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EFFECTS OF OTHER AGENTS ON THE BIOLOGIC RESPONSES TO VINCALEUKOBLASTINE

J. HARRY CUTTS

Cancer Research Laboratory and Department of Medical Research,
University of Western Ontario, London, Canada

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Abstract—The drug VLB (vinblastine) failed to show synergism when therapy of tumored animals (L1210 and P1534 leukemias) was combined with antifolics, cortisone, colchicine, 6-mercaptopurine, 8-azaguanine, or 2,6-diaminopurine. 'Reversal' of the antitumor effect was achieved by glutamic acid or tryptophan. Mortality from lethal doses of VLB could be reduced by administration of glutamic and aspartic acids, but this protection could not be related to any effect upon alleviation of peripheral leukopenia, decrease in packed cell volume (hematocrit), or reduction of weight loss.

VINBLASTINE (vincaleukoblastine, VLB), an alkaloid isolated from *Vinca rosea* L., has been shown to suppress normal bone marrow activity,[1, 2] to inhibit some experimental tumors in animals,[3–6] and to modify the growth of some human tumors.[7–11] Reversal of tumor inhibition in animals and in tissue culture[12] by various agents has been reported, and mitotic arrest in normal and neoplastic tissue has been described.[13, 14] The present report concerns attempts to modify the biologic activities of VLB by the administration of a variety of other agents.

MATERIALS AND METHODS

The P1534 and L1210 leukemias, carried in BDF_1 hybrid mice, were used to evaluate the usefulness of combination therapy with other known anticancer agents and for reversal studies.

In the studies of combination therapy, treatment with VLB was begun 24 hr after tumor inoculation, at a dose that produced a definite but minimal prolongation of survival. The second antitumor agent was administered simultaneously with VLB, at doses previously found to produce increased survival times. Both drugs were given intraperitoneally for 10 consecutive days or until the prior death of the animals.

For reversal studies, treatment with VLB was begun 24 hr after tumor inoculation, at doses that gave increased survival. The first dose of 'reversing' agent was given immediately after tumor inoculation or 24 hr later when therapy with VLB was begun. Treatment with VLB and reversing agent was continued for 10 days, and all materials were administered intraperitoneally. Those agents that showed any reversal of VLB activity were re-investigated at lower doses or were withheld for 72 to 96 hr after initiating VLB therapy.

Further study of the effective reversing agents was carried out in normal BDF_1 male mice. Single doses of VLB were injected intraperitoneally, and the reversing agent was administered simultaneously as a single dose. In other experiments the amino acids

and VLB were given daily. Total leukocyte counts, hematocrit (PCV) and body weights were measured daily for the first seven days. The animals remained under close observation for an additional seven days to determine the two-week mortality, but were not finally discarded for 30 days.

RESULTS

A. Combination therapy with other antitumor drugs

The P1534 leukemia, in our hands, has been the tumor most sensitive to treatment with VLB, and many animals showed indefinite survival times ('cures'). On the other

