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Binding of Vinblastine to Recrystallized Human α_1-Acid Glycoprotein

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Summary. *The binding of vinblastine (VLB) to recrystallized human α_1-acid glycoprotein (α_1-AGP) dissolved in phosphate-buffered saline (pH 7.4) was determined at different drug concentrations using the technique of continuous ultrafiltration. Vinblastine was extremely highly bound (> 99.0%) at a drug concentration of 4.0 $\mu mol \cdot l^{-1}$, dropping to under 60% at 65.0 $\mu mol \cdot l^{-1}$. Binding was best described by a two-class model with higher- (9.4 μM^{-1}) and lower- (0.1 μM^{-1}) affinity sites but with a similar number of binding sites (1.5 as against 1.1 lower-affinity sites). These results strongly suggest that α_1-AGP would be a major binding protein for VLB in plasma.*

Introduction

Vinblastine (VLB) is a vinca alkaloid used widely in the chemotherapy of neoplastic disease [5, 13]. It is highly bound in serum, binding predominantly to α-globulins, as shown by cellulose acetate electrophoresis [17, 18] or by binding to the individual protein fractions [6]. Although the identity of the binding protein or proteins is unknown vinblastine is a weak base [12] and may bind to α_1-acid- glycoprotein (α_1-AGP) to some other basic drugs [14]. This hypothesis is strengthened when it is considered that α_1-AGP separates on cellulose acetate electrophoresis with the α_1-globulin fraction. In this study the binding of vinblastine to recrystallized human α_1-AGP, reconstituted in phosphate buffer, was examined.

Methods

Recrystallized human α_1-AGP (Sigma) (18.0 $\mu mol \cdot l^{-1}$) was reconstituted in 0.1 mol . l^{-1} phosphate-buffered saline (pH 7.4). Protein binding was measured by continuous ultrafiltration with the Amicon Multimicroconcentrator (MMC) system and Diaflo XM50 ultrafiltration membranes at 37° C. A standard reservoir solution of 2 $\mu mol \cdot l^{-1}$ VLB permitted 12 consecutive estimates of percentage bound drug, each pertaining to a different total drug concentration within the range 4.0–65.0 $\mu mol \cdot l^{-1}$. {^{3}H}-VLB (sp. act. 13.4 Ci/nmol) was added to the reservoir (10,000,000 dpm) and VLB concentrations were measured by comparing the radioactivity in the ultrafiltrate with standard drug solution. The radioactivity was measured by liquid scintillation counting.

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Unbound drug concentration were related to the increase in total drug concentration in the MMC chamber by application of eq (1):

$$\uparrow\{D_t\} = \frac{1}{V}[(vi\,\{R\}) - (vi\,\{Df\})] \quad (1)$$

where:

$\uparrow\{D_t\}$ = increase in total drug concentration during the ith collection period ($\mu mol \cdot l^{-1}$)
V = volume of solution in the MMC chamber (μl)
v_i = the volume of ultrafiltrate collected in the ith period (μl)
$\{R\}$ = reservoir concentration of VLB ($\mu mol \cdot l^{-1}$)
$\{D_f\}$ = concentration of drug in the ultrafiltrate in the ith period ($\mu mol \cdot l^{-1}$).

Estimates of the protein binding within the concentration range referred to above were obtained by application of the following equations:

$$D_t = \frac{1}{V}\sum_{i=1}^{i=n}[(v_i\,\{R\}) - (v_i\,\{Df\})] \quad (2)$$

and

$$\{D_b\} = \{D_t\} - \{D_f\} \quad (3)$$

where:

$\{D_t\}$ = total VLB concentration ($\mu mol \cdot l^{-1}$)
$\{D_b\}$ = the concentration of bound VLB ($\mu mol \cdot l^{-1}$).

No protein was detected in the ultrafiltrates on the addition of sulphosalicylic acid (1.0 mol $\cdot l^{-1}$), showing that α_1-AGP did not pass through the ultrafiltration membranes. Binding of VLB to ultrafiltration membranes accounted for < 8% of the overall binding.

Data Analysis. Estimates of the apparent binding parameters for α_1-AGP-VLB binding were made using a non-linear least-squares regression of D_b on D_f [2] using the models shown in Table 1. Provision was made for binding to one class and two classes of binding sites. The two-class model was selected by examination of the asymptotic standard deviation observed after fitting the data to the different models and also comparing the residual sum of squares using the 'F' ratio test [3].

Results

The percentage binding of VLB to α_1-AGP was non-linear between drug concentrations of 4.0 $\mu mol \cdot l^{-1}$ and

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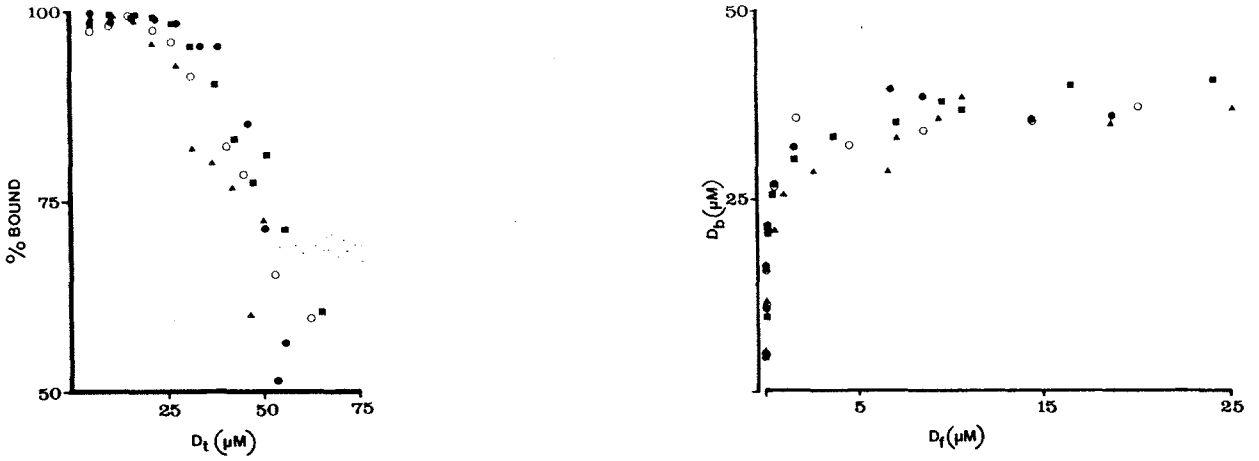

