Biochemical Pharmacology, Vol. 22, pp. 2113–2119. Pergamon Press, 1973. Printed in Great Britain.

INTERACTION OF VINBLASTINE, VINCRISTINE AND COLCHICINE WITH SERUM PROTEINS

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(*Received* 17 *November* 1972; *accepted* 28 *February* 1973)

Abstract—Equilibrium dialysis has been used to demonstrate that vincristine (VCR), vinblastine (VLB) and colchicine (CLC) adsorb to serum proteins. In addition, there is about 19 per cent more binding of CLC to plasma than serum. The extent of adsorption for all three alkaloids is approximately proportional to both the free alkaloid concentration and the serum protein concentration, with no evidence of saturation at the concentrations used. At physiologic concentrations of protein, the per cent of drug bound to serum protein is about 75 per cent for VLB and VCR and 50 per cent for CLC, indicating that the drugs are not tightly bound, and that there is no impediment to rapid extravascular distribution. On a mole per g basis, VLB and VCR adsorb ten times more extensively to commercially obtained α- and β-globulins than to albumin or γ-globulin. CLC binds nonselectively to these components.

TRITIUM-labeled vinblastine (^{3}H-VLB) and vincristine (^{3}H-VCR) have been used recently in several pharmacologic studies.[1–8] After intravenous injection of ^{3}H-VCR in the rat, the tritium is cleared from the blood with a $T_{\frac{1}{2}}$ of 70 min, during the first 4 hr, then more slowly, and is still present after 24 hr.[1] Hebden *et al.*[2] have shown that after an intraperitoneal injection, ^{3}H-VLB is initially cleared from the blood of a rat at about one-half the rate measured for VCR and appreciable levels are still present after 24 hr. Initially, most of the ^{3}H-VLB is carried in platelets and cellular components, but 24 hr after injection, over 50 per cent of the blood radioactivity is carried in the plasma.[2]

Our purpose in this report is to characterize the interaction of these alkaloids with serum and plasma proteins using equilibrium dialysis *in vitro* and to compare this interaction with that of a biologically similar but un-ionized compound, colchicine (CLC).

MATERIALS AND METHODS

Tritium-labeled VCR (84·8 Ci/mole) and VLB (64·9 Ci/mole) were prepared as reported earlier[1,3] and stored at 4° in 0·05 M NaH_2PO_4 solution. The pH of an aliquot of this stock solution was adjusted to 7·4 with 10 N NaOH prior to each experiment. Tritium-labeled colchicine (142 Ci/mole) was purchased from New England Nuclear Corp., and stored in distilled water at 4°.

Purity of the radiolabeled alkaloids was measured by thin-layer chromatography (TLC). Kodak chromagram Silica gel plates were spotted with radioactive alkaloid ($\sim 10^{-3}$ mCi) and pure unlabeled alkaloid (20 μg), exposed to ammonia vapor for 5 min, and developed with acetone. Radiolabeled alkaloid purity was determined as the per cent of applied radioactivity traveling in the pure alkaloid spot.

For a serum and drug diluent, phosphate-buffered saline (PBS) containing $1{\cdot}34 \times 10^{-1}$ M NaCl, $5{\cdot}36 \times 10^{-3}$ M KCl, $1{\cdot}46 \times 10^{-3}$ M NaH_2PO_4, $8{\cdot}16 \times 10^{-3}$ M Na_2HPO_4, $9{\cdot}01 \times 10^{-4}$ M $CaCl_2$, $1{\cdot}04 \times 10^{-3}$ M $MgCl_2$ was used.

Normal human plasma was obtained from heparinized blood after centrifugation at 1500 *g* for 10 min. Its protein concentration was calculated from the corresponding serum value.[9]

Pooled normal serum was obtained from the blood of eight volunteers and stored at −70°. The protein concentration of this serum, measured by the biuret method,[10] was 69·5 mg/ml. When a portion of this serum was centrifuged at 100,000 *g* for 60 min, a floating lipid-like layer formed. This layer was discarded and the remaining solution utilized as "lipid-free" serum.

The relative amounts of α-, β- and γ-globulins, and albumin in the serum were measured using cellulose acetate zonal electrophosesis.[11] The cleared strips were recorded with an integrating Photovolt Densicord densitometer. The α-, β- and γ-globulins and albumin accounted for 19·8, 11·7, 12·0 and 56·5 per cent of the staining respectively.

