The Effects of 5-Fluorodeoxycytidine, 5-Fluorodeoxyuridine, and Related Compounds on Transplanted Mouse Leukemias*

JOSEPH H. BURCHENAL, EZEQUIEL A. D. HOLMBERG,† JACK J. FOX, SANDRA C. HEMPHILL, AND JERE ANNE REPPERT

(Divisions of Experimental and Clinical Chemotherapy and Nucleoprotein Biochemistry, Sloan-Kettering Institute for Cancer Research, and Cornell University Medical College, New York, N.Y.)

The anti-tumor activity of the fluorinated uracil derivatives, 5-fluorouracil (FU), 5-fluorouridine (FUR), 5-fluorodeoxyuridine (FUDR), and 5-fluoroorotic acid (FO), prepared by Duschinsky, Pleven, Malbica, and Heidelberger (9)[1] have been demonstrated in mice and rats by Heidelberger *et al.* (11–13). These results have been confirmed and extended by Law (14), Liebling and Humphreys (15), and our own group (3). McIver *et al.* (16) and Curreri *et al.* (7) have reported objective evidence of regression of carcinomas in patients following intravenous injection of 5-fluorouracil. Since these results were usually accomplished only with considerable toxic manifestations, it was hoped that other fluorinated pyrimidines might be less toxic for the same degree of therapeutic activity. Heidelberger *et al.* (13) reported that 5-fluorocytosine (FC) at doses of 25 or 35 mg/kg/day was without effect against the Flexner-Jobling carcinoma in rats, the Ehrlich ascites tumor, and Sarcoma 180 in mice, and Law (14) reported it ineffective on mouse leukemia. Schnitzer and Grunberg *et al.*[2] have noted no anti-tumor effects in Sarcoma 180, even at doses as high as 500 mg/kg daily. Fox, Wempen, and Duschinsky (10) have recently synthesized 5-fluorocytidine (FCR) and 5-fluorodeoxycytidine (FCDR) (Chart 1). These compounds have been studied for their effect against a spectrum of transplanted mouse leukemias, and the results are herewith reported.

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† Present Address: Academy of Medicine, Buenos Aires, Argentina.

[1] R. Duschinsky, E. Pleven, J. Malbica, and C. Heidelberger. Synthesis of 5-Fluorouracil Nucleosides. "Abstr.," Am. Chem. Soc. meeting, pp. 19C–20C, Sept. 8–13, 1957.

[2] R. J. Schnitzer and E. Grunberg, personal communication, 1958.

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MATERIALS AND METHODS

The technic for evaluation of the chemotherapeutic activity of a given drug by means of its capacity to prolong the survival time of mice with transplanted leukemia has been described previously (4). In a typical experiment approximately 100 mice were given injections intraperitoneally of 0.1 cc. of a saline suspension of leukemic cells so diluted that 0.1 cc. contained 1,000,000 cells. Twenty-four hours after inoculation, the mice were divided into comparable groups of ten mice each, with one set of controls, and the remaining nine groups treated intraperitoneally daily or 3 times weekly for a 20-day period with the compounds under study. The mice were observed for the development of leukemia and autopsied at death. If gross evidence of leukemia was not conclusive, microscopic sections were taken. The mean survival times of treated and control mice were compared. The rationale behind the various steps of this technic has been discussed in detail in prior publications (4, 5).

