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Comparative Activity of 1-β-D-Arabinofuranosyl-5-fluorocytosine and Related Compounds against Transplanted Mouse Leukemias *in Vivo* and *in Vitro*[1]

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Summary

1-β-D-Arabinofuranosyl-5-fluorocytosine (5-fluorocytosine arabinoside; ara-FC)[2] possesses a high degree of chemotherapeutic activity against transplanted mouse leukemia. It is more active on a molar basis than 1-β-D-arabinofuranosylcytosine (cytosine arabinoside; ara-C), 5-fluorouracil (FU), or 5-fluoro-2′-deoxyuridine (FUDR) against leukemia P815. Ara-FC is somewhat more active than ara-C against leukemia P388. In leukemia L1210, ara-FC is approximately equal to ara-C, but more effective than FU or FUDR. It is less effective than either FU or FUDR, however, against leukemia B82. Both ara-FC and ara-C are highly effective against a line of leukemia P815 made resistant to FU.

Ara-FC and ara-C are equally inhibitory against cells of leukemias P815Y and P388SK in tissue culture. The inhibitory effects of both are blocked by deoxycytidine (CDR) but not by thymidine (TDR). It is concluded that ara-FC is a somewhat more active derivative of ara-C that merits clinical trials.

Introduction

The synthesis of FU by Heidelberger *et al.* (12) and FUDR by Duschinsky *et al.* (8) and the demonstration of their clinical value by Curreri and Ansfield (5, 6) provided the 1st type of pyrimidine antimetabolites useful in clinical cancer chemotherapy. Changes in the sugar moiety of pyrimidine ribonucleosides alone, however, by the substitution of a 3′-amino group, as in the purine-containing aminonucleoside of puromycin, were less successful (14). The substitution of arabinose for deoxyribose, however, created pyrimidine nucleoside antagonists of great interest (9, 18, 22), and Evans *et al.* (9) demonstrated the antitumor activity of ara-C. Chu and Fischer (3) suggested that ara-C inhibits the growth of leukemia L5178Y cells by depriving them of CDR or a phosphorylated derivative thereof, presumably by blocking the conversion of cytidylate to deoxycytidylate. Their later studies demonstrated an incorporation of ara-C into RNA and DNA (4). Although occasional partial remissions have been noted in acute leukemia, ara-C has so far not had a high degree of clinical success (13, 16, 17, 21).

In an attempt to enhance the antitumor activity and decrease the toxicity of a 5-fluorinated pyrimidine, Yung *et al.* (23) 1st synthesized 1-β-D-arabinofuranosyl-5-fluorouracil (ara-FU). Although ara-FU was active against transplanted mouse leukemia B82 in C58 mice (23), no beneficial effects were noted in preliminary trials in patients (D. A. Karnofsky, personal communication). A more recent derivative, ara-FC, has been synthesized by Fox *et al.* (11). The antileukemic activity of this particular derivative is herewith reported.

Materials and Methods

The technic for evaluating the chemotherapeutic activity of a drug by its ability to prolong the survival time of mice with transplanted leukemia has been reported previously (1). The experiments described here were done with leukemias P815 (7), P388 (19), and L1210 (15) in F_1 hybrids of the C57BL/6 × DBA/2 cross (BDF_1) and with leukemia B82 (2) in F_1 hybrids of the Bagg albino × C58 cross ($C58F_1$). One million leukemic cells in saline suspension were inoculated i.p. into each animal, which produced an ascitic leukemia that later progressed to generalized disease. The mice were divided into groups of 10 mice each, and treatment was initiated 24 hr after the inoculation with leukemia and continued once daily to a total of 10 doses unless otherwise noted. All pyrimidine antagonists were dissolved in saline and injected i.p. Mice were weighed once weekly and autopsied at death for gross evidence of leukemia.

For the studies in tissue culture the leukemic cells were grown according to the Fischer technic in liquid medium containing 10% horse serum (10). Cell lines used were mouse leukemia P815Y, adapted to tissue culture by Schindler *et al.* (20), and P388SK, adapted to tissue culture by our own group in February, 1962 (J. H. Burchenal, V. C. Gregg, and S. P. Lancaster, unpublished observations), and carried in tissue culture since that time.