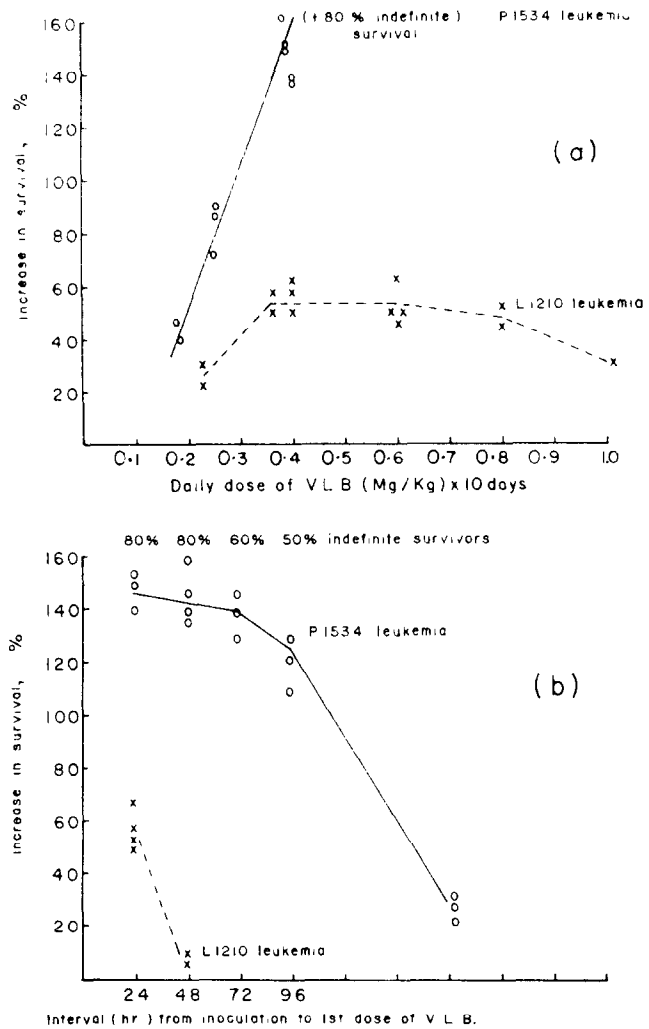

FIG. 1. Tumor response to VLB treatment. (a) Dose response to intraperitoneal treatment. Each point represents the mean increase in survival for 10 mice. (b) Effect of delaying treatment with VLB. All animals treated with 0·4 mg VLB/kg daily for 10 days or until prior death. Each point represents the mean increase in survival for 10 animals. Both tumors maintained in DBF_1 male mice.

hand, prolongation of survival of mice with L1210 tumors could be achieved with VLB therapy, but the tumor was much less responsive, and the survival times were significantly increased only at the maximal doses tolerated. Indefinite survival of treated L1210 leukemic mice has not been observed. Prolongation of survival of mice bearing the P1534 leukemias could be achieved also, even when treatment was withheld until late in the disease. Although the effectiveness of therapy decreased rapidly if not instituted until 96 hr or later after tumor transplant, increases in survival in excess of 130 per cent with 60–80 per cent 'cures' could be achieved when therapy was delayed until 48 or 72 hr. However, delaying treatment of L1210-tumored animals nullified any effect of treatment, even at so short a time as 48 hr. This effect, and the dose response of the two tumors, is seen in Fig. 1.

Survival times in these two tumor systems was no greater when VLB therapy was combined with antifolics (amethopterin and aminopterin), cortisone, colchicine, 6-mercaptopurine, 8-azaguanine, and 2,6-diaminopurine. The results of such treatment are seen in Table 1.

TABLE 1. COMBINATION THERAPY WITH VLB AND OTHER ANTITUMOR AGENTS*

Tumor	Drug	Dose VLB mg/kg	Dose Other drug, mg/kg × days	Mean survival days	Change in survival, %
P1534	VLB	0·2 × 10		32·7 ± 0·8	+41
	Aminopterin		0·4 × 10	28·9 ± 0·9	+33
		0·2 × 10	0·4 × 10	31·2 ± 0·8	+43
	Cortisone		50·0 × 10	31·6 ± 0·7	+39
		0·2 × 10	50·0 × 10	29·9 ± 0·4	+36
	6-MP		5·0 × 10	23·6 ± 0·4	+ 7
		0·2 × 10	5·0 × 10	30·5 ± 0·6	+38
	8-AG		50·0 × 10	24·2 ± 0·7	+24
		0·2 × 10	50·0 × 10	28·1 ± 0·9	+44
L1210	VLB	0·2 × 10		8·8 ± 0·23	**+20**
	Amethopterin		4·0 × 10	8·6 ± 0·19	+18
		0·2 × 10	4·0 × 10	8·9 ± 0·20	+23
	Colchicine		1·0 × 10	7·9 ± 0·21	+ 8
		0·2 × 10	1·0 × 10	8·8 ± 0·23	+19
	2,6-DAP		30·0 × 10	7·8 ± 0·28	+ 4
		0·2 × 10	30·0 × 10	9·0 ± 0·21	**+21**
	6-MP		5·0 × 10	7·5 ± 0·23	+ 3
		0·2 × 10	5·0 × 10	8·9 ± 0·26	**+22**

* 6-MP = 6-mercapturine; 8-AG = 8-azaguanine; 2,6-DAP = 2,6-diaminopurine. Mean values ± S.E.M. for 12 animals in each group. All injections made intraperitoneally, beginning 24 hr after inoculation; treatment continued for 10 days or until prior death.

