4. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, The Adaptive Modification of Biorhythms [in Russian], Moscow (1975).
5. H. Druckrey, in: Potential Carcinogenic Hazards from Drugs (UICC Monograph Series, Vol. 7), New York (1967), pp. 60-77.
6. H. V. Gelboin, Advances Cancer Res., 10, 1 (1967).
7. T. Narisawa and J. H. Weisburger, Proc. Soc. Exp. Biol. (New York), 148, 166 (1975).
8. T. Omura and R. Sato, J. Biol. Chem., 239, 2370 (1964).
9. E. A. Smuckler, E. Arrhenius, and T. Hultin, Biochem. J., 103, 55 (1967).

SELECTIVE THERAPY OF LEUKEMIA L1210 BY A COMBINATION OF DEOXYCYTIDINE AND LETHAL DOSES OF CYTOSINE ARABINOSIDE

V. M. Bukhman, M. R. Lichinitser, G. Ya. Svet-Moldavskii, and D. M. Mkheidze

UDC 616.155.392.092.9-085.277.3

Peroral administration of deoxycytidine (dC) to mice with leukemia L1210 simultaneously with intraperitoneal injections of toxic doses of cytosine arabinoside (araC) reduces the severity of toxicosis and prevents the death of the animals by poisoning. A marked antitumor effect is observed in these animals. The mean life span of such mice is much longer than that of untreated mice and also of mice receiving dC or araC alone. With the optimal scheme of treatment about 23% of mice live longer than 60 days. Protection with dC weakens the antileukemic effect of araC when the latter is given in nontoxic doses. This combination is not effective against transplantable myeloid leukemia of mice.

KEY WORDS: leukemia L1210; cytosine arabinoside; 2'-deoxycytidine hydrochloride; selective therapy.

Deoxycytidine (dC) is an antagonist of cytosine arabinoside (araC) and reduces both the severity of its various toxic manifestations and its antitumor effect [1-8]. The writers' observations show that dC, given by mouth to normal mice simultaneously with intraperitoneal injections of araC, weakens the manifestations of the toxicosis and prevents the lethal effect. Analysis of the action of a combination of dC plus araC on the different branches of medullary hematopoiesis shows that the lymphocyte count in the "protected" animals is the same as that observed in mice receiving the antimetabolite alone. This observation suggested that the use of a combination of dC plus araC could lead to selective inhibition or disappearance of lymphoid tumors and leukemias. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Leukemia L1210 was maintained in the ascites form by daily intraperitoneal passage in male DBA/2 mice. In the chemotherapeutic experiments adult female DBA/2 and male BDF_1 hybrids with a body weight of 22-28 g were used as recipients of leukemia L1210. A transplantable strain of myeloid leukemia, obtained in the writers' laboratory from virus-induced Graffi's leukemia was maintained by weekly intravenous passage in C57BL/6j mice. Experiments were carried out on mice of the same strain. All the mice were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. Cytosar (from Upjohn, USA) and deoxycytidine hydrochloride (from Reanal, Hungary) were used. The compounds were dissolved in physiological saline. In all the experiments treatment began not before the 5th day after inoculation of the mice with tumor cells. The doses, days, and mode of administration of the compounds are indicated in the tables. All compounds were given in a volume of 0.2 ml. dC was given perorally through a curved metal tube. The antitumor

Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Blokhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 3, pp. 340-343, March, 1978. Original article submitted June 13, 1977.

 0007-4888/78/8503-0366$07.50

TABLE 1. Effect of dC on Effectiveness of Nontoxic Regimes of araC Administration for Lymphoblastic Leukemia L1210

Experiment No	Group	Number of animals	Compound, mg/kg per injection		Scheme of treatment (days after injection of tumor cells)	Survival period days (M ± m)	Increase in survival period, %
			dC	ara C			
1	1	9	—	—	—	11,9±0,2**	—
	2	9	—	20	4—10	17,4±0,4	46
	3	9	40	20	4—10	15,6±0,4**	31
	4	8	40	—	4—10	11,9±0,2**	0
2	1	7	—	—	—	13,3±0,2**	—
	2	15	—	40	10—17	20,3±0,4	53
	3	15	120	40	10—17	18,1±0,5*	36
	4	9	120	—	10—17	13,0±0,3**	—2
3	1	30	—	—	—	14,6±0,2**	—
	2	9	—	4	10—17	26,7±0,3	83
	3	9	12	4	10—17	23,1±0,3**	58
	4	8	12	—	10—17	14,2±0,4**	—3

Legend. 1. Data for which differences between means differ significantly from mean for mice of group 2, receiving araC alone, marked by asterisks (one and two).
2. Ascites tumor cells were injected on day 0: in experiment No. 1 10^3 cells were injected intraperitoneally into DBA/2 females, in experiment No. 2 10^3 cells were injected subcutaneously into DBA/2 females, and in experiment No. 3 10^5 cells were injected subcutaneously into BDF_1 males.
3. The compounds were administered simultaneously once a day in experiments Nos. 1 and 2 and 4 times a day in experiment No. 3; dC was given perorally and araC injected subcutaneously in experiment No. 1 and intraperitoneally in experiments Nos. 2 and 3.

effect was assessed from the length of survival with calculation of the percentage increase in the survival period (%ISP) by the equation:

$$\%ISP = \frac{T_{exp} - T_c}{T_c} \times 100,$$

where T_{exp} and T_c represent the mean survival period of mice of the experimental (treated) and control (untreated) groups respectively.