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[2] The abbreviations used are: ara-FC, 1-β-D-arabinofuranosyl-5-fluorocytosine; ara-C, 1-β-D-arabinofuranosylcytosine; FU, 5-fluorouracil; FUDR, 5-fluoro-2′-deoxyuridine; CDR, deoxycytidine; TDR, thymidine; and ara-FU, 1-β-D-arabinofuranosyl-5-fluorouracil.

Cells for experiments were taken from stock cultures and diluted with the medium to give a concentration of 10^5 cells/ml. One-half-ml aliquots of this suspension were added to screw-cap culture tubes containing 4.5 ml of the medium, which resulted in a final count of 10^4 cells/ml. The compounds were dissolved in sterile distilled water and diluted to a concentration 50 times greater than the experimental level desired, so that 0.1 ml of the compound solution could be added to 5 ml of growth medium. In each experiment 2 tubes were inoculated, incubated at 37°C, and counted after 72 hr of incubation. Fresh medium was not added during this time. All cell counts were done by diluting the cell culture 1:20 and then counting with an electronic cell counter (Coulter counter, Model A). The concentrations of cells in the control tubes of P815Y and P388SK after 72 hr were approximately 750,000/ml and 500,000/ml, respectively.

TABLE 1

EFFECT OF ARA-FC[a] AND RELATED COMPOUNDS ON SURVIVAL TIME OF MICE WITH P815 LEUKEMIA

Compound	Dose (mg/kg/day × 10)	Weight change (gm)	Survival time (days)	Treated/control (%)	50-day survivors	50-day wt. change (gm)
Control		+2.4	9.3			
ara-FC	25	−0.4	44.2[b]	475[b]	7	+3.3
	12.5	+0.4	43.7[b]	470[b]	6	+2.9
	6.25	+0.5	23.7	255		
ara-C	25	+0.2	42.7[b]	459[b]	5	+3.3
	12.5	+0.9	27.6	297		
	6.25	+2.0	12.1	130		
FUDR	100	−0.2	14.8	159		
	50	+1.3	10.9	117		
	25	+2.1	11.3	122		
FU	25	−1.5	24.7	265		
	12.5	+1.5	19.8	213		
	6.25	+0.8	13.1	141		

[a] ara-FC, 1-β-D-arabinofuranosyl-5-fluorocytosine; ara-C, 1-β-D-arabinofuranosylcytosine; FUDR, 5-fluoro-2′-deoxyuridine; and FU, 5-fluorouracil.

[b] 50-day survivors.

TABLE 2

EFFECT OF ARA-FC[a] AND ARA-C ON SURVIVAL TIME OF MICE WITH LEUKEMIA P388

Compound	Dose (mg/kg/day × 10)	Weight change (gm)[b]	Survival time (days)	Treated/control (%)
Control		+1.8	10.9	
ara-FC	25	−1.2	21.2	194
	12.5	−1.4	19.6	180
	6.25	−0.5	20.6	189
ara-C	25	−0.7	20.4	187
	12.5	−0.1	18.1	166
	6.25	−0.2	17.5	160
Control		+2.8	10.8	
ara-FC	25	0.0	21.4	198
	12.5	+0.2	19.3	179
	6.25	+0.7	16.5	153
	3.12	+0.7	13.7	127
ara-C	25	−1.6	17.4	161
	12.5	+0.3	18.7	173
	6.25	+1.0	14.5	134
	3.12	+2.6	13.2	122

[a] ara-FC, 1-β-D-arabinofuranosyl-5-fluorocytosine; ara-C, 1-β-D-arabinofuranosylcytosine.

[b] Weight change calculated at 9 days for 1st experiment, at 7 days for 2nd experiment.

TABLE 3

EFFECT OF ARA-FC[a] AND RELATED COMPOUNDS ON SURVIVAL TIME OF MICE WITH LEUKEMIA L1210

Compound	Dose (mg/kg/day × 10)	6-day wt. change (gm)	Survival time (days)	Treated/control (%)	50-day survivors	50-day wt. change (gm)
Control		+2.0	9.1			
ara-FC	25	−0.7	32.4[b]	356[b]	3	+2.6
	12.5	+0.8	39.4[b]	433[b]	3/9	+1.5
	6.25	+0.8	26.9[b]	296[b]	2	+6.5
	3.12	−0.4	16.9	186		
ara-C	25	−0.4	39.0[b]	428[b]	5	+2.3
	12.5	+1.3	45.5[b]	500[b]	8	+3.3
	6.25	+1.1	16.1	177		
	3.12	+1.7	11.6	127		
FU	25	−0.7	23.0	253		
	12.5	−0.1	17.7	195		
	6.25	+0.6	16.1	177		
	3.12	−0.1	12.4	136		
FUDR	50	+0.2	10.8	119		
	25	+1.2	10.7	118		
	12.5	+1.8	10.1	111		
	6.25	+2.3	9.1	100		

