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CORRELATION OF BIOLOGIC DATA WITH PHYSICO-CHEMICAL PROPERTIES AMONG THE VINCA ALKALOIDS AND THEIR CONGENERS

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Abstract—Displacement of [^{3}H]vinblastine binding to tubulin by other Vinca alkaloid derivatives has been demonstrated to be a competitive process, allowing for determination of the association constant of each drug. Correlation of LD_{50} data and anti-*P*-388 activity was found with log *P* and log K_a according to the equations: $\log LD_{50} = 0.129\ (\log P)^2 - 0.522\ \log P - 0.479\ \log K_a + 4.652$ $\log P - 388 = 0.222\ (\log P)^2 - 1.059\ \log P - 0.520\ \log K_a + 5.366$. Vincristine and desacetylvinblastine were the two most active agents in this series. That the latter drug had significant biologic activity was of considerable interest, since it is known to be a human metabolite.

Our laboratory has been engaged in studies on the pharmacology of the Vinca alkaloids, and as part of our continuing interest we have studied the correlation of the biologic activity of these drugs with certain of their physico-chemical properties. We evaluated two particular parameters: the binding affinity of these drugs for tubulin, their purported site of intracellular action [1–3]; and the partition coefficient of Hansch *et al.* [4–6], which relates the lipophilicity of a closely related group of drugs to their biologic response.

We have previously described the interaction of vinblastine (VLB) and vincristine (VCR) with the microtubular protein, tubulin, at low (10^{-7} to 10^{-6} M) concentrations [1, 2]. Within the cell, such interaction with tubulin is important, since the drug-protein complex can no longer form intact microtubules [1–3, 7]. This interaction in effect reduces the size of the available soluble pool of tubulin, leading to a break-down and/or prevention of formation of critical microtubular elements. This action, in turn, disrupts intracellular processes dependent upon these microtubular elements. Thus, the mitotic spindle apparatus in dividing cells and the neurotubules in neural tissue are disrupted, leading to cell death or malfunction.

In our present work, we have expanded these studies to correlate antitumor and LD_{50} activity of these drugs in mice with their affinities for tubulin, as measured by the K_a, and their partition coefficients.

MATERIALS AND METHODS

Protein preparations. Fresh pig brain was homogenized 1:1 by weight with a Lyton-glass homogenizer in either 0.24 M sucrose, 0.01 M Na_2HPO_4/NaH_2PO_4 (pH 6.5), 0.1 mM GTP, 0.01 M $MgCl_2$ (SPGM buffer); or 0.10 M 2(*N*-morpholino)-ethane sulfonic acid (adjusted to pH 4.6 with NaOH), 1.0 mM EGTA, 1.0 mM GTP, and 0.5 mM $MgCl_2$ (MEGM buffer); or 0.10 M piperazine-*N*-*N*'-bis[2-ethane sulfonic acid], 1.0 mM EGTA, pH 6.95 (PE buffer). The homogenate was centrifuged at 100,000 *g* for 60 min and the supernatant was used as a crude tubulin solution. Purification of crude tubulin was accomplished by three cycles of reassembly-disassembly in MEGM buffer, as described earlier [2]. All operations were carried out at 4°.

Alkaloids. (See Fig. 1) VLB sulfate, VCR sulfate, vinrosidine (VRD) sulfate, vinleurosine (VLR) sulfate, dihydrovinblastine (HVLB) sulfate, desformylvincristine (DVCR) sulfate, vinglycinate (VGL) sulfate, and vindoline were kindly supplied by the Eli Lilly Company. Vindesine (desacetylvinblastine amide) (DVA) sulfate, desacetylvinblastine hydrazide (DVH) sulfate and desacetylvinblastine hydroxyethylamide (DVEA) sulfate were prepared and supplied to us by Dr. K. Gerzon and G. Cullinan of the Eli Lilly Research Laboratories [8–10]. Desacetylvinblastine (DVLB) was prepared by the method of Hargrove *et al.* [11]. Radiolabeled VLB was prepared as described earlier [2].

