

Biological Properties of Vincalukoblastine, an Alkaloid in *Vinca rosea* Linn, with Reference to Its Antitumor Action

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SUMMARY

Some biological effects of a new alkaloid, Vincalukoblastine (VLB), are described. In mice bearing the transplantable leukemias L1210, P1534, and AKr or Ehrlich ascites tumor, treatment with VLB effectively prolonged survival. The effectiveness of treatment depended on the amount and spacing of dosage and the time of the institution of treatment after transplantation. A single injection of VLB was highly active, but hourly doses were probably the most effective. VLB was also active when incubated with tumor cells.

Transplanted or spontaneous mammary tumors in C3H mice also showed a reduced growth during treatment with VLB.

Mice "cured" of AKr or P1534 transplanted leukemia by treatment with VLB were subsequently resistant to repeated challenges by the tumor. AK mice, so treated, have to date not developed spontaneous leukemia.

Fischer rats bearing transplanted IRC 741 leukemia also responded to VLB treatment. Circulating tumor cells were particularly affected.

VLB did not inhibit regeneration of the liver of the partially hepatectomized rat. Bone marrow depression was striking, although megakaryocytes were little affected. Intestinal lesions were absent. The mouse tolerated greater doses of VLB than did the rat or the guinea pig.

An outline of our interest, over a number of years, in the biological activities of extracts of *Vinca rosea* Linn has been published previously (9). From a study of the effects of purified extracts on the hemopoietic system of rats (1, 3) it was possible to isolate from one active fraction a crystalline alkaloid named Vincalukoblastine (VLB) (9). The chemical and physical evidence has since indicated that VLB has an empirical formula $C_{46}H_{58}O_9N_4$ and is a member of a new class of dimeric alkaloids which contain both indole and dihydroindole moieties (4, 8). This substance, when injected into rats, depressed the bone marrow, leading to a severe granulocytopenia. Tissues such as the gut were apparently unaffected, although other fractions of the plant extracts contained additional substances, not yet isolated, which caused marrow, gut, and other changes

(2, 9). Because of the striking biological action of this new type of compound, testing was conducted from time to time against experimental cancer. It soon was apparent that extracts possessed anti-tumor activity (9), and a more detailed preliminary study has already been published (10).

Following our report of the isolation of VLB we learned that a group of workers at the Eli Lilly Co., Indianapolis, were interested in the screening of extracts of *Vinca rosea* against leukemia (7).¹ With the isolation of a sufficient quantity of VLB, and in view of the experimental data, the Indianapolis group commenced preliminary testing on patients suffering from cancer (6). They generously provided us with material so that clinical studies could then be made in Canada through collaboration with a group of workers in Toronto. This paper will describe some of the biological

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¹ I. S. Johnson, personal communication.

properties of VLB characterized in animals, and the subsequent paper will report on its effects on cancer in man (11). The mechanism of action, although not yet definitely determined, appears to differ from those presently established for other chemotherapeutic agents.

METHODS AND MATERIALS

The tumors used in screening VLB for possible antitumor properties have included the L1210 leukemia (ascitic and nonascitic forms) and the P1534 leukemia carried in the BDF₁ hybrid mouse, the AKr leukemia in AK mice, the Ehrlich ascites tumor in Swiss mice, and the C3H mammary tumor (spontaneous and transplanted). In addition, one rat tumor has been employed—the IRC 741/139B acute leukemia in Fischer line 344 rats. Suspensions of spleen or tumor containing a stand-

injections. The doses and schedules have been varied from time to time as indicated in the text. On occasions when VLB sulfate was used it was dissolved in saline as indicated. The mice used were selected for healthy appearance and uniformity of weight. Throughout the experimental period control and experimental animals were maintained in equal groups, weighed daily, and were given unlimited access to a commercial chow and tap water. Doses of the drug were calculated on the individual animal weight. Animals surviving more than 100 days after tumor inoculation were considered to be indefinite survivors.

RESULTS

EFFECT OF DAILY INJECTION OF VLB

Treatment with VLB has prolonged the survival time of mice bearing L1210, P1534, AKr,

TABLE 1
EFFECT OF VLB INJECTIONS ON SURVIVAL OF TUMOR-BEARING MICE

TUMOR	DOSE (MG/KG) × DAYS	ROUTE	SURVIVAL (DAYS)		PER CENT INCREASE T/C	PER CENT INDEFINITE SURVIVOR	No. MICE
			Controls	Treated			
L1210	0.35 × 10	I.P.	7.5 ± 0.5	11.9 ± 1.2	+ 59	0	10
	0.8 × 10	S.C.	8.8 ± 0.6	12.0 ± 0.9	+ 37	0	5
P1534	0.4 × 10	I.P.	16.5 ± 2.1	41.7 ± 5.5	+153	0	5
	0.8 × 10	S.C.	24.0 ± 2.5	230+		100	15
AKr	0.4 × 10	I.P.	16.1 ± 1.4	23.0 ± 3.3	+ 43	40	10
	0.4 × 10	S.C.	18.8 ± 2.3	24.3 ± 2.6	+ 29	20	10
	0.4 × 10	I.V.	20.2 ± 2.6	30.5 ± 4.0	+ 51	20	5
	0.8 × 10	S.C.	20.1 ± 1.8	230+		100	15
Ehrlich ascites	0.8 × 10	I.P.	27.0 ± 2.1	43.0 ± 2.6	+ 59	0	5
	0.8 × 10	S.C.	19.8 ± 1.2	18.0 ± 1.7	- 10	0	5

The deviation from the mean values was calculated according to conventional statistical methods.

ard number of cells were used for transplantation. All the tumors used have consistently given 100 per cent takes, with little change in survival time through succeeding transplant generations.

The BDF² and Swiss mice³ were obtained from reliable commercial suppliers, while the AK and C3H mice and the Fischer rats were raised at the laboratory by selected brother × sister matings.

For routine screening the VLB was administered as the free base in 50 per cent aqueous propylene glycol. Control animals received an equivalent amount of the medium only. Usually treatment was begun 24 hours after inoculation of the animals and was continued for a total of ten daily

² BFD mice from Jackson Memorial Laboratory, Bar Harbor, Maine.

³ Swiss mice from Woodlyn Farms, Guelph.

and Ehrlich ascites tumors. Treatment was started 24 hours after tumor inoculation and was continued daily for 10 days. Various routes of administration and doses were used, as indicated in Table 1.

The AKr and the P1534 leukemias were particularly sensitive to VLB, and many of the treated animals have survived indefinitely. Although the L1210 leukemia showed some response to VLB treatment, all animals eventually died of the disease. The Ehrlich ascites tumor was insensitive to subcutaneously administered VLB but responded to intraperitoneal administration of the drug. The subcutaneous injection was usually less effective than the intraperitoneal. Although toxicity was not marked at the doses used, the animals appeared to tolerate subcutaneous injection better than intraperitoneal. Limited experience with the

intravenous route would indicate this to be equivalent to, or slightly better than, intraperitoneal injection.

EFFECT OF DELAYED INJECTION OF VLB

The effect of delaying the start of treatment on tumor growth may be seen in Table 2. The more sensitive tumors (AKr and P1534) permitted greater delay between inoculation of tumor and the start of treatment, although a decreased effectiveness was apparent. On the other hand, the survival time of mice bearing the L1210 was not prolonged if the dose was delayed for 48 hours.

The effect of VLB on survival of tumor-bearing mice was not due to a permanent modification of the host. For example, a group of five normal mice was treated with VLB (0.8 mg/kg) daily for 10 days. One week after the last dose each mouse was given an inoculation of P1534 leukemia. The pretreated mice showed no significant increase

in survival over nontreated controls (16.5 ± 1.0 days for treated animals vs. 15.8 ± 1.2 days for controls).

OTHER SCHEDULES OF ADMINISTRATION

Administration of VLB as a single dose, or when divided into hourly injections, also proved effective in prolonging survival times. As shown in Table 3, an equivalent increase in survival could be achieved by administering, as a single dose, one-quarter to one-half of the total amount of VLB administered over a 10-day period.

Likewise, administration of this single effective dose as a number of smaller injections spread over the first 24 hours following tumor implantation resulted in survival times for L1210 leukemia which were superior to those achieved by any other schedule of administration. Good survival times were obtained even when the drug was withheld for 48 hours.

TABLE 2
EFFECT OF DELAYING VLB TREATMENT

TUMOR	DOSE (MG/KG) × DAYS	ROUTE	TIME OF 1ST DOSE	SURVIVAL (DAYS)		PER CENT INCREASE T/C	PER CENT INDEFINITE SURVIVORS	No. MICE
				Control	Treated			
L1210	0.4 × 6	I.P.	48 hrs.	7.3 ± 4.0	7.7 ± 0.6	+ 5	0	5
	0.8 × 7	S.C.	48 hrs.	8.8 ± 0.6	9.2 ± 0.9	+ 4	0	5
P1534	0.4 × 10	I.P.	48 hrs.	16.5 ± 2.1	34.6 ± 4.1	+110	0	5
	0.8 × 10	S.C.	72 hrs.	16.9 ± 0.5	41.0	+142	80	5
	0.8 × 10	S.C.	7 days	16.9 ± 0.5	17.6 ± 1.5	+ 4	0	5
AKr	0.4 × 10	I.P.	48 hrs.	20.2 ± 2.6	24.0 ± 7.0	+ 19	20	5
	0.8 × 10	S.C.	48 hrs.	" "	31.2 ± 3.4	+ 56	20	10

TABLE 3
EFFECT OF A SINGLE INJECTION OF VLB

TUMOR	DOSE* (MG/KG)	SURVIVAL (DAYS)		PER CENT INCREASE T/C	PER CENT INDEFINITE SURVIVOR	No. MICE
		Control	Treated			
L1210	1.0	7.2 ± 0.40	8.9 ± 1.5	+24	0	10
	1.5	" "	10.7 ± 1.3	+48	0	10
	2.3	8.5 ± 0.5	14.4 ± 2.1	+68	0	20
	3.0	8.1 ± 0.6	12.8 ± 0.7	+58	0	5
AKr	1.0	14.9 ± 3.7	21.0 ± 4.0	+41	20	5
	1.5	" "	26.5 ± 0.5	+78	60	5
	2.3	" "	20.7 ± 1.7	+38	20	10
Ehrlich ascites	1.0	19.2 ± 1.0	26.5 ± 1.0	+38	0	10
	1.5	" "	28.1 ± 2.6	+46	20	10
	2.3	" "	31.7 ± 4.7	+63	30	15
	3.0	" "	22.3 ± 2.5	+20	0	5

* Dose of VLB administered 24 hours after tumor inoculation.

THE EFFECT OF VLB SULFATE
ON TUMOR INOCULI

Death of mice following inoculation of tumor could be delayed by pretreatment of the inoculi with VLB sulfate. The latter was added to a suspension of the tumor, and the volumes were adjusted so that 0.20 ml. would contain the standard inoculum for the tumor being used, plus an amount of VLB in the dose range previously found effective on daily injection. The pretreated and control inoculi were incubated for 30 minutes at room temperature before inoculation.

The survival times of mice receiving pretreated L1210, P1534, and AKr leukemia exceeded those of their controls by 130, 120, and 70 per cent, respectively. C3H mammary tumor suspensions, pretreated with VLB sulfate, failed to elicit tumor in recipient animals by 32 days, whereas similar nontreated suspensions produced tumors with diameters in excess of 1.00 cm. by 28 days, and death by 50 days. The tumor-resistant animals were

TABLE 4
EFFECT OF HOURLY INJECTIONS OF VLB
ON L1210 LEUKEMIA

HOURLY DOSES (MG/KG) × HOURS	SURVIVAL (DAYS)		PER CENT INCREASE T/C	NO. MICE
	Controls	Treated		
0.25×8	7.4±0.3	12.7±1.2	+72	10
0.25×8*	6.8±0.5	9.0±0.9	+32	10
0.50×4†	7.8±0.4	14.2±0.9	+82	5
0.50×4‡	8.0±0.1	12.1±0.7	+51	5

* Doses given on 2 successive days.

† Doses given at 2-hour intervals.

‡ Doses given at 2-hour intervals but started 48 hours after tumor inoculation.

subsequently re-inoculated with nontreated tumor suspensions and died at 51 ± 2.8 days, bearing tumors with diameters in excess of 1.75 cm.

EFFECT OF VLB ON MAMMARY
TUMORS IN C3H MICE

Spontaneous tumors.—A number of C3H mice bearing spontaneous mammary tumors have been treated with VLB. Daily treatment for 10 days with doses of VLB less than 0.5 mg/kg had no effect on tumor diameter. Continuous daily treatment for periods of 20–30 days with doses of VLB between 0.6 and 0.8 mg/kg reduced tumor diameters by 35–45 per cent. Cessation of treatment was followed by further tumor growth after a short latent period, and the tumor appeared to be somewhat more resistant to a second course of therapy. Subsequent growth was rapid. Histological sections of the regressing tumors showed much necrosis and hemorrhage, but viable tumor was present at the periphery.

Transplanted tumors.—Female C3H mice bearing fifth- to seventh-generation transplants of mammary tumor received VLB, subcutaneously, daily for the first 10 days following transplantation. The animals were examined daily, and tumor growth was estimated by measuring four to six diameters for each tumor. Administration of 0.8 mg/kg of VLB increased the “take time” (arbitrarily set as the number of days between implantation and production of a tumor 1.0 cm. in diameter) by 38 per cent and the survival time by 36 per cent, with corresponding increases of 26 and 23 per cent when half this dose was used.

EFFECT ON P1534 “SOLID” TUMORS

The carcinostatic activity of VLB was also demonstrated by its effects on a sub-line of the

TABLE 5
EFFECT OF VLB ON ORGAN WEIGHTS OF LEUKEMIC MICE

DAYS OF VLB TREATMENT	WT. OF LIVER*		WT. OF SPLEEN*		WT. OF MESENTERY*	
	Control (mg.)	Treated (mg.)	Control (mg.)	Treated (mg.)	Control (mg.)	Treated (mg.)
3	1025	960	94	95	380	228
5	1070	985	102	92	386	232
10	1100	1025	175	106	585	320
5 days after cessa- tion of treatment	1365	950	181	95	2540	235
10 days after cessa- tion of treatment	1080	1010	295	102	2185	330

* Average of two mice for each figure.

P1534 leukemia. In addition to a disseminated leukemia, with gross infiltration of spleen and liver, this line also grew as a solid tumor along the mesentery to produce a large mass of firm tumor tissue in the peritoneal cavity. VLB was administered subcutaneously at a dose level of 0.8 mg/kg/day to BDF animals given inoculations of this line of leukemia. During and after treatment animals were autopsied, and weights of liver, spleen, and mesentery noted. The mesenteric weight listed in Table 5 indicates the amount of mesentery, or mesentery plus tumor that could be stripped from the gut. It can be noted that as treatment was continued there was less increase in the weight of the various organs due to tumor cell infiltration.

EFFECT OF RE-INOCULATION OF "CURED" AK AND P1534 MICE

AK mice which showed indefinite survival following treatment with VLB have subsequently received repeated re-inoculation with large numbers of AKr leukemic cells. Twenty mice, successfully treated with VLB, survived in excess of 250 days and withstood from three to five "challenges" with AK tumor inoculi containing from 6 to 8 times the number of cells which produced leukemic deaths in control animals. One animal developed multiple tumors, probably lymphoid, in the skin of the chest and abdomen. Ten other mice were sacrificed at 200 days, but transplantation of liver spleen, and lymph nodes failed to elicit leukemia in the recipient mice.

Similarly, BDF mice, successfully treated with VLB, have resisted repeated challenges with P1534 leukemia, but remained susceptible to challenges with L1210 leukemia.

Although both of these tumors have consistently given 100 per cent takes through a number of transplant generations, neither has arisen in the animals used as carriers. The original AK leukemia arose as a spontaneous tumor in a line of AK mice maintained by Dr. A. W. Ham of Toronto. The first and subsequent transplants were made into AK mice raised at the Collip Laboratory from an original breeding nucleus obtained from Millerton Farms. Similarly, the P1534 leukemia, although originating in the DBA line 3, has been maintained in this laboratory in the BDF₁ hybrid mouse. Although none of the surviving AK mice had developed a spontaneous tumor at 250–300 days, no control mice of this line of corresponding age are yet available from which to determine the natural incidence of leukemia.

EFFECT OF VLB ON IRC 741/1398 LEUKEMIA IN FISCHER RATS

This tumor grows as a localized mass at the site of inoculation and also produces a leukemic infiltration of blood, spleen, and liver. The tumor can be passed by inoculation of any of these tissues, by any route. The effect of different dose regimens of VLB on this tumor are included in Table 6.

At the doses used there was suppression of the hematopoietic organs, particularly at the higher dose levels. Consequently, an interrupted schedule of treatment was sometimes employed, the time of administration being determined from the total leukocyte count.

TABLE 6
EFFECT OF VLB ON IRC-741 TUMOR*

DOSE (MG/KG) × DAYS	ROUTE	SURVIVAL TIME (DAYS)		PER CENT INCREASE T/C
		Control	Treated	
0.4×7	I.P.	16.3±2.4	19.0±1.4	+17
0.2×10	S.C.	13.3±2.2	17.3±1.1	+30
0.2×10	I.P.	14.0±1.1	18.3±1.1	+31
0.1×10	I.P.	14.0±1.6	18.3±1.1	+31
0.6×2	I.P.	14.0±1.1	19.0±4.2	+36
1.3×1	I.P.	15.2±1.2	20.6±1.7	+36
0.1×8 (hourly)	I.P.	16.1±0.9	24.6±1.9	+53

* Kindly supplied by Dr. W. Dunning, Miami.
Treatment commenced 24 hours after inoculation.
Each group representing five rats.

A single dose of 1.3 mg/kg was apparently as active as a similar dose spread over 10 days. The longest survival time followed treatment with hourly injections of VLB.

If treatment was delayed for 7–14 days when a leukemic blood picture was present, less protection was afforded by VLB. Single or multiple doses of VLB, from 0.3 to 0.8 mg/kg/day given intraperitoneally, had little effect on the survival time of the animals but did reduce the leukemic peripheral blood count and number of circulating tumor cells.

At the time of treatment the animals showed a peripheral leukemoid blood picture (total WBC, 45,000–60,000/cm. with 7–20 per cent tumor cells and excessive myeloid cells). Within 24 hours of administering a single dose of 0.65 mg/kg of VLB, or after two injections of 0.3 mg/kg, the peripheral white count had returned to normal values (13–20,000), tumor cells had disappeared, and the number of circulating myeloid cells was

within normal limits. Continued administration of VLB resulted in a severe leukopenia (WBC, 3000–5000/cm.). Cessation of treatment was followed by an abrupt return to a leukemoid state, often within 49–96 hours, regardless of the degree of leukopenia. At autopsy all animals showed much less enlargement of the spleen and liver than did the nontreated controls, and in some animals (at the higher doses) there was a decrease in the size of the subcutaneous tumors.

The livers of treated animals weighed 17–56 per cent less, and the spleens 48–64 per cent less than those of their respective nontreated control. The extent of this decrease was related to the time interval between tumor inoculation and the be-

OTHER BIOLOGICAL EFFECTS OF VLB

Effects of VLB on liver regeneration.—Sprague-Dawley rats (weight 200–520 gm.) were partially hepatectomized (5). The animals were maintained before and after the operation on a laboratory chow diet and water ad libitum. In most cases the animals of the treated groups were given daily intraperitoneal injections of VLB starting on the day following partial hepatectomy. At intervals ranging from 1 to 9 days the animals were sacrificed, and the livers were removed for weighing and metabolic and histological study. In one or two experiments, i.e., those of 1–2 days' duration, the alkaloid was administered either a few hours before or 24 hours before the operation.

TABLE 7

EFFECT OF VLB ON THE REGENERATION OF LIVER OF PARTIALLY HEPATECTOMIZED RATS

Exp. no.		Day										
		-1	0	1	2	3	4	5	6	7	8	9
1	Control			2.6(5)	4.1(6)	4.7(2)	5.2(2)	5.9(3)				
2	Treated		1.0*	2.25(2)								
3	"	1.0			4.35(2)							
4	"		0.7†		4.05(2)							
5	"			1.0	4.3(3)							
6	"			0.6	0.6	4.3(2)						
7	"			0.5	0.5	0.5	6.4(3)					
8	"			0.5			6.7(2)					
9	"				0.6	0.6		5.0(4)				
10	"			0.3	0.3	0.3	0.25	0.25	5.9(3)			
11	"			0.15	0.15	0.15	0.15	0.15		9.85(1)		
12	"			0.2	0.2	0.2	0.2	0.2	0.2			9.9(2)

* VLB administered 3 hours before operation.

† VLB administered 1½ hours before operation.

Rats were partially hepatectomized on day 0.

Figures in ordinary type are mean liver weights at autopsy.

Figures in italics are the daily doses of VLB in mg/kg.

Figures in parentheses indicate the number of animals used.

ginning of therapy. The greater the delay in the institution of treatment, the less was the difference in the weights of the treated vs. nontreated rats. The spleens of control rats showed a complete loss of normal architecture and exhibited a gross infiltration of tumor cells. The spleens of treated animals were less cellular and, while showing some infiltration by tumor, contained many areas devoid of tumor cells. The livers of nontreated animals showed dense, closely packed accumulations of tumor cells which were especially marked around the portal triad and extended deeply along the liver sinuses into the parenchyma. Livers of treated rats, on the other hand, showed large open areas between the sinuses, devoid of cells, and the areas of tumor infiltration in the portal triads were less extensive and contained fewer tumor cells.

Those animals sacrificed by the 4th or 5th day usually received on each injection 2–3 times the minimum dose of VLB (0.15–0.3 mg/kg), causing leukopenia in control animals. Since large doses were lethal beyond this period, the experiments of longer duration were made with smaller daily doses of VLB which were, however, sufficient to cause leukopenia.

The dosage and times of administration of VLB and the weights of the regenerating livers are shown in Table 7. In the earlier stages of regeneration (i.e., days 1–4, nos. 2–7), doses of VLB which eventually cause symptoms of marked toxicity did not inhibit liver regeneration significantly. The VLB-treated animals rapidly lost weight and had developed a pronounced leukopenia by the 3d or 4th day after the first injection. In some cases when treatment was continued

to the 4th or 5th day, there was a decrease in the amount of liver obtained on autopsy relative to the control (no. 9, Table 7). By this time the animals were in most cases sick and had lost about one-quarter of their original weight.

At the lower dose levels the animals lost weight less rapidly, although they developed leukopenia well before the end of the experiment (Table 8). In such cases, however, liver regeneration was uninhibited (Table 7, nos. 10, 11, 12).

The respiratory quotient of slices of the regenerating liver from the control and the VLB-treated animals has been measured in a number of experiments, e.g., in nos. 3, 5, 7, 9 (Table 7). No significant differences have been found between

in both mice and rats treated with VLB. Microscopic examination of the intestine failed to show any abnormal areas.

Tumor-bearing mice were apparently considerably more sensitive to VLB than the normal. Single intraperitoneal doses of 5–9 mg/kg in Swiss or C57 mice was followed by profound depression of peripheral blood, weight loss, but not death. Half the dose produced a moderate hematologic response only. Mice bearing tumors, however, showed signs of toxicity (weight loss, lethargy) at single doses of 2.0 mg/kg, and deaths occurred at doses at 3 mg/kg and above. The rat was apparently considerably more sensitive to the hemopoietic action of VLB and could not tolerate doses com-

TABLE 8
EFFECT OF VLB ON WEIGHT AND WHITE BLOOD COUNT IN PARTIALLY HEPATECTOMIZED RATS

Exp. no.	DAY										
	0	1	2	3	4	5	6	7	8	9	
Relative wt. % of pre-operative value											
1	100	93	92	93	95	97					
7	100	93	89	85	82						
9	100	90	84	79	76						
10	100	93	91	87	83	81	76				
12	100	94	89	87	86	84	81	79	77.5	76	
Relative WBC % of pre-operative value											
1	100	91	108	115	130	118					
7	100	203	110	68	47.5						
9	100		155	95	36	27.5					
10	100	133	72	56	29.5	32	16.5				
12	100	91	101	95	94	66	43.5	43	29	58.5	

The dose and period of administration of drug for Exp. nos. 7, 9, 10, and 12 are given in Table 7.

the treated and the untreated groups (to be published).

Effects of VLB on other tissues.—The antitumor activity of VLB could be demonstrated in mice without evidence of weight loss, toxicity, or damage to the hemopoietic system. The only side effect noted has been loss of hair at the site of subcutaneous injection in the BDF mice. Local hair loss was produced after injections of 0.8 mg/kg/day \times 10, and in 2 weeks the lost black hair had been replaced by white. These patches have remained white throughout the period of observation. The inadvertent intramuscular injection in some animals usually led to the development of a localized induration which later broke down and ulcerated. Such ulcers healed quickly, leaving an area of scar tissue devoid of hair. Changes in the gut have been strikingly absent

parable to those used in mice. Profound marrow depression has been observed in the rat with a single dose of 0.3 mg/kg of VLB. No evidence of developed resistance to the hemopoietic effects has been observed. Recovery after doses of VLB repeated at intervals has been prompt and is usually associated with a markedly elevated WBC. In a limited number of experiments the guinea pig has been found, like the rat, to be highly sensitive to the hemopoietic effects of VLB. Although the injection of VLB is usually well tolerated, in the experiments in which it was mixed with tumor cells and injected a severe tissue reaction and ulceration was frequently noted.

