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Comparison of the Actions of 9-β-D-Arabinofuranosyl-2-fluoroadenine and 9-β-D-Arabinofuranosyladenine on Target Enzymes from Mouse Tumor Cells[1]

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ABSTRACT

9-β-D-Arabinofuranosyl-2-fluoroadenine (2-F-ara-A), a derivative of 9-β-D-arabinofuranosyladenine (ara-A) that is resistant to deamination, selectively inhibits DNA synthesis and has activity against mouse leukemia L1210 comparable to that of ara-A plus the adenosine deaminase inhibitor, 2′-deoxycoformycin. To determine if these two nucleosides have similar modes of action, comparisons were made of their effects and those of their triphosphates on enzymes known to be inhibited by ara-A or 9-β-D-arabinofuranosyladenine 5′-triphosphate. 9-β-D-Arabinofuranosyl-2-fluoroadenine 5′-triphosphate was more effective than 9-β-D-arabinofuranosyladenine 5′-triphosphate in inhibiting the reduction of adenosine 5′-diphosphate and cytidine 5′-diphosphate by ribonucleotide reductase from HEp-2 cells or L1210 cells. DNA polymerase α from L1210 cells was equally sensitive to 9-β-D-arabinofuranosyl-2-fluoroadenine 5′-triphosphate and 9-β-D-arabinofuranosyladenine 5′-triphosphate, and DNA polymerase β from L1210 cells was much less sensitive to both triphosphates. *S*-Adenosylhomocysteine hydrolase from L1210 cells was inactivated by 2-F-ara-A and ara-A, but higher concentrations of the fluoro derivative were required. These results are consistent with 2-F-ara-A and ara-A inhibition of DNA synthesis by inhibition of ribonucleotide reductase and DNA polymerase α.

INTRODUCTION

The use of ara-A[3] as an antitumor agent is limited by its susceptibility to deamination with the consequence that it is effective only in the presence of inhibitors of adenosine deaminase (5, 15, 16, 22). 2-F-ara-A is a derivative of ara-A that is resistant to deamination, that selectively inhibits DNA synthesis, and that has activity against mouse leukemia L1210 and against human lymphoblastoid cells comparable to that of ara-A plus 2′-deoxycoformycin, an inhibitor of adenosine deaminase (4, 5, 11, 21). The efficacy of 2-F-ara-A raises the question as to whether this derivative has the same biochemical sites of action as ara-A. Three enzymes that are inhibited by ara-A or ara-ATP at low concentrations are DNA polymerase α (9, 10, 27), ribonucleotide reductase (7, 9, 27), and *S*-adenosylhomocysteine hydrolase (8, 13, 14). We report here, as part of a continuing study (4, 5) of the mode of action of 2-F-ara-A, a comparison of 2-F-ara-ATP and ara-ATP or 2-F-ara-A and ara-A, as inhibitors of these enzymes. A preliminary report of some of these data has been presented (32).

MATERIALS AND METHODS

Chemicals and Radioisotopes. 2-F-ara-A and 2-F-ara-AMP were synthesized in the Organic Chemistry Department, Southern Research Institute, by Anita Shortnacy and Sarah Jo Clayton. Phosphorylation of 2-F-ara-AMP to 2-F-ara-ATP was accomplished by Dr. Robert Naylor, P-L Biochemicals, Inc., Milwaukee, Wis. The following compounds were obtained from the indicated sources: ara-A, Drug Development Branch, Division of Cancer Treatment, National Cancer Institute; ara-ATP, P-L Biochemicals; [8-^{3}H]dATP (7.0 Ci/mmol), Schwarz/Mann, Spring Valley, N. Y., or Moravek Biochemicals, City of Industry, Calif.; [8-^{14}C]adenosine (57.6 mCi/mmol), Amersham Corp., Arlington Heights, Ill.; [2-^{14}C]CDP (46.6 mCi/mmol) and [8-^{14}C]ADP (40 mCi/mmol), Schwarz/Mann; CDP, ADP, ATP, dGTP, other biochemicals, and reagent grade chemicals, Schwarz/Mann, Sigma Chemical Co., St. Louis, Mo., or P-L Biochemicals.

Enzyme Assays. The standard assay for ribonucleotide reductase (EC 1.17.4.1) is based on the method of Moore (18). The modified assay is described in detail in published work (6).

DNA polymerase (EC 2.7.7.7) was assayed by the method described by others (2, 12, 17). The reaction mixture (final volume, 120 μl) contained 62.5 μM of each deoxyribonucleoside 5′-triphosphate, 0.39 mg "activated" salmon sperm DNA per ml, 0.5 mg bovine serum albumin per ml, 6.8 mM $MgCl_2$, 3 mM β-mercaptoethanol, 5×10^8 dpm [^{3}H]dATP, 7.4 mM Tris-HCl (pH 8), and 20 μl of enzyme. The reaction mixture was incubated at 25° for 60 min, and then 100 μl were spotted on a glass microfiber filter. The filters were washed in ice-cold 5% TCA containing 1% $Na_4P_2O_7 \cdot 10H_2O$, followed by 5% TCA, 95% ethanol, and finally acetone. The filters were dried, and the amount of radioactivity on each filter was determined.