Many of these studies were done on the 50th to 96th transplant generations of leukemia B82, which originated as a spontaneous leukemia in a C58 mouse in October, 1953. This transplanted acute lymphatic leukemia kills in 10–15 days, with an elevation of the white blood count to the 50,000–100,000 level and marked enlargement of liver and spleen, and some enlargement of lymph nodes. This leukemia in many experiments was injected subcutaneously to give local tumors; in such case it is designated B82T. In this case, the mice were sacrificed at 14 days and the tumors weighed. In this form Leukemia B82T lends itself particularly well to quantitative measurement of effect and was particularly useful in quantitating the molecular equivalents of the various fluorinated pyrimidines. Leukemias B82 and B8174 were carried in F_1 hybrids of the BALB female × C58 male cross. Leukemia L1210, originally supplied by Dr. Lloyd Law, and L1210/A, L1210/MP, L1210/AG, L1210/ADMP (3), and a line of L1210 made resistant to Actinobolin were carried in DBA mice or F_1 hybrids thereof. In addition to these some of the fluorinated pyrimidines were tested in the same mice against the following leukemias, kindly supplied by Dr. Michael Potter: Chloroleukemia P1081; mast-cell leukemia P815; reticulum-cell leukemia P329; and acute lymphocytic leukemia P388.[3]

RESULTS AND DISCUSSION

As can be seen from a scatter diagram (Chart 2), FU caused a definite increase in the survival

[3] M. Potter, personal communication, 1958.

time of mice inoculated with leukemia L1210. The median survival time of the controls was 10.3 days, as against 18.6 days for those treated with FU, 25 mg/kg 3 times weekly. Table 1 shows the relative effectiveness of FU, FUR, and FUDR against leukemia B82T. It can be seen that, on a molecular basis, FUR was 5–10 times as effective as FU, whereas FUDR was about as effective as FU. Charts 3 and 4 demonstrate that FU was active against an amethopterin-resistant strain of L1210 leukemia (L1210/A) and a strain made resistant to both amethopterin and the combination of mercaptopurine and DON (L1210/ADMP) (2). This strain is completely resistant to mercaptopurine but not to high doses of 6-diazo-5-oxo-norleucine (DON).

Table 2 shows the relative activity of the various fluorinated pyrimidines studied against a spectrum of transplanted leukemias in C58 or DBA mice or F_1 hybrids thereof. The first column gives the numerical designation, type, resistance status, and strain of origin of each leukemia. It can be seen that FU has a wide spectrum of activity but that FO appears considerably less effective against P388, P1081, and L1210/A. FUR and FCR were not tested against the complete spectrum. FUDR was particularly active against P1081 (as previously noted by Law)[4] and B82, and showed some significant effect against the remainder of the spectrum. FCDR was in very short supply and could not be tested adequately against the whole spectrum; there was no significant effect, however, against L1210 and L1210/A, at doses which were half of the maximum tolerated dose.

Table 3 shows the relative activity of the 5-fluorinated cytosine derivatives against leukemia B82T. Only a slight effect was seen with FC at 500 mg/kg daily for 5 days, whereas FCR at 1.2 mg/kg/day and FCDR at 24.5 mg/kg/day were extremely active against this particular form of leukemia.

Table 4 shows the dose-response curve with the two deoxyribosides. FUDR at $\frac{1}{4}$th–$\frac{1}{16}$th and FCDR at $\frac{1}{12}$th the maximum tolerated dose still maintained significant anti-leukemic activity.

