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PRELIMINARY COMMUNICATIONS

BIOLOGIC ACTIVITY OF 9-β-D-ARABINOFURANOSYL-2-FLUOROADENINE, A METABOLICALLY STABLE ANALOG OF 9-β-D-ARABINOFURANOSYLADENINE

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The antitumor activity of 9-β-D-arabinofuranosyladenine (AraA) is enhanced by inhibition of adenosine deaminase.[1-4] Inhibition of DNA synthesis in tumor cells by AraA also is enhanced by concurrent inhibition of adenosine deaminase.[2,5,6] Chemotherapeutic activity and inhibition of DNA synthesis can be correlated with increased levels of AraATP in leukemia cells in mice treated with adenosine deaminase inhibitors in combination with AraA.[5,7,8]

An equally valid and simpler approach is the design of adenosine analogs that are not substrates for adenosine deaminase. The observation that 2-fluoroadenosine[9] was not a substrate for adenosine deaminase[10] led to the synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine (2-F-AraA).[11] We have now observed that 2-F-AraA also is not a substrate for this catabolic enzyme (EC 3.5.4.4.) from calf intestinal mucosa or from P388 and L1210 mouse leukemia cells. An examination of the phosphorylation of 2-F-AraA in L1210 cells in vivo (Figure 1) revealed that the intracellular pools of 2-F-AraATP attained in L1210 cells from BDF1 mice treated with 2-F-AraA alone were comparable to levels of AraATP found in L1210 cells treated with AraA in combination with an adenosine deaminase inhibitor.[8] The intracellular concentration of 2-F-AraATP in L1210 cells was 45, 156 and 220 nanomoles per 10^9 cells three hours after intraperitoneal treatment with 80, 150 and 400 mg/kg of 2-F-AraA (Figure 2). 2-F-AraATP was not found in L1210 cells 9 and 18 hours after treatment of leukemic mice with a single dose of 80 and 150 mg/kg of 2-F-AraA but significant levels were present 18 hours after treatment with a dose of 400 mg/kg of 2-F-AraA.

2-F-AraA inhibited DNA synthesis in L1210 cells in culture (Table 1). Protein synthesis was less affected and RNA synthesis was not inhibited under the same experimental conditions.

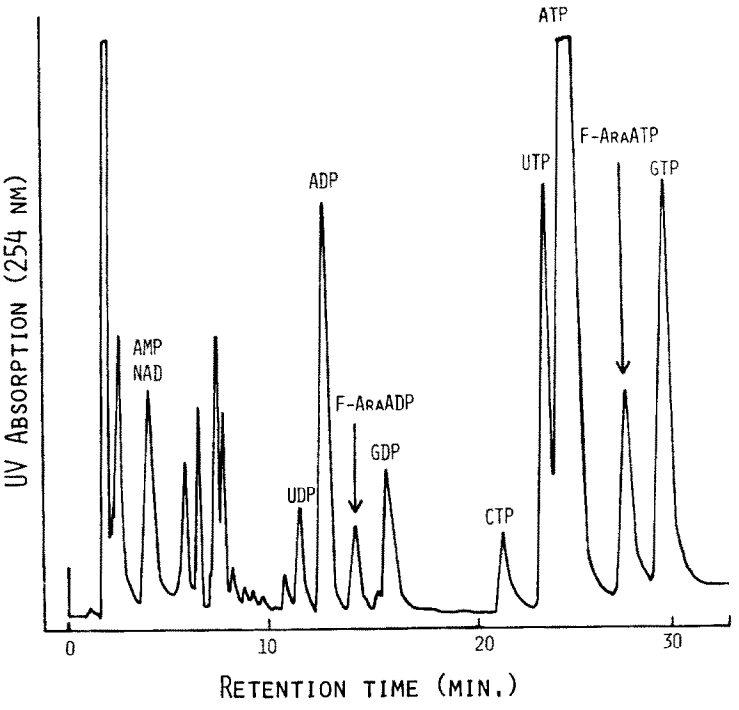

Figure 1. HPLC analysis of cold perchloric acid extracts of L1210 ascites tumor cells 3 hours after treatment of mice with 150 mg/kg of 2-F-AraA. A Waters ALC 202 high-pressure liquid chromatograph equipped with a Partisil-10 SAX column (Whatman) was used to separate nucleotides at ambient temperature using a linear gradient (40 min.) from 5 mM (pH 2.8) to 750 mM ammonium dihydrogen phosphate (pH 3.7) at a flow rate of 2 ml/min (1200 p.s.i.).[8]