S-Adenosylhomocysteine hydrolase (EC 3.3.1.1) was measured, based on published methods (8, 14), in a reaction mixture consisting of 12.5 mM DL-homocysteine, 112 μM adenosine, 25 mM potassium phosphate buffer (pH 7.5), 5 mM β-mercaptoethanol, 1×10^5 dpm [^{14}C]adenosine, and 3 μl of enzyme in a final volume of 10 μl. The mixture was incubated for 20 min at 37° and stopped by the addition of 5 μl of ice-cold 10% TCA. Ten μl were spotted on Whatman No. 3MM chromatography paper, and 5 μl of a standard solution containing *S*-adenosylhomocysteine and adenosine were added to the same spot. The paper was developed overnight in *n*-butyl alcohol:ethanol:H_2O (2:1:1), and after visualization under uv the spots containing the substrate and product were cut out and counted.

Enzyme activities for all charts except the column chromatography of DNA polymerase are an average of 3 assays. All constants and experiments presented in the charts and table were determined from a minimum of 2 experiments.

Enzyme Preparation. Enzymes were prepared from L1210 ascites tumor cells that were harvested on the sixth day after implantation of 10^5 L1210 cells in the i.p. cavity of BALB/c × DBA/2 F_1 mice. Tumor

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[3] The abbrevations used are: ara-A, 9-β-D-arabinofuranosyl-adenine; 2-F-ara-A, 9-β-D-arabinofuranosyl-2-fluoroadenine; ara-ATP, 9-β-D-arabinofuranosyladenine 5′-triphosphate; 2-F-ara-ATP, 9-β-D-arabinofuranosyl-2-fluoroadenine 5′-triphosphate; 2-F-ara-AMP, 9-β-D-arabinofuranosyl-2-fluoroadenine 5′-monophosphate; TCA, trichloroacetic acid.

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cells were removed from 25 mice with a heparinized syringe, washed with Puck's Saline G buffer (23), suspended in Tris-buffered isotonic ammonium chloride (pH 7.2) to lyse RBC (3), and washed again with Puck's Saline G. The cells were either processed immediately or stored at −20° until needed.

To prepare ribonucleotide reductase, leukemia cells (6.5 g wet weight) were suspended in 0.05 M Tris buffer (pH 7.5) containing dithiothreitol (0.025 M), homogenized in a glass homogenizer with a Teflon pestle, and centrifuged at 100,000 × *g* for 1 hr. Ammonium sulfate fractionation of the supernatant solution gave enzyme activity in the 40% ammonium sulfate precipitate. Preparation of ribonucleotide reductase by ammonium sulfate fractionation of the 100,000 × *g* supernatant solution derived from human epidermoid carcinoma cells (HEp-2) grown in culture has been described in detail (6). Enzyme activity was linear with time for 60 min and was proportional to enzyme concentration. Activities for reduction of CDP and ADP were 7.9 and 5.2 nmol per mg protein per 30 min, respectively.

DNA polymerases α and β were prepared and separated by published procedures (2, 12, 17). Frozen L1210 cells were homogenized in 20 mM potassium phosphate buffer, 5 mM β-mercaptoethanol, 1 mM EDTA, 0.5 M KCl (pH 7.5), centrifuged, dialyzed to remove the KCl, and applied to a column of DEAE-Sepharose CL-6B (Pharmacia Fine Chemicals, Uppsala, Sweden). Both polymerases were further purified by chromatography over phosphocellulose (Sigma). The samples were dialyzed against 50% glycerol and stored at −20°. The assay was linear with respect to enzyme concentration in the range used. The K_m for dATP was 9.4 μM.

S-Adenosylhomocysteine hydrolase was prepared from L1210 cells as described by Hershfield (14). The final preparation of enzyme showed no adenosine deaminase activity when tested in the standard *S*-adenosylhomocysteine hydrolase assay. The assay was linear with respect to time and enzyme concentration in the range used in this paper. The K_m for adenosine was 7 μM.

RESULTS

Inhibition of Ribonucleotide Reductase by ara-ATP and 2-F-ara-ATP. Both nucleoside triphosphates inhibited the reduction of ADP and CDP catalyzed by enzyme preparations from HEp-2 and L1210 cells (Table 1). 2-F-ara-ATP clearly was the more effective inhibitor. The concentration of 2-F-ara-ATP for 50% inhibition of ADP reduction was 10 to 15 μM with enzyme from HEp-2 and L1210 cells. The concentration of 2-F-ara-ATP for 50% inhibition of CDP reduction was 20 μM with enzyme from HEp-2 cells and 95 μM with enzyme from L1210 cells. Concentrations of ara-ATP more than 10 times those of 2-F-ara-ATP were required to attain 50% inhibition of reduction of ADP or CDP for both enzymes.