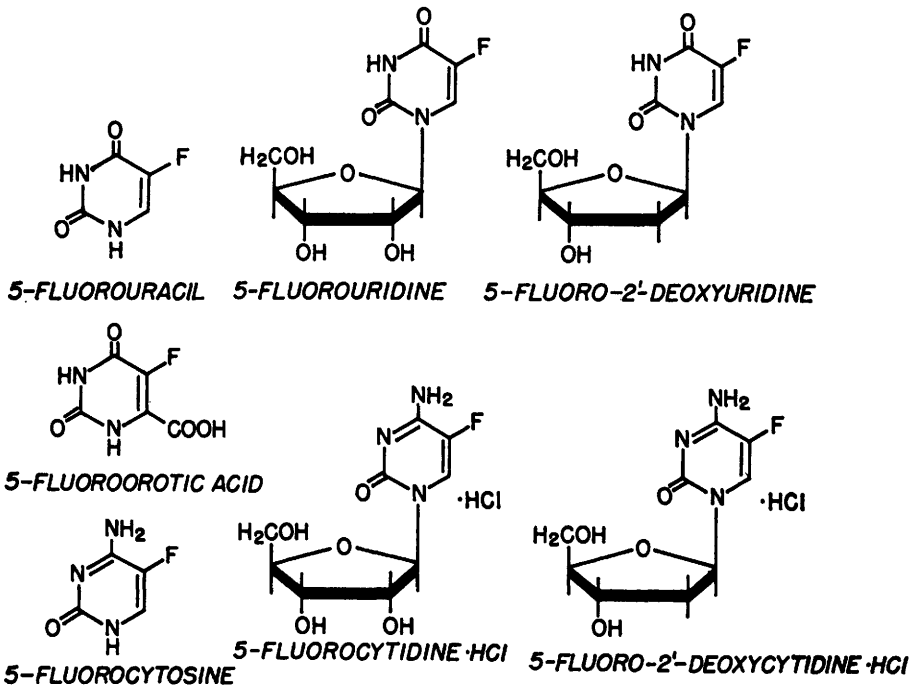

CHART 1.—Fluorinated pyrimidine derivatives

The probable sites of action of the various fluorinated uracil derivatives have been demonstrated by Heidelberger *et al.* (1, 6, 8) and by Rich *et al.* (17) (Chart 5). FU and FUR appear to act in three loci: (*a*) prevention of incorporation of uridine monophosphate (UMP) into ribonucleic acid (RNA); (*b*) incorporation directly into RNA to give a fraudulent RNA; and (*c*) prevention of methylation of deoxyuridine monophosphate (DUMP) to give thymidine monophosphate (TMP) of deoxyribonucleic acid (DNA). FUDR, on the other hand, seems to act only on the methylation reaction. Since all three compounds have definite anti-leukemic action and have but

[4] L. W. Law, personal communication, 1958.

one of these pathways in common, it would appear that the methylation of DUMP to TMP is an important reaction for the leukemic cell. Preliminary studies by Eidinoff and Rich[5] have suggested

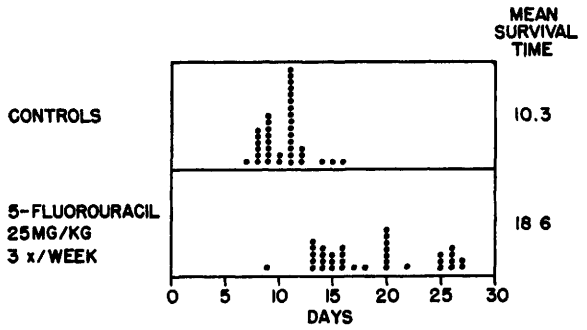

CHART 2.—The effect of 5-fluorouracil on the survival time of mice with Leukemia L1210.

perience of other investigators who, in bacteria (11) and in developing marine embryos,[6] have shown that thymidine will completely prevent the toxicity of FU and FUDR. Studies are under way at the present time in an attempt to explain this apparent paradox.

The fact that the fluorinated pyrimidines are effective against L1210 leukemias made resistant both to amethopterin and to mercaptopurine suggests that, whatever the mechanism of action of these compounds, it must be somewhat different from that of amethopterin, even though one of the important sites of amethopterin activity is the prevention of a donation of a one-carbon fragment to DUMP to form TMP. The fact that these compounds are effective in leukemias resistant to mercaptopurine and amethopterin suggests also to the clinician that they might be of

TABLE 1

RELATIVE ANTI-LEUKEMIC ACTIVITY OF 5-FLUORINATED URACIL DERIVATIVES AGAINST LEUKEMIA B82T

Treatment	Dose		Wt. change*	Tumor wt.†	Per cent inhibition
	(mmole/kg)	(mg/kg daily)	(gm.)	(mg.)	
None (controls)			+1.1	453	
5-Fluorouracil	0.1	13	+0.5	29	93
	0.05	6.5	+1.2	151	66
	0.025	3.25	+1.3	437	3
5-Fluorouridine	0.013	3.25	−3.6	35	92
	0.006	1.62	+0.1	199	56
	0.003	0.81	+1.7	341	24
5-Fluorodeoxyuridine	0.2	49.2	−1.4	12	97
	0.1	24.6	−0.4	61	86

* Weight change, 12th to 14th days.