At the doses of drug used in Table 1, overt toxic symptoms, either in normal or in tumored mice, were not noted. When given in combination with VLB, there was no apparent increase in toxicity. Only when one or both drugs were given at doses that were toxic per se were such signs as diarrhea, marked weight loss, listlessness, etc. noted.

B. 'Reversal' of antitumor activity

Several compounds of diverse nature were examined for ability to reverse the antitumor activity of VLB. Of these, only glutamic and aspartic acids and tryptophan were effective in any way. Table 2 shows some typical results. No evidence of reversal, as judged by their effects on survival time, was seen with folic or folinic acids, vitamin B_{12}, crude liver extracts, nicotinic acid, adenine, serine, or glycine. Cysteine, cystine, and methionine gave extremely equivocal results and were considered to be ineffective.

At the doses used, glutamic acid and tryptophan appeared equivalent in their ability to reverse the antitumor properties of VLB. Aspartic acid was much less effective. At the lower doses of aspartic acid there appeared to be a slightly better response to VLB, and from the gross appearance of the mice, VLB appeared to be better tolerated when given in conjunction with aspartic acid.

TABLE 2. INHIBITION OF ANTITUMOR ACTIVITY OF VLB BY AMINO ACIDS

	Amino acid,	Daily dose: Amino acid, mg/kg	Daily dose: VLB,	Mean survival, days	Change in survival %	"Cures" (indef. survival) %
P1534				16·9 ± 0·9		
			0·4 × 10	39·6 ± 1·4	+134	40
	Glutamic acid	600 × 11*	0·4 × 10	22·7 ± 0·9	+33	nil
		300 × 10	0·4 × 10	24·6 ± 0·8	+44	nil
		600 × 11		17·2 ± 0·55		
	Aspartic acid	600 × 11*	0·4 × 10	27·1 ± 1·6	+68	10
		300 × 10	0·4 × 10	28·7 ± 1·0	+72	30
		600 × 11		16·1 ± 0·67		
	Tryptophan	600 × 11*	0·4 × 10	24·6 ± 1·8	43	nil
		300 × 10	0·4 × 10	26·1 ± 1·5	+52	nil
		600 × 11		15·8 ± 0·75		
L1210				7·7 ± 0·12		
			0·6 × 10	12·0 ± 0·13	+56	nil
	Glutamic acid	600 × 11*	0·6 × 10	9·4 ± 0·28	+22	nil
		300 × 10	0·6 × 10	10·2 ± 0·22	+33	nil
		600 × 11		7·8 ± 0·14		nil
	Aspartic acid	600 × 11*	0·6 × 10	10·6 ± 0·24	+41	nil
		300 × 10	0·6 × 10	11·8 ± 0·18	+57	nil
		600 × 11		7·5 ± 0·17		nil
	Tryptophan	600 × 11*	0·6 × 10	9·2 ± 0·22	+20	nil
		300 × 10	0·6 × 10	10·4 ± 0·32	+35	nil
		600 × 11		7·2 ± 0·09		nil

* Total dose for 24-hr period. Amino acid given as 200 mg/kg, i.p., every 8 hr. The first dose was given on day of inoculation with tumor and continued for an additional 10 days or prior death. Mean ± S.E.M. for 12 animals in each group.

'Reversal' of the antitumor activity by tryptophan and glutamic acid could be shown when the reversing agent was withheld for 24 hr after beginning VLB treatment. With further delay, e.g. at 72 or 96 hr after the first dose of VLB, the effectiveness of the reversing agent diminished, even when continued beyond the period of VLB treatment (Table 3).

