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SYNERGISM OF THE ANTINEOPLASTIC ACTIVITY OF CYTOSINE ARABINOSIDE BY PORFIROMYCIN

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Abstract—When mice bearing ascitic L5178Y or L1210 lymphoblasts were treated with a combination of 1-β-D-arabinofuranosylcytosine (cytosine arabinoside) and porfiromycin, the median survival time and the number of survivors were increased. The combination is most effective when treatment is started within 24 hr after implanting the leukemic cells. The synergistic effect is noted only when the dosage level of the cytosine arabinoside component of the combination is optimal. The same synergistic effects were noted when the combination of mitomycin C and cytosine arabinoside was used.

ONE-β-D-arabinofuranosylcytosine (cytosine arabinoside; CA) exerts activity *in vivo* on recently transplanted and well-established tumors and leukemias of mice.[1, 2] In a preliminary study, the inhibitory activity of CA on the growth of certain solid tumors in terminal human cases was demonstrated.[3] In addition to its antienoplastic activity, the drug also possesses antiviral activity as demonstrated both in culture and *in vivo*.[4–6]

Cytosine arabinoside intereferes with the formation of deoxyribonucleic acid (DNA) by inhibiting the conversion of cytidine to deoxycytidine (CdR) in *in-vitro* systems, using either L5178Y murine lymphoblast cells[7] or viral systems.[4] The same mechanism applies *in vivo*, since the concurrent administration of CdR and CA inhibits the expected response of the tumor to CA.[8]

Porfiromycin, isolated as an antibacterial agent,[9–11] possesses both antitumor and antileukemic activity.[12] It was shown later that porfiromycin is identical with methylmitomycin C.[13] Mitomycin C has been studied clinically, especially by Japanese workers,[14] and their work has been reviewed critically and favorably by American clinicians.[14] Wagner and Gitterman[15] have reported that porfiromycin possesses about 70% of the activity of mitomycin C, but has only about one third the toxicity. Wagner's results, if they can be transferred to the clinical situation, suggest that porfiromycin should have a therapeutic index superior to that of mitomycin C.

The mechanism(s) of action of either porfiromycin or mitomycin C has not been definitely established. There is some evidence[16] that each of the compounds may act by inhibiting nucleic acid metabolism; however, there is also evidence[16] that porfiromycin may affect cellular organization. The physiological effect observed is dependent upon the drug concentration at the cellular level.

Studies on the use of the combination of CA and porfiromycin for the treatment of murine leukemias are summarized in this paper.

EXPERIMENTAL

Murine lymphoblast L5178 Y

The murine lymphoblast was received as a cell suspension from Dr. Glenn A. Fischer of Yale University. It was successfully transferred to female BDF_1 mice and has been carried as an ascitic tumor for over forty generations in our laboratory. In the following studies, female BDF_1 mice, weighing about 15–18 g, were each injected i.p. with 0·25 ml of a suspension of L5178Y cells (approximately 5–7 $\times$ 10^6 cells). Suitable numbers of control mice each received intraperitoneally 0·2 ml of saline daily for seven days. Under these conditions the control mice developed ascites and died; the median survival time of these animals was 12·5 to 15 days.

CA prolonged the median survival time of mice bearing the lymphoblast L5178Y (Table 1). The mice in these studies were treated with CA for seven days, starting

TABLE 1. EFFECT OF CYTOSINE ARABINOSIDE (CA) AND PORFIROMYCIN ON THE SURVIVAL OF MICE WITH TRANSPLANTED L5178Y (ASCITES) NEOPLASMS

