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TUMOR-INHIBITORY EFFECTS OF COMBINATIONS OF THE VINCA ALKALOIDS WITH ACTINOMYCIN D*

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Abstract—Combinations of vinblastine and actinomycin D exhibited synergistic tumor-inhibitory activity against the ascitic form of Ehrlich carcinoma and sarcoma-180 when given daily to mice by intraperitoneal injection for 6 to 10 days. Against L1210 ascites tumor the combination produced approximately additive increases in survival time. Single large doses of vinblastine were much less effective than chronic treatment. Antitumor effects of combinations of single doses of vinblastine and actinomycin D were not significantly greater than those produced by actinomycin D alone, unless the drugs were administered in a sequence, 24 hr apart, initiated with vinblastine. Vincristine was not a very effective inhibitor of the three tumors examined, and in combination with actinomycin D, showed additive or subadditive effects against L1210 and sarcoma-180 ascites tumors. Biochemical studies using the Ehrlich ascites carcinoma indicated that therapy with vinblastine produced an increase in cell size coupled with elevation of the cellular contents of DNA, RNA, and protein. Superimposition of actinomycin D did not affect the increases in protein or cell size, but largely prevented the increase in DNA per cell and reduced the level of RNA. Incorporation of ^{3}H-uridine into RNA was depressed by treatment with actinomycin D; further reduction in the uptake of isotope by superimposing single doses of vinblastine was achieved only if treatment with the vinca alkaloid preceded actinomycin therapy by 24 hr.

AMONG the large number of indole derivatives isolated from the periwinkle plant, *Vinca rosea* Linn. (see for example the list in Ref. 1), are the dimeric alkaloids, vinblastine and vincristine, which markedly inhibit the growth of a variety of experimental and human tumors.[2] Studies carried out in this laboratory,[3–5] have demonstrated that in Ehrlich ascites mouse carcinoma, vinblastine and vincristine inhibit the synthesis of RNA, particularly the soluble or transfer fraction, and interfere with the uptake of glutamic acid. Since soluble RNA is the fraction whose synthesis is least inhibited by actinomycin D,[6, 7] it appeared possible that combined therapy with the latter agent and a vinca alkaloid might establish a more complete inhibition of RNA synthesis leading to enhanced antineoplastic activity. This report indicates that combinations of vinblastine and actinomycin D possess synergistic tumor-inhibitory activity against certain tumors, and describes some of the biochemical features of treated cells.

MATERIALS AND METHODS

Chemicals. ^{3}H-Uridine was obtained from the New England Nuclear Corp. Vinblastine and vincristine sulfates were generously provided by Doctors I. S. Johnson

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and J. G. Armstrong of the Eli Lilly Co. Actinomycin D was the gift of Merck Sharp & Dohme.

Tissues and treatment. Ehrlich ascites carcinoma and sarcoma-180 (S-180) cells in the ascites form were maintained in Swiss mice (CD-1) obtained from the Charles River Breeding Laboratories; a vinblastine-resistant strain of the Ehrlich carcinoma was developed by repeated passage of cells through mice treated daily with vinblastine (0·1 mg/kg). L1210 cells, also in the ascites form, were maintained in B6D2F1 mice (C57BL × DBAF1, Cumberland View Farms, Tenn.). Transplantation of tumors was carried out by withdrawing ascitic fluid from a donor mouse bearing a 7-day-old tumor. After centrifuging for 2 min in a clinical centrifuge (1600 × *g*), the supernatant fraction was discarded and a 10-fold dilution of the cells with istonic saline was made; 0·1 ml of cell suspension containing approximately 3×10^6 cells was inoculated intraperitoneally into each animal. Mice were distributed into groups of comparable weight and maintained during experiments on Purina rat chow pellets and water *ad libitum*. All chemicals were dissolved in isotonic saline and administered intraperitoneally. Therapy was initiated 24 or 48 hr after tumor implantation, and treatments were continued once daily for 4 to 10 days, except in the case of animals receiving single injections. Tumor-bearing mice injected with saline served as controls, and all groups were weighed daily. Survival time was used as one criterion of tumor inhibition and was calculated only for those mice that died within 100 days of tumor implantation. The number of 50-day survivors, and the number of animals that survived more than 100 days and that were shown on autopsy to be free from solid tumors in the peritoneal cavity, served as the other criteria. Deaths were ascribed to drug toxicity if the animals died before the controls and without marked accumulation of ascitic fluid. When cells were to be studied biochemically, the ascitic fluid was withdrawn and centrifuged, the erythrocytes in the cell pellet were lysed by exposure to 4 volumes of hypotonic sodium chloride solution (0·2%), and the tumor cells washed with normal saline.