TABLE 3. EFFECT OF DELAYING TREATMENT WITH AMINO ACIDS

Tumor	Amino acid	Daily dose VLB, mg/kg	Daily dose AA, mg/kg	Time from implant to 1st dose, VLB	Time from implant to 1st dose, AA	Survival, days	Change in survival, %
P1534		0·60		24		39·1 ± 1·9	+133 (+30% indef.)
	Glutamic	0·60	600 × 10*	24	72	29·2 ± 0·8	−68
		0·60	600 × 10*	24	96	33·4 ± 1·2	−92 (+10% indef.)
	Tryptophan	0·60	600 × 10*	24	72	31·0 ± 0·6	−80
		0·60	600 × 10*	24	96	34·7 ± 0·9	−98 (+10% indef.)
	Aspartic	0·60	600 × 10*	24	72	38·4 ± 1·8	+130 (+30% indef.)
		0·60	600 × 10*	24	96	39·7 ± 2·2	+147 (+50% indef.)
						16·6 ± 0·53	
L1210		0·80		24		10·9 ± 0·22	49
	Glutamic	0·80	600†	24	72	9·6 ± 0·23	−32
		0·80	600†	24	96	10·4 ± 0·10	−42
	Tryptophan	0·80	600†	24	72	9·8 ± 0·08	35
		0·80	600†	24	96	10·6 ± 0·20	−45
	Aspartic	0·80	600†	24	72	10·6 ± 0·27	−46
		0·80	600†	24	96	11·3 ± 0·31	−54
						7·3 ± 0·22	

* Amino acid given as 200 mg/kg every 8 hr for 10 days. Mean ± S.E.M. for 12 animals in each group. Treatment with VLB began 24 hr after tumor inoculation.

† Amino acid given as 200 mg/kg every 8 hr until 10 days or death.

TABLE 4. EFFECT OF SIMULTANEOUS ADMINISTRATION OF AMINO ACID ON VLB TOXICITY*

Amino acid	Dose: Amino acid, mg/kg	Dose: VLB, mg/kg	Initial values for: Body wt	Initial values for: Total WBC	Initial values for: PCV	Lowest values for: Body wt	Lowest values for: Total WBC	Lowest values for: PCV	Relative change in: Body wt %	Relative change in: WBC %	Relative change in: PCV %
Nil		5·0	26·4 ± 0·42	10,525 ± 325	49·9 ± 0·45	24·1 ± 0·44	5,296 ± 571	37·3 ± 0·91	−10	−50	−26
		8·0	23·2 ± 0·47	10,668 ± 680	52·3 ± 0·5	15·1 ± 1·47	4,660 ± 680	33·7 ± 1·33	−25	−56	−36
		12·0	25·3 ± 0·30	11,465 ± 489	50·8 ± 0·41	16·7 ± 0·35	2,614 ± 521	34·7 ± 1·29	33	−77	33
Tryptophan	300	5·0	26·3 ± 0·39	10,358 ± 528	50·1 ± 0·57	23·3 ± 0·67	4,817 ± 431	34·9 ± 0·83	−12	−55	−30
	300	8·0	20·9 ± 0·16	12,792 ± 735	51·9 ± 0·72	16·5 ± 0·42	3,762 ± 662	32·2 ± 1·41	−21	−76	−38
	300	12·0	21·1 ± 0·24	13,133 ± 821	51·1 ± 0·63	14·6 ± 0·35	2,757 ± 226	39·7 ± 0·93	31	−79	32
	300		23·3 ± 0·19	11,047 ± 652	50·5 ± 0·68	22·6 ± 0·22	11,047† ± 652	50·1 ± 0·54			
Glutamic	300	5·0	20·5 ± 0·49	10,613 ± 371	48·8 ± 0·55	17·3 ± 0·94	5,688 ± 486	37·5 ± 0·78	−14	−48	−24
	300	8·0	20·3 ± 0·22	13,338 ± 573	53·2 ± 0·57	15·9 ± 0·73	3,267 ± 372	36·6 ± 0·81	−22	−76	−32
	300	12·0	22·3 ± 0·19	14,375 ± 847	49·8 ± 0·50	16·1 ± 0·69	3,225 ± 406	34·6 ± 0·66	−29	−78	−32
	300		21·6 ± 0·32	12,104 ± 516	51·9 ± 0·62	21·0 ± 0·53	11,987 ± 581	49·7 ± 0·79			
Aspartic	300	5·0	21·8 ± 0·42	11,867 ± 295	51·9 ± 0·35	19·0 ± 0·58	5,196 ± 970	39·0 ± 1·07	13	−57	−25
	300	8·0	21·6 ± 0·76	11,038 ± 1,119	51·5 ± 0·49	18·2 ± 1·58	4,167 ± 314	35·6 ± 1·01	−14	−63	−29
	300	12·0	21·7 ± 0·97	12,854 ± 231	50·8 ± 0·41	16·8 ± 0·54	4,082 ± 103	38·6 ± 0·75	−23	−76	−25
	300		22·3 ± 0·67	10,958 ± 448	49·3 ± 0·39	21·3 ± 0·72	10,958† ± 448	49·3 ± 0·39			