Characterization of DNA Polymerases α and β. Two peaks of DNA polymerase activity were eluted from the DEAE-Sepharose column (Chart 1). The salt concentrations used to elute these 2 polymerases from the DEAE and phosphocellulose columns were similar to those used by Allaudeen and Bertino (2) and Graham *et al.* (12). The more acidic protein was designated DNA polymerase α, and the more basic one was designated DNA polymerase β (31). The effect of KCl and *N*-ethylmaleimide on both polymerases was examined. DNA polymerase α was inhibited 50% by 60 mM KCl whereas DNA polymerase β was not inhibited at concentrations up to 160 mM. Ten mM *N*-ethylmaleimide was 100% inhibitory for DNA polymerase α and 20% inhibitory for DNA polymerase β (data not shown).

The assay for DNA polymerase α was nonlinear with respect to time when run at 37° (Chart 2). However, lowering the incubation temperature to 25° produced a linear reaction up to 60 min. DNA polymerase β was linear for 1 hr at both temperatures. When the polymerases were preincubated at 45° and then assayed at 25°, DNA polymerase α had a $t_{1/2}$ of 6 min (Chart 3). DNA polymerase β retained 100% of its activity after 40 min of incubation at 45°.

Effect of ara-ATP and 2-F-ara-ATP on DNA Polymerase. Both ara-ATP and 2-F-ara-ATP are reversible inhibitors of DNA polymerase α and β. ara-ATP, tested at concentrations of 5, 15, and 30 μM in the DNA polymerase α assay with varying concentrations of dATP, gave a K_i of 11 μM (Chart 4). The kinetics indicated that the reaction was competitive. When 2-F-ara-ATP was tested under similar conditions, a K_i of 11 μM was also obtained (Chart 5). However, the curves intersected in the first quadrant, indicating that the mechanism was not competitive.

DNA polymerase β was much less sensitive to both ara-ATP and 2-F-ara-ATP. For both compounds, the concentration that gave 50% inhibition of the enzyme activity was greater than 200 μM (data not shown).

Effect of ara-A and 2-F-ara-A on *S*-Adenosylhomocysteine Hydrolase. ara-A and 2-F-ara-A were compared for their capacity to inactivate the hydrolase when incubated in the ab-

Table 1

Inhibition of ribonucleotide reductase by ara-ATP and 2-F-ara-ATP

	Concentration (μM) for 50% inhibition of enzyme activity[a]			
	HEp-2		L1210	
Inhibitor	ADP[b]	CDP[c]	ADP[b]	CDP[c]
ara-ATP	165	550	175	>1000
2-F-ara-ATP	10	20	15	95

[a] Ribonucleotide reductase was assayed by the method of Moore (18) with modifications described previously (6).

[b] The concentration of [8-^{14}C]ADP was 0.4 mM (0.1 μCi/0.2 ml reaction mixture) and that of dGTP as activator was 0.05 mM.

[c] The concentration of [8-^{14}C]CDP was 0.4 mM (0.1 μCi/0.2 ml reaction mixture) and that of ATP as activator was 4.4 mM.

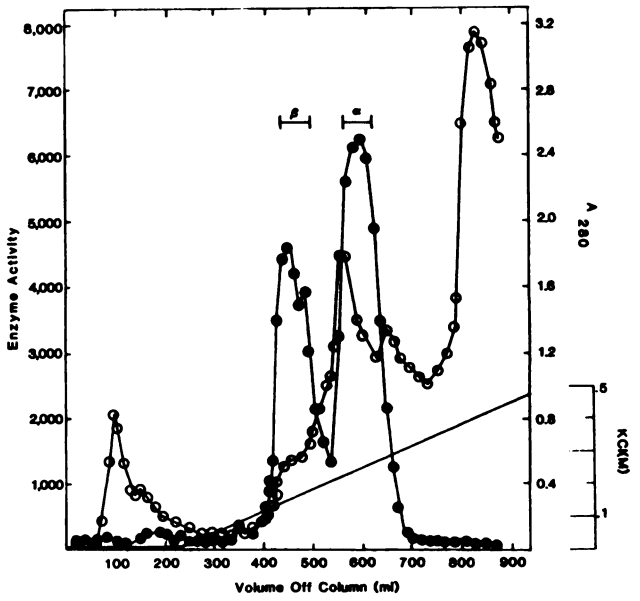

Chart 1. Separation of DNA polymerases α and β on DEAE-Sepharose CL-6B. DNA polymerase α and β were pooled as indicated. ●, enzyme activity (dpm/10 μl); ○, protein (A_{280}); ——, KCl (M).