Fig. 1. The percentage of drug bound to α_1-acid glycoprotein over a range of vinblastine concentrations between 4.0 μmol · l^{-1} and 65.0 μmol · l^{-2}. *Symbols* refer to separate experiments

Fig. 2. The concentration of vinblastine bound (D_b) plotted against the concentration of free vinblastine. *Symbols* refer to separate experiments

Table 1. Models used in the analysis of protein binding data

Model	Equation	Description
I	$\{D_b\} = \frac{(N \cdot P)K\{D_f\}}{1 + K\{D_f\}}$	One class of binding site
II	$\{D_b\} = \frac{(N_1 \cdot P)K_1\{D_f\}}{1 + K_1\{D_f\}} + \frac{(N_2 \cdot P)K_2\{D_f\}}{1 + K_2\{D_f\}}$	Two classes of binding sites

$\{D_b\}$ = concentration of vinblastine bound
$\{D_f\}$ = concentration of vinblastine free
K = affinity constant
N.P = hybrid capacity factor
(Subscripts 1 and 2 refer to higher- and lower-affinity sites, respectively)

Table 2. Binding parameters decrived from model II

Sample	$N_1 \cdot P$ (μM)	N_1	K_1 (μM^{-1})	$N_2 \cdot P$ (μM)	N_2	K_2 (μM^{-1})
1	20.12	1.12	14.78	22.84	1.27	0.13
2	24.80	1.38	6.58	17.92	1.00	0.16
3	26.70	1.48	9.63	18.21	1.01	0.09
4	33.19	1.84	6.44	17.32	0.96	0.03
Mean	26.20	1.46	9.36	19.07	1.06	0.10
± SD	5.42	0.30	3.90	2.54	0.14	0.06

K_1 and K_2: affinity constants
$N_1 \cdot P$ and $N_2 \cdot P$: hybrid capacity factors
N_1 and N_2: number of binding sites
The number of binding sites was derived by dividing the hybrid capacity factor by the α_1-AGP concentration

65.0 μmol · l^{-1}, dropping from greater than 99.0% bound to under 60% bound at the higher concentration (Fig. 1). Estimates of the apparent binding parameters were made by plotting the concentration of bound drug (D_b) against the concentration of unbound or free drug (D_f) (Fig. 2). These results were best described by model II (Table 1). Selection of model II was supported by a significantly smaller residual sum of squares ($P < 0.01$) and smaller asymptotic standard deviations of the parameters than with model I. The binding parameters for the high- and low-affinity sites obtained from model II are shown in Table 2.

Discussion

This study shows that VLB is extremely highly bound to α_1-AGP and that the nature of this binding is best described by a two-class binding model. The extensive binding to α_1-AGP occurs even at higher concentrations than those attained during therapy. This strongly suggests that in therapeutic use of VLB, α_1-AGP will be a major binding component of plasma.

It is well established that the plasma concentration of α_1-AGP can increase in neoplastic disease [4, 15, 16, 18]. In these disease states it is likely that VLB binding in plasma would be affected, since the intrinsic capacity factor for the binding of VLB to α_1-AGP would be altered. This may be an important consideration for therapy since VLB is more than 99% bound to plasma proteins [17]; thus small perturbations in binding would have profound effects on the concentration of free drug present, which may necessitate a change in dosage regimen. This indicates a need for pharmacokinetic studies in patients when their disease state is active. Also following successful therapy there is evidence that the concentration of α_1-AGP returns from its elevated values towards normal levels [9, 11, 15]. The need for further dosage adjustments during states of remission is suggested.

α_1-Acid glycoprotein is not only present in plasma but may also be a constituent of platelet cell walls and may be involved in the mediation of platelet function in a similar way to that postulated for other plasma protein fractions [1, 7]. It is, therefore, not surprising that VLB binds reversibly to platelets [8]. Indeed, VLB has been administered after it has been bound to a platelet suspension [10]. Also, compared with vincristine (VCR), VLB is toxic to the reticuloendothelial system and this may be a manifestation of its binding to platelets. Finally, in this context, VLB may influence its own toxicity by causing a thrombocytopenia, thus reducing its binding in blood.

Vinblastine is generally administered with other drugs [5, 13] in the treatment of neoplastic disease. It is possible that drug displacement or competition for binding sites may occur and that this may be of particular therapeutic significance for a highly bound drug. In particular, antiemetics are often incorporated at random into the therapeutic regimen. These drugs, e.g., phenothiazines, are basic in character and would be expected to bind, together with VLB, to α_1-AGP. Potential drug displacement interactions should be investigated as they may influence the toxic reactions which have been reported with VLB.

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