Uptake of precursors in vivo. Tumor-bearing mice received 4 μc of ^{3}H-uridine (20 μc/μmole) i.p., 4·5 hr after drug treatment, and were sacrificed 30 min later. Ascites cells were removed and treated as described above. Radioactivity was determined with a Packard Tri-Carb liquid scintillometer.

Determinations. Packed cell volumes were determined by centrifuging cell suspensions for 5 min at 1600 *g* in Shevky-Stafford and McNaught tubes; no corrections were made for trapped extracellular fluid. The number of cells was determined with a Coulter model A particle counter. Cell preparations were washed 5 to 7 times with cold perchloric acid (0·5 M), and heated for 30 min at 90° with 0·2 M NaOH to hydrolyze RNA; DNA and protein were then precipitated by bringing the hydrolysate to 0·5 M with respect to perchloric acid. DNA was estimated by the method of Burton,[8] with deoxyadenosine as a standard. RNA was determined by measuring absorbance at 260 mμ of the acidified supernatant from alkaline hydrolysis; this measurement was closely correlated with RNA determinations by the orcinol method.[9] Protein was measured by the biuret reaction;[10] bovine serum albumin fraction V served as the standard.

RESULTS

Tumor inhibition studies

Since the earlier biochemical studies had been carried out on Ehrlich ascites cells, this was the first tumor examined for possible synergism between vinca alkaloids and actinomycin D. As seen in Table 1, chronic treatment of Ehrlich ascites carcinoma with vinblastine produces significant inhibition of tumor growth, with a regression rate reaching a maximum of 33%. Superimposition of actinomycin D, however, which does not itself produce regressions, enables a maximum of 64% of the mice to survive indenfinitely and gives a high survival incidence over a wide range of doses. Weight

TABLE 1. EFFECT OF COMBINATIONS OF VINCA ALKALOIDS AND ACTINOMYCIN D ON SURVIVAL TIME OF MICE BEARING EHRLICH ASCITES CARCINOMA

Daily dose*			No. of mice	Average survival† (days ± S.E.)	50-Day survivors (%)	Regressions† (%)	Avg. Δ weight‡ (%)	Toxic deaths
VLB	VCR (mg/kg)	ACT						
0	0	0	45	10·8 ± 0·8	0	0	+11·6	0
0	0	0·005	12	14·6 ± 1·2	0	0	+19·0	0
0	0	0·01	20	20·5 ± 1·3	0	0	+27·1	0
0	0	0·02	18	24·4 ± 2·0	17	0	+16·6	0
0	0	0·04	12	29·8 ± 4·5	8	0	+ 3·3	1
0	0	0·06	12	23·9 ± 2·9	0	0	−15·0	6
0·1	0	0	24	25·5 ± 3·9	37	26	+ 2·7	0
0·2	0	0	14	28·6 ± 5·9	43	28	+ 5·9	0
0·3	0	0	18	33·8 ± 3·3	44	33	− 3·1	0
0·4	0	0	12	14·7 ± 3·1	42	25	− 7·4	3
0	0·1	0	10	12·7 ± 1·8	0	0	+ 9·2	0
0	0·2	0	10	14·0 ± 1·3	0	0	−10·0	2
0·1	0	0·005	11	32·8 ± 6·0	73	64	+ 2·4	0
0·1	0	0·01	12	24·0 ± 2·9	58	58	+ 4·4	0
0·1	0	0·02	12	40·0 ± 12·4	58	42	− 0·7	0
0·1	0	0·04	12	19·8 ± 3·1	8	0	− 5·9	3
0·2	0	0·005	12	41·8 ± 8·3	67	50	+ 1·0	0
0·2	0	0·01	14	40·8 ± 7·4	72	50	+ 0·8	0
0·2	0	0·02	12	37·3 ± 6·1	58	42	− 2·1	0
0	0·1	0·01	10	18·0 ± 1·4	0	0	+ 5·8	0
0	0·1	0·02	10	19·7 ± 3·2	0	0	− 3·3	0
0	0·2	0·01	10	18·3 ± 3·7	0	0	− 0·4	0
0	0·2	0·02	10	15·2 ± 3·0	0	0	− 4·2	1