† Tumors were dissected and weighed on the 14th day following inoculation. There were 10 mice in each group.

that growth inhibition by FCDR involves this methylation step as an important site of its antitumor action.

On the other hand, in chemotherapeutic studies *in vivo*, thymidine at $\frac{1}{5}$th the maximum tolerated dose increased markedly the toxicity of otherwise tolerated doses of FUDR, FCDR, and FU (Table 5). There was no prevention of the anti-leukemic effect at any dose level tried (25–100 mg/kg daily of thymidine), and, if anything, there was some suggestion that the anti-leukemic effect was increased. This was in direct contrast to the ex-

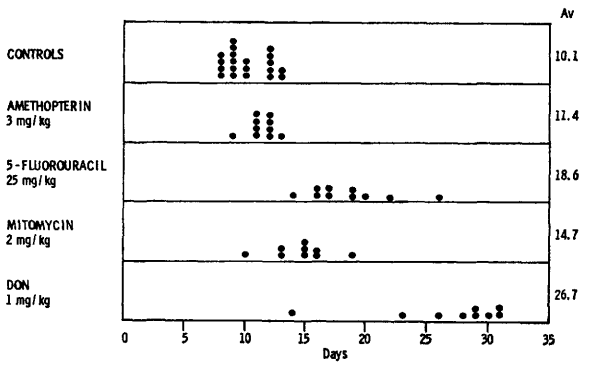

CHART 3.—Effect of 5-FU on amethopterin-resistant line of Leukemia L1210. All doses given 3 times weekly to total of ten doses.

[5] M. L. Eidinoff and M. A. Rich, personal communication, 1958.

[6] D. A. Karnofsky, personal communication, 1958.

TABLE 2

EFFECT OF FLUORINATED PYRIMIDINES ON A SPECTRUM OF MOUSE LEUKEMIAS

Leukemic strain	Type	Mouse strain	5-Fluorouracil				5-Fluoroorotic Acid				5-Fluorouridine				5-Fluorodeoxyuridine				5-Fluorocytidine				5-Fluorodeoxycytidine			
			Dose	No. doses	T/C*	Per cent response†	Dose	No. doses	T/C*	Per cent response†	Dose	No. doses	T/C*	Per cent response†	Dose	No. doses	T/C*	Percent response†	Dose	No. doses	T/C *	Per cent response†	Dose	No. doses	T/C*	Per cent response†
			(mg/kg)		(days)		(mg/kg)		(days)		(mg/kg)		(days)		(mg/kg)				(mg/kg)				(mg/kg)			
B82	Lymphoid	C58	25	3×/wk	42/1455	97	12.5	3×/wk	194/1093	82	5	3×/wk	242/1455	83	75	daily	17/1455	99	7.5	3×/wk	14.3/1455	+99	25	daily	75/1061*	93
L1210	Lymphoid	DBA	25	3×/wk	25.8/12.1	113	25	3×/wk	19.2/11	75	6.5	3×/wk	20.9/9.4	120	75	daily	16.3/11	48	1.8	daily	14.3/11	30	37.5	daily	12.1/11.0	10
L1210/A	Resist. amethopterin		25	3×/wk	16.6/9.5	70	25	3×/wk	12.2/10.1	20					75	daily	20.6/14.2	45	7.5	3×/wk	10.1/10.1	0	37.5	daily	16.8/14.2	18
L1210/MP	Resist. 6-mercaptopurine		12.5	daily	17.9/7.7	132	25	3×/wk	15.4/7.3	110					75	3×/wk	13.2/8	65	7.5	3×/wk	14.7/8	83				
L1210/Actinobolin	Resist. actinobolin		25	3×/wk	16.1/10.1	59	25	3×/wk	24.2/13.6	77	5	3×/wk	17.4/13.6	27	75	3×/wk	27.2/15.9	71								
P388	Lymphoid	DBA	25	daily	21.1/11.6	82	12.5	3×/wk	15.5/12.9	20					75	3×/wk	24.1/13.7	76	10	3×/wk	14/13.5	3	50	daily	7.3/7.8	− 6.4
P1081	Chloroleukemia	DBA	12.5	daily	84.3/27.6	205	12.5	daily	37.8/27.6	36	5	3×/wk	46.8/27.6	69	75	daily	47.5+/18.7	154+								
P815	Mast cell	DBA	12.5	daily	16.0/8.1	98									75	3×/wk	13.9/8.4	65					50	daily	13.2/8.7	52
P329	Reticulum cell	DBA	12.5	daily	31.4/17.3	82	25	3×/wk	19/11.3	68																
B8174T	Lymphoid	C58	25	3×/wk	169/919	81	12.5	3×/wk	308/919	66																