Competition experiments. These experiments were performed by adding an isotopically unlabeled alkaloid to displace [^{3}H]VLB from its tubulin binding sites. To a 100 μl portion of the protein solution (approximately 1 mg/ml for the crude tubulin solutions, and 0.1 mg/ml for the purified tubulin solutions) diluted with 850 μl of 0.1 M Na_2HPO_4/NaH_2PO_4 buffer (pH 6.5), was added 25 μl of the first alkaloid solution (7.2×10^{-5} M), and the mixture was incubated for 15 min at 37°. At this point, 25 μl of the second alkaloid solution (7.2×10^{-5} M) was added, and incubation was continued at 37° for an additional 60 min. Two sets of determinations were run for each alkaloid, one with the order of addition reversed, so that [^{3}H]VLB was added first in one experiment, and the unlabeled alkaloid added first in the second experiment. The alkaloid-protein complex was adsorbed onto DEAE (Whatman DE81) paper [1, 2], and after three 5 ml rinses with the above PO_4 buffer, the paper

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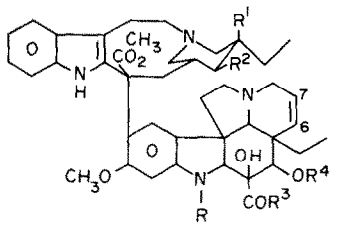
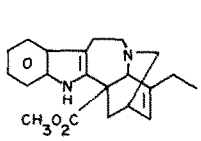
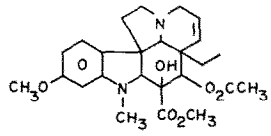


Drug	R	R^1	R^2	R^3	R^4	
VLB	CH_3	OH	H	OCH_3	$COCH_3$	
VCR	CHO	OH	H	OCH_3	$COCH_3$	
DVLB	CH_3	OH	H	OCH_3	H	
DVA	CH_3	OH	H	NH_2	H	
DVH	CH_3	OH	H	$NHNH_2$	H	
DVEA	CH_3	OH	H	$NHCH_2CH_2OH$	H	
VGL	CH_3	OH	H	OCH	$COCH_2N(CH_3)_2$	
VRD	CH_3	H	OH	OCH_3	$COCH_3$	
VLR	CH_3	—O—		OCH_3	$COCH_3$	
HVLB	CH_3	OH	H	OCH_3	$COCH_3$	$(6,7\text{-}H_2)$
DVCR	H	OH	H	OCH_3	$COCH_3$	



Catharanthine



Vindoline

Fig. 1. Structures of the Vinca alkaloids.

was counted directly in 10 ml of Bray's scintillation fluid. To obtain data for the modified Dixon plots, varying amounts of [^{3}H]VLB and the competing drug were added simultaneously to 100 μl of the protein solution, as above, and after 60 min of incubation at 37°, adsorbed onto DEAE paper and counted in the same manner.

Partition coefficients. 1-Octanol was purified by three successive washes each with dilute $NaHSO_3$, 1 N HCl, 1 N NaOH, 1 N HCl, and distilled water, followed by filtration through activated charcoal, and vacuum distillation at 20 mm Hg. The center fraction was collected and provided 1-octanol with no u.v. absorption (vs. H_2O) from 200 to 350 nm.

Each alkaloid, as its free base, was dissolved in octanol and equilibrated with 0.1 M sodium phosphate buffer, where in separate determinations, the pH of the buffer was varied from 6.0 to 8.0 at intervals of 0.2 pH units. The amount of drug in each layer was determined by comparison of the u.v. spectrum with standard solutions in the same solvent. In general, the concentration in the aqueous phase approximated 10^{-5} M. The octanol layer was diluted approximately 20-fold with fresh octanol for the u.v. measurements to be on scale. From the reported [12, 13] pKa values for VLB, VCR, VLR and VRD, and our measured value for VGL, we calculated the amount of free base available in the aqueous phase. All the values were then corrected to reflect only the distribution of nonionized drug as log P^*. For the alkaloids, HVLB, DVLB, DVA, DVEA, and DVCR, the log P^* values were calculated from the value for either VLB or VCR as the parent, using standard π values for the functional group differences as shown in the Appendix [14–17].

LD_{50} data. Graded doses of each alkaloid, dissolved in saline, were administered i.p. to 6–12 week old BD_2F_1 female mice. The LD_{50}'s were calculated in the standard manner. The mice were observed for evidence of neurotoxicity, as described by Uy *et al.* [18].