* Administered once daily for 10 consecutive days beginning 48 hr after tumor implantation; combined treatments were given simultaneously. Abbreviations: VLB, vinblastine; VCR, vincristine; ACT, actinomycin D (in all tables).

† Average survival was calculated only for mice which died; regressions are those animals surviving more than 100 days and found to be free of visible tumor on autopsy, and percentages of survivors are calculated to the nearest whole number (same for all tables).

‡ Average percentage change in weight from onset to termination of drug treatment (same for all tables).

losses resulting from combined therapy are relatively small. Vincristine, on the other hand, is only a poor inhibitor of this tumor, and apparently antagonizes rather than synergizes with actinomycin D. When mice bearing Ehrlich ascites tumor are given single large doses of vinblastine, the increase in survival that is obtained (Table 2) compares very unfavorably with the results of chronic therapy (Table 1). Of special interest, however, is the finding that in combination with actinomycin D, the sequence in which the latter and vinblastine are administered greatly affects the survival times

TABLE 2. EFFECT OF DIFFERENT SEQUENCES OF TREATMENT WITH VINBLASTINE AND ACTINOMYCIN D ON SURVIVAL OF MICE BEARING EHRLICH ASCITES CARCINOMA

Drugs and doses* (mg/kg) VLB	ACT	Sequence*	No. of mice	Avg. Δ weight† (%)	Toxic deaths	Avg. survival time (days ± S.E.)
0	0		37	+11·2	0	12·6 ± 0·8
1	0		10	+ 2·4	0	16·5 ± 3·0
2	0		26	− 0·4	0	13·6 ± 0·7
4	0		11	− 8·8	3	12·7 ± 1·5
0	0·1		16	− 0·8	0	16·9 ± 0·5
0	0·2		16	− 5·3	0	19·2 ± 0·8
1	0·1	simultaneous	10	− 5·1	0	22·3 ± 1·4
1	0·2	simultaneous	10	−10·5	2	19·7 ± 2·5
1	0·1	ACT first	10	− 7·2	0	20·8 ± 1·7
1	0·2	ACT first	10	− 6·0	0	22·6 ± 1·6
1	0·1	VLB first	10	− 3·8	0	23·3 ± 1·9
1	0·2	VLB first	10‡	− 7·2	0	25·8 ± 3·0

* Administered as single i.p. injections 72 or 96 hr after tumor implantation. Combined treatments were given simultaneously 72 hr after tumor implantation, or drugs were given in sequence in the order indicated at 72 and 96 hr; single treatments were performed at 72 hr.
† Maximal changes which occurred after treatment.
‡ This group includes one regression.

(Table 2); in general the greatest prolongation in survival is obtained when vinblastine, given as a single dose, is administered 24 hr before actinomycin D; it was in such a sequence that the only example of a regression was encountered.

A vinblastine-resistant subline of the Ehrlich ascites tumor was developed in mice treated daily with vinblastine (0·1 mg/kg) as an aid to studies of mechanism of action. It is interesting that, apart from almost complete resistance to the level of vinblastine (0·3 mg/kg/day) that is most effective against the parent strain, the tumor is also resistant to vincristine and is almost unaffected by actinomycin D (Table 3), suggesting

TABLE 3. EFFECT OF TREATMENT WITH VINCA ALKALOIDS AND ACTINOMYCIN D ON THE SURVIVAL OF MICE BEARING A VINBLASTINE-RESISTANT STRAIN OF EHRLICH ASCITES