Ultrafiltrate was obtained by filtering the serum through Amicon ultrafiltrate membrane cones (2100 CF50) designed to remove all components with molecular weight higher than 50,000. This ultrafiltrate contained 1·5 mg/ml of protein according to the method of Lowry *et al.*[12]

Human albumin and γ-globulin were obtained from Sigma Chemical Company; α-globulin and β-globulin were obtained from Pfaltz & Bauer. Each component was dissolved in ultrafiltrate at a concentration which matched that in our pooled normal serum. The albumin and γ-globulin migrated very similarly to their corresponding normal serum components during cellulose acetate electrophoresis. The α- and β-globulins migrated properly, but the α_1, α_2, β_1 and β_2 peaks were blurred.

The equilibrium dialysis cells were machined from pairs of small Lucite blocks. The compartment volume on each side of the membrane was 140 μl and the membrane surface area was 0·5 cm^2. Cellulose dialysis membrane was obtained from A. Thomas & Co. (retention at 12,000 mol. wt).

In equilibrium dialysis experiments, protein solution and PBS were added to one side of the cell, PBS alone to the other, and equal amounts of drug to both sides. The cells were rotated at 6 rev/min at 37° until the drug concentrations on each side reached steady state (18 hr), then aliquots from each side were counted in 5 ml of Bray's scintillation fluid in a Packard Tri-Carb scintillation spectrophotometer (25 per cent counting efficiency). The concentration of alkaloid on the PBS side was the unbound or free drug concentration. The difference between this and the protein side concentration was the absorbed drug concentration. The ratio (γ) of adsorbed molarity to protein concentration (γ = moles/g) was calculated from these data.

Controls. When PBS and $7{\cdot}0 \times 10^{-10}$ moles of radioactive drug were added to one side of a dialysis cell and PBS alone to the other, equal concentrations of drug were obtained on both sides within 4 hr. After 18 hr, 85 per cent of VCR and VLB total radioactivity was recovered in the liquid, and 5 per cent was found on the dialysis membrane.

In the presence of serum, the same final distribution resulted whether radioactive drug was added to the PBS compartment, the serum compartment, or in equal quantities to both compartments. In each situation, 18 hr was required to reach equilibrium.

Aliquots removed from both the drug and PBS compartments at 18 hr revealed no decrease in drug purity on TLC examination. In all cases, 85–90 per cent of the radioactivity was recovered with the liquid contents and 5 per cent was found on the dialysis membrane.

When undiluted serum and PBS were put on opposite sides of the membrane and incubated for 24 hr, the PBS side contained 0·14 mg/ml of protein according to the method of Lowry *et al.*[12]

We compared fresh and frozen-thawed serum with all three drugs and found no difference in adsorption, and thereafter used frozen-thawed serum.

Equilibrium dialysis comparing serum with "lipid-free" serum resulted in the same ratio of adsorbed to free drug.

RESULTS

By the use of equilibrium dialysis, we have demonstrated that the alkaloids, VCR, VLB and CLC, bind to serum proteins. When the concentration of serum protein was held constant, it was found that the amount of adsorbed alkaloid varied in direct proportion to the concentration of free alkaloid for VLB and CLC (Fig. 1). VCR showed proportionality at low and at high concentrations, but there was a break in the curve. This proportionality at high concentrations did not indicate saturation when plotted linearly. Though binding was linear for VLB up to 2×10^{-3} M, at higher concentrations precipitation occurred in the protein compartment making further evaluation impossible.[13]

There was no difference between plasma and serum binding of VLB and VCR (Table 1). CLC was bound more to plasma than serum by 19 per cent. This difference is significant at the $P = 0{\cdot}06$ level. None of the alkaloids was significantly bound to plasma ultrafiltrate.