Antitumor activity. Female BD_2F_1 mice (6–12 weeks old) were inoculated i.p. with 1×10^6 *P*-388 lymphocytic leukemia cells. Three days later, the appropriate drug was administered i.p. at various dose levels, centering around a dose corresponding to one-half of the LD_{50}. Survival in days was determined for each group of treated animals, and compared to the control group (ILS = treated/control × 100).

PKa values. A 10 mg solution of VGL in 10 ml of 25% DMF was titrated with 1 N HCl in the usual manner, measuring the pH with a Radiometer pH meter.

RESULTS

Data on the partitioning of VLB, VCR, VLR, VRD and VGL between octanol and water are presented in Fig. 2. Straight lines resulted for all compounds when the logarithm of the ratio of octanol to water concentrations was plotted vs. pH.

Since only the nonionized portion of a drug is available for aqueous-to-lipid transfer, the partitioning data in Fig. 2 were corrected to reflect the distribution of free base alone, assuming that the octanol phase contained only free base (reasonable, since

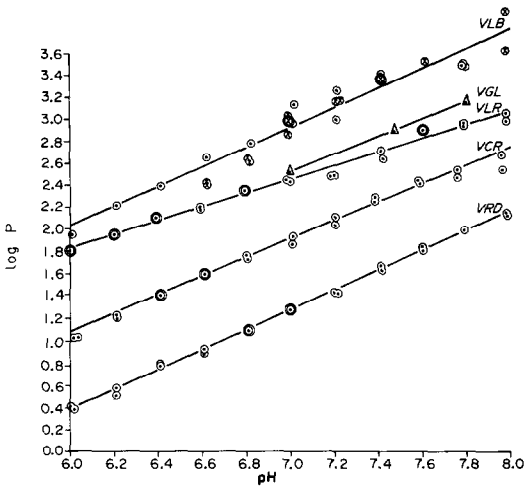


Fig. 2. Octanol-water partition coefficients of VLB, VGL, VLR, VCR and VRD vs. pH. The drugs were equilibrated with octanol and 0.1 M sodium phosphate buffer adjusted to the respective pH values. Both phases were examined by u.v. spectral absorption to determine the concentration of drug present.

< 5% of the octanol concentration was found in octanol equilibrated with crystalline drug sulfate). The pKa values used were: VLB, 5.4 and 7.5 [12]; VCR, 5.0 and 7.4 [13]; VLR, 5.5 and 7.5 [12]; VRD, 5.0 and 8.8 [13]; and VGL, 5.5 and 8.5 (measured). The fraction $[B]/[HB^+] + [HB^{2+}]$ was calculated from these pKa values for each alkaloid at each pH value. The log P value was then divided by the log of this fraction to give log P^*, and the data are plotted in Fig. 3. As expected, these lines were relatively flat, with log P^* values that varied little over the pH range. The two drugs VCR and VRD, however, showed minor breaks from linearity. Though these breaks are not fully explained, they may relate to inaccuracies in determining the pKa's, as the values for these two drugs were determined in aqueous-dimethylformamide solution, as opposed to aqueous solution alone for VLB and VLR. The value for DVH was measured at pH 7.4 only. Starting with the log P^* values of VLB or VCR at pH 7.4, the log P^* values for DVLB, HVLB, DVA, DVEA and DVCR were calculated. Values for the log P^* at pH 7.4 for all the drugs are listed in Table 2.

We have demonstrated that VLB binds tightly to porcine tubulin with a Ka of from 5.0 to 6.3 $\times 10^6$ L/M [1, 2], when measured under specific conditions. Assuming that the other Vinca derivatives bind to the same site on this protein, it should be possible to measure the tightness of binding of each of the Vinca derivatives to tubulin, by measuring the extent to which they will competitively displace [^{3}H]VLB from this site, and a preliminary report of this finding has appeared [19]. To evaluate this system, we first studied the competition of isotopically unlabeled VLB for the binding of [^{3}H]VLB. A crude tubulin preparation was incubated with [^{3}H]VLB for 15 min at 37 (this reaction is essentially complete in < 7 min [1]). The mixture was then diluted with isotopically cold VLB, such that the concentration of labeled and unlabeled drug was equal at 1.80×10^{-6} M (total concentration = 3.60×10^{-6} M). An identical set of samples was prepared in which the isotopically unlabeled drug was added first, followed by the [^{3}H]VLB. The final mixtures were again incubated at 37° for varying periods of time, and the amount of bound alkaloid was determined by adsorption onto DEAE filter paper, as reported earlier [1, 2]. It was found that 60 min were required to establish equilibrium. Longer times did not alter the equilibrium, but led to gradual decreases in bound counts, as the protein-drug complex slowly degraded. At the final concentration of 3.6×10^{-6} M, 93.8% of the tubulin is bound by VLB.