		Drugs and doses (mg/kg)*				
	Control	VLB	VCR	VCR	ACT	ACT
		0·3	0·1	0·2	0·02	0·04
No. of animals	14	14	10	10	10	10
Average Δ weight (%)	+9·8	+12·6	+8·3	+3·0	+6·1	−4·8
Survival time (days ± S.E.)	15·1 ± 0·9	16·5 ± 1·1	10·3 ± 0·3	12·1 ± 0·7	16·5 ± 1·3	13·4 ± 1·4

* Administered daily for 6 consecutive days beginning 24 hr after tumor implantation.

that there may possibly be some similarity in the mode of action of the three drugs. The results obtained by daily treatment of mice bearing the sarcoma-180 ascites tumor resemble those described for Ehrlich ascites carcinoma. Vinblastine is again more active as a tumor inhibitor than is vincristine, and when combined with actino-

TABLE 4. EFFECT OF COMBINATIONS OF VINCA ALKALOIDS AND ACTINOMYCIN D ON SURVIVAL TIME OF MICE BEARING S-180 CELLS

Daily dose*			No. of mice	Average survival (days ± S.E.)	50-Day survivors (%)	Regressions (%)	Avg. Δ weight (%)	Toxic deaths
VLB	VCR (mg/kg)	ACT						
0	0	0	30	13·8 ± 0·4	0	0	+21·1	0
0	0	0·005	10	17·6 ± 1·2	0	0	+13·1	0
0	0	0·01	10	20·0 ± 3·3	0	0	+ 5·4	0
0	0	0·02	10	17·3 ± 1·1	0	0	− 0·8	0
0	0	0·04	10	16·8 ± 1·7	0	0	− 3·3	1
0	0·05	0	10	17·8 ± 0·7	0	0	+11·3	0
0	0·1	0	10	18·4 ± 1·0	0	0	+ 4·8	0
0	0·2	0	10	17·8 ± 1·8	0	0	+ 5·7	0
0	0·3	0	10	17·7 ± 0·9	0	0	− 4·9	1
0·1	0	0	10	25·5 ± 1·7	0	0	+ 4·3	0
0·2	0	0	10	39·4 ± 6·3	20	0	+ 0·6	0
0·3	0	0	10	39·5 ± 6·1	30	20	+ 0·2	0
0·4	0	0	10	32·8 ± 2·1	10	0	− 1·4	1
0·1	0	0·005	10	38·4 ± 4·9	30	0	+ 0·1	0
0·1	0	0·01	10	49·1 ± 9·6	60	30	+ 1·1	0
0·1	0	0·02	10	25·5 ± 2·1	40	40	− 1·6	0
0·2	0	0·005	10	35·1 ± 4·6	30	10	− 1·8	0
0·2	0	0·01	10	46·8 ± 5·7	50	20	− 0·3	0
0·2	0	0·02	10	30·0 ± 3·0	50	40	− 2·5	0
0·3	0	0·01	10	32·9 ± 5·5	30	20	− 1·1	1
0	0·05	0·005	10	19·8 ± 1·1	0	0	+ 6·2	0
0	0·05	0·01	10	18·7 ± 0·9	0	0	+ 3·9	0
0	0·05	0·02	10	21·4 ± 0·5	10	10	− 5·3	0
0	0·1	0·005	10	21·5 ± 2·1	0	0	+ 3·7	0
0	0·1	0·01	10	21·0 ± 0·9	0	0	+ 3·1	0
0	0·1	0·02	10	21·5 ± 1·8	0	0	− 4·4	0

* Administered once daily for 6 consecutive days beginning 24 hr after tumor implantation; combined treatments were given simultaneously.

mycin D, produces regressions in up to 40% of the mice (Table 4). Combinations of vincristine and actinomycin D gave subadditive increases in survival, and only one regression. With L1210 leukemia cells (Table 5), vinblastine does not possess as great an antineoplastic activity, although the relative activity of vincristine is greater than in Ehrlich ascites cell tumors. Combination of either of the two drugs with actinomycin D gives approximately additive increases in survival time, but the increase in toxicity as expressed by losses in body weight appears to be greater in B6D2F1 than in the Swiss mice.