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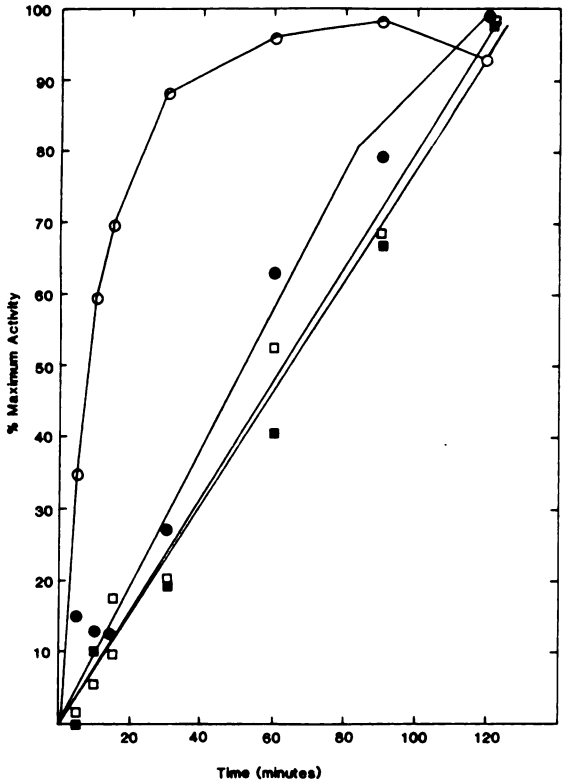

Chart 2. Temperature effects on the linearity of DNA polymerase-catalyzed reactions. ○, DNA polymerase α at 37°; ●, DNA polymerase β at 37°; □, DNA polymerase α at 25°; ■, DNA polymerase β at 25°.

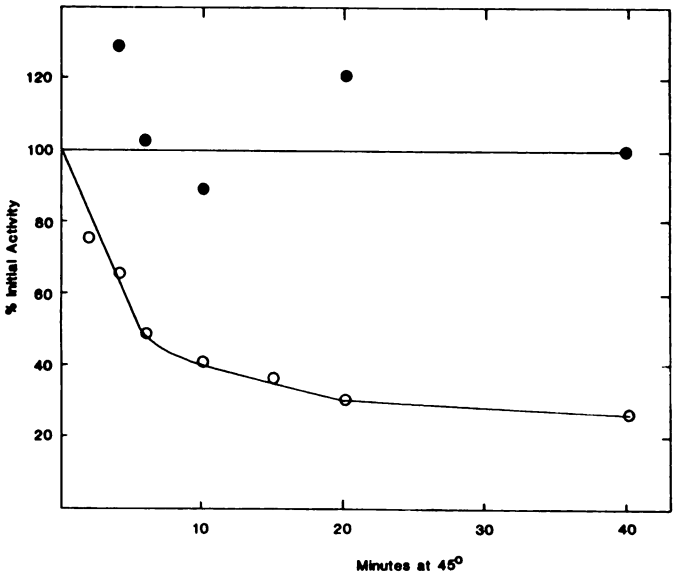

Chart 3. Effect of heating on DNA polymerases. ○, DNA polymerase α; ●, DNA polymerase β.

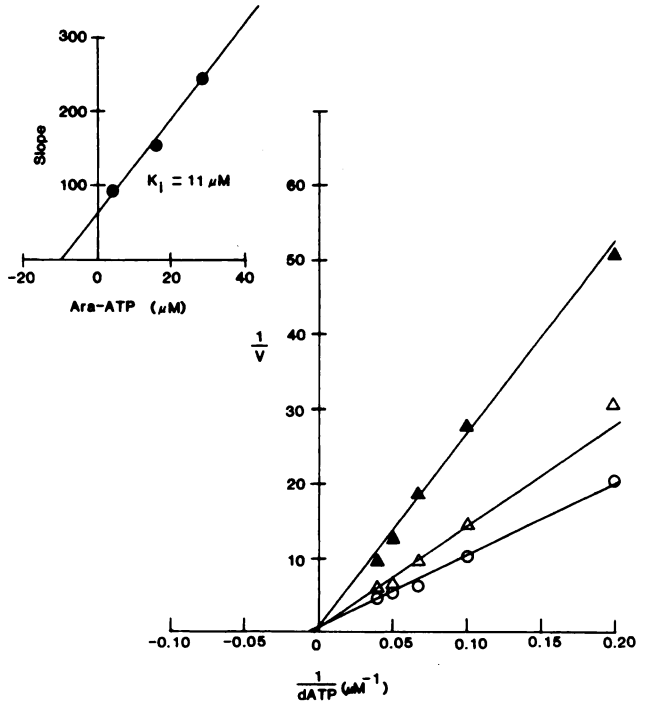

Chart 4. Determination of K_i for ara-ATP for DNA polymerase α. Concentrations of ara-ATP: 5 μM (○); 15 μM (△); and 30 μM (▲).

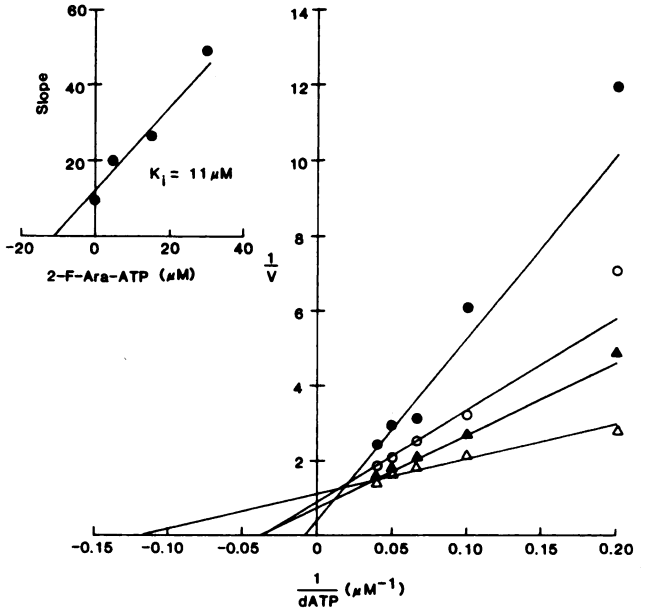

Chart 5. Determination of K_i for 2-F-ara-ATP for DNA polymerase α. Concentrations of 2-F-ara-ATP: 0 μM (△); 5 μM (▲); 15 μM (○); and 30 μM (●).

sence of adenosine. Both arabinosyl compounds inactivated the enzyme (Charts 6 and 7). The method described by Schaeffer (25) and Walsh (30) was used to calculate K_I, an index of the affinity of inactivator for the enzyme, and k_2, the limiting rate constant for inactivation. For ara-A, the K_I was 19 μM and k_2 was 0.31 min^{-1}. For 2-F-ara-A, the values were 122 μM and 0.10 min^{-1}. Inactivation by 2-F-ara-A and ara-A differed in that for ara-A the semilog plot was linear for only 12 min whereas that for 2-F-ara-A was linear for 30 min. In calculating the constants for ara-A, the data used were from the linear portion of the plots.

To determine if *S*-adenosylhomocysteine could convert nucleosides to the corresponding bases, as has been observed by others (1, 8, 28), the hydrolase was incubated with adenosine, ara-A, or 2-F-ara-A (45 or 450 μM) at 30° for 1 hr. The samples were applied to paper chromatograms and developed overnight. Each chromatogram was segmented into 2-cm strips

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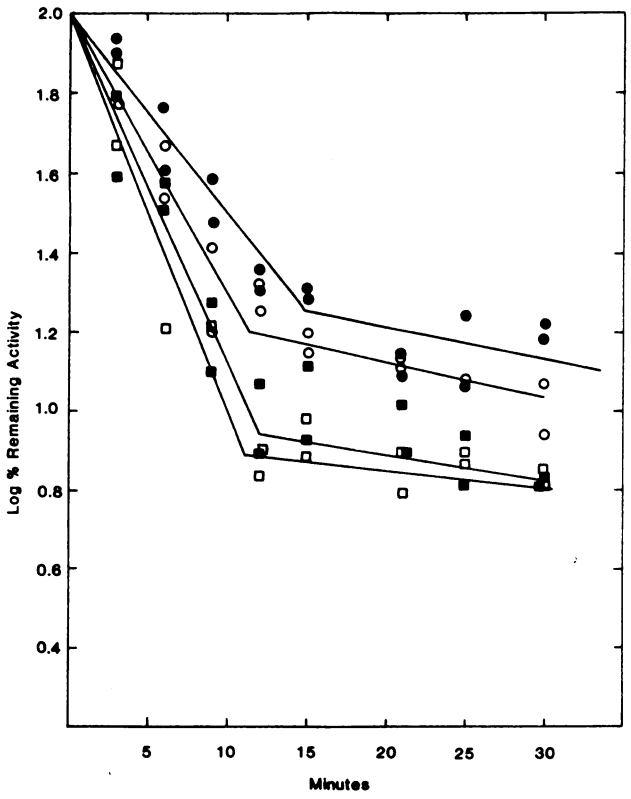

Chart 6. Irreversible inactivation of *S*-adenosylhomocysteine hydrolase by ara-A. Concentrations of ara-A in incubation mixtures: 11 μM (●); 23 μM (○); 34 μM (■); and 45 μM (□).