Having demonstrated that [^{3}H]VLB could be competitively displaced to equilibrium by unlabeled VLB, we then proceeded to evaluate the other available Vinca derivatives by measuring the displacement of [^{3}H]VLB at equimolar concentration. Each sample was run in duplicate pairs, with the order of addition of the alkaloids reversed in the second set. Thus, two samples were run with [^{3}H]VLB added first, and after 15 min of incubation, the second alkaloid was added. Another pair of samples was prepared with other alkaloids added first, followed by [^{3}H]VLB. After 60 min of further incubation, there was no statistically significant difference between the pairs, and all the data were averaged.

In these experiments, we varied both the method of preparation and the purity of the tubulin protein, and the results obtained are shown in Table 1. The data in the first three columns were determined as the percent of dis/min bound vs. the dis/min bound by a sample that was 3.60×10^{-6} M in [^{3}H]VLB alone. This value was not identical to the dis/min bound with 1.80×10^{-6} M [^{3}H]VLB alone, because of the variation in binding vs. concentration seen earlier in Scatchard plots [1, 2]. The data in the remaining columns were normalized, taking as 100% a value twice the dis/min obtained for the mixture of equimolar [^{3}H]VLB and unlabeled VLB. Note that crude brain extracts behaved identically with reassembly purified tubulin, and that buffer changes (MEGM vs. SPGM), aging for 8 hr, and freeze-thawing the brain beforehand, had no significant effect upon the competitive binding data, even though the number of

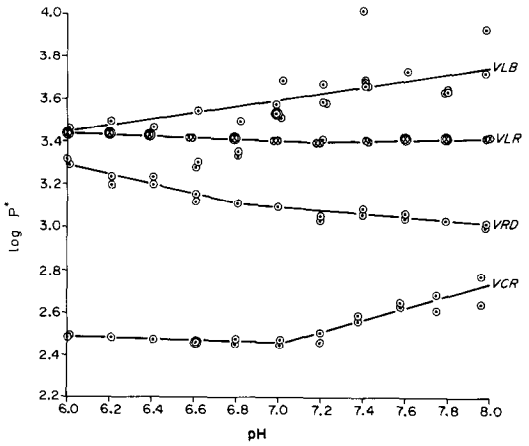


Fig. 3. Corrected octanol-water partition coefficients. The values in Fig. 2 were corrected to reflect the amount of free base in the aqueous phase, and assuming all drug in the octanol phase was the free base.

Table 1. Competition between [^{3}H]VLB and Vinca congeners for the porcine tubulin binding site

Drug	Tubulin preparation MEGM*	SPGM*	MEGM*	MEGM†	MEGM*	MEGM*‡	MEGM*§	SPGM*	PE*
VLB	42.7‖	49.7	53.6	50.0	50.0	50.0	50.0	50.0	
DVLB	33.1	31.2	36.4	38.3	37.9	37.8			
VCR	24.4	23.3	15.1¶	24.3	24.6	23.9	22.0	27.6	
VLR	83.2	61.7	75.1				79.7		
VRD	67.9	69.3	82.7				85.3		
VGL	69.8	70.2	67.0				79.4		
HVLB	74.3	62.9	79.2	82.3	80.0	85.6			
DVCR	70.6	70.8	85.1				79.6		
DVA									37.9
DVH									35.5
DVEA									48.6
Catharanthine									100
Vindoline								97.6**	
Protein conc. (mg/ml)	0.83			0.140	0.752	0.859			

Porcine brain was homogenized in a Teflon-glass homogenizer as described in the text, with the listed buffer on a wt/wt basis (e.g., 1/1 or 3/1), then centrifuged for 1 hr at 100,000 *g*. The supernatant was then used as the protein source. Pure tubulin was prepared as described, by the repolymerization technique. A 100 μl sample of protein solution diluted with 850 μl of buffer was incubated with 25 μl of 7.2×10^{-5} M [^{3}H]VLB for 15 min at 37°, after which 25 μl of 7.2×10^{-5} M unlabeled alkaloid was added and incubation continued for 60 min. Bound [^{3}H]VLB was adsorbed onto DEAE filter paper and counted directly. Repeat determinations were made for each alkaloid pair with the order of addition reversed (i.e., unlabeled alkaloid followed by [^{3}H]VLB). The values reported are the percent of [^{3}H]VLB bound compared to that of 50 μl of 7.2×10^{-5} M [^{3}H]VLB alone.

* Crude protein preparation.
† Protein purified by three polymerization cycles.
‡ Crude protein preparation aged 8 hr.
§ Crude protein preparation frozen-thawed.
‖ (dis/min of drug + [^{3}H]VLB/dis/min of [^{3}H]VLB alone) × 100.
¶ This data point was excluded in the averaging (see text).
** Vindoline molarity 4 fold that of [^{3}H]VLB.

bound dis/min (i.e., bound pmoles) was less for the latter two preparations, and higher for the purified tubulin. It was found that all of the alkaloids tested, save for vindoline and catharanthine, displaced [^{3}H]VLB from tubulin to a greater or lesser extent at equimolar concentration. Since there were no statistically significant differences for the displacement of [^{3}H]VLB by the various alkaloids with each of the protein preparations, except for VCR in Column C, the averages for each drug were determined and appear in Table 2 with the standard error of the mean. VCR and DVLB were good competitors of

Table 2. Correlation of biologic with physico-chemical data for the Vinca alkaloids

Drug	[^{3}H]VLB Competition (% remaining radioactivity)†	log K_a	K_a × 10^6 L/M	log P*†	P-388‡ Dose (mg/kg)	ILS (%)	LD_{50}§ (mg/kg)	Ratio P-388/LD_{50}
VLB	50.0	6.7782	5.3 − 6.0‖	3.65	9.0	152	17.2 ± 3.0	0.52
VCR	24.3 ± 0.6	6.9031	8.0‖ 5.2¶	2.57	3.0	146	5.3 ± 1.5	0.57
DVLB	35.8 ± 1.2	6.5798	3.8‡‡	2.76‡‡	2.0	140	5.9 ± 0.4	0.34
DVA	37.9	6.5185	3.3¶	1.27‡‡	6.0	158	8.8 ± 2.5	0.68
DVH	35.5	6.6021	4.0¶	1.45	18.0	156	22.8 ± 6.5	0.79
DVEA	48.6	6.2553	1.8¶	1.11‡‡	10.0	143	10.2 ± 1.6	0.98
VGL	71.6 ± 2.7			4.02	20.0	111	42 ± 6	0.48
VRD	76.3 ± 4.5			3.05	60.0	106	127 ± 20	0.47
VLR	74.9 ± 4.7	5.1761	0.15¶	3.40	45.0	139	76 ± 6	0.59
HVLB	77.4 ± 3.3			3.95‡‡	100.00	108	142 ± 40	0.70
DVCR	76.5 ± 3.5			2.89‡‡	not tested		85 ± 50	
Vindoline	97.6††				—0—		>800	
Catharanthine	100				—0—		>800	
CLC	100							

† Average of values from Table 1, ±1 standard error.
‡ mg/kg for most effective dose/increased life span, as (days survived treated divided by days survived control) × 100.
§ ±1 standard deviation.
‖ from Scatchard plots.
¶ from modified Dixon plots.
†† vindoline in 4-fold excess.
‡‡ calculated.

VLB and gave very consistent data, while wider variations occurred with the drugs which were poor competitive inhibitors: VLR, VRD, VGL, HVLB and DVCR. The new drugs, DVA, DVH and DVEA, were also good competitors, but only a single run was done for each (see Dixon plot data later). The noncompetitive behavior of vindoline and catharanthine was very significant, indicating that the effects seen were specific for the dimeric compounds. In another set of incubations, colchicine (CLC), as has been shown before, had no effect upon the binding of [^{3}H]VLB to tubulin.

We next measured the binding of [^{3}H]VLB to tubulin in the presence of several concentrations of VCR, and the data appear in Fig. 4 as a modified Dixon plot. From this plot, the inhibition constant, K_i, for VCR was calculated at 1.93×10^{-6} M/L, and the association constant, K_a, as 5.2×10^6 L/M. The data for [^{3}H]VLB alone, when graphed as a Scatchard plot, gave a K_a of 4.5×10^6 L/M. In a similar manner, Dixon plots were done for DVA, DVH, DVEA and VLR, and the values obtained for the K_a's are listed in Table 2.

VLR is the only Vinca with an epoxide in the top half of the molecule, and we therefore questioned whether it might function by binding to the same site as CLC. This could explain why it is a poor competitor for VLB while still blocking tubulin polymerization by binding to this alternate site. We therefore evaluated the ability of the drug to displace [^{3}H]CLC, and found that no displacement occurred.

LD_{50} data are presented in Table 2. Their determinations were straightforward, except for VLB, which exhibited very anamolous behavior, with an unexplained early peak and fall occurring with both i.v. and i.p. routes of administration (Fig. 5). This behavior was consistently observed with VLB, but not with any of the other alkaloids. The LD_{50} for VLB in Table 2 is an average value for all the i.p. data

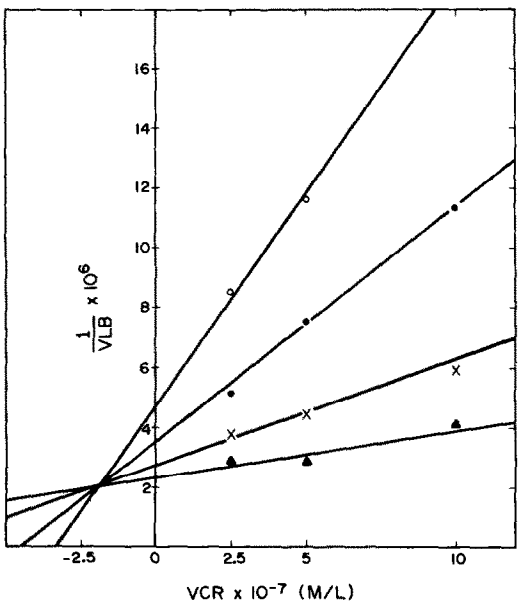


Fig. 4. Modified Dixon plot of the competition between [^{3}H]VLB and VCR for the tubulin binding site.

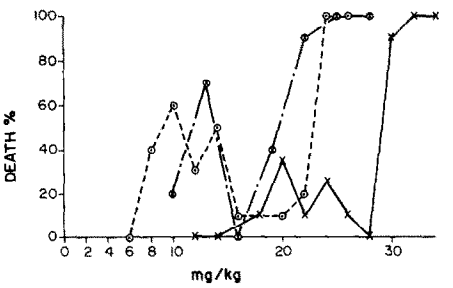


Fig. 5. Toxicity of VLB in BD_2F_1 female mice. The drugs were delivered i.p. (⊙-----⊙ and ⊗———⊗) or i.v. (×——×), and total deaths determined at 2 weeks.

points, and appears reasonable, since a parabolic dose–response curve was obtained for anti-*P*-388 activity, with progressive toxicity appearing at doses >10 mg/kg. The difference in LD_{50}'s between the i.v. and i.p. routes of administration of about 10 mg/kg has been noted before [20]. No evidence of neurotoxicity was seen, save with VCR, where the typical hind limb palsy regularly occurred at toxic dose levels [18].

The values reported in Table 2 for anti-*P*-388 activity reflect the highest increase in life span (ILS) obtained, and the dose level giving that result.

DISCUSSION

The binding of radiolabeled VLB to the protein tubulin has been well described [1–3, 7] and in the present work, we have demonstrated that competition for this binding site may be employed as a useful tool in evaluating similar binding activity of the other Vinca alkaloids. The displacement of VLB from tubulin binding sites was demonstrated to be a truly competitive process by the behavior of VCR, VLR, DVA, DVH and DVEA, when the data was displayed as modified Dixon plots, and analysis of these plots allowed calculation of individual K_a's. Simple equimolar competition reactions between [^{3}H]VLB and the other Vinca congeners were then employed to estimate the relative intensity of binding of these drugs to tubulin.

When the percent of [^{3}H]VLB displaced was plotted vs. the log of the K_a (as determined by the Dixon plots), a straight line was obtained, as shown in Fig. 6, with an r value of 0.9937 for the linear regression analysis. The value of the K_a for DVLB could be determined from this line, and it is listed in Table 2.