Biochemical studies

The biochemical composition of treated Ehrlich ascites cells was studied in an attempt to determine the basis for the marked antitumor effects of combinations of vinblastine and actinomycin D. Treatment was delayed until 72 hr after tumor implantation, to allow accumulation of sufficient cells for the analytical procedures, and was continued until the changes in weight indicated that regression of the tumor was occurring; this required four consecutive days of therapy. The results of determinations of the major cellular components at this time are shown in Table 6. As has been reported previously,[11, 12] actinomycin D produces a decrease in cellular RNA and protein. Vinblastine, on the other hand, causes a marked increase (66%) in packed

TABLE 5. EFFECT OF COMBINATIONS OF VINCA ALKALOIDS AND ACTINOMYCIN D ON SURVIVAL OF MICE BEARING L1210 ASCITES CELLS

Daily dose*			No. of mice	Average survival (days ± S.E.)	50-Day survivors (%)	Avg. Δ weight (%)	Toxic deaths
VLB	VCR (mg/kg)	ACT					
0	0	0	46	7·8 ± 0·1	0	+17·4	0
0	0	0·01	10	10·9 ± 0·6	0	+ 5·1	0
0	0	0·02	10	14·8 ± 0·6	0	+ 1·1	0
0	0	0·03	10	17·4 ± 1·7	0	− 9·1	0
0	0	0·04	10	18·2 ± 1·3	0	−12·1	0
0·05	0	0	10	11·5 ± 0·6	0	+10·4	0
0·1	0	0	10	12·1 ± 0·8	0	+ 5·4	0
0·2	0	0	10	14·2 ± 1·1	0	+ 2·7	0
0·3	0	0	10	11·4 ± 0·6	0	− 2·7	0
0	0·1	0	15	9·7 ± 0·2	0	+ 4·7	0
0	0·2	0	15	10·3 ± 0·3	0	− 0·4	1
0·05	0	0·01	10	13·3 ± 0·9	0	+ 5·4	0
0·05	0	0·02	14	21·2 ± 3·6	7	− 0·7	0
0·05	0	0·03	10	22·5 ± 4·8	10	− 4·6	1
0·1	0	0·01	10	12·6 ± 1·2	0	+ 1·0	0
0·1	0	0·02	15	18·4 ± 1·9	0	− 1·2	0
0·2	0	0·02	11	17·7 ± 1·2	0	− 4·7	1
0	0·1	0·01	10	14·0 ± 1·1	0	− 2·2	0
0	0·1	0·02	10	13·3 ± 1·0	0	− 4·4	1
0	0·2	0·01	10	13·3 ± 0·9	0	− 5·5	0
0	0·2	0·02	10	18·1 ± 2·3	0	− 6·2	1

* Administered once daily for 6 consecutive days beginning 24 hr after tumor implantation; combined treatments were given simultaneously.

TABLE 6. THE EFFECTS OF CHRONIC TREATMENT WITH VINCA ALKALOIDS AND ACTINOMYCIN D ON THE COMPOSITION OF EHRLICH ASCITES CELLS

Daily dose*		Packed cell volume† (ml/10^8 cells)	RNA† ($A_{260m\mu}$/10^8 cells)	DNA† (μmole/10^8 cells)	Protein† (mg/10^8 cells)
VLB (mg/kg)	ACT (mg/kg)				
0	0	0·32 ± 0·01 (7)	114·6 ± 10·8 (7)	2·82 ± 0·29 (7)	45·0 ± 1·6 (4)
0	0·01	0·31 ± 0·02 (8)	70·8 ± 6·8 (6)	2·54 ± 0·33 (6)	42·4 ± 5·5 (5)
0	0·02	0·31 ± 0·03 (9)	57·6 ± 6·3 (5)	2·97 ± 0·57 (5)	39·4 ± 5·7 (5)
0·2	0	0·53 ± 0·04 (7)	140·7 ± 12·4 (6)	4·43 ± 0·51 (7)	110·5 ± 10·3 (6)
0·2	0·01	0·55 ± 0·05 (8)	109·9 ± 11·0 (6)	3·23 ± 0·43 (8)	109·1 ± 15·6 (5)
0·2	0·02	0·62 ± 0·07 (6)	92·6 ± 6·5 (5)	3·41 ± 0·34 (5)	101·9 ± 13·6 (6)

* Aministered daily for 4 consecutive days beginning 72 hr after tumor implantation. Animals were killed and the cells collected 5 hr after the last treatment with drug.