The results were subjected to statistical analysis. Differences between means were regarded as significant at the $P \leq 0.05$ level.

EXPERIMENTAL RESULTS

dC had neither toxic nor antitumor effect (Tables 1 and 2). In the experiments of series I the regime of administration of araC was such that it did not lead to the development of toxicosis (Table 1). Under these conditions dC weakened the antitumor effect of araC. The mean survival period was significantly shorter for mice treated with a combination of dC plus araC than in those receiving the antimetabolite alone. All the animals in this series of experiments died as a result of the development of leukemia. This fact is in good agreement with data of other workers [3, 4, 8].

In the experiments of series II (Table 2) the regime of administration of araC led to the development of lethal toxicosis. At autopsy on the mice receiving araC alone no subcutaneous tumor was found at the site of inoculation of the L1210 leukemia cells and the mean weight of the spleen and liver was significantly less than in the healthy animals (not shown in Table 2). Administration of dC prevented the toxic effect of araC. Meanwhile the dC plus araC combination had a strong antitumor action on leukemia L1210 (Table 2, experiments Nos. 4-6). In leukemia of myeloid type (Table 2, experiment No. 7), however, the dC plus araC combination proved to be nontoxic but it likewise had no marked antileukemic effect. In experiment No. 5 dC prevented the development of toxicosis in 12 of the 19 experimental mice, and in the other experiments it did so in all mice. The dC plus araC combination thus proved effective for the treatment of leukemia L1210 if the regime of araC administration was such that the antimetabolite alone caused the development of lethal toxicosis. The results

TABLE 2. Effect of dC on Effectiveness of Toxic Regimes of araC Administration for Graffi's Myeloblastic Leukemia and Lymphoblastic Leukemia L1210

Experiment No.	Group	Compound, mg/kg per injection		Scheme of treatment (days after injection of tumor cells)	Survival period, days (M ± m)	Increase in survival period, %	l/n
		dC	ara C				
4	1	—	—	—	10,4±0,2**	—	0/9
	2	—	40	8, 9, 13—15	15,6±0,8	50	0/11
	3	120	40	8, 9, 13—15	20,7±0,3**	99	0/9
	4	120	—	8, 9, 13—15	10,6±0,2**	1	0/11
5	1	—	—	—	14,6±0,2**	—	0/30
	2	—	40	14—18	18,4±0,1	26	0/20
	3a	120	40	14—18	20,3±0,3**	39	0/7
	3b	120	40	14—18	27,7±0,4**	90	0/12
	4	120	—	14—18	14,1±0,2**	—3	0/20
6	1	—	—	—	15,0±0,4**	—	0/10
	2	—	40	5—7, 12—14, 19, 20	10,0±0,1	—50	0/32
	3	120	40	5—7, 12—14, 19, 20	38,4±1.3**	156	8/35
	4	120	—	5—7, 12—14, 19, 20	15,3±0,5**	2	0/10
7	1	—	—	—	16,1±0,3	—	0/9
	2	—	40	10—12, 15, 16	14,2±0,2	—12	0/10
	3	120	40	10—12, 15, 16	19,0±2,7*	19	0/10
	4	120	—	10—12, 15, 16	16,1±0,4	0	0/11

Legend. 1. l/n: numerator represents number of "long-surviving" (over 60 days) mice, denominator total number of mice.
2. Data for which differences between means differ significantly from mean for mice of group 2, receiving araC alone, marked by asterisks (one and two).
3. 10^5 L1210 ascites tumor cells were injected subcutaneously on day 0 into DBA/2 females in experiment No. 4 and into BDF_1 males in experiments Nos. 5 and 6. 5×10^3 cells of Graffi's transplantable myeloid leukemia were injected intravenously on day 0 into C57BL/6j males in experiment No. 7. In experiment No. 5 the mice of group 3 were divided into sub-groups a and b, for in seven mice the lethal toxicosis was not prevented.

of the present investigation and the writers' earlier study of the effect of the dC plus araC combination on hematopoiesis in the bone marrow of normal mice suggest that the selective effect is linked with absence of protection of the lymphoid cells.

The next step should be to study the clinical effectiveness of the dC plus araC combination both in acute and chronic leukemias and also in other forms of neoplastic diseases and also, perhaps, in autoimmune processes.

LITERATURE CITED

1. V. Biro and D. M. Goldenberg, Chemotherapy, 16, 29 (1971).
2. S. Chaube, W. Kreis, K. Uchida, et al., Biochem. Pharmacol., 17, 1213 (1968).
3. M. R. Dollinger, J. H. Burchenal, W. Kreis, et al., Biochem. Pharmacol., 16, 689 (1967).
4. J. S. Evans and G. D. Mengel, Biochem. Pharmacol., 13, 989 (1964).
5. D. S. Fisher, E. P. Cassidy, and A. D. Welch, Biochem. Pharmacol., 15, 1013 (1966).
6. R. J. Papac, P. Calabresi, J. W. Hollingworth, et al., Cancer Res., 25, 1459 (1965).
7. R. J. Papac, W. A. Creasey, P. Calabresi, et al., Proc. Am. Assos. Cancer Res., 6, 50, Abstract 197 (1965).
8. A. W. Schecker, J. A. R. Mead, and M. U. Urshel, Biochem. Pharmacol., 15, 1443 (1966).