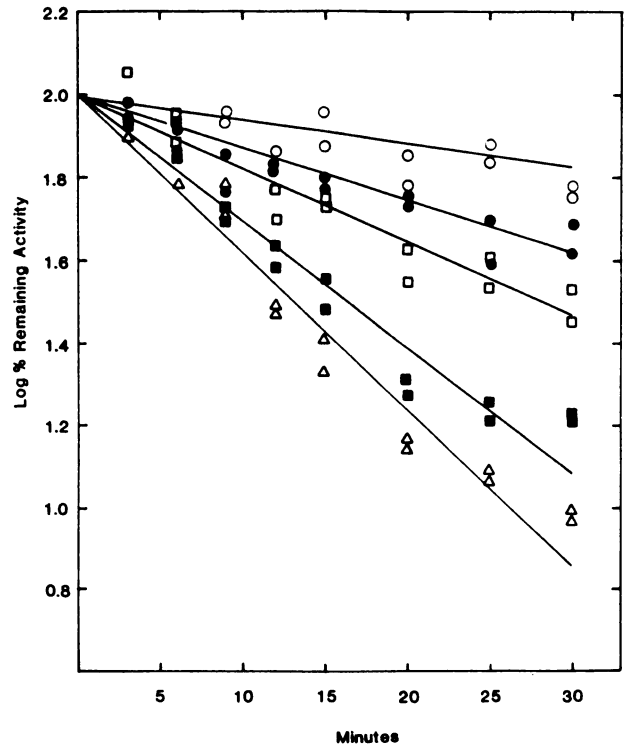

Chart 7. Irreversible inactivation of *S*-adenosylhomocysteine hydrolase by 2-F-ara-A. Concentrations of 2-F-ara-A in incubation mixtures: 23 μM (○); 56 μM (●); 113 μM (□); 169 μM (■); and 225 μM (△).

and counted. Under these conditions, no production of free base was observed.

DISCUSSION

The biological activity of ara-A is generally ascribed to inhibition of DNA synthesis, which results principally from inhibition by ara-ATP of DNA polymerase α and to a lesser extent from inhibition of ribonucleotide reductase (7, 9). The results of the present study show that 2-F-ara-ATP is an effective inhibitor of both of these enzymes. As an inhibitor of DNA polymerase α from L1210 cells (Charts 4 and 5), 2-F-ara-ATP was equal to ara-ATP, and as an inhibitor of ribonucleotide reductase from HEp-2 and L1210 cells, it was clearly superior to ara-ATP (Table 1). Similar results have been reported for DNA polymerase α and ribonucleotide reductase from HeLa cells (27). Inhibition of sequential enzyme reactions of DNA synthesis by 2-F-ara-ATP may increase its effectiveness.

The DNA polymerase α preparation from L1210 cells differed in behavior from polymerases from other sources. The reaction was not linear at 37° but was linear at 25° (Chart 2). The departure from linearity at 37° most probably reflects the unusual temperature sensitivity of the enzyme, which, as shown in Chart 3, rapidly loses activity at elevated temperatures. Such behavior may be characteristic of the polymerase from L1210 cells rather than any property, such as nonhomogeneity, of our particular preparation. In the only other reported study (2) with the enzyme from L1210 cells, some departure from linearity was also noted.

A second instance of unexpected behavior of the L1210 polymerase is its difference in response to ara-ATP and 2-F-ara-ATP (Charts 4 and 5). Whereas ara-ATP exhibited competitive inhibition with dATP, inhibition by 2-F-ara-ATP was not competitive, as indicated by intersection of the lines in the first quadrant (Chart 5). This was a reproducible phenomenon with the enzyme from L1210 cells. The result was unexpected both because ara-ATP and 2-F-ara-ATP are competitive inhibitors of DNA polymerase α from HeLa cells (27) and because any mechanism would have to explain the qualitative differences caused by 2 compounds with such similar structures. We have no explanation for this anomalous result with the L1210 enzyme. Contamination with an exonuclease, which has not been ruled out, might be responsible for these differences if the exonuclease should remove incorporated 9-β-D-arabinofuranosyladenine 5′-monophosphate and 2-F-ara-AMP at different rates. However, exonuclease activity is not generally associated with mammalian DNA polymerases (31). Another possible explanation for this result that has not yet been examined could reside in differential incorporation of the 2 nucleotides into the DNA chain with resultant differences in effects on the kinetics of the reaction.

2-F-ara-A and ara-A differed in their effectiveness as inhibitors of *S*-adenosylhomocysteine hydrolase. When incubated with the enzyme in the absence of adenosine, both arabinosyl compounds inactivated the enzyme, but 2-F-ara-A was clearly inferior to ara-A as an inactivator (Charts 6 and 7). The K_i for inactivation of the L1210 enzyme by ara-A (19 μM) was of the same magnitude as that obtained by Helland and Ueland (13) for the mouse liver enzyme and Hershfield (14) for the enzyme from human lymphoblasts. Although it is of interest, as a structure-activity relationship, that the substitution of a fluorine

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at position 2 decreases the capacity of ara-A to inactivate *S*-adenosylhomocysteine hydrolase, it is unlikely that effects on this enzyme contribute significantly to the biological action of either ara-A or 2-F-ara-A. The reasons for this conclusion are: (*a*) 2-F-ara-A and ara-A plus an adenosine deaminase inhibitor have similar toxicities *in vivo*; and (*b*) cells that have lost capacity to phosphorylate these nucleosides are highly resistant to them (29).[4]

Thus, it appears that, for both ara-A and 2-F-ara-A, the 5′-triphosphates are the active inhibitors, and presently available data are consistent with inhibition of DNA polymerase α and ribonucleotide reductase as sites of action. ara-A is known to be incorporated into DNA in internucleotide linkage (9, 19, 20), and its inhibition of DNA synthesis may result from a slowing of chain extension as well as from competition with dATP for the polymerase. For 2-F-ara-A, we have as yet no information on the extent of its incorporation into DNA and resultant effects on chain extension.

Another potential action of 2-F-ara-A that has not yet been examined is inhibition of polyadenylate polymerase. This enzyme from a mammalian source is inhibited by ara-ATP at low concentrations (24), but the contribution of such inhibition to the biological activity of ara-A is not known.

We have observed that in the mouse 2-fluoroadenine is a urinary metabolite of 2-F-ara-A (26). Since purine nucleoside phosphorylase did not act upon 2-F-ara-A even after prolonged incubation,[5] some other enzyme is likely to be responsible for the formation of 2-fluoroadenine. Because *S*-adenosylhomocysteine hydrolase may form some free base from nucleosides (1, 8, 28) and because the production of adenine from ara-A was greater than its production from adenosine (8), we considered it possible that this enzyme might be responsible for the formation of 2-fluoroadenine from 2-F-ara-A. The enzyme preparations from L1210 cells, however, did not convert adenosine, ara-A, or 2-F-ara-A to free base. The possibility obviously remains that there may be differences in the hydrolases from various tissues or that, although the enzyme from L1210 cells did not act on 2-F-ara-A, the enzyme from mouse tissues may do so.

REFERENCES

1. Abeles, R. H., Tashjian, A. H., Jr., and Fish, S. The mechanism of inactivation of *S*-adenosylhomocysteinase by 2′-deoxyadenosine. Biochem. Biophys. Res. Commun., *95:* 612–617, 1980.
2. Allaudeen, H. S., and Bertino, J. R. Inhibition of activities of DNA polymerase α, β, γ, and reverse transcriptase of L1210 cells by phosphonoacetic acid. Biochim. Biophys. Acta, *520:* 490–497, 1978.
3. Boyle, W. An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins. Transplantation (Baltimore), *6:* 761–764, 1968.
4. Brockman, R. W., Cheng, Y-C., Schabel, F. M., Jr., and Montgomery, J. A. Metabolism and chemotherapeutic activity of 9-β-D-arabinofuranosyl-2-fluoroadenine against murine leukemia L1210 and evidence for its phosphorylation by deoxycytidine kinase. Cancer Res., *40:* 3610–3615, 1980.
5. Brockman, R. W., Schabel, F. M., Jr., and Montgomery, J. A. Biologic activity of 9-β-D-arabinofuranosyl-2-fluoroadenine, a metabolically stable analog of 9-β-D-arabinofuranosyladenine. Biochem. Pharmacol., *26:* 2193–2196, 1977.
6. Brockman, R. W., Shaddix, S., Laster, W. R., Jr., and Schabel, F. M., Jr. Inhibition of ribonucleotide reductase, DNA synthesis, and L1210 leukemia by guanazole. Cancer Res., *30:* 2358–2368, 1970.
7. Chang, C-H., and Cheng, Y-C. Effects of deoxyadenosine triphosphate and 9-β-D-arabinofuranosyladenine 5′-triphosphate on human ribonucleotide reductase from Molt-4F cells and the concept of "self-potentiation." Cancer Res., *40:* 3555–3558, 1980.
8. Chiang, P. K., Guranowski, A., and Segall, J. E. Irreversible inhibition of *S*-adenosylhomocysteine hydrolase by nucleoside analogs. Arch. Biochem. Biophys., *207:* 175–184, 1981.
9. Cohen, S. S. The lethality of aranucleotides. Med. Biol. (Helsinki), *54:* 299–326, 1976.
10. Dicioccio, R. A., and Srivastava, B. I. S. Kinetics of inhibition of deoxynucleotide-polymerizing enzyme activities from normal and leukemic human cells by 9-β-D-arabinofuranosyladenine 5′-triphosphate and 1-β-D-arabinofuranosylcytosine 5′-triphosphate. Eur. J. Biochem., *79:* 411–418, 1977.
11. Dow, L. W., Bell, D. E., Poulakos, L., and Fridland, A. Differences in metabolism and cytotoxicity between 9-β-D-arabinofuranosyladenine and 9-β-D-arabinofuranosyl-2-fluoroadenine in human leukemic lymphoblasts. Cancer Res., *40:* 1405–1410, 1980.
12. Graham, D. G., Tye, R. W., and Vogel, F. S. Inhibition of DNA polymerase from L murine leukemia by a sulfhydryl reagent from *Agaricus bisporus*. Cancer Res., *37:* 436–439, 1977.
13. Helland, S., and Ueland, P. M. Interaction of 9-β-D-arabinofuranosyladenine, 9-β-D-arabinofuranosyladenine 5′-monophosphate, and 9-β-D-arabinofuranosyladenine 5′-triphosphate with *S*-adenosylhomocysteinase. Cancer Res., *41:* 673–678, 1981.
14. Hershfield, M. S. Apparent suicide inactivation of human lymphoblast *S*-adenosylhomocysteine hydrolase by 2′-deoxyadenosine and adenine arabinoside. J. Biol. Chem., *254:* 22–25, 1979.
15. Lee, S. H., Caron, N., and Kimball, A. P. Therapeutic effects of 9-β-D-arabinofuranosyladenine and 2′-deoxycoformycin combinations on intracerebral leukemia. Cancer Res., *37:* 1953–1955, 1977.
16. LePage, G. A., Worth, L. S., and Kimball, A. P. Enhancement of the antitumor activity of arabinofuranosyladenine by 2′-deoxycoformycin. Cancer Res., *36:* 1481–1485, 1976.
17. Lewis, B. J., Abrell, J. W., Smith, R. G., and Gallo, R. C. DNA polymerases in human lymphoblastoid cells infected with simian sarcoma virus. Biochim. Biophys. Acta, *349:* 148–160, 1974.
18. Moore, E. C. Mammalian ribonucleoside diphosphate reductases. Methods Enzymol. *12* (Part A): 155–164, 1967.
19. Müller, W. E. G., Zahn, R. K., Bittlingmaier, K., and Falke, D. Inhibition of herpesvirus DNA synthesis by 9-β-D-arabinofuranosyladenine in cellular and cell-free systems. Ann. N. Y. Acad. Sci., *284:* 34–48, 1977.
20. Pelling, J. C., Drach, J. C., and Shipman, C., Jr. Internucleotide incorporation of arabinosyladenine into herpes simplex virus and mammalian cell DNA. Virology, *109:* 323–335, 1981.
21. Plunkett, W., Chubb, S., Alexander, L., and Montgomery, J. A. Comparison of the toxicity and metabolism of 9-β-D-arabinofuranosyl-2-fluoroadenine and 9-β-D-arabinofuranosyladenine in human lymphoblastoid cells. Cancer Res., *40:* 2349–2355, 1980.
22. Plunkett, W., and Cohen, S. S. Two approaches that increase the activity of analogs of adenine nucleosides in animal cells. Cancer Res., *35:* 1547–1554, 1975.
23. Puck, T. T., Cieciura, S. J., and Robinson, A. Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. J. Exp. Med., *108:* 945–956, 1958.
24. Rose, K. M., and Jacob, S. T. Selective inhibition of RNA polyadenylation by ara-ATP *in vitro*: a possible mechanism for antiviral action of ara-A. Biochem. Biophys. Res. Commun., *81:* 1418–1424, 1978.
25. Schaeffer, H. J. Factors in the design of reversible and irreversible enzyme inhibitors. *In:* E. J. Ariens (ed.), Drug Design, Vol. 2, pp. 129–160. New York: Academic Press, Inc., 1971.
26. Struck, R. F., Shortnacy, A. T., Kirk, M. C., Thorpe, M. C., Brockman, R. W., Hill, D. L., El Dareer, S. M., and Montgomery, J. A. Identification of metabolites of 9-β-D-arabinofuranosyl-2-fluoroadenine, an antitumor and antiviral agent. Biochem. Pharmacol., in press, 1982.
27. Tseng, W-C., Derse, D., Cheng, Y-C., Brockman, R. W., and Bennett, L. L., Jr. "Self potentiation" mechanism of action of 9-β-D-arabinofuranosyl-2-fluoroadenine (2-F-ara-A). Fed. Proc., *40:* 666, 1981.
28. Ueland, P. M., and Saebo, J. Cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase from mouse liver. A fraction of adenosine bound is converted to adenine. Biochim. Biophys. Acta, *585:* 512–526, 1979.
29. Verhoef, V. L., Sarup, J., and Fridland, A. Isolation and biochemical characterization of mutant human leukemic cell lines resistant to 9-β-D-arabinofuranosyladenine (ara-A). Proc. Am. Assoc. Cancer Res., *22:* 206, 1981.
30. Walsh, C. Recent developments in suicide substrates and other active site-directed inactivating agents of specific target enzymes. Horiz. Biochem. Biophys., *3:* 36–81, 1977.
31. Weissbach, A., Baltimore, D., Bollum, F., Gallo, R., and Korn, D. Nomenclature of eukaryotic DNA polymerases. Science (Wash. D. C.), *190:* 401–402, 1975.
32. White, L., Shaddix, S. C., Cheng, Y-C., Brockman, R. W., and Bennett, L. L., Jr. Inhibition of ribonucleotide reductase and DNA polymerases of tumor cells by 9-β-D-arabinofuranosyl-2-fluoroadenine 5′-triphosphate (2-F-ara-ATP). Proc. Am. Assoc. Cancer Res., *22:* 33, 1981.

[4] R. W. Brockman, B. Bowdon, and L. L. Bennett, Jr., unpublished results.

[5] S. C. Shaddix and R. W. Brockman, unpublished results.

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