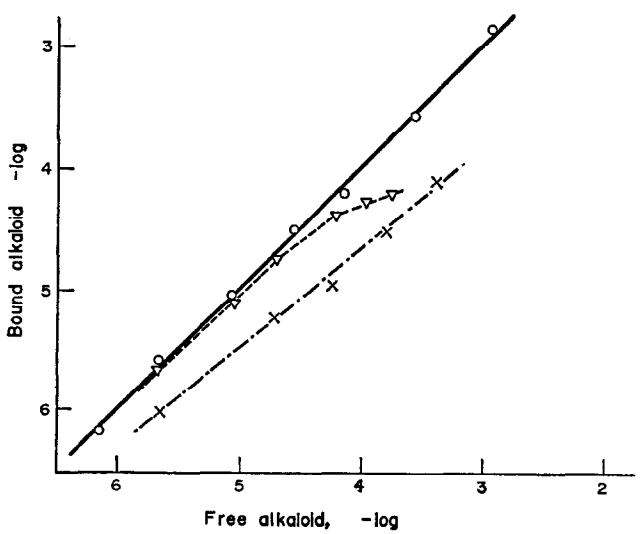

FIG. 1. Equilibrium dialysis binding of VLB, VCR and CLC to serum protein obtained by varying the alkaloid concentrations against a serum protein concentration of 34·5 mg/ml. (○) VLB, (▽) VCR, (×) CLC.

TABLE 1. INTERACTION OF VLB, VCR AND CLC WITH PLASMA, SERUM AND ULTRAFILTRATE*

Alkaloid	Protein solution	Protein concn (mg/ml)	Unbound alkaloid concn (moles/l. $\times 10^{-6}$)	Bound alkaloid concn (moles/l. $\times 10^{-6}$)	γ (moles/g $\times 10^{-8}$)
VLB	Plasma	65·0	1·2 ± 0·1	2·3 ± 0·1	3·5
VLB	Serum	62·0	1·2 ± 0·1	2·3 ± 0·1	3·7
VLB	Ultrafiltrate	1·1	10·0 ± 0·1	0·02 ± 0·01	1·8
VCR	Plasma	65·0	0·59 ± 0·05	0·82 ± 0·05	1·3
VCR	Serum	62·0	0·58 ± 0·05	0·82 ± 0·05	1·3
VCR	Ultrafiltrate	1·1	7·3 ± 0·1	0·2 ± 0·02	1·8
CLC	Plasma	54·0	2·0 ± 0·1	0·91 ± 0·07	1·7
CLC	Serum	51·7	2·1 ± 0·1	0·76 ± 0·04	1·5
CLC	Ultrafiltrate	1·1	4·3 ± 0·1	0·02 ± 0·01	1·8

* Equilibrium concentrations of drug are given with their standard errors. The serum concentration was measured by the biuret method, that of the ultrafiltrate by the method of Lowry *et al.*,[12] and that of plasma was calculated. Data were obtained in duplicate; γ is moles of drug bound/g of protein.

The adsorption of all three alkaloids was found to be proportional to the serum protein concentration (Table 2). Of interest was the relative constancy of γ (moles of alkaloid/g of protein).

TABLE 2. INTERACTION OF VLB, VCR AND CLC WITH VARYING SERUM PROTEIN CONCENTRATIONS*

Serum protein concn (mg/ml)	Alkaloid	Unbound alkaloid concn (moles/l. $\times 10^{-6}$)	Bound alkaloid concn (moles/l. $\times 10^{-6}$)	Bound alkaloid (%)	γ (moles/g $\times 10^{-8}$)
6·9	VLB	2·7 ± 0·3	0·4 ± 0·1	13	5·8
13·8	VLB	2·7 ± 0·3	1·0 ± 0·2	27	7·4
27·6	VLB	2·4 ± 0·3	2·3 ± 0·3	50	8·6
55·2	VLB	1·9 ± 0·3	3·1 ± 0·2	62	5·6
6·9	VCR	3·1 ± 0·2	0·5 ± 0·1	14	7·8
13·8	VCR	2·9 ± 0·2	1·0 ± 0·1	26	7·3
27·6	VCR	2·3 ± 0·2	2·2 ± 0·1	51	8·3
55·2	VCR	1·9 ± 0·2	3·5 ± 0·2	65	6·3
6·9	CLC	1·8 ± 0·1	0·09 ± 0·03	5	1·2
13·8	CLC	1·8 ± 0·1	0·17 ± 0·02	9	1·2
27·6	CLC	1·7 ± 0·1	0·45 ± 0·06	22	1·6
55·2	CLC	1·6 ± 0·1	0·66 ± 0·17	29	1·2

* Equilibrium concentrations are listed with their standard errors from duplicate measurements. Per cent bound is 100 × (concn of bound drug/concn of bound plus unbound drug); γ is moles of drug bound/g of protein.

