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Preclinical Studies with Vinglycinate, One of a Series of Chemically Derived Analogs of Vinblastine

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Summary

The antitumor activity of a series of 4-acyl and α-aminoacetyl analogs of vinblastine is described. Marked differences in activity and toxicity were observed. One of the α-aminoacetyl analogs, vinglycinate, produced therapeutic effects in experimental tumors similar to those observed with vincristine, including 100% "cure" of the P-1534 leukemia, but with a greatly reduced toxicity.

Introduction

The carbon skeletons of the clinically active dimeric alkaloids VLB[1] and VCR are known to be identical (9) and similar to 2 other experimentally active oncolytic Vinca alkaloids, vinleurosine (5) and vinrosidine (10). These 4 compounds represent naturally occurring modifications of the same basic structure, which lead to differences in tumor spectrum both experimentally and clinically as well as to differences in toxicologic manifestations. The difference in clinical spectrum and lack of cross resistance at the clinical level suggested that chemical modification of this basic structure might lead to other clinically active antitumor compounds with different, and perhaps more desirable, oncolytic activity and toxicologic effects. VLB was selected for initial chemical modification studies in a continuing program.

Materials and Methods

The chemistry involved in these studies has been presented in some detail (6) but is of such pertinence to the present study that it might be briefly summarized. The skeleton of VLB and VCR is seen in Chart 1. A number of 4-acyl analogs were made by substitution at *R* by the action of acid anhydrides on 4-deacetyl VLB, the starting material having been prepared from the parent alkaloid by the action of methanol and anhydrous hydrogen chloride. These ester analogs are known to have the same stereochemical configuration as the parent alkaloid in that VLB is regenerated from 4-deacetyl VLB by acetic anhydride.

A series of 4-aminoacetyl ester analogs of VLB was prepared from the 4-chloroacetate of deacetyl VLB by the action of various amines, replacing the α-chloro atom with amino functions.

All of these compounds were screened against the P-1534 leukemia, an acute lymphocytic neoplasm carried in DBA/2 mice. This tumor originally detected the oncolytic activity of the Vinca alkaloids and has been the most sensitive tumor to their activities. It has changed in sensitivity to vinblastine without changing the response to the other naturally occurring compounds (8).

The procedures used in antitumor testing in these laboratories have been previously described (7) but may be summarized as follows. Maximum tolerated levels of drugs to be administered daily for 10 days are determined in non-tumor-bearing mice based on a 5-day subacute toxicity test. Solid tumors are transplanted s.c. by trocar, and ascitic tumors and most leukemias are implanted by the i.p. injection of a standard cell suspension. Treatment is initiated 24 hr after implantation and is continued daily for 10 days. The effect of drug therapy against solid tumors is evaluated by comparing mean tumor diameters of treated groups with those of saline-treated control groups. In leukemias and ascitic tumors, the comparison is of average survival times to those of control groups. Animals which die earlier than saline controls are considered toxic deaths and are not used in calculating the % prolongation.

Results

The activity of the 4-acyl analogs against the P-1534 leukemia is seen in Table 1. For the most part, there was no significant change in activity compared with the parent compound VLB. The 2 possible exceptions were the propionyl and chloroacetyl derivatives. While their activity is slightly superior to the current responses of this tumor to VLB, they were not as effective as VLB prior to the P-1534 changing sensitivity to VLB.

The responses of the P-1534 leukemia to the α-aminoacetyl analogs of VLB are seen in Table 2. There were marked changes in toxicity and activity. The 3 most toxic derivatives, diethylaminoacetyl, piperazinoacetyl, and *N*-phenylpiperazinoacetyl, were inactive. The 3 most effective were the dimethylaminoacetyl, methylcyclopropylaminoacetyl, and methylaminoacetyl derivatives. The dimethylaminoacetyl derivative, VGL, was chosen for more extensive evaluation because of the high incidence of "cures" or indefinite survivors and excellent oral activity (Tables 3 and 4). Oral levels at least 10-fold higher than the i.p. levels were required with the other active derivatives to demonstrate any or comparable activity. Vinglycinate gave good p.o. activity at levels essentially equal to the optimal i.p. dose. The optimal p.o. level was approximately 1.5 times the optimal i.p. dosage. Dose-related response and reproducibility of activity are also demonstrated in Tables 3 and 4.

[1] The following abbreviations are used: VLB, vinblastine; VCR, vincristine; VGL, vinglycinate; CNS leukemia, central nervous system leukemia; and SGOT, serum glutamate oxaloacetate transaminase.

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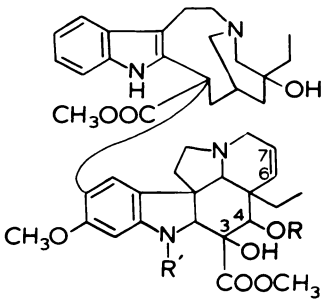

R = Acetyl, R' = CH_3 Vinblastine
R = Acetyl, R' = CHO Vincristine

CHART 1. Carbon skeleton of active Vinca alkaloids.

TABLE 1

ACTIVITY OF 4-ACYL ANALOGS OF VINBLASTINE AGAINST THE P-1534 LEUKEMIA

R (Chart 1)	Dose (mg/kg)	Av. life (T/C)[a]	% prolongation	Indefinite survivors
Propionyl	0.55	27.0/15.2	>77 (1 survivor)	1/5
Butyryl	0.75	21.7/14.2	52 (1 survivor)	1/5
Isobutyryl	0.9	19.2/14.2	35	0/5
Phenylacetyl	2.2	17.8/14.2	25	0/5
Benzoyl	0.75	17.2/13.0	32	0/5
Chloroacetyl	0.35	34.6/15.2	127	0/5
Dichloroacetyl	0.3	19.8/14.4	37	0/5
Cyanoacetyl	0.3	19.0/14.4	31	0/5
Acetyl (VLB)[b]	0.45	27.4/14.6	87	0/5

[a] T/C, treated/control.
[b] VLB, vinblastine.

Most clinically effective antitumor drugs must be capable of affecting or moderating the disease in its terminal stages. Experimentally, this is mimicked by delaying therapy until the implanted malignancy is well established. Activity was demonstrable by delayed therapy (Table 5). Smaller repetitive treatment appeared superior to fewer larger doses even at the same total dosage. Efficacy was demonstrated against the s.c. implanted P-1534 tumor as well as the intracranially implanted tumor. The latter is used to mimic both CNS leukemia and tumors of the brain as either primary or secondary lesions.

Clinically active antitumor drugs are also usually active against a spectrum of tumors, although greatest activity is frequently demonstrable against a fairly limited spectrum. Vinglycinate was active against a series of other leukemias and ascitic tumors, including the virus-induced Rauscher leukemia (Table 6). Vinglycinate was ineffective against a series of solid tumors except for the Gardner lymphosarcoma (Table 7).

TABLE 3

ACTIVITY OF VINGLYCINATE AGAINST THE P-1534 LEUKEMIA

Dose (mg/kg) and frequency	Av. wt. change (gm) (T/C)[a]	Av. survival time (days) (T/C)	% prolongation	Indefinite survivors
1.0 × 10	0.4/−1.7	20.7/14.0	47	0
1.5 × 10	1.7/−0.6	18.0/13.2	36	0
2.0 × 10	1.0/−1.7	21.2/14.0	51	0
3.0 × 10	2.3/0.5	32.6/14.6	123	0
3.0 × 10	−1.6/−1.7	>49.0/14.0	>200	5/5
3.75 × 10	2.1/0.5	41.6/14.6	184	0
4.0 × 10	0.4/−1.7	22.0/14.0	57	3/5
4.26 × 10	2.6/0.5	38.2/14.6	161	1/5
5.0 × 10	1.7/0.5	>45.0/14.6	>200	5/5
7.5 × 10	−0.8/−0.6	24.5/13.2	85	1/5
10.0 × 10	Toxic	Toxic	0	0/5

[a] T/C, treated/control.

TABLE 4

ORAL ACTIVITY OF VINGLYCINATE AGAINST THE P-1534 LEUKEMIA

Dose (mg/kg) and frequency	Av. wt. change (gm) (T/C)[a]	Av. survival time (days (T/C)	% prolongation	Indefinite survivors
6.0 × 10	−1.2/−2.0	22.8/14.0	62	0/5
7.5 × 10	0.6/−1.0	27.0/13.0	107	0/5
8.0 × 10	−2.4/−2.0	28.8/14.0	105	0/5
10.0 × 10	−5.2/−2.0	35.5/14.0	153	3/5
15.0 × 10	Toxic	Toxic	0	0/5

[a] T/C, treated/control.

TABLE 2

ACTIVITY OF 4-AMINOACETYL ANALOGS OF VINBLASTINE AGAINST THE P-1534 LEUKEMIA

R (Chart 1)	Dose (mg/kg)	Av. life (T/C)[a]	% prolongation	Indefinite survivors
Methylaminoacetyl	3.0	36.5/13.0	187	3/5
Dimethylaminoacetyl	5.0	>45.0/14.6	∞	5/5
Methylcyclopropylaminoacetyl	3.75	41.0/13.6	201	4/5
Diethylaminoacetyl	1.5	14.4/15.8	0	0/5
Pyrrolidinoacetyl	7.5	27.6/13.8	100	0/5
Piperidinoacetyl	3.0	16.2/13.0	24	0/5
Morpholinoacetyl	15.0	29.6/13.0	127	0/5
Piperazinoacetyl	1.5	15.0/14.2	0	0/5
N-Methylpiperazinoacetyl	15.0	18.6/14.2	30	0/5
N-Phenylpiperazinoacetyl	1.5	16.4/14.2	0	0/5
β-Hydroxyethylpiperazinoacetyl	30.0	35.0/12.6	177	2/5

[a] T/C, treated/control.

TABLE 5

DELAYED, SINGLE AND TRIPLE DOSE i.p. THERAPY OF P-1534 WITH VINGLYCINATE INCLUDING INTRACRANIALLY AND s.c. IMPLANTED P-1534

Dose (mg/kg) and frequency	Days after tumor implantation treatment initiated	Av. wt. change (gm) (T/C)[a]	Av. survival time (days) (T/C)	% prolongation	Indefinite survivors
3.0 × 10	3	0.2/−1.7	30.7/14.0	119	1/5
3.0 × 10	6	−4.2/−1.7	24.2/14.0	72	0
3.0 × 10	10		13.6/14.0	0	0
5.0 × 10	3	−3.1/−2.0	30.0/14.0	114	0
5.0 × 10	6	−3.1/−2.0	17.0/14.0	21	0
5.0 × 10	10	−3.1/−2.0	12.8/14.0	0	0
20.0 × 1	1	−1.1/−1.7	23.4/14.0	67	0
30.0 × 1	1	Toxic			
30.0 × 1	3	−3.0/0.2	29.5/14.6	102	0
30.0 × 1	6	−1.2/−2.0	13.8/14.0	0	0
30.0 × 1	10	−3.0/−2.0	13.0/14.0	0	0
10.0 × 3	1, 6, and 10	−0.2/−1.7	31.0/14.0	121	4/5
3.0 × 10[b]	1	−2.9/−1.7	21.2/14.0	51	0
4.5 × 10[c]	1	−1.0/0.8	35.0/14.2	140	1/5
15.0 × 3[c]	1, 6, and 10	−1.5/0.8	10.0/14.2	0	4/5

[a] T/C, treated/control.
[b] Intracranially implanted P-1534.
[c] Subcutaneously implanted P-1534.

Vinglycinate was tested for metaphase arresting capacity since this biologic activity is common to all of the experimentally active Vinca alkaloids. The results were obtained by exposing the cultures to the drugs for 16 hr. In the results given in Tables 8 and 9, the cells were stained by hematoxylin and eosin, and in those recorded in Table 10, by a chromosome preparation according to the method of Puck and Steffen (11). The duration of metaphase arrest was not studied.

Toxicologic studies of vinglycinate have not been published in detail, but some comparisons to vinblastine and vincristine can be made. In general, the action of vinglycinate is similar to that exerted by the parent compound, vinblastine.

The acute i.v. LD_{50} in mice was approximately 17.0 and 50.0 mg/kg for VLB and VGL, respectively. The acute i.v. LD_{50} for VGL was more variable than for VLB. The acute i.v. LD_{50} of VGL in rats and rabbits was approximately 45.0 and 4.4 mg/kg, respectively, and between 2.0 and 7.5 mg/kg for dogs.

In subacute studies in rats, 0.3, 0.75, and 1.5 mg/kg were administered 5 times weekly over 25 days to groups of 10 animals of each sex on each level. There was no mortality or significant differences in growth rates except that the males on the 2 higher dosages were moderately retarded. Hematologically, anisocytosis, hypochromia, and polychromasia were frequently observed. Leukocyte counts varied on all dosage levels. Hb and HTC were often depressed, but recovery occurred. Changes in erythrocyte counts did not always parallel those in Hb. Histologically, tissues of all animals were normal at necropsy except for thymic atrophy in animals of each sex on the highest dose. These findings are similar to those with VLB (7) except that, with the latter compound, males rather than females were either less affected or unaffected in terms of growth and hematologic effects.

Dogs received VGL i.v. daily at 0, 0.1, 0.25, and 0.5 mg/kg for 28 days and at 0.5, 1.0, and 2.0 mg/kg for 14 days. One male and 1 female were on each dosage in the 14-day study, and 2 animals of each sex, in the 28-day study. In the higher level 14-day study, 1 dog died after 4 days, 2 were killed after 6 days, 1 after 10 days, and the remaining 2 after 14 days. In the lower level 28-day regimen, 1 animal was killed after 22 days, and all other animals survived until termination of the study. In the 14-day study, all animals lost from 5 to 10% body weight. In the 28-day study, both drug-treated and saline-treated animals registered gains and losses. The higher gains and losses were in the drug-treated group. There were no changes in organ weight to body weight ratio except in testes of 4 dogs in the 28-day study. All dogs developed

TABLE 6

ACTIVITY OF VINGLYCINATE AGAINST OTHER LEUKEMIAS AND ASCITIC TUMORS

Tumor	Host	Dose (mg/kg) and frequency	Av. wt. change (gm) (T/C)[a]	% prolongation	Indefinite survivors
L-1210 leukemia	DBA/2	4.5 × 10	−0.2/3.5	34	0/5
L-5178Y leukemia	DBA/2	4.5 × 10	−5.9/0.2	0	0/5
C-1498 leukemia	C57BL/6	4.5 × 10	1.2/0.4	0	0/5
C-1498 leukemia	C57BL/6	15.0 × 3	0.2/0.4	25	0/5
B-82A leukemia	C58	4.5 × 10	1.2/2.4	36	2/10
B-82A leukemia	C58	15.0 × 3	0.2/2.4	50	3/10
Leukemia	AKR	4.5 × 10	−1.4/1.1	33	0/10
Leukemia	AKR	15.0 × 3	−3.0/1.1	0	1/5
Rauscher	BALB/c	4.5 × 10	0.8/2.4	81	0/5
Rauscher	BALB/c	15.0 × 3	−2.3/−	86	2/5
Ehrlich ascitic	ICR	4.5 × 10		0	2/5
Ehrlich ascitic	ICR	15.0 × 3		0	2/5
Freund ascitic	ICR	4.5 × 10	4.1/4.9	0	2/5
Freund ascitic	ICR	15.0 × 3	1.2/4.9	57	3/5
Sarcoma 180 ascitic	ICR	4.5 × 10	3.8/7.4	∞	5/5
Sarcoma 180 ascitic	ICR	15.0 × 3	2.7/7.4	∞	5/5

[a] T/C, treated/control.

TABLE 7

ACTIVITY OF VINGLYCINATE AGAINST SOLID TUMORS

Tumor	Host	Dose (mg/kg) and frequency	Av. wt. change (gm) (T/C)[a]	% inhibition
Sarcoma 180	ICR	4.5 × 10	3.5/3.7	0
Sarcoma 180	ICR	15.0 × 3	−0.2/3.7	31
Adenocarcinoma 755	C57BL/6	4.5 × 10	0.1/1.0	0
Adenocarcinoma 755	C57BL/6	15.0 × 3	0.2/1.0	0
Mecca lymphosarcoma	AKR	4.5 × 10	1.4/1.4	0
Mecca lymphosarcoma	AKR	15.0 × 3	0.6/1.4	0
Ridgeway osteogenic sarcoma	AKR	4.5 × 10	1.0/0.8	0
Ridgeway osteogenic sarcoma	AKR	15.0 × 3	−0.7/0.8	0
Gardner lymphosarcoma	C3H	4.5 × 10	5.1/7.7	59 (+4 regressions)
Gardner lymphosarcoma	C3H	15.0 × 3	1.5/7.7	0 (+2 regressions)
Lilly mammary	C3H	4.5 × 10	0.6/2.3	0
Plasmacytoma X5563	C3H	15.0 × 3	−0.6/1.0	0
Plasmacytoma X5563	C3H	4.5 × 10	−0.9/1.0	0
High malignancy clone	C3H	4.5 × 10	−2.7/−1.2	0
Melanoma S91	DBA/1	4.5 × 10	−2.1/1.9	0
Melanoma S91	DBA/1	15.0 × 3	0.2/1.9	0
Lilly mammary	DBA/1	4.5 × 10	1.4/0.1	0
Lilly mammary	DBA/1	15.0 × 3	1.4/0.1	0
Walker 256 sarcoma-carcinoma	Rat	1.2 × 10	68/78	0
Lilly rhabdomyosarcoma	Rat	1.2 × 10	60/70	42

[a] T/C, treated/control.

TABLE 8

EFFECT OF ALKALOIDS ON MITOSIS OF HELA CELLS (HEMATOXYLIN-EOSIN STAIN)

Compound[a]	Concentration (μg/ml)	Metaphases/1000 cells		% control metaphases	
		Normal	Ball type	Normal	Total
VLB	200	TT[b]			
VLB	20	288	345	560	1300
VLB	2	267	192	524	900
VLB	0.2	147	16	288	330
VCR	200	TT			
VCR	20	155	149	300	590
VCR	2	28	170	55	400
VCR	0.2	40	264	78	590
VGL	200	445	324	875	1500
VGL	20	500	240	1000	1470
VGL	2	448	242	880	1360
VGL	0.2	232	29	455	835
Control		51	0		

[a] VLB, vinblastine; VCR, vincristine; VGL, vinglycinate.
[b] Too toxic.

TABLE 9

EFFECT OF ALKALOIDS ON MITOSIS OF CHINESE HAMSTER OVARY CELLS (HEMATOXYLIN-EOSIN STAIN)

Compound[a]	Concentration (μg/ml)	Metaphases/1000 cells		Total metaphase (% of control)
		Normal	Ball type	
VLB	2.0	Toxic		
VLB	0.2	Toxic		
VLB	0.02	Toxic 60	70	148
VLB	0.002	80	6	100
VCR	2.0	Toxic		
VCR	0.2	Toxic 72	132	244
VCR	0.02	Toxic 20	260	318
VCR	0.002	Toxic 108	64	195
VGL	2.0	Toxic 16	232	280
VGL	0.2	0	152	173
VGL	0.02	100	56	180
VGL	0.002			
Control		68	20	

[a] VLB, vinblastine; VCR, vincristine; VGL, vinglycinate.

leukopenia in this study. In the 28-day study, there were no major hematologic changes at the 0.1-mg/kg level; 1 of 4 showed moderate marrow depression with recovery on weekends; at 0.5 mg/kg, 3 of 4 dogs showed marrow depression, 2 of them markedly.

Some elevation of SGOT and alkalinephosphatase was observed in animals on the 28-day regimen and appeared to be dose related. Histologically, some animals showed slight epithelial cell proliferation in small- and medium-sized bile ducts. In the 28-day treatment group, 4 males showed arrest of spermatogenesis.

Discussion

The efficacy of vinglycinate in treatment of the P-1534 leukemia suggested a therapeutic effect similar to vincristine and superior to vinblastine. With vinglycinate and vincristine, we were able to achieve 100% "cures" or indefinite survivors. With vinblastine, only a rare "cure" was achieved. The optimal dose

TABLE 10

EFFECT OF ALKALOIDS ON CHINESE HAMSTER OVARY CELLS (CHROMOSOME PREPARATION)

COMPOUND[a]	CONCENTRATION (μg/ml)	METAPHASES/1000 CELLS	
		Number	% of controls
VLB	2.0	64	114
VLB	0.2	140	250
VLB	0.02	104	217
VLB	0.002	52	93
VCR	2.0	138	247
VCR	0.2	160	288
VCR	0.02	160	288
VCR	0.002	126	224
VGL	2.0	90	160
VGL	0.2	146	300
VGL	0.02	148	310
VGL	0.002	84	175
Control		56	100

[a] VLB, vinblastine; VCR, vincristine; VGL, vinglycinate.

required for "cures" was approximately 20-fold higher than that of vincristine and 10 times the effective dose of vinblastine.

Vinglycinate was slightly less effective than vincristine in delayed therapy studies. "Cures" of the P-1534 leukemia could frequently be achieved with treatment initiated as late as 6–10 days after inoculation with leukemic cells. Vinglycinate generally produced prolongation but not cures, except when larger but less frequent dosage regimens were used.

There were significant differences from the other 2 clinically active alkaloids in spectrum of tumors affected by vinglycinate. Vinblastine was completely effective against the Ehrlich, Freund, and S-180 ascites. Vincristine was ineffective against the Ehrlich, moderately effective against the Freund, and completely effective against the S-180 ascites (7). Vinglycinate appeared more like vincristine in this series. Like vincristine, vinglycinate affected the virus-induced Rauscher leukemia, which is insensitive to vinblastine. Slight activity was seen against the L-1210 leukemia in contrast to both vincristine and vinblastine, which are ineffective. A further difference between VLB and VGL was observed in the L-5178Y leukemia, in which vinblastine was active and vinglycinate was not.

In the solid tumor systems studied, vinglycinate was essentially ineffective except against the Gardner lymphosarcoma, a tumor markedly sensitive to L-asparaginase (1). This limited experimental solid tumor spectrum more nearly resembles vinblastine, although vinblastine was ineffective against the Gardner tumor.

Mechanism studies have not been made. However, vinglycinate is a potent arrester of metaphase, as are the other clinically active and inactive dimeric Vinca alkaloids. Studies in polyploid HeLa cells and diploid Chinese hamster ovary cells indicate that vinglycinate is a more potent but less cytotoxic inducer of metaphase arrest than either vinblastine or vincristine. While duration of metaphase arrest was not studied in this series, several investigators have reported that cells exposed to Vinca alkaloids are able to recover from the metaphase arrest and that the duration varies with the alkaloid.

The relationship of metaphase arrest to systemic antitumor activity of these compounds is still not clear. In spite of the fact that all of the "active" alkaloids studied are nearly equivalent in mitotic activity, they differ in tumor spectrum and pharmacologic effects. Tumors which are exquisitely sensitive to VLB may be resistant to VCR as reported by Hutchinson in the paper of Johnson *et al.* (7). Creasey and Markiw (3, 4) have reported essentially similar metabolic findings with VLB, VCR, and colchicine. Colchicine, however, does not give the systemic antitumor responses of either VLB or VCR. The experimental tumor most sensitive to the Vinca alkaloids (P-1534) is insensitive to colchicine.

While it is difficult to think that such minor chemical differences will provide compounds of entirely different mechanisms of action, we are still struck by the lack of cross resistance at the clinical level and differences in sensitivity and resistance at the experimental level. There are precedents for lack of cross resistance among closely related compounds such as the phthalanilides. In addition, in Burchenal *et al.*'s study of these compounds (2), they demonstrated cross resistance between 1 phthalanilide and VLB but not with VCR.

There is also the precedent in the case of steroids of a large multiringed nucleus being required for biologic activity but relatively minor chemical changes around the nucleus resulting in major differences in biologic activity (androgenic, estrogenic, anabolic, catabolic, anti-inflammatory, K^+ retention, Na^+ excretion, etc.) and distribution. It is attractive, by analogy to the steroids, to consider the dimeric skeleton of these alkaloids as being necessary for biologic activity with minor changes around the skeleton, resulting in marked changes in biologic and toxicologic manifestations. The naturally occurring alkaloids suggested toxicity, and antitumor activity was not necessarily inseparable; the synthetically derived compounds tend to confirm this observation (Table 2). These experimental studies and clinical experience continue to support the probable utility of a program of molecular modification of these unique compounds.

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