* Survival time, in days, of treated/controls, except for leukemia B82 and B8174T, for which tumor weight (in mg.) is given. In the latter two cases tumors were dissected and weighed on the 14th day following inoculation with leukemia. There were ten mice in each group.

† Response is given as per cent increase in survival time, except for leukemias B82 and B8174T, for which the values represent tumor inhibition.

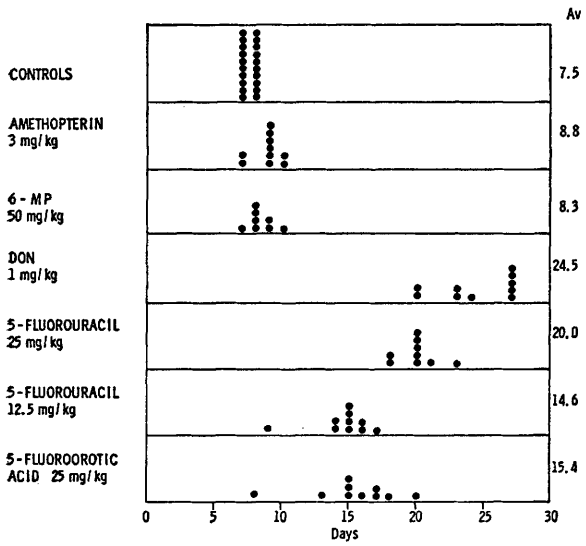

CHART 4.—Effect of 5-FU on line of Leukemia L1210 resistant to amethopterin and mercaptopurine. All doses given 3 times weekly to total of ten doses.

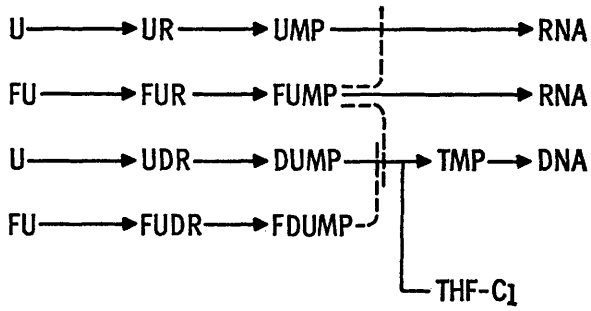

CHART 5.—Probable sites of action of fluorinated pyrimidines.

U = uracil.
UR = uridine.
UMP = uridine monophosphate.
UDR = deoxyuridine.
DUMP = deoxyuridine monophosphate.
TMP = thymidine monophosphate.
RNA = ribonucleic acid.
DNA = deoxyribonucleic acid.
THF-C_1 = 1-carbon fragment transported by tetrahydrofolic acid.
FU = 5-fluorouracil, etc.

TABLE 3

RELATIVE ANTI-LEUKEMIC ACTIVITY OF 5-FLUORINATED CYTOSINE DERIVATIVES AGAINST LEUKEMIA B82T

Treatment	Dose		Weight change*	Tumor weight†	Per cent tumor inhibition
	(mmoles/kg)	(mg/kg daily)	(gm.)	(mg.)	
None (controls)			+2.6	903	
5-Fluorocytosine	3.9	500(×5)	+2.7	504	44
5-Fluorocytidine	0.005	1.19	+0.4	62	93
5-Fluorodeoxycytidine	0.1	24.5	−0.2	23.3	97
	0.05	12.25	+1.1	181	80
5-Fluorouracil	0.1	13	0	51	94
	0.05	6.5	+1.5	417	54
5-Fluorodeoxyuridine	0.2	49.2	−1.5	0.5	99
	0.1	24.6	+0.4	61	93

* Weight change, 12th–14th day.

† Tumors were dissected and weighed on the 14th day following inoculation. There were ten mice in each group.

value in patients with leukemias which have become resistant to those conventional agents.

SUMMARY

The 5-fluorinated pyrimidine derivatives, 5-fluorouracil (FU), 5-fluoroorotic acid (FO), 5-fluorouridine (FUR), 5-fluorodeoxyuridine (FUDR), 5-fluorocytidine (FCR), and 5-fluorodeoxycytidine (FCDR), showed activity against various transplanted mouse leukemias. Because of shortness of supply, 5-fluorocytosine (FC) was not tested adequately but had relatively little effect even at the massive doses employed.

FU and FUDR were active against amethopterin- and mercaptopurine-resistant lines of leukemia L1210.

In leukemia B82, the ribonucleosides, FUR and FCR, were approximately 10–20 times as active on a molecular basis as their corresponding deoxyribonucleosides (FUDR and FCDR) or than fluorouracil.

Dose-response curves on Leukemia B82 would suggest that FUDR and FCDR have a relatively high chemotherapeutic index against Leukemia B82 and for this reason merit clinical trial.

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TABLE 4

DOSE-RESPONSE OF LEUKEMIA B82T TO FLUORODEOXYURIDINE AND FLUORODEOXYCYTIDINE

All doses were given every day. There were ten mice in each group.

Dose	Fluorodeoxyuridine			Fluorodeoxycytidine		
	Wt. loss T/C*	Tumor wt. T/C	Per cent tumor inhibition	Wt. loss* T/C	Tumor wt. T/C	Per cent tumor inhibition
(mg/kg)	(gm.)	(mg.)		(gm.)	(mg.)	
100	−4.4/+1.0	24/1129	98			
75	−2.3/−0.5	17/1455	98	−4.9/−0.4	0/994	100
50	−0.8/+0.8	80/805	90			
37.5	−1.0/−0.5	69/1455	95	−0.2/+3.5	139/777	82
24.5	−0.4/+1.2	61/903	93	−0.3/+1.2	23/903	97
12.5	+0.5/+0.9	554/977	43	−0.9/+0.4	160/948	82
6.25	+0.8/+1.5	601/1247	52	−0.9/−0.4	288/994	71
3.12	+1.5/+1.5	810/1247	35	+2.7/+1.0	945/1128	16
1.6	+1.0/+1.5	1242/1247	0			

* Mice weighed and sacrificed on the 14th day. T/C = treated/control animals.

TABLE 5

EFFECT OF THYMIDINE ON ANTI-TUMOR ACTIVITY AND TOXICITY OF FLUORODEOXYURIDINE

FUDR	Thymidine	Per cent tumor inhibition	Mortality*
(mg/kg/day)	(mg/kg/day)		
.....	...	0	0/10
50	...	76	0/10
50	...	89	1/10
50	100	90	9/10
50	50	92	4/10
50	25	94	0/10
25	50	98	0/10
6.25	...	73	0/10
6.25	50	69	0/10
FCDR			
25	...	93	0/10
25	100	97	6/10
FU			
12.5	...	60	0/10
12.5	...	49	0/10
12.5	100	93	8/10
12.5	50	94	3/10
12.5	25	73	0/10

* Mortality from toxicity before the experiment was terminated at the 14th day.

Ten mice in each group.

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