DISCUSSION

The findings which have been presented outline in some detail certain aspects of our experiences

with VLB. From the responses of all the tumors described it is apparent that this alkaloid has the property of profoundly affecting malignant cells. Although its primary action might have been anticipated to be on malignancy of the blood-forming organs, it has also shown definite effects on solid tumors. In our experience, with suitable doses, we have found only one tumor which was unaffected, and that was a rat sarcoma. The results of clinical testing on patients have confirmed that different types of tumors may be influenced (11). The response of tumor-bearing animals was influenced by the amount of VLB administered, and particularly by the spacing of the dosage. Curiously enough, single large injections proved to be almost as efficient as did spreading the doses over ten daily administrations. It seemed that doses given at hourly intervals were probably the most effective form of treatment. Other experiments, to be reported later, indicate that VLB as such may disappear extremely quickly from the blood so that its action on tumor cells may be very rapid or possibly exerted through some metabolite. The effectiveness of VLB in clearing the blood of circulating tumor cells in the leukemic Fischer rats was striking, even though its effect on the general progress of the disease was less dramatic. Such observations might suggest its possible practical value in destroying circulating tumor cells and so minimizing the spread of malignancy through this medium. With transplanted tumors, as might be expected, treatment was most effective when started 24 hours after tumor inoculation, although the development of some tumors was also delayed when treatment was withheld for 48 hours. On the other hand, the growth of spontaneous mammary tumors in C3H mice could be checked by suitable daily treatment with VLB. When administered orally to rats, VLB had little or no leukopenic action. In addition, crude fractions may be followed by severe convulsions and death when given by mouth. Such effects were not prevented by glucose administration. Injected VLB may cause a local reaction which is apparently accentuated under some conditions, such as when it is mixed with tumor cells. Although the injection of crude extracts frequently led to septicemia and multiple abscesses in rats in earlier experiments (9), no such effects have been observed after VLB administration.

The limiting factor to the employment of increased dosage of VLB in tumor-bearing animals has been the profound depression of the bone marrow. This action would appear to be curiously specific, affecting chiefly the myeloid leukocytes and having little effect on megakaryocytes. The

intestinal mucosa and regenerating liver cells were apparently little, if at all, affected. In a search for a mechanism of action of VLB it is difficult to envision a common metabolic factor in tumor and bone marrow cells which is not shared by other rapidly proliferating cells. However, biochemical investigations now in progress indicate that VLB may markedly affect metabolic processes which are peculiar to certain tissues. The experiments presented have shown that much larger doses of VLB are required to affect the hemopoietic tissue of the mouse than the rat so that it has allowed the more satisfactory treatment of tumors in this species. The guinea pig also appears to be relatively sensitive to the marrow-depressant action of VLB. This may prove to be a serious limiting factor in an attempt to treat malignancy in humans, unless effective chemical or physical means of protecting bone marrow can be found. Although an extensive study has not yet been made of the acute toxicity of VLB, evidence suggests that toxicity other than that referable to bone marrow depression may be shown by large doses. Deaths have occurred, particularly in rats, within 48 hours after VLB injection. Although the cause of death was not accurately determined, the circulating WBC and bone marrow appeared to be adequate for survival.

The observations on the apparent immunity of "cured" AK and BDF mice are of interest, even though their significance cannot be assessed at this time. The results with the inbred AK mice showed that mice which had been successfully treated with VLB could subsequently resist enormous challenging doses of the AKr leukemia. Although this resistance is obviously of a high magnitude, its specificity may be questioned, since the AKr leukemia arose in an AK mouse in a different laboratory. Of possibly greater interest is the apparent failure, so far, of AK mice resistant to the transplanted leukemia to develop the spontaneous disease. These observations, however, obviously require confirmation with comparable control groups.

Studies with VLB are still very limited, and only preliminary observations suggest possible mechanisms for its action, metabolism, and excretion. It would appear, however, from these observations, that VLB may offer new leads in the search for more effective chemotherapeutic agents. In our experience VLB represents only one of the active substances present in *Vinca rosea*. Other crude fractions have shown activity of a different nature (9), and one apparently exhibited antifolic action. The latter activity, however, has not been consistently obtained. A more recently obtained

fraction, devoid of VLB, depressed the blood count and also extended the survival time of mice with L1210 leukemia.

The rather unorthodox approach leading to the isolation of VLB has been recorded (9), but it is perhaps of interest to note that the assay of material for its capacity to depress the bone marrow of a normal animal led to the eventual isolation and use of VLB as an antitumor agent.

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