[a] ara-C, 1-β-D-arabinofuranosyl-5-fluorocytosine; ara-C, 1-β-D-arabinofuranosylcytosine; FU, 5-fluorouracil; and FUDR, 5-fluoro-2′-deoxyuridine.

[b] 50-day survivors.

TABLE 4

EFFECT OF ARA-FC[a] AND RELATED COMPOUNDS ON SURVIVAL TIME OF MICE WITH LEUKEMIA B82

Compound	Dose (mg/kg/day × 10)	8-day wt. change (gm)	Survival time (days)	Treated/control (%)
Control		−0.2	9.4	
ara-FC	25	−1.2	14.3	152
	12.5	−0.9	11.6	123
	6.25	−0.3	12.1	129
ara-C	25	−1.2	15.0	160
	12.5	+0.1	11.0	117
	6.25	−0.6	11.6	123
FU	25	−3.6	12.9	137
	12.5	−2.3	14.4	153
	6.25	−0.4	15.0	160
FUDR	50	−3.5	15.3	163
	25	−2.4	18.4	196
	12.5	−1.5	15.7	167

[a] ara-FC, 1-β-D-arabinofuranosyl-5-fluorocytosine; ara-C, 1-β-D-arabinofuranosylcytosine; FU, 5-fluorouracil; and FUDR, 5-fluoro-2′-deoxyuridine.

Results

As can be seen from Table 1, ara-FC is highly active against transplanted mouse leukemia P815. It is approximately 2 times

TABLE 5

EFFECT OF ARA-FC[a] AND ARA-C ON SURVIVAL TIME OF MICE WITH A LINE OF LEUKEMIA MADE RESISTANT TO FU

Compound	Dose (mg/kg /day × 10)	Wt. change (gm)	Survival time (days)	Treated/ control (%)	50-day survivors	50-day wt. change (gm)
Control		−0.4	9.2			
ara-FC	25	−0.1	48.0[b]	522[b]	8	+3.2
	12.5	+0.3	43.6[b]	474[b]	6	+2.7
	6.25	−0.7	34.2[b]	372[b]	5	+3.7
ara-C	25	+1.4	38.4[b]	418[b]	2	+2.2
	12.5	−0.6	37.2[b]	404[b]	2	+4.1
	6.25	0.0	21.3	232		
FU	13	−0.5	10.0	109		

[a] ara-FC, 1-β-D-arabinofuranosyl-5-fluorocytosine; ara-C, 1-β-D-arabinofuranosylcytosine; and FU, 5-fluorouracil.

[b] 50-day survivors.

more active on a molar basis than ara-C and considerably more active than FU or FUDR. It is also somewhat more active against leukemia P388 (Table 2). There is no significant difference between the 2 compounds in leukemia L1210 (Table 3), but both appear more active than FU or FUDR. In leukemia B82, however, both ara-C and ara-FC are less active than the fluorinated pyrimidines FU or FUDR (Table 4). Ara-FC is highly active and somewhat more active than ara-C against a line of leukemia P815 made resistant to FU (Table 5).

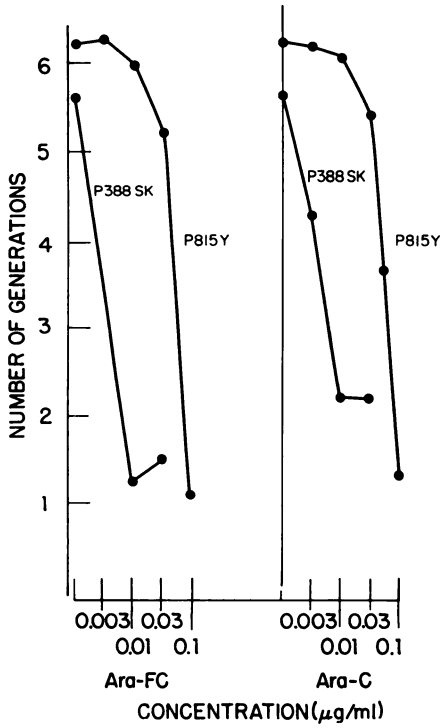

CHART 1. Inhibitory effects of 1-β-D-arabinofuranosyl-5-fluorocytosine (ara-FC) and 1-β-D-arabinofuranosylcytosine (ara-C) on the growth of mouse leukemia cells *in vitro*. The leukemic cells were grown from an inoculum of 10^4 cells/ml for a period of 72 hr in the presence of various levels of ara-FC and ara-C.