* All values are mean ± S.E.M. for 12 animals; PCV = packed cell volume (hematocrit).
† The initial values for these groups of mice were also the lowest values.

TABLE 5. PATTERN OF RESPONSE AFTER VLB PLUS AMINO ACIDS*

Amino acid	Dose		Day on which weight loss			Day on which total WBC decrease			Day on which PCV decrease		
	Amino acid mg/kg	VLB,	Began	At maximum	Ret. to normal	Began	At maximum	Ret. to normal	Began	At maximum	Ret. to normal
Nil		5·0	1·4 ±0·15	3·00±0·06	7·3±1·41	1·38±0·07	2·27±0·23	3·9 ±0·52	1·5 ±0·18	3·27±0·18	6·3 ±1·41
		8·0	1·3 ±0·07	3·40±0·19	7·2±0·52	1·30±0·07	3·10±0·18	5·6 ±0·24	1·3 ±0·18	3·6 ±0·14	7·4 ±0·11
		12·0	1·08±0·04	4·00±0·14	8·1±0·80	1·25±0·14	4·09±0·16	6·0 ±0·19	1·00±0·00	4·09 ±0·00	7·5 ±0·15
Trypto-phan	300	5·0	1·58±0·25	3·50 ±0·05	6·9±0·38	1·35±0·12	2·83±0·25	6·08 ±0·32	1·35±0·13	3·17±0·22	7·8 ±0·16
	300	8·0	1·08±0·09	3·75 ±0·07	7·2±0·32	1·30±0·18	2·75±0·17	6·6 ±1·10	1·25±0·02	3·25±0·13	8·1 ±0·08
	300	12·0	1·00±0·00	4·09 ±0·09	8·2 ±0·46	1·00±0·00	4·17 ±0·22	7·2 ±0·25	1·00±0·00	4·17 ±0·37	7·3 ±0·22
Glutamic	300	5·0	1·53±0·15	3·08±0·18	6·8±0·29	1·33±0·14	2·00±0·17	4·17 ±0·82	1·60±0·21	3·08±0·46	6·8 ±0·31
	300	8·0	1·16±0·18	3·50±0·40	7·5±0·82	1·50±0·11	2·92±0·22	6·4 ±0·81	1·17±0·15	3·58±0·24	7·9 ±0·33
	300	12·0	1·00±0·00	3·80 ±0·65	8·3 ±0·16	1·02±0·11	3·67±0·17	7·3 ±1·50	1·00±0·00	4·07±0·43	7·67±0·37
Aspartic	300	5·0	1·5 ±0·15	2·93 ±0·75	6·4±0·26	1·17±0·27	2·50±0·19	3·75 ±0·24	1·12±0·07	3·17±0·75	5·4 ±0·25
	300	8·0	1·16±0·23	3·17±0·18	6·8±0·46	1·25±0·14	2·58±0·14	4·9 ±0·69	1·08±0·15	3·4 ±0·07	6·8 ±0·35
	300	12·0	1·00±0·00	3·50±0·71	7·4±0·30	1·00±0·00	3·09±0·54	5·8 ±0·85	1·00±0·00	3·42±0·54	7·5 ±0·15

* All values are the mean ± S.E.M. for 12 animals; PCV = packed cell volume (hematocrit)

C. Effect of amino acids on VLB toxicity

As can be seen from Table 4, the degree of response to VLB was dependent upon the dose administered. None of the amino acids, at the doses given, was capable of alleviating the toxic symptoms of VLB when measured as changes in body weight, hematocrit, or total leukocyte count.