† These values are accompanied by their standard errors and by the number of determinations tions (given in parentheses). Each determination was carried out on pooled cells from 3 mice.

cell volume, which is accompanied by an elevation in the cell content of DNA RNA, and protein. Combination therapy appears to accentuate the increase in cell size, although the increments are not statistically significant; but the vinblastine-induced increase in RNA content is clearly prevented, and the levels of DNA rise only slightly. The incorporation of uridine into the RNA and DNA of Ehrlich ascites cells which had been treated for 4 days is shown in Table 7; synthesis of DNA does not appear to be significantly affected. Vinblastine stimulates RNA synthesis, presumably

TABLE 7. INCORPORATION OF ^{3}H-URIDINE INTO THE RNA OF EHRLICH ASCITES CELLS TREATED WITH VINBLASTINE AND ACTINOMYCIN D

Treatment*		^{3}H-uridine incorporation		
		RNA		DNA
ACT (mg/kg)	VLB (mg/kg)	(cpm/10^8 cells)	(cpm/A_{260})	(cpm/10^8 cells)
0	0	17,500	142·3	217
0·01	0	13,260	149·6	209
0·02	0	6,780	116·4	230
0	0·2	26,540	178·2	236
0·01	0·2	14,909	128·1	254
0·02	0·2	9,480	102·3	189

* Administered daily for 4 consecutive days at 10 a.m.; radioactive uridine (4 μc; 0·2 μmole) was injected 4·5 hr after the last drug treatment, and the animals were sacrificed 30 min later. Data are the means of two or three experiments involving cells from 3 mice each.

through an effect on the rapidly-labeling fraction described previously,[4] whereas actinomycin D, whether alone or in combination with vinblastine, inhibits incorporation of precursor into RNA. Although the combination of drugs does not depress RNA synthesis per cell as much as actinomycin D alone, the uptake of uridine estimated on the basis of tissue volume or specific activity of the RNA is depressed further by

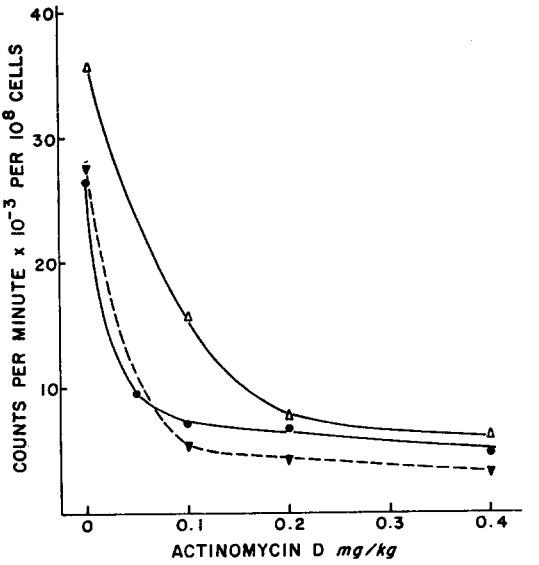

FIG. 1. The incorporation of ^{3}H-uridine into RNA of Ehrlich ascites cells *in vivo*. Mice were treated with actinomycin D alone 96 hr after tumor transplantation (●——●); with vinblastine (2 mg/kg) at 72 hr and the indicated dose of actinomycin at 96 hr (▼- - -▼); or with vinblastine (2 mg/kg) and actinomycin together at 96 hr (△——△). The mice were sacrificed 5 hr after the last drug treatment and 30 min after having received ^{3}H-uridine (4 μc; 0·2 μmole). Each point is the mean of two experiments involving 4 mice each. It should be noted that only the point —●— at zero level of actinomycin represents control animals, the zero actinomycin points —▼— and —△— are for mice receiving only vinblastine.