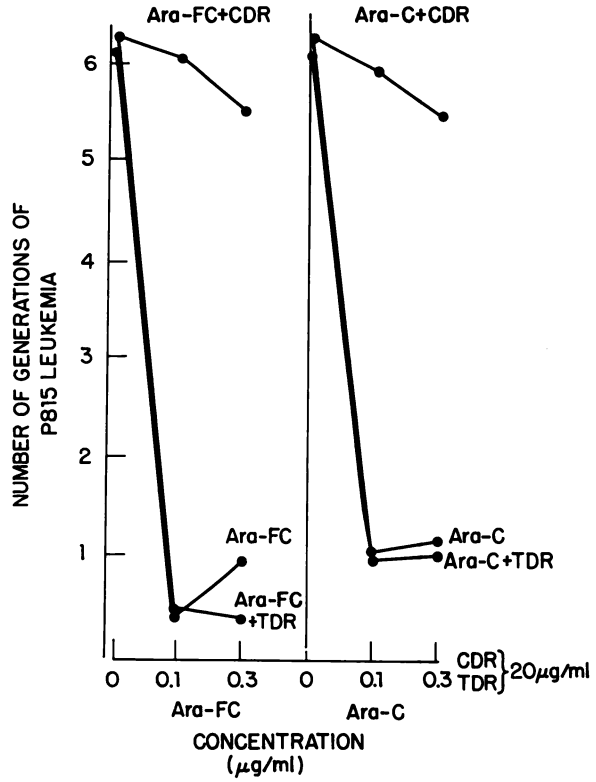

CHART 2. Blocking of the inhibitory effects of 1-β-D-arabinofuranosyl-5-fluorocytosine (ara-FC) and 1-β-D-arabinofuranosylcytosine (ara-C) on P815Y leukemic cells *in vitro* by deoxycytidine (CDR) but not by thymidine (TDR). The leukemic cells were grown from an inoculum of 10^4 cells/ml for a period of 72 hr in the presence of 2 concentrations of ara-FC and ara-C with or without 20 μg/ml of CDR or TDR.

When ara-C and ara-FC were compared for inhibitory activity against 2 cell lines of mouse leukemia, P815Y and P388SK, in tissue culture, the 2 compounds were equally effective, but there were significant differences in the concentrations necessary to inhibit the 2 leukemias. With P815Y, 50% inhibition was noted with concentrations of 0.03 μg/ml of either drug, but with P388SK only approximately 0.1 that concentration, 0.003 μg/ml, was necessary for the same degree of inhibition. The effect of various levels of the compounds on the growth of the 2 cell lines can be seen in Chart 1.

In studies on the mechanism of action of the 2 derivatives, highly inhibitory concentrations (0.1 and 0.3 μg/ml) were added alone with 20 μg/ml of CDR or TDR. As can be seen in Chart 2, CDR blocked the inhibitory effects of both ara-FC and ara-C, whereas similar concentrations of TDR blocked neither. These studies in tissue culture would suggest that ara-FC is working by the same mechanism as ara-C, namely, by interfering with deoxycytidylate synthesis rather than by blocking thymidylate synthetase, as do the fluorinated pyrimidines. A similar blocking of the inhibitory effects of these 2 compounds in the chick embryo has also been noted by Karnofsky (D. A. Karnofsky, personal communication). In addition, the high degree of activity *in vivo* against a line of leukemia made resistant to FU and cross-resistant to FUDR indicates for ara-FC and ara-C a mechanism

of action different from that of the fluorinated pyrimidines. Chu and Fischer (3) have also demonstrated this lack of cross-resistance to ara-C in a FUDR-resistant line of P815Y in tissue culture.

Ara-FC appears to be a somewhat more active derivative of ara-C and as such merits clinical trial. Preclinical pharmacologic studies to that end are now in progress.

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