The displacement process is specific, since neither CLC, catharanthine, nor vindoline (even when the latter is in 4-fold molar excess) will displace [^{3}H]VLB. The inability of vindoline to displace VLB is particularly important since this molecule, while biologically inactive, comprises the entire bottom half of the dimeric Vinca alkaloids. Catharanthine, while not identical to the top half of the dimeric compounds, is closely related structurally, and therefore is also important yet is also biologically inert. That neither of these compounds displaces [^{3}H]VLB from its binding site is a further indication that non-specific inhibition of the binding process has not occurred.

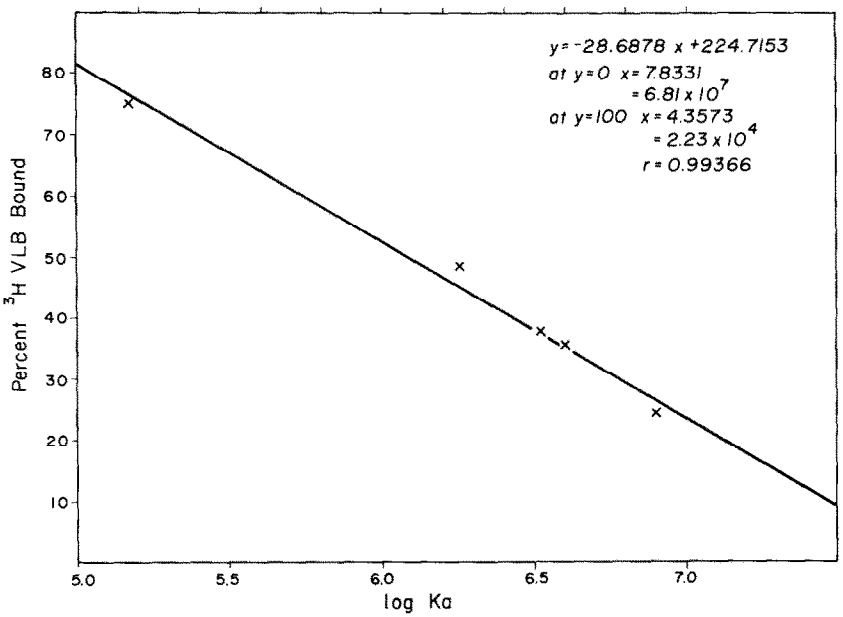


Fig. 6. Correlation of log K_a with percent [^{3}H]VLB bound to tubulin after equilibration with equimolar unlabeled Vinca drug.

A rough correlation of the binding affinity of the Vinca derivatives to tubulin with their antitumor activity was apparent. Those drugs which displace [^{3}H]VLB by at least 50 per cent, i.e. VCR, DVLB, DVA, DVH and DVEA, all had significant antitumor activity against *P*-388. Those derivatives which did not displace [^{3}H]VLB by at least 50 per cent, i.e. VGL, VRD, HVLB, DVCR†, with the exception of VLR, had little or no anti-*P*-388 activity. The LD_{50} values also correlated with the binding to tubulin, with low values where the displacement of [^{3}H]VLB was greater than 50 per cent.

Correlation of the biologic (LD_{50} and anti-*P*-388) and physico-chemical data was tried using the data for only those drugs which had significant antitumor activity. The values for log LD_{50} and log *P*-388 were correlated with log *P** and log K_a, using least squares regression analysis (done on an IBM 370/145 computer). The equations derived are listed below along with their *r* values.

Eq. 1. $\log LD_{50}\ (\mu M/kg) = 0.129\,(\log P^*)^2 = 0.522 \log P^* - 0.479 \log K_a + 4.652$
$r = 0.841$

Eq. 2. $\log P\text{-}388\ (\mu M/kg) = 0.222\,(\log P^*)^2 - 1.059 \log P^* - 0.520 \log K_a + 5.366$
$r = 0.805$

From this we saw that both the binding to tubulin and the partition coefficient, as expressed by the log *P** were important parameters in determining biologic activity. That there was an optimal value for log *P* was not surprising, indicating that there is an optimal value for the lipid-water solubility ratio relating to the distribution and ease of transfer of the drug across biologic membranes resulting in drug access into the cell providing its given biologic effect.

The affinity of binding is another important parameter, determining how much drug is required to block normal tubulin polymerization [21]. The value of the coefficient of this term (log K_a) is negative in both equations, correlating, as expected, higher binding with lower dose levels to achieve a given effect. A word of caution should be included at this point, since the number of points that were used to derive these equations is relatively small when compared to the number of degrees of freedom used in the form of the equation. Further evaluation in this manner of other active congeners, as they become available, should provide better and perhaps even more statistically meaningful correlation parameters.

The two drugs which lie closest to the minimal dose level predicted by these curves are VCR and DVLB. VCR is known in humans as the most effective and toxic drug in terms of dose (mg/kg) of the clinically useful Vincas, but DVLB has never undergone clinical evaluation.

Implicit in our above analysis of LD_{50} and *P*-388 correlations is the fact that they do not relate to the therapeutic index, i.e. the ratio of effective to toxic dose levels. This latter parameter is expressed in the last column of Table 2. DVLB, of the drugs tested, had the best therapeutic index with a ratio of 0.34. This is particularly interesting and important, since we have recently found this drug to be a significant human metabolite of VLB, and to date, the only metabolic material shown to have cytotoxic activity‡[22]. Our data on DVLB indicate that it is one of the more active drugs, since it lies near VCR

† K. Gerzon, personal communication.

‡ R. J. Owellen, F. O. Hains and C. A. Hartke, manuscript in preparation.

and close to the optimal point on the correlation curve and has the highest therapeutic index of the drugs tested. The question of whether DVLB is *the* active metabolite of VLB is intriguing and important. We realize that data obtained on murine systems do not always correlate with human data. However, in light of the facts presented, we think that DVLB may be a useful agent, and should be evaluated in the clinic.

We do not understand why the other Vinca derivatives VGL, VRD, HVLB and DVCR do not have anti-*P*-388 activity, since most of them displaced VLB in the same range as VLR.

It should be appreciated that VLR is unique among the derivatives, as it is the only one with an epoxide linkage. The possibility exists that metabolic processes may open this epoxide ring, yielding a derivative closely related to VLB.

The correlation obtained in equation 2 is only valid for *P*-388, and an analysis using another tumor system may yield a correlation that would include some of the other drugs, such as VGL and VRD, both of which have been shown to be active drugs in certain other murine tumor systems.

There was an obvious, not unexpected, gross correlation of LD_{50} dose with anti-*P*-388 dose, where a simple linear regression analysis for the 7 active compounds gave an r value of 0.9872 for the expression $P\text{-}388 = 0.5883\ LD_{50}\ \text{dose} + 0.9984$.

Neurotoxicity is an important clinical effect of the Vinca alkaloids, and for VCR, and under certain circumstances for VLB [23], it is the limiting factor in dose level selection in humans. To correlate neurotoxicity among the Vincas with the physico-chemical properties we had measured was difficult, as we had only a crude observational method for evaluating neurotoxicity in mice [18]. Only VCR induces a detectable hind limb palsy in this system, while none of the other derivatives evaluated had a similar effect. Thus within the series of drugs examined, VCR, with the greatest affinity for tubulin, is the most neurotoxic drug.

We conclude that the affinity of the Vinca alkaloids for the protein tubulin can be evaluated by their ability to displace [^{3}H]VLB from this protein in an *in vitro* test system. Correlation of the log of the association constant and the log of the partition coefficient, for these Vinca derivatives showing antitumor activity, was found with both the log LD_{50} and log anti-*P*-388 antitumor activity, when expressed in a quadratic relationship (See Equations 1 & 2). The two drugs, VCR and DVLB, were found to be the most active of the series. The observation that DVLB has the best therapeutic index of all the agents, the knowledge that it is a metabolite in man, and its lack of neurotoxicity in the mouse, prompts us to suggest that this drug deserves evaluation in clinical trials.

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APPENDIX

The calculation of log *P** data for those compounds not directly measured is illustrated below for DVLB.

log *P** of VLB = 3.65 (measured)

DVLB = VLB minus O_2CCH_3, $\pi = -0.27$

plus OH, $\pi = -1.16$

log *P** DVLB = 2.76 (calculated)

The values used [12–15] to calculate the various log *P** data are listed:

Function	π
O_2CCH_3	−0.27
OH	−1.16
$N(CH_3)_2$	0.18
CO_2CH_3	−0.27
$CONH_2$	−1.71
CH=CH†	−0.30
NHCHO (aromatic)	−0.98
NH_2 (aromatic)	−1.23

† Difference of *n* propyl and allyl groups.