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THE REVERSAL OF CYTOSINE ARABINOSIDE ACTIVITY *IN VIVO* BY DEOXYCYTIDINE

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Abstract—The reversal of 1-β-D-arabinofuranosylcytosine (cytosine arabinoside) activity by deoxycytidine, which has been shown in cell culture by Renis and Johnson (*Bact. Proc.* **45**, 140, 1962) and Chu and Fischer (*Biochem. Pharmacol.* **11**, 432, 1962), was demonstrated in mice in three tumor systems. Reversal of activity was shown in two ascites systems (L5178Y and Ehrlich carcinoma) and in one solid tumor system (T-4 lymphoma). Preliminary studies showed that in certain tumor systems the availability of deoxycytidine may be one of the limiting factors in tumor growth.

ONE-β-D-arabinofuranosylcytosine (cytosine arabinoside; CA) exerts antitumor activity *in vivo* against recently transplanted and well-established Ehrlich's carcinoma, sarcoma 180, and L1210 leukemia.[1] In preliminary studies in man, CA has induced objective, but temporary, decreases in the size of tumor masses of three patients with lymphosarcoma and a slight decrease in some lesions in two of ten patients with disseminated carcinomatosis.[2] Human toxicity was characterized by megaloblastic changes in the bone marrow, accompanied by depression of hemoglobin as well as the leukocyte and platelet counts in the peripheral blood. Talley and Vaitkevicius attributed the megaloblastic alterations and the associated peripheral blood changes to a relative DNA deficiency.[2]

Pizer and Cohen[3] and Slechta[4] showed that mutants of *Escherichia coli* deaminate CA to uracil arabinoside and can degrade CA to uracil.

Renis and Johnson[5] showed that inhibition by CA of the production of vaccinia virus in cell culture could be prevented by the addition of deoxycytidine (CdR). Chu and Fischer[6] confirmed and extended the observation of Renis and Johnson in a series of experiments *in vitro* using murine lymphoblast cells, L5178Y; these workers showed that the primary action of CA is to inhibit the formation of CdR-derivatives from cytidine, a reduction believed to occur at the level of cytidine diphosphate, with the production of deoxycytidine diphosphate;[7] an inhibition of DNA synthesis results. Secondary sites of CA action may involve competitive inhibition of the conversion of CdR to phosphorylated derivatives.

This paper presents evidence that the antitumor activity of CA can be prevented by deoxycytidine *in vivo*, and possible applications of this phenomenon are discussed.

MATERIALS AND METHODS

Cytosine arabinoside was supplied by Dr. James H. Hunter. It was dissolved in 0·9% sodium chloride for administration. Deoxycytidine hydrochloride was

purchased from Cyclo Chemical Corp. (Los Angeles, Calif.) and was dissolved in 0·9% sodium chloride for use.

The murine lymphoblast, L5178Y, was obtained from Dr. Glenn A. Fischer as a cell suspension. It was transferred successfully to female BDF_1/Jax mice weighing 16–18 g each and has been carried as an ascitic tumor. The leukemic cells were removed aseptically from the peritoneal cavity of a donor mouse, diluted with saline, and 0·25 ml (5×10^6 cells) were injected i.p. into each of the mice on test. Activity of the drug was demonstrated by an increase in the median survival time. Average survival time also was recorded for calculating significance of the data.

The T-4 lymphoma was obtained through the courtesy of Dr. S. Livingston, Los Angeles, Calif. The lymphoma was carried as a solid tumor in male or female A/Heston mice (16–18 g) supplied by Cumberland Farms. The tumor was transferred as a cell suspension and injected s.c. into the groin.

The sarcoma 180 and Ehrlich carcinoma lines have been carried in our laboratories for several years as ascitic tumors. Known numbers ($2·5 \times 10^6$) of cells were injected either s.c. or i.p. into Swiss mice derived from pathogen-free stock. Experiments in which the ascitic form of S180 or Ehrlich carcinoma was used were evaluated on the basis of the median or average survival times. All solid tumors (T-4 lymphoma, S180 or Ehrlich carcinoma) were measured in two diameters and the average of the two measurements used for evaluation.

The standard error and the Student 't' test were calculated by E. Markovich of the Research Statistical Section.

EXPERIMENTAL AND RESULTS

Mice that have received approximately 5×10^6 L5178Y cells i.p. have a median survival time of 12–13 days. When mice with L5178Y, after a delay of either 1 or 5 days post implantation, were treated for seven days with CA (40 mg/kg per day), they survived for a significantly longer period of time under either circumstance.

TABLE 1. EFFECT OF DEOXYCYTIDINE ON THE SURVIVAL OF MICE WITH ASCITIC POPULATIONS OF L5178Y, AND SUBJECTED TO EARLY TREATMENT WITH CYTOSINE ARABINOSIDE

Cytosine arabinoside (mg/kg per day)*	Deoxycytidine (mg/kg per day)*	No. of mice	Av. body wt. (g)	Average survival (days ± S.E.)
		20	17·4	13·2 ± 0·28
40		20	17·3	18·7 ± 0·15
	80	10	17·2	13·0 ± 0·63
40	5	10	17·7	17·1 ± 0·31
40	10	10	17·8	17·2 ± 0·33
40	20	10	18·6	16·1 ± 0·41
40	40	10	17·8	15·3 ± 0·33
40	80	10	17·3	14·6 ± 0·22

* CA and CdR were given i.p. once daily for 7 days starting 24 hr after implanting the leukemic cells.

When both CA and CdR were given i.p. to mice with L5178Y, the antileukemic activity of CA was inhibited. The degree of inhibition depended on the quantitative relationship of CA to CdR (Table 1). There was still some inhibitory effect of CA remaining at the highest dose of CdR used. When the CA and CdR treatments were

delayed until the leukemia was well established, the antileukemic activity of CA still could be abolished completely by CdR (Table 2).

When CA and CdR were given i.p. to mice bearing established T-4 lymphomas (Table 3), the immediate regression of the tumor produced by CA alone could be prevented by CdR therapy. The effect of CdR on the response of the T-4 lymphoma to CA was indicated by the number of measurable tumors found 16 days after implanting

TABLE 2. EFFECT OF DEOXYCYTIDINE ON THE SURVIVAL OF MICE WITH ESTABLISHED MURINE LYMPHOBLAST (L5178Y) ASCITIC NEOPLASMS AND SUBJECTED TO DELAYED TREATMENT WITH CYTOSINE ARABINOSIDE (CA)

CA (mg/kg per day)*	Supplement and dosage (mg/kg per day)*	No. of mice	Body wt. (g)	Average survival (days ± S.E.)
		30	18·7	13·9 ± 0·17
20		10	19·1	16·4 ± 0·37
40		10	18·5	17·6 ± 0·67
	Deoxycytidine 80	10	18·5	14·3 ± 0·26
40	Deoxycytidine 80	10	17·9	13·9 ± 0·67

* Drugs were given i.p. once daily for 7 days starting 5 days after implanting the leukemic cells.

the tumor (T_{16}) and was reflected in the average tumor measurement. The inhibitory effect of CdR on the response to CA was noticeable even one week after discontinuing therapy. The administration of CA prolonged the survival time of mice with T-4 lymphomas. Concurrent administration of CdR with CA decreased the expected survival time resulting from the CA treatment; however, the median survival time was never shortened to that of the untreated or CdR controls. If CdR was given before CA therapy, the tumor (T-4) still responded to CA treatment. When CdR was given after CA therapy, there was an apparent stimulation of antitumor activity at one dosage level (5 mg CA/kg and 80 mg CdR/kg;) however, this apparent stimulation ($P = 0{\cdot}05$) disappeared within three days and was not considered to be significant.

A similar effect of CdR on CA therapy was shown in a preliminary experiment using the ascitic form of Ehrlich carcinoma. Concurrent administration of CdR with either amethopterin or 5-iododeoxyuridine (IUdR) had no significant effect on the response to the antitumor agent.

Chu and Fischer[6] have shown in studies *in vitro* that CA inhibits the growth of L5178Y cells; the effect of the drug could be nullified by the concurrent administration of CdR. On the basis of these studies and the work of Reichard *et al.*,[7] Chu and Fischer felt that the primary site of action of CA was the inhibition of the conversion of cytidine diphosphate to deoxycytidine diphosphate. We have shown that the action of CA can be nullified *in vitro* by the concurrent administration of CdR to mice, using three different tumor systems, L5178Y, T-4 lymphoma, and Ehrlich carcinoma. Administration of CdR before or after CA therapy does not prevent the effect on the response to therapy of the T-4 lymphoma with CA, except for a partial inhibition of the response to CA when dosages were initiated 16 days after implantation of the tumor cells.

Complete abolition of the response to CA therapy by CdR was not always obtained with L5178Y and S180 neoplasms. This effect may be attributed either to insufficient

TABLE 3. EFFECT OF A COMBINATION OF CYTOSINE ARABINOSIDE AND DEOXYCYTIDINE ON THE GROWTH OF ESTABLISHED T-4 LYMPHOMAS IN MICE

Therapy		T_9*			T_{16}			T_{22}			
CA	Deoxy-cytidine	T/S†	Average body wt. (g)	Av. tumor meas. (mm ± S.E.)	T/S	Average body wt. (g)	Av. tumor meas. (mm ± S.E.)	T/S	Average body wt. (g)	Av. tumor meas. (mm ± S.E.)	Mean survival (days)
		20/20	20·9	11·9 ± 0·37	20/20	22·8	24·5 ± 0·51	3/3	24·4	31·8 ± 1·17	20·5
10		20/20	20·4	12·7 ± 0·25	0/20	19·6	0·0	20/20	22·4	20·3 ± 0·62	32·0
20		20/20	19·1	12·3 ± 0·32	0/20	17·1	0·0	5/19	18·3	2·9 ± 1·07	>37
10	20	18/18	19·5	11·9 ± 0·30	16/18	18·7	10·8 ± 0·97	18/18	22·9	24·5 ± 0·77	31·0
10	80	20/20	19·9	12·2 ± 0·32	20/20	20·3	16·9 ± 0·64	20/20	25·4	29·6 ± 0·46	27·0
20	20	20/20	20·7	12·2 ± 0·35	0/20	19·9	0·0	19/20	23·2	19·3 ± 1·20	33
20	80	20/20	19·8	11·7 ± 0·44	19/20	19·4	11·3 ± 0·64	20/20	24·2	26·3 ± 0·60	30·0
	20	20/20	20·5	12·2 ± 0·28	20/20	22·6	23·6 ± 0·42	4/4	22·2	28·5 ± 1·02	20·5
	80	20/20	19·9	12·1 ± 0·33	20/20	21·8	23·2 ± 0·42	5/5	22·5	28·3 ± 0·64	21·0

The drugs were given i.p. once daily for 7 days starting 9 days after implanting the lymphoma cells.

* Subscripts refer to the number of days after implanting the cells.

† Number of mice with tumors/number of survivors.

dosages of CdR or the effect of CA at other metabolic sites, as suggested by Chu and Fischer, such as the conversion of CdR to phosphorylated derivatives.

Administration of CdR to mice with Ehrlich ascites carcinoma led to a reduction in the expected survival time (median survival time, 15·0–11·5 days). Analogous experiments, using either the Ehrlich tumor or sarcoma 180 (Table 4), demonstrated the

TABLE 4. EFFECT OF TREATMENT WITH DEOXYCYTIDINE ON THE WEIGHT OF MICE WITH ASCITIC S180 NEOPLASMS

Deoxycitidine (mg/kg per day)*	T_0		T_8		T_{13}		%
	No. of mice	Body wt. (g)	No. of surv.	Body wt. (g)	No. of surv.	Body wt. (g)	Average survival (days ± S.E.)
	20	19·3	20	25·2	6	25·4	12·7 ± 0·58
20	20	19·5	19	23·9	11	25·6	12·7 ± 0·65
80	20	19·5	16	27·1	3	18·6	10·0 ± 0·52

* Deoxycytidine was given i.p. once daily for seven days starting 24 hr after implanting the tumor cells (i.p.).

same trend—namely, a reduction in the median survival time. This observation strongly suggested that the rate of reproduction of Ehrlich carcinoma and S180 cells, growing as free cells in the peritoneal cavity, may be limited by the availability of CdR. The growth of L5178Y *in vivo* does not appear to be limited by the availability of CdR, since there was no difference in the average survival times of saline controls as compared to that of mice treated with CdR. Similarly, there appears to be no significant difference in tumor size of T-4 lymphoma-bearing mice treated with either saline or CdR. The expected differences may be too small to measure because of compression of the cells in the solid tumors. The effect of CdR on the reproduction of tumor cells appears at this time to depend on the type of tumor cell and location, i.e., ascitic vs. solid.

Tally and Vaitkevicius[2] emphasized the relationship of agents that affect DNA metabolism to bone marrow toxicity. Mark and Calabresi[8, 9] have shown that appropriate intra-arterial administration of small amounts of thymidine can prevent the major portion of the leukopenia-inducing effect of IUdR, without compromising the effect of IUdR on a neoplasm in man. The reversal *in vivo* of CA activity by CdR may make possible analogous protection of the host's bone marrow from the toxic effects of the CA, as has been suggested by Welch.[10]

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