There tended to be some correlation between dose of VLB and the pattern of response with increasing dosage; reduction of leukocytes, loss of body weight, and decrease in hematocrit began slightly earlier, reached the maximal depression later, and showed a slight tendency to persist longer. If any effect was produced by administration of the amino acids, it was to exaggerate the duration of response, but in general no change in the overall pattern of response to VLB was noted (Table 5).

In spite of this almost complete lack of effect on body weight, hematocrit, and total leukocyte count, these amino acids had a marked effect upon the mortality resulting from injection of single doses of VLB (Table 6). With the same dose of amino acid,

TABLE 6. EFFECT OF GLUTAMIC, ASPARTIC AND TRYPTOPHAN ON VLB MORTALITY*

Amino acid	Dose VLB	Dose Amino mg/kg acid	Mortality, (%)
	12		54 (13/24)
	8		26 (9/35)
	5		9 (3/35)
Trytophan	12	300	46 (11/24)
	8	300	25 (6/24)
	5	300	0 (0/24)
Glutamic	12	300	46 (11/24)
	8	300	17 (4/24)
	5	300	0 (0/24)
Aspartic	12	300	13 (3/24)
	8	300	0 (0/26)
	5	300	0 (0/24)

* All mice were male BDF_1 hybrids, ranging from 20 to 25 g.

the protection given varied not only with the dose of VLB, but also with the amino acid used. Thus, tryptophan gave little protection except at the lowest doses of VLB, whereas glutamic acid was effective at doses of VLB which were less than the LD_{50}. Aspartic acid was effective at all doses of VLB.

Dividing the same total dose of VLB and administering it daily did not alter the total response of body weight, hematocrit, or leukocyte count but did produce a marked increase in mortality. Whereas the death rate of BDF_1 male mice after a single i.p. injection of 5·0 mg/kg was 9 per cent, when the same dose was administered over a five-day period, mortality rose to 83 per cent. The concurrent administration of glutamic acid, tryptophan, or aspartic acid markedly reduced mortality but again had no effect on the other parameters measured (Table 7).

TABLE 7. CHRONIC ADMINISTRATION OF VLB AND AMINO ACIDS*

Amino acid	Dose		Initial values for			Lowest values for			Relative change in			Mortality
	Amino acid, mg/kg	VLB, days	Body wt.	WBC	PCV	Body wt.	WBC	Body PCV	wt %	WBC %	PCV %	%
Nil		1·0 × 5	19·7±0·23	10,729±314	51·6±0·37	16·9±0·34	4,788±321	34·7±0·91	−15	−56	−33	83 (11/12)
Trypto-phan	120 × 5	1·0 × 5	18·3±0·19	10,175±327	51·7±0·22	15·7±0·16	4,550±540	35·2±0·66	−15	−55	−32	50 (6/12)
	60 × 5	1·0 × 5	19·9±0·33	10,159±426	49·8±0·41	16·6±0·22	4,191±476	35·5±0·74	−14	−59	−28	100 (12/12)
Glutamic acid	120 × 5	1·0 × 5	18·8±0·24	10,433±528	52·0±0·44	15·5±0·12	5,408±368	34·1±0·99	−17	−51	−35	17 (2/12)
	60 × 5	1·0 × 5	18·6±0·29	10,466±475	48·3±0·62	16·1±0·24	4,033±651	38·3±0·71	−15	−52	−20	75 (9/12)
Aspartic acid	120 × 5	1·0 × 5	21·0±0·72	11,000±929	52·0±0·57	18·5±0·20	4,950±232	33·0±0·31	−17	−55	−37	0 (0/12)
	60 × 5	1·0 × 5	19·4±0·67	10,516±626	49·1±0·91	16·5±0·36	5,860±392	34·3±0·36	−16	−44	−32	58 (7/12)

*All values are mean ± S.E.M. for 12 animals.