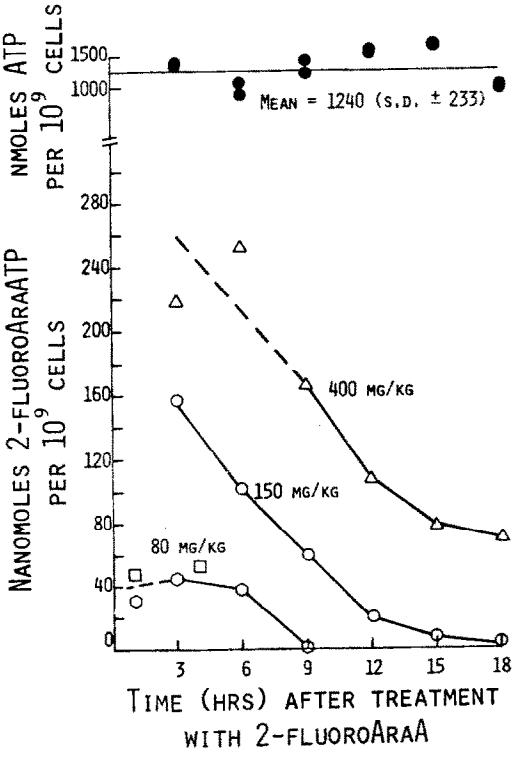

Figure 2. 2-F-AraATP levels in L1210 cells in vivo after treatment of mice with 80, 150 and 400 mg/kg of 2-F-AraA.

Table 1. Effects of 2-F-AraA (NSC-118218) on Macromolecular Synthesis in L1210 Cells in Culture

Conc. (μg/ml)	Treated as % of Control[a] DNA[b] TdR-^{3}H	DNA[b] UR-^{3}H	RNA[c] UR-^{3}H	Protein[d] Leu-^{3}H
1.0	102	84	135	119
3.0	49	36	163	86
10.0	18	11	137	66

[a]Inhibitor was added to L1210 cells growing in culture; radioactive substrate was added 30 min later. These data are based on samples taken 4 hrs after addition of radioactive substrate to culture medium.[12]

[b]Incorporation of [C^{3}H$_3$]-thymidine or [5-^{3}H]-uridine into DNA.

[c]Incorporation of [5-^{3}H]-uridine into RNA.

[d]Incorporation of [4,5-^{3}H]-L-leucine into protein.

Table 2. Therapeutic Activity of 2-F-AraA Against Leukemia L1210[a]

Expt.	2-F-AraA Treatment mg/kg/dose[b]	Schedule; Route[c]	Median Day of Death (Dying Mice Only) Treated/Control	Median % ILS[d]	60-Day Survivors	Estimated No. L1210 Cells Surviving Therapy[e]
1	100	q3hx8, days 1,5,9; i.p.	19/8.5	123	2/6	$4x10^4$ (1.1)
2			23/9	155	1/6	$5x10^2$ (1.8)
2	100	q6hx4, days 1,5,9; i.p.	16.5/9	83	0/6	$3x10^6$
2	400	q12hx2, days 1,5,9; i.p.	22/9	144	1/6	$2x10^3$ (1.8)
2	150	q3hx8, days 1,5,9; oral	15/9	66	0/6	$2x10^7$

[a]10^5 L1210 ascites cells implanted i.p. on day 0.

[b]Treatments reported were selected from a range of dose levels from lethally toxic to apparently nontoxic. Those included here resulted in no more than 16% (1/6 treated mice) dying without gross evidence of progressive leukemia (splenomegaly), except those treated orally, in which the dose range tested contained no toxic levels.

[c]i.p. = intraperitoneal; oral = drug administered directly into stomach by catheter.

[d]% ILS = % increase in life span, compared to untreated control; excluding 60-day survivors.

[e]Based on median day of death in dying animals only. Figures in parentheses based on % survivors.

DNA synthesis in L1210 cells *in vivo* was markedly inhibited (10 to 20% of controls) for a period of 6 hours after treatment with 2-F-AraA (80 mg/kg).

AraA, in the absence of adenosine deaminase inhibitors, has no significant therapeutic activity against L1210 leukemia.[2-4] Since 2-F-AraA is not deaminated it was tested alone in BDF1 mice implanted i.p. with 10^5 L1210 cells. 2-F-AraA was given i.p. or orally on the basis of average mouse body weight. Therapeutic activity based on observed

increase in median life span of treated animals over untreated controls and on estimates of the number of viable L1210 cells present at the end of treatment[13] is summarized in Table 2. The data presented allow the conclusion that 2-F-AraA has reproducible therapeutic activity against L1210 and suggest, in agreement with the observed maintenance of 2-F-AraATP levels in L1210 cells following a single dose of 400 mg/kg of 2-F-AraA (Figure 2), that repeated doses of the drug at short intervals may not be required for optimal therapeutic activity. 2-F-AraA appears to have some therapeutic activity against L1210 following oral administration. Studies to determine the optimal doses and schedule for both parenteral and oral treatment are planned and will be conducted as 2-F-AraA becomes available.

Our findings show (a) that 2-F-AraA is not a substrate for adenosine deaminase; (b) that it is phosphorylated to 2-F-AraATP; (c) that it inhibits DNA synthesis in L1210 cells in culture and *in vivo*; and (d) that 2-F-AraA alone is therapeutically effective against L1210 leukemia.

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