the combined therapy. However, interpretation of radioactive uptake studies is difficult in situations in which varying degrees of cell accumulation, growth, and death occur, as in chronic treatment with regimens of differing efficacy. For this reason the effects of single large doses of drugs were studied in the same tumor line (Fig. 1). It appears that simultaneous administration of vinblastine and actinomycin D is less effective in inhibiting the synthesis of RNA than is actinomycin D alone. On the other hand, sequential combination therapy starting with vinblastine appears to give a small extra depression in RNA synthesis beyond that given by actinomycin D. This finding is correlated with the increased survival times for such a sequence, given in Table 2.

DISCUSSION

The data presented here show that, in certain cell lines, combinations of vinca alkaloids with actinomycin D may possess enhanced antineoplastic activity. This is most clearly seen in Ehrlich ascites and S-180 cells when vinblastine is the alkaloid used, but effects which are at least additive are seen in L1210, a more resistant tumor. Vincristine, in these studies, does not seem as promising for use in combination with actinomycin D. This may, however, be due to the relative insensitivity to this agent of the three tumors examined, for the best results were obtained with L1210 and S-180, against which vincristine alone showed its greatest activity. Vincristine exhibited minimal inhibitory action for Ehrlich ascites, on the other hand and, as shown, combination therapy was completely unsuccessful in this tumor line. It would appear essential, therefore, that the vinca alkaloid alone show activity in order that the combination exhibit any degree of additive or synergistic action.

The present biochemical studies do not in themselves offer a convincing explanation for the enhanced antitumor effect. Any increased depression of RNA synthesis, in terms of reduced specific activities, is marginal, and unless we assume that the level of synthesis in actinomycin-treated cells is somehow especially critical for survival (a suggestion advanced for combined therapy with actinomycin D and ribonuclease[11]), it would appear unlikely that such small effects would give such great increases in cell death. What does clearly emerge, however, is that we are dealing with a phenomenon of unbalanced growth. Treatment with vinblastine produces enlarged cells reminiscent of those found in tumor-cell populations exposed to alkylating agents.[13, 14] Thus there is an increase in the amount of DNA, tending toward the premitotic level, a finding not unexpected for an antimitotic agent, together with a less pronounced increase in RNA and a much larger accretion of protein. The mechanism for such an effect is unknown, but it is of interest that vinblastine, or possibly a derivative formed intracellularly, associates with DNA, RNA, and protein,[15] in a manner similar to the alkylating agents. Whatever its mechanism, this "unbalancing" of the cellular constituents is further aggravated by exposure to actinomycin D, which prevents most of the increase in DNA induced by vinblastine and causes a reduction in RNA content, while leaving the cells enlarged and with abnormal protein contents. Apparently sequential exposure to the two agents is necessary to achieve the full effect, and two possible explanations might be advanced for this. First, the secondary stimulation of RNA synthesis 18–24 hr after exposure to vinblastine,[3, 4] which appears to be a recovery phenomenon, is suppressed by the treatment with actinomycin D, thereby

reducing the likelihood of cells recovering from the metabolic insult. Second, treatment with vinblastine may result in vinblastine-bound protein and RNA, the subsequent replacement of which is inhibited by actinomycin D, thus falling into the pattern of "complementary inhibition".[11] In this case, simultaneous administration of actinomycin D and vinblastine may produce antagonism through competition for binding sites on DNA at which actinomycin D normally acts.[16, 17]

In considering possible clinical applications of the present findings, the data suggest that only tumors sensitive to the vinca alkaloids and actinomycin are suitable; among these would be lymphomas and choriocarcinoma. However, two factors need to be considered: first, the markedly greater efficacy of vinblastine, both alone and in combination, when given in divided doses than when administered as a large single injection, which raises the question of whether the usually recommended regimen[2] is actually the most effective. Second, great care should be exercised in combining two potent depressants of hematopoiesis such as vinblastine and actinomycin D, even if, in accordance with the present results, the level of the latter drug is kept low.

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