DISCUSSION AND SUMMARY

The failure of combination therapy to potentiate antitumor effect or to increase the toxicity of VLB would seem to indicate a mode of action for VLB which differs from that of the compounds studied. In this our data agree with those of Johnson[12] who, in a similar study, was unable to show better survival of treated mice when VLB was administered in combination with any one of 15 other agents.

Administration of VLB as a single dose, while productive of the same blood response and loss of weight, caused fewer deaths than the same total dose spread over a number of days. Thus the mortality from 5·0 mg/kg was increased ninefold when divided into five daily doses. Administration of amino acids had no effect on the peripheral blood values or on body weight loss, whether the VLB and amino acids were given in a single dose or fractionated into multiple doses. The mortality from either mode of administration, however, was decreased markedly when glutamic or aspartic acids were given concurrently, and to a lesser extent when tryptophan was administered. From the studies of the effects of these amino acids on VLB activity, it is apparent that these agents differ in their capacity to reverse the antitumor properties or to ameliorate the toxic effects of VLB. Glutamic acid appeared to reverse both, equally. Tryptophan on the other hand, was much less effective in reducing mortality but was as effective as glutamic acid in reversing the antitumor effect. Aspartic acid was the most effective agent for reducing mortality due to VLB intoxication but was the least effective in reversing antitumor response.

In general there was a wide range between a 'protective' dose of amino acid and a 'reversing' dose. The amino acids were given to leukemic mice at total doses up to 1,600 times the dose of VLB, yet even at this amount tumor reversal was not complete. One-tenth of this quantity would protect animals from lethal effects of chronically administered VLB.

The greater toxicity of fractionated doses of VLB was reflected in the larger doses of amino acid required to protect normal animals. Whereas a total dose of 300 mg of aspartic or glutamic/kg would protect mice from a dose of 8 mg/kg (ratio 37:1) when given as a single injection, three times this amount was required to protect animals from 5 mg of VLB/kg spread over five days (ratio of total doses, 120:1). Gross symptoms of toxicity, from either schedule of administration, included diarrhea, listlessness, ruffing of the fur, and a generally unkempt appearance. Those animals protected by aspartic acid especially, but also by glutamic acid, showed no (or only transient) diarrhea, maintained their normal activity, and appeared normally sleek and tidy.

We have reported previously[15] a decrease in the mitotic arrest induced by VLB in the bone marrow of animals treated with glutamic acid or tryptophan, and Vaitkevicius[16] has noticed a similar result in human marrow after combining VLB therapy with administration of L-glutamic acid. Although a more detailed report of the effects of amino acids on VLB mitosis will be presented later, various tissues of amino acid-treated animals showed modification of the mitotic arrest induced by VLB. This was particularly true of bone marrow. However, the reduction in the mitotic arrest did not seem to be sufficiently pronounced to account wholly for the protection afforded by these agents and, in any event, this protection was not accompanied to any great extent by lessening the suppression of circulating leukocytes and erythrocytes.

Protection with aspartic acid could be achieved even when the amino acid was administered simultaneously with VLB but by a different route, or when the amino acid was injected at some time before or after the dose of VLB. Thus, 30mg of VLB /kg given *subcutaneously* resulted in 85 per cent mortality in BDF_1 male mice, whereas the same dose of VLB followed immediately by 300 mg of aspartic acid/kg *intraperitoneally* produced a 66 per cent, mortality. The mean survival time for the latter group was increased by 33 per cent over those receiving VLB alone. Similarly, pretreatment with aspartic acid at 4 hr and 1 hr before administration of VLB allowed 75 per cent of the mice to survive 16 mg of VLB/kg—ordinarily LD_{100} for BDF mice. The mortality from a similar dose of VLB was reduced to 32 per cent even when aspartic acid was withheld for 2 hr after injecting VLB. It would seem, then, that the protective action of aspartic acid administered simultaneously with VLB was not the result merely of decreased absorption because of interaction of the amino acid with VLB.

Inasmuch as the protective action of glutamic and aspartic acids could not be associated with changes in values for peripheral leukocytes and red cells, it seems that depression of these elements need not be the instrument of VLB mortality. The actual cause of acute deaths after VLB is unknown at present.

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