Having determined that the alkaloids are bound to serum proteins, we then explored which of these serum proteins is responsible for the majority of the adsorption. Commercial preparations of albumin and α-, β- and γ-globulins dissolved in ultrafiltrate were analyzed for their ability to adsorb the three alkaloids. The results (Table 3)

TABLE 3. INTERACTION OF VLB, VCR AND CLC WITH SERUM PROTEIN COMPONENTS*

Protein solution	Protein concn (mg/ml)	Alkaloid	Unbound alkaloid concn (moles/l. $\times 10^{-6}$)	Bound alkaloid concn (moles/l. $\times 10^{-6}$)	Serum binding (%)	γ (moles/g $\times 10^{-8}$)
Serum	34·5	VLB	4·6 ± 0·1	3·0 ± 0·1	100	8·7
Albumin	19·5	VLB	4·4 ± 0·1	0·41 ± 0·1	14	2·1
α-Globulin	6·8	VLB	4·2 ± 0·1	1·1 ± 0·1	37	16·0
β-Globulin	4·1	VLB	4·3 ± 0·1	0·95 ± 0·1	31	23·0
γ-Globulin	4·2	VLB	4·6 ± 0·1	0·21 ± 0·03	7	5·0
Serum	34·5	VCR	4·7 ± 0·1	4·0 ± 0·1	100	11·0
Albumin	19·5	VCR	4·9 ± 0·1	0·40 ± 0·1	10	1·9
α-Globulin	6·8	VCR	4·4 ± 0·1	1·4 ± 0·1	36	22·0
β-Globulin	4·1	VCR	4·6 ± 0·1	1·2 ± 0·1	28	28·0
γ-Globulin	4·2	VCR	5·0 ± 0·1	0·24 ± 0·02	6	5·7
Serum	34·5	CLC	4·3 ± 0·1	1·2 ± 0·1	100	3·4
Albumin	19·5	CLC	4·3 ± 0·1	0·77 ± 0·16	64	3·9
α-Globulin	6·8	CLC	4·2 ± 0·1	0·23 ± 0·01	19	3·4
β-Globulin	4·1	CLC	4·2 ± 0·1	0·07 ± 0·03	6	1·7
γ-Globulin	4·2	CLC	4·4 ± 0·1	0·15 ± 0·07	12	3·6

* Serum concentration was measured by the biuret method. Component concentrations were calculated from weighed amounts dissolved in known volumes of ultrafiltrate. Equilibrium concentrations of drug are given with their standard errors. Samples were run in duplicate. Per cent of serum binding of a component is 100 × (concn bound to component/concn bound to serum); γ is moles of bound drug/g of protein.

indicate clearly that VLB and VCR are adsorbed more to the α- and β-globulins than to any of the other serum components, as seen in the higher γ and per cent bound values. Colchicine is adsorbed to all the serum components with approximately the same degree of affinity, as reflected by the relative constancy of the γ values. This alkaloid then is adsorbed in major proportion by albumin.

DISCUSSION

We have demonstrated that VLB, VCR and CLC bind to serum proteins in proportion to both alkaloid and protein concentrations. That VLB and VCR bind primarily to α- and β-globulins is particularly interesting in view of the relatively small number of drugs that have been found to bind to these serum components.[14] Though no evidence of saturation was found under these conditions, undoubtedly at higher concentrations, a break in this relationship should occur. An attempt to determine this for VLB resulted in precipitation in the protein compartment, precluding direct confirmation. Since the curve for VCR has two linear segments, we might infer that there are two types of binding sites for this material, but further investigation of this has not been undertaken.

From Table 1, we see that 66 per cent of the VLB and 59 per cent of the VCR are bound to serum proteins at the concentrations utilized ($\sim 10^{-6}$ M in alkaloid). If these drugs are assumed to equilibrate with total body water at this concentration (which would be at the upper limits of pharmacologic levels *in vivo*), then less than 20 per cent of the total amount of drug would be retained in the plasma.[15] By similar extrapolation, CLC would be expected to have less than 10 per cent of the total drug bound up in plasma. This information would indicate that the binding to serum proteins is not a significant deterrent to the distribution of these drugs in other body compartments *in vivo*. Indeed, studies have shown that VCR, VLB and CLC* are all rapidly distributed in the rat.[1,2] Furthermore, there is significant tissue binding of these drugs, with levels much higher than in blood, in all but a very few of the organs of the rat. This latter would then appear to be more significant in the distribution of these drugs in the whole body than is their binding to serum proteins.

Acknowledgements—Our gratitude is extended to Dr. Richard Humphrey for his advice, and to Miss Carol Hartke for technical assistance. We also acknowledge the support of NIH Grant CA-6973 and the Eli Lilly Company.

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