CA (mg/kg per day)*	Porfiromycin (mg/kg per day)*	T_0 No.	T_0 Av. body wt (g)	T_8 No. of surv.	T_8 Δ Body wt (g)	Median survival (days)	No. of 30-day surv.
Saline controls		10	16·8	10	+3·8	12·5	0
	2·5	10	16·3	10	+1·1	14·0	0
	5·0	10	16·5	10	−0·7	17·5	0
	10·0	10	16·8	10	−1·9	12·5	0
5		10	16·9	10	+3·1	14·0	0
10		10	15·9	10	+2·2	15·5	0
20		10	17·0	10	+0·8	18·5	0
5	2·5	10	16·6	10	−0·7	17·0	0
10	2·5	10	17·3	10	−0·9	19·0	0
20	2·5	10	15·8	10	−1·9	>35	7
5	5·0	10	16·9	10	−1·3	21	2
10	5·0	10	16·4	10	−1·4	22	1
20	5·0	10	16·1	9	−1·1	>28	5
5	10·0	10	17·2	10	−1·3	13	0
10	10·0	10	17·3	9	−2·0	11·5	0
20	10·0	10	16·0	10	−2·0	11·5	0
Saline controls		30	16·1	30	+1·8	13·0	0
	2·5	10	16·0	10	0·0	18·0	1
	5·0	10	15·8	10	−1·5	21	0
	10·0	10	15·2	10	−3·1	10·0	0
5		10	15·8	10	+0·9	17·0	1
10		10	16·1	10	+0·3	17·0	0
20		10	15·7	10	−0·8	21·5	0
5	2·5	10	15·4	10	−1·6	23·5	0
10	2·5	10	16·7	10	−2·0	27·0	2†
20	2·5	10	16·4	10	−1·5	39·0	7‡
5	5·0	10	16·3	10	−2·1	27·5	0
10	5·0	10	17·4	10	−2·5	23·5	0
20	5·0	10	16·2	10	−1·8	18·5	0

* Mice were injected i.p. once daily for 7 days starting 24 hr after implanting the leukemic cells.
† Died on days 32 and 34, respectively.
‡ Three mice survived for 100 days.
The following abbreviations are used in this and subsequent tables:
T_0 = Day of implanting the leukemia.
T_x = Subscript x refers to the number of days after implanting the leukemia.
Δ Body wt. = Average change in body weight from the start of the experiment (T_0).

24 hr after implanting the tumor cells. Only one mouse survived for 30 days after treatment with CA alone in doses up to 20 mg/kg per day.

Porfiromycin (Table 1) increased the survival time of mice bearing L5178Y cells, when given i.p. at 2·5 or 5·0 mg/kg per day, but was toxic at 10 mg/kg, as shown by the loss in body weight during the injection period and by the decrease in survival time; there were no 30-day survivors among the porfiromycin-treated mice.

When mice bearing L5178Y cells were treated i.p. with both CA and porfiromycin, there was an increase in the median survival time, as compared to those observed with either component alone (except in the highest doses, when toxicity was observed). The effectiveness of the combination chemotherapy also was shown by an increase in the number of mice that survived for a 30-day period (Table 1). When the two experiments are combined, the increase in the number of 30-day survivors in highly significant.

Leukemia L1210

Leukemia L1210 was received as a solid tumor from Dr. A. Goldin of the National Cancer Institute. The leukemia was transferred to BDF_1 mice and has been carried as either ascitic or solid tumor. We have shown in an earlier study[2] that when mice bearing the ascitic form of L1210 are treated with CA, the ratio of the treated to control median survival times (T/C) reached a maximum of 3·0. The T/C ratio was essentially

TABLE 2. EFFECT OF CYTOSINE ARABINOSIDE (CA) AND PORFIROMYCIN ON THE SURVIVAL OF MICE WITH RECENTLY TRANSPLANTED L1210 (ASCITES) NEOPLASMS

(mg/kg per day)*	(mg/kg per day)*	T_0 No.	T_0 Av. body wt. (g)	T_8 No. of surv.	T_8 Δ Body wt. (g)	Median survival (days)	T/C	No. of 50-day surv.
Saline controls		10	16·5	7	0·0	9·0		0
20		10	16·6	10	−0·5	27·0	3·0	2
40		10	17·0	10	−2·3	25·0	2·8	2
60		10	16·7	9	−1·6	22·0	2·4	0
	2·5	10	16·0	10	+0·3	12·0	1·3	0
	5·0	10	17·0	10	−1·3	14·5	1·6	0
20	2·5	10	16·9	10	−1·4	>50	>5·6	8
20	5·0	10	16·7	10	−2·1	45	5·0	3
40	2·5	10	16·6	10	−2·5	>50	>5·6	10
40	5·0	10	16·4	10	−1·9	>47	>5·2	5
60	2·5	10	16·5	9	−3·4	11·0	1·2	1
60	5·0	10	17·3	9	−3·7	10·0	1·1	2

* Mice were injected i.p. once daily for 7 days starting 24 hr after implanting the L1210 cells.

the same whether treatment was started at 24 or 96 hr after implantation of the leukemia cells. The T/C ratio reached its maximum when the dosage of CA was between 20 and 30 mg/kg per day. When the dosage of CA exceeded 40 mg/kg per day, the T/C ratio began to fall, and other signs of toxicity became evident. The number of mice that survived for at least 50 days after 7 days of CA was less than the 20% figure shown in the experiment illustrated in Table 2.

When leukemic mice were treated with porfiromycin, the maximal T/C ratio observed was 1·9, and this was observed only when treatment was started within 24 hr after leukemic cells were implanted. Chemotherapy with porfiromycin alone resulted in no long-term survivors.[2]

When mice bearing L1210 cells were treated with a combination of CA and porfiromycin (Table 2), there was an increase in the median survival time that was additive when the amount of CA used was less than 20 mg/kg per day. When the dosage of CA was regulated to give its maximal effect and nontoxic doses of porfiromycin also were given, an increase in the median survival time, that was more than additive, was observed. The T/C ratio achieved by the proper combination of the two drugs has never been observed with any dose of either drug alone. Furthermore, the number of mice that survived for a maximum of 50 days greatly exceeded that observed with either drug alone, given by itself at any dosage level.

In the treatment of the well-established ascitic neoplasm, a T/C ratio that is more than additive can be achieved when nontoxic combinations of the two drugs are used (Table 3). Under these conditions up to 40% of the mice treated with the combination survived for a minimum of 50 days. Porfiromycin was the more toxic of the two drugs, and the combination used should be one that has the lowest porfiromycin content that will still give the desired effect.

TABLE 3. EFFECT OF CYTOSINE ARABINOSIDE AND PORFIROMYCIN ON THE SURVIVAL OF MICE WITH ESTABLISHED L-1210 (ASCITES) NEOPLASMS

CA (mg/kg per day)*	Porfiromycin (mg/kg per day)*	T_0 No.	T_0 Av. body wt. (g)	T_{11} No. of surv.	T_{11} Δ Body wt. (g)	Median survival (days)	T/C	No. of 50-day surv.
Saline controls		10	16·5	1	+1·1	9·0	1·00	0
20		10	17·7	10	0·0	27·5	3·06	0
40		10	17·5	10	−0·6	24·0	2·67	0
30		10	17·1	10	−0·4	26·0	2·89	0
60		10	17·0	10	−1·8	25·5	2·84	0
	2·5	10	16·9	3	−2·1	10·5	1·17	0
	3·75	10	16·3	1	−0·8	10·0	1·11	0
	5·0	10	17·2	3	−1·3	11·0	1·22	0
	7·5	10	16·7	6	−1·1	14·5	1·61	0
20	2·5	10	17·1	10	−0·4	26·5	2·94	1
20	5·0	10	16·8	10	−1·5	30·5	3·39	2
30	3·75	10	17·2	10	−1·7	35·5	3·94	2
40	2·5	10	16·7	10	−1·8	24·0	2·67	1
40	5·0	10	17·1	10	−2·6	25·0	2·78	1
60	2·5	10	16·9	8	−3·0	33·5	3·72	4
60	5·0	10	17·3	9	−2·9	27·0	3·00	4
60	7·5	10	16·8	8	−2·9	12·5	1·39	1

* Drugs were given i.p. once daily for 7 days starting 4 days after implanting the leukemic cells.

An experiment was designed to disclose whether mitomycin C would have the same effect on survival as porfiromycin; the results (Table 4) show that mitomycin C and porfiromycin react the same in this test system. Porfiromycin has been studied more intensively than mitomycin by us because of its lower toxicity and its more favorable therapeutic ratio.

DISCUSSION

Goldin and Mantel[17] have used the expression 'therapeutic synergism' when combination treatment provides an improved form of therapy. Evaluation of chemotherapeutic agents on experimental leukemia can be based upon either increasing the survival time or, what is more important, increasing the number of long-time survivals. We have shown that the use of the combination of CA and porfiromycin will increase the median survival time and the number of 50-day survivors over those expected from either drug alone. More than additive effects on the median survival time or on the number of long-term survivors are noted only when the level of CA is optimal and when nontoxic levels of the combination are used. Since both drugs will reduce the number of circulating white cells, excessive amounts of the drugs may cause deaths attributable not to neoplastic disease but to bacterial or viral infections that may occur in the mice.

TABLE 4. EFFECT OF CYTOSINE ARABINOSIDE (CA) AND MITOMYCIN C ON THE SURVIVAL OF MICE WITH RECENTLY TRANSPLANTED L1210 (ASCITES) NEOPLASMS

Group	CA (mg/kg per day)*	Mitomycin (mg/kg per day)*	T_0 Av. body wt. (g)	T_8 No. of surv.	T_8 Δ Body wt. (g)	Median survival (days)
1	Saline controls		16·8	1	+0·9	8·0
2	20		17·7	10	0·0	21·0
3		0·1	17·0	6	+1·4	9·0
4		0·3	17·4	10	+1·3	11·5
5		1·0	17·3	10	−0·1	14·0
6	20	0·1	17·5	10	+0·1	23·5
7	20	0·3	17·6	10	−0·3	28·5
8	20	1·0	17·4	10	−0·4	>42

* Drugs were given i.p. once daily for 7 days starting 4 days after implanting the leukemia cells.

Cytosine arabinoside inhibits the conversion of cytidine 5′-diphosphate to deoxycytidine 5′-diphosphate.[18] Administration to mice of deoxycytidine at the same time as CA abolished the antitumor activity of CA, confirming the studies *in vitro*.[8] The primary effect of CA therapy is an interference with pyrimidine synthesis that results in an inhibition of DNA synthesis.

Studies by Pittillo and Quinnelly[19] suggested that one of the modes of action of porfiromycin may be an interference with purine metabolism. Their conclusion was based upon a modest reversal of porfiromycin inhibition by purines, especially guanylic acid, and by studies using bacteria resistant to purine analogues and antagonists. They showed evidence of cross-resistance between azaserine, mitomycin C, and porfiromycin, using resistant bacteria. The similarity in activity shown by porfiromycin and mitomycin C was placed on a firmer basis with the discovery that porfiromycin was the same as methylmitomycin C.[13]

Grula and Grula,[20] in their studies on cell division in a species of Erwinia, suggest that one mode of action of mitomycin C may be on the synthesis of inosine *de novo*, but the primary site of action may be by the activation of a DNase or by damage to the

osmotic barrier. The work of Magee and Miller[16] with porfiromycin and mitomycin C on the multiplication of vaccinia virus in HeLa cells supports the view that the effect of low doses of either drugs may be on a mechanism involving cellular organization, such as cell division or chromosomal integrity.

Iyer and Syzbalski[21] showed that the DNA isolated from bacterial cells that had been treated with mitomycin C has complementary strands of DNA bound together by covalent linkages. Schwartz *et al.*[22] presented evidence that mitomycin C acts more like an alkylating agent. They proposed that mitomycin C is reduced enzymatically under anaerobic conditions to an intermediate that is believed to act by intracellular alkylation. It is still unknown whether or not mitomycin C or some metabolite is actively involved in the cross-linking of the DNA by virtue of its alkylating activity.

The explanation for the increase in the number of survivors when leukemic mice are treated with a combination of cytosine arabinoside and either porfiromycin or mitomycin C, as compared to that observed with these agents given singly, is still unknown. One possibility is that, in combining these two agents, interferences with both purine and pyrimidine metabolism result. Another possibility is that with the combination of the two drugs, CA interferes with a critical pathway in pyrimidine metabolism, whereas either porfiromycin or mitomycin C interferes with cellular organization by virtue of its cross-linkage of DNA.

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