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SHORT COMMUNICATION

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Comparative serum protein binding of anthracycline derivatives

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Abstract The binding of doxorubicin, iododoxorubicin, