinosyl nucleotides to modulate the cellular metabolism of different arabinosyl nucleosides in K562 cells. The maximum rate of accumulation of the respective 5′-triphosphate (TP) was observed in cells incubated with 10 μM arabinosylcytosine (ara-C), 10 μM arabinosylguanine (ara-G), 300 μM arabinosyl-2-fluoroadenine (F-ara-A), and >1000 μM arabinosyladenine (ara-A). Cell extract fractionation studies demonstrated that ara-C and F-ara-A were phosphorylated by dCyd kinase, whereas ara-A was phosphorylated by dCyd kinase and Ado kinase; ara-G phosphorylation was attributed to dGuo kinase. When nucleoside kinase was rate limiting to arabinosyl nucleotide accumulation, cells preloaded with F-ara-ATP showed increased rates of ara-CTP and ara-GTP accumulation, whereas cells preloaded with ara-CTP had decreased rates of F-ara-ATP and ara-GTP accumulation. Preloading cells with ara-GTP had little effect on arabinosyl nucleoside triphosphate accumulation. F-ara-ATP accumulation was inhibited in cells containing all other arabinosyl nucleotides, whereas ara-ATP metabolism was not affected by pre-incubation with any other arabinosyl nucleoside. Cells incubated with ara-C and ara-G had a general rise in dNTP, whereas F-ara-A incubation was associated with a decrease in cellular dNTP. The differential effects of arabinosyl nucleotides and cellular metabolism of other arabinosyl nucleosides are due to phosphorylation by distinct nucleoside kinases that likely have characteristic sensitivities to cellular dNTP levels.

The arabinosyl nucleosides have had a substantial impact on chemotherapy. The most prominent of these analogues is 1-β-D-arabinofuranosylcytosine (ara-C‡), which is regarded as the major drug with curative potential in adult acute leukemia [1, 2]. However, ara-C has only weak antiviral activity [3]. Investigations of 9-β-D-arabinofuranosyladenine (ara-A) indicated that, when formulated as soluble monophosphate, the drug demonstrated substantial antiviral activity [4, 5]. Large doses of this drug were needed, however, since it is deaminated rapidly. The adenosine deaminase-resistant analogue, 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A) was developed to circumvent this problem [6]. Preliminary investigations indicated that the drug was active against hematologic malignancies [7, 8]. Finally, several laboratories have demonstrated the selective toxicity of 9-β-D-arabinofuranosylguanine (ara-G) against T cell lines [9–11].

Although these antimetabolites differ slightly from the natural deoxy or ribosyl counterparts, this simple alteration has generated nucleoside analogues that have diverse biological activities. The diversity manifests itself as differences in clinical activity as indicated above, and these can be studied as a range of responses in cell lines in culture. dCTP is known to inhibit the phosphorylation of ara-C [12], and ara-CTP may also limit phosphorylation of ara-C and other nucleosides that require the activity of dCK [13, 14]. Conversely, the phosphorylation of ara-C is enhanced in cells that contain F-ara-ATP [14]. Nothing is known, however, of the interrelationships of ara-C and F-ara-A with ara-G and ara-A.

The goal of this study was to evaluate the biochemical basis for the metabolic diversity of interactions among arabinosyl nucleosides. At the cellular level, it is likely that variations in response result from both quantitative and qualitative differences in the intracellular metabolism among these analogues. Therefore, the metabolism of each of these drugs was compared in a single cell line (human leukemia, K562). This investigation focused on the anabolism of individual arabinosyl nucleosides to their active triphosphates, the effect of one arabinosyl nucleotide on the metabolism of another, and the influence of deoxynucleotides on these processes.

MATERIALS AND METHODS

Chemicals. Ara-C, ara-CTP and ara-ATP were purchased from the Sigma Chemical Co. (St Louis, MO). Ara-G and ara-GTP were purchased from the Calbiochem-Behring Corp. (La Jolla, CA). F-ara-A

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‡ Abbreviations: ara-C, ara-CTP, 1-β-D-arabinofuranosylcytosine and its 5′-triphosphate; ara-G, ara-GTP, 1-β-D-arabinofuranosylguanine and its 5′-triphosphate; ara-A, ara-ATP, 9-β-D-arabinofuranosyladenine and its 5′-triphosphate; F-ara-A, F-ara-ATP, 9-β-D-arabinofuranosyl-2-fluoroadenine and its 5′-triphosphate; ara-N, ara-NTP, an arabinofuranosyl nucleoside and an arabinofuranosyl 5′-triphosphate; and DEAE, diethylaminoethyl.

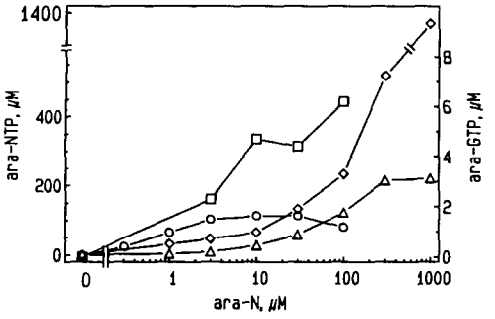

Fig. 1. Concentration–response of ara-NTP accumulation by K562 cells. Cells were incubated with the indicated concentrations of ara-C (○), ara-G (□), or ara-A (◊) for 3 hr or F-ara-A (△) for 2.5 hr. Intracellular levels of arabinosyl triphosphates were determined as described in Materials and Methods. Data are the means of at least two separate experiments and the standard errors were less than 8%.

was provided by Dr V. L. Narayanan, Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD). F-ara-ATP was synthesized chemically as described previously [15]. Ara-A and deoxycoformycin were obtained through the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). All other chemicals were of the highest reagent grade available.

For enzyme assays, the natural nucleosides [2,8-^{3}H]Ado (39 Ci/mmol), [8-^{3}H]dAdo (18 Ci/mmol), [5-^{3}H]dCyd (26 Ci/mmol), and [8-^{3}H]dGuo (7 Ci/mmol) were purchased from ICN Biochemicals (Costa Mesa, CA). [5,6-^{3}H]ara-C (20 Ci/mmol), [8-^{3}H]ara-G (2.5 Ci/mmol), and [2,8-^{3}H]ara-A (20 Ci/mmol) were purchased from Moravek Biochemicals, Inc. (Brea, CA). [8-^{3}H]F-ara-A (12 Ci/mmol) was purchased from the Amersham Corp. (Arlington, IL). All nucleosides were purified by reverse-phase HPLC to greater than 99% purity.

Cell line. The K562 cell line, derived from a patient with chronic myelogenous leukemia, was obtained from the American Type Culture Collection (Rockville, MD) and used throughout the study [16]. Cell cultures were periodically certified free of mycoplasma by the American Type Culture Collection. The cells were maintained in suspension culture in exponential growth phase in RPMI-1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Gibco) at 37° in a humidified atmosphere containing 5% CO_2. Cell number and mean cell volume were determined before, during, and after the incubation using a Coulter counter equipped with a model C-1000 particle size analyzer (Coulter Electronics, Hialeah, FL). The population doubling time was between 24 and 28 hr.

Metabolism of arabinosyl nucleosides. Exponentially growing cells were incubated with 1–1000 μM arabinosyl nucleosides for 2.5 to 3 hr to determine the concentration that saturated the accumulation of the respective nucleoside triphosphates. To investigate the influence of ara-NTP on the anabolism of a different ara-N, cells were first loaded with the triphosphates by incubation with ara-C (10 μM, 3 hr), ara-G (10 μM, 3 hr), F-ara-A (300 μM, 2.5 hr), or ara-A (100 μM, 3 hr). The cells were washed into drug-free medium (zero time), and the second arabinosyl nucleoside was added in the same concentrations as indicated above. Aliquots were removed at hourly intervals for 5 hr and analyzed for ara-NTP concentrations.

Nucleotides were extracted using perchloric acid [17]. Analogue triphosphates were separated from natural nucleotides by HPLC using an anion-exchange partisil 10 SAX column (Whatman, Clifton, NJ) and different gradient elution schemes [11, 17–19]. The identity of nucleoside analogue triphosphates was confirmed by their coelution with authentic triphosphates, by their resistance to periodate oxidation, and by their UV absorbance. Quantitation of the nucleotides in $HClO_4$ extracts was determined by electronic integration and reference to preprogrammed response factors. The intracellular concentration of nucleotides was calculated by dividing the quantity of nucleotides contained in an $HClO_4$-soluble fraction by the number of cells analyzed and then multiplying this value by the mean cell volume (6.7×10^8 cells/ml cell H_2O). This calculation assumes that nucleotides are uniformly distributed in total cell water.

Table 1. Effect of preincubation with arabinosyl nucleosides on the rates of different ara-NTP accumulation in K562 cells

Preincubation nucleoside (μM ara-NTP at zero time)	Initial rate of ara-NTP accumulation (μM/hr)			
	ara-CTP	ara-GTP	F-ara-ATP	ara-ATP
None	41	2.4	80	85
ara-C (98)		1.4	47	71
ara-G (4.4)	43		35	84
F-ara-A (208)	128	5.1		103
ara-A (261)	80	4.5	54	

Cells were preincubated for 3 hr with ara-N concentrations that saturated the rate of ara-NTP accumulation (ara-C, 10 μM; ara-G, 10 μM; F-ara-A, 300 μM; and ara-A, 100 μM), washed into fresh medium, and incubated with a different ara-N. Cellular concentrations of ara-NTP were determined by HPLC, as described in Materials and Methods. The initial rates of ara-NTP accumulation were calculated by linear regression from 0 to 3 hr (ara-CTP, 0 to 2 hr, and ara-GTP, 0 to 1 hr).

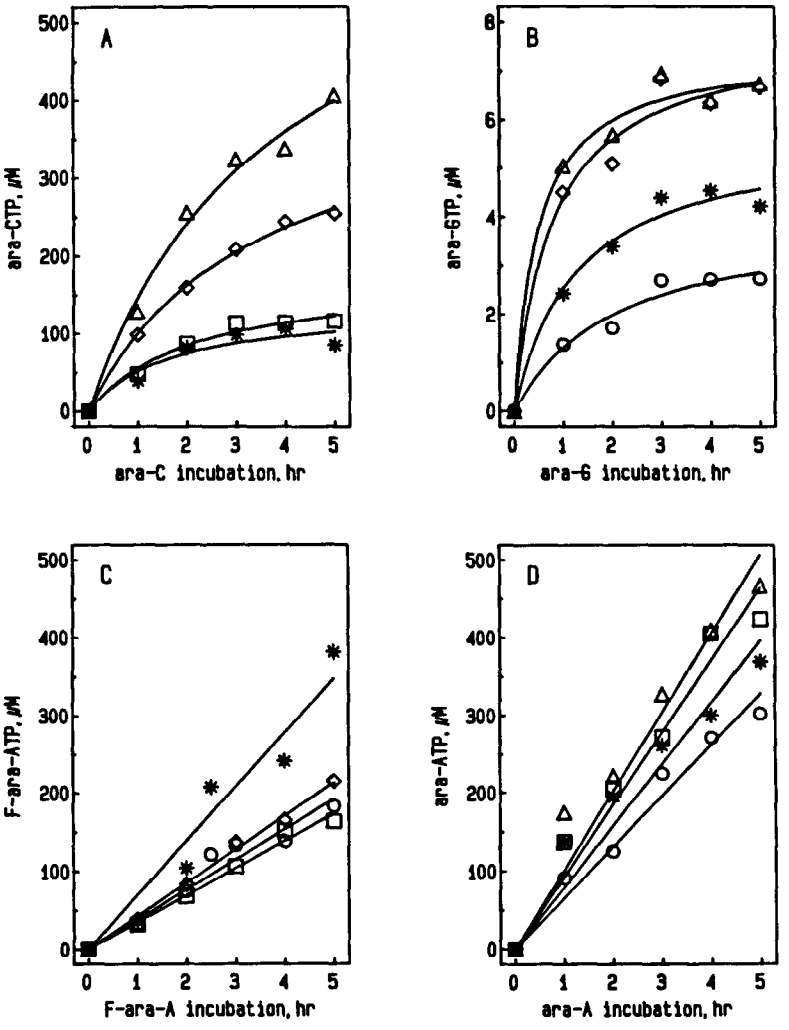

Fig. 2. Effect of preincubation with ara-N on the accumulation of ara-NTP in K562 cells. Data points are means of at least two separate experiments and the standard errors were less than 15%. (A) Accumulation of ara-CTP. Cells preincubated with none (*), 10 μM ara-G for 3 hr (□), 300 μM F-ara-A for 2.5 hr (△), or 100 μM ara-A for 3 hr (◊) were washed into fresh medium and incubated with 10 μM ara-C. (B) Accumulation of ara-GTP. Cells preincubated with none (*), 10 μM ara-C for 3 hr (○), 300 μM F-ara-A for 2.5 hr (△), or 100 μM ara-A for 3 hr (◊) were washed into fresh medium and incubated with 10 μM ara-G. (C) Accumulation of F-ara-ATP. Cells preincubated with none (*), 10 μM ara-C for 3 hr (○), 10 μM ara-G for 3 hr (□), or 100 μM ara-A for 3 hr (◊) were washed into drug-free medium and incubated with 300 μM F-ara-A. (D) Accumulation of ara-ATP. Cells preincubated with none (*), 10 μM ara-C for 3 hr (○), 10 μM ara-G for 3 hr (□), or 300 μM F-ara-A for 2.5 hr (△) were washed and incubated with 100 μM ara-A.

Determinations of dNTP. Neutralized $HClO_4$ extracts from treated or untreated cells were evaporated to dryness in an Evapomix volume reduction apparatus (Buchler Instruments, Fort Lee, NJ). Ribonucleotides in the extracts were degraded by treatment with $NaIO_4$ by the method of Neu and Heppel [20]. dNTPs were separated by an anion-exchange column on a Waters HPLC system (Waters Chromatography, Milford, MA) as described previously [14], except that for the analyses of ara-GTP and ara-ATP the pH of buffer B was 3.68 and 3.72, respectively.

Enzyme assays. Cells were harvested and stored at −70°. Frozen cells were thawed in a buffer containing the protease inhibitors phenylmethylsulfonyl fluoride and *o*-phenanthroline (250 μM each). The cells were then subjected to bomb-cavitation for 30 min at 1000 psi of nitrogen. Kinases were separated and eluted by passing the cell extract through a DE-52 column with a linear gradient of 0–600 mM KCl (300 ml). Fractions (3 ml) were collected, and every third fraction was assayed for the kinase activity against four natural and four arabinosyl nucleosides.

The phosphorylation activities of natural and arabinosyl nucleosides were determined with the anion exchange filter disc method described by Saunders and Lai [21]. All the nucleosides were used at a final concentration of 25 μM (1 Ci/mmol). 8-Aminoguanosine, 2′-deoxycoformycin, and 3,4,5,6-tetrahydrouridine were added to a final concentration of

5 μM to inhibit purine nucleoside phosphorylase, adenosine deaminase, and cytidine deaminase respectively. All reactions were carried out for 1 hr and were found to be linear. The activity was expressed as picomoles of product formed per milligram of protein. Protein content was determined with the Bio-Rad Bradford assay dye (Bio-Rad Laboratories, Richmond, CA) using bovine plasma gamma globulin as a standard.

RESULTS

Accumulation of arabinosyl nucleoside 5'-triphosphates. The maximum rate of ara-NTP accumulation was determined by incubating K562 cells with increasing concentrations of ara-N. The accumulation of ara-CTP was saturated by 10 μM ara-C (Fig. 1). Higher exogenous ara-C concentrations (30–100 μM) resulted in a decrease in intracellular ara-CTP. The cellular concentration of F-ara-ATP increased in proportion to the F-ara-A concentration to 300 μM. A higher concentration (1000 μM) of F-ara-A did not substantially augment triphosphate accumulation. Ara-ATP also accumulated in proportion to the exogenous nucleoside, however, no saturation of the phosphorylation rate was evident even at 1000 μM ara-A. K562 cells exhibited a marginal ability to accumulate ara-GTP.

Effect of arabinosyl nucleoside preincubation on the accumulation of other ara-NTP. The activity of nucleoside kinases may be affected by arabinosyl nucleotides [14]. To evaluate these effects, separate cultures of exponentially growing K562 cells were incubated with or without an ara-N for 2.5 to 3 hr to accumulate the respective ara-NTP. Cells were then washed into fresh medium, and portions of each culture were incubated with a different ara-N for 5 hr. The calculated initial rates of accumulation are shown in Table 1, and the kinetics of cellular accumulation of the second ara-NTP are presented in Fig. 2.

Cells incubated with ara-C alone accumulated ara-CTP for 3 hr before the onset of a slight but reproducible decrease of the ara-CTP level (Fig. 2A). Preloading with F-ara-ATP or ara-ATP enhanced the rate of ara-CTP accumulation (Table 1), and no saturation of ara-C phosphorylation was evident through 5 hr. Cells loaded with ara-GTP exhibited no change in either the rate or the plateau of ara-CTP accumulation. Cellular ara-GTP concentrations increased until 3 hr, when a plateau was reached (Fig. 2B). Cells preloaded with ara-CTP showed a decreased rate of ara-GTP accumulation, and the plateau occurred at a lower cellular ara-GTP concentration. As observed for ara-CTP, the initial rate of ara-GTP accumulation was potentiated by the presence of F-ara-ATP and ara-ATP in the cells (Table 1). However, plateau kinetics were observed under all conditions. F-ara-ATP accumulation was linear for up to 5 hr (Fig. 2C). Preincubation with each arabinosyl nucleoside resulted in a decreased rate of F-ara-ATP accumulation, although the kinetics remained linear. Ara-ATP accumulation was linear and was not affected significantly by preincubation with ara-N (Fig. 2D).

Effects of arabinosyl nucleotides on dNTP pools. Previous studies have demonstrated that F-ara-ATP and ara-ATP inhibit ribonucleotide reductase assayed in cell extracts. The fact that dCyd and dGuo kinase activities are affected by various dNTP indicated the importance of establishing the effects of arabinosyl nucleotides on the dNTP of K562 cells. Separate cultures were incubated with each ara-N as detailed in Table 1, and the dNTP levels were determined, as described in Materials and Methods (Table 2). The 3-hr incubation with 10 μM ara-C resulted in a slight increase in the concentration of all dNTP. Likewise, ara-G treatment increased all dNTP except dGTP, which was decreased slightly. As previously reported [14], cells loaded with F-ara-ATP experienced a decrease in cellular dCTP, dATP, and dGTP, while dTTP was increased. Minimal changes were seen in the dNTP pools of cells incubated with ara-A except for the dATP peak which was not detectable. Separate determinations on extracts from these cells demonstrated that the concentrations of ribonucleoside 5'-triphosphates were not affected by incubation with any of the arabinosyl nucleosides (data not shown).

Table 2. Effects of arabinosyl nucleoside triphosphates on cellular dNTP concentration in K562 cells

dNTP	Control* (μM)	dNTP (% of control) ara-C	ara-G	F-ara-A	ara-A
dCTP	3.5 ± 1.3	142	128	63	111
dTTP	9.9 ± 2.7	155	147	137	126
dATP	8.6 ± 1.4	131	125	71	ND†
dGTP	3.3 ± 0.4	114	85	69	101

Cells were incubated with a saturating concentration of analogue, as indicated in Table 1. dNTP levels were quantitated by HPLC analysis, as described in Materials and Methods.

* Values are the means ± SD of three to six determinations in separate experiments.

† Not detectable, < 20% of control.

Kinase activity in cell extracts. Nucleoside kinases present in K562 cells were analyzed to identify those enzymes and to establish their roles in the phosphorylation of arabinosyl nucleosides. Figure 3A shows a typical elution profile of kinase activities from a cell extract fractionated by DEAE-cellulose chromatography using a linear gradient of KCl from 0 to 600 mM. Adenosine kinase eluted first from the column. Deoxyadenosine phosphorylating activity coincided with that of adenosine kinase. In the absence of an adenosine deaminase inhibitor (deoxycoformycin), however, the dAdo phosphorylating activity was resolved in two peaks: one coincident with Ado kinase and another coincident with dGuo kinase (data not shown). Deoxyguanosine was phosphorylated by only one peak, termed deoxyguanosine kinase. This peak was distinct and separable from the peak that eluted last and phosphorylated deoxycytidine.

These fractions were used to specify the activities responsible for the phosphorylation of arabinosyl nucleosides (Fig. 3B). Ara-C and F-ara-A were phosphorylated by the same peak that was responsible for the phosphorylation of deoxycytidine.

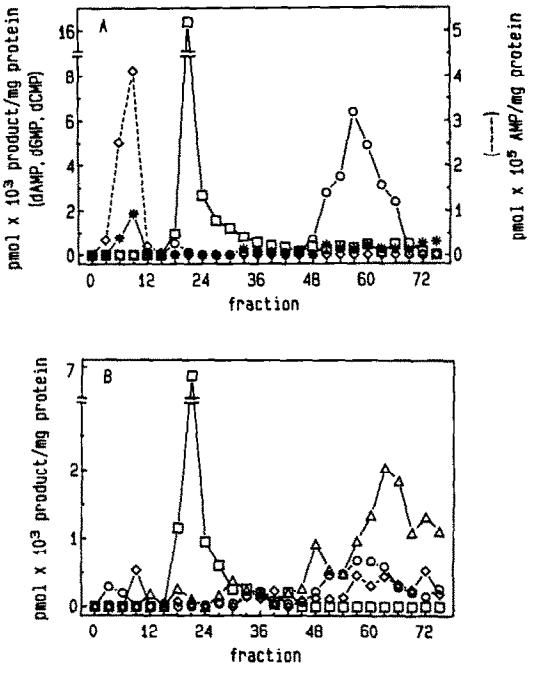

Fig. 3. DEAE-cellulose chromatography of K562 cell kinases. Cell extract was applied to a column of DEAE cellulose. Elution was with a 300-ml linear gradient of KCl. Each third fraction was assayed for (A) natural nucleosides, adenosine (◊), deoxyadenosine (*), deoxyguanosine (□), and deoxycytidine (○) or (B) arabinosyl nucleosides, ara-C (○), ara-G (□), F-ara-A (△), and ara-A (◊).

Ara-G was phosphorylated exclusively by the deoxyguanosine kinase peak. Addition of 8-aminoguanosine in the reaction mixture to inhibit purine nucleoside phosphorylase did not change this pattern. Ara-A phosphorylating activity was located mainly in two peaks, one coincident with adenosine kinase and the other with deoxycytidine kinase. The products retained on DE-81 filter discs were analyzed by HPLC and found to be the respective monophosphates. Triphosphates were less than 10% in each case.

DISCUSSION

We previously reported the potentiation of ara-CTP accumulation in intact cells that had been treated with F-ara-A. Experiments with cell extracts indicated the stimulation and inhibition of dCyd kinase activity by F-ara-ATP and ara-CTP respectively [14]. An understanding of the mechanism by which this combination exerts its effect suggested that interactions might affect combinations of other arabinosyl nucleosides activated by the same or different pathways. Furthermore, in intact cells, metabolic pathways of nucleoside analogues are not isolated entities but interact with each other and with endogenous nucleotides. Perturbations in the cellular concentrations of natural nucleotides may also affect the metabolism of other arabinosyl nucleosides.

Ara-NTP accumulation. The rate of cellular accumulation of ara-NTP varied between 2.4 μM/hr and 85 μM/hr (Fig. 2 and Table 1). These arabinosyl nucleosides enter the cell by a high-capacity facilitated diffusion mechanism [22, 23]. At the concentrations used, the transport of the ara-N was unlikely to limit their metabolism. The differences in the rates of ara-NTP elimination are unlikely to be crucial determinants of nucleotide accumulation because the individual ara-NTP varied by only 4-fold (unpublished data).

Ara-C and F-ara-A are initially phosphorylated by dCyd kinase [24–28] (Fig. 3, A and B). At lower substrate concentrations, the intracellular accumulation of ara-CTP was much higher than that of F-ara-ATP (Fig. 1). This may be due to the fact that ara-C is a better substrate for dCyd kinase than F-ara-A [29]. Feedback inhibition of ara-C phosphorylation by intracellular ara-CTP and inhibition by endogenous dCTP may be responsible for the plateau level of ara-CTP. The lack of a plateau in F-ara-ATP accumulation may be attributable to reduction of deoxynucleotide pools by F-ara-ATP [30] (Table 2) and a less effective feedback inhibition of dCyd kinase by F-ara-ATP.

K562 cells accumulated ara-GTP at a slower rate than did other ara-NTP, and reached a plateau after 2 hr of incubation (Fig. 2). Accumulation of ara-GTP by K562 cells was saturated at about 10 μM ara-G (Fig. 1). Except in T cells, studies with other cell types have indicated similar results [9–11]. This may be due to a higher level of kinase activity in T cells [31, 32], faster elimination of the triphosphates in other cell types [33], or both factors [34]. The significant difference in the rate of phosphorylation of the substrates that use dCyd kinase and ara-G phosphorylation may reflect the possibility that ara-G is phosphorylated by dGuo kinase (the enzyme specific for deoxyguanosine) [21, 35–37] or by both dCyd kinase and dGuo kinase [38]. The present study used DEAE column chromatography to separate nucleoside kinase activities. When the concentration of ara-G was 25 μM in the reaction mixture, dGuo kinase was the only activity that phosphorylated ara-G in extracts of K562 cells (Fig. 3).

The initial rate of ara-A phosphorylation was similar to that of F-ara-A and lacked a plateau. As with F-ara-A, this may be attributed to the inhibition of ribonucleotide reductase and self-potentiation. Unlike other ara-N, ara-A uses primarily Ado kinase for its initial phosphorylation to ara-AMP [39]. Ara-A is a poor substrate for Ado kinase [40], but the relatively high specific activity of this enzyme (Fig. 3A) may account for the greater accumulation of ara-ATP at higher exogenous concentration of ara-A (Fig. 1).

Effect of ara-N preincubation on other ara-NTP accumulation. To discover whether these biochemical modulations, produced by the intracellular arabinosyl nucleotides, affect the phosphorylation of other substrates and whether these modulations are specific for phosphorylating enzymes, we used cells loaded with different arabinosyl nucleoside triphosphates to phosphorylate substrates that do (ara-C, F-ara-A) or do not (ara-G, ara-A) primarily use dCyd kinase. The observations using combinations of these substrates can be summarized as follows.

First, ara-CTP and ara-GTP accumulation was

stimulated by the presence of intracellular F-ara-ATP and ara-ATP (Fig. 2, A and B). The presence of ara-GTP had no significant effect on ara-C phosphorylation (Fig. 2A). Second, ara-CTP-loaded cells inhibited phosphorylation of all nucleoside analogues that use dCyd kinase or dGuo kinase for phosphorylation (Fig. 2, B and C). Third, F-ara-ATP accumulation was inhibited by preincubation with any arabinosyl nucleosides tested in the current study (Fig. 2C). Finally, ara-ATP accumulation was not affected significantly by the presence of any intracellular arabinosyl nucleoside triphosphate (Fig. 2D).

We tried to uncover the mechanism behind these observed effects. F-ara-ATP and ara-ATP resulted in potentiation of the arabinosyl nucleosides that use dCyd kinase or dGuo kinase but not ara-A, indicating that this potentiation is specific to these enzymes. Furthermore, these triphosphates inhibit ribonucleotide reductase [30, 41], resulting in lower dNTP (dATP by ara-ATP) levels (Table 2). This action may also potentiate dCyd kinase and dGuo kinase, which are inhibited by dCTP and dGTP respectively [35, 37, 42, 43]. Thus, reduction of dATP pools is an indirect effect of ara-NTP on nucleoside kinase activities. In fact, the indirect effect seems to be the reason for the stimulation of ara-CTP and ara-GTP accumulation in cells loaded with ara-ATP because ara-A primarily uses a different kinase than ara-C or ara-G.

Maximum potentiation of ara-C or ara-G phosphorylating activity was achieved when cells were loaded with F-ara-ATP. This seems to be a combination of indirect and direct effects of F-ara-ATP on the activity of dCyd and dGuo kinases. Another possibility is that F-ara-ATP serves as a phosphate donor. Stimulation of ara-CTP accumulation and a positive effect on cytotoxicity have been reported in cells that were incubated with deoxyguanosine [44]. In this study, also, the induction was reported to be due to the effect of dGTP on ribonucleotide reductase [44, 45]. Further experiments using cell lysates suggested that the elevation of ara-C nucleotide accumulation may represent induction of enzyme synthesis [46]. Our studies using cell extracts supplemented with NTP and dNTPs indicated that lower dNTP levels stimulated ara-C phosphorylation; however, the presence of F-ara-ATP along with lower dNTP had the maximum stimulatory effect, indicating a direct effect of F-ara-ATP on the potentiation of dCyd kinase activity [14].

Ara-GTP did not show any significant effect on the rate of ara-CTP accumulation. This may be because the concentration of ara-GTP achieved in K562 cells was very low. Similar experiments using T cells that accumulate high levels of ara-GTP [11, 33] may provide information regarding this issue. Cells loaded with ara-CTP exhibited a slower rate of F-ara-A phosphorylation, indicating the inhibitory effect of ara-CTP on dCyd kinase as observed in cell extracts [13, 14]. Inhibition of ara-GTP accumulation may be due to the indirect effect of increased dNTP pools [35, 37] associated with ara-C treatment.

F-ara-ATP phosphorylation was inhibited by all the nucleoside analogues tested in this study. Except for the inhibitory effect of ara-CTP on dCyd kinase, the mechanism for this effect seems to be complex, especially because not all preincubated substrates use dCyd kinase. Also, there seems to be no relationship between the amount of preincubated arabinosyl nucleoside triphosphate and the extent of inhibition.

Ara-ATP accumulation served as a negative control to see the effects of different arabinosyl nucleoside triphosphates. None of the arabinosyl nucleoside triphosphates showed a significant effect on the rate of ara-ATP accumulation, indicating that the observed effects on dCyd kinase and dGuo kinase and the perturbations in the dNTP levels do not affect Ado kinase. A slight inhibition by ara-CTP and stimulation by F-ara-ATP of the rate of ara-ATP accumulation suggest the involvement of dCyd kinase in ara-A phosphorylation.

An understanding of the interactions of drugs in combination is necessary for their rational clinical use. Our previous studies indicated that lymphocytes isolated from patients with chronic lymphocytic leukemia had a 1.7-fold increase in ara-CTP accumulation when pretreated with F-ara-A *in vitro*. Also, leukemic lymphocytes recovered from patients who received an infusion of 25 mg/m^2 of F-ara-A monophosphate accumulated 1.5-fold more ara-CTP when incubated *in vitro* with 100 μM ara-C for 2 hr, compared to a similar incubation before treatment [47]. These results are consistent with the present studies and demonstrate that this approach can be useful in the selection of preferred sequences for administration of arabinosyl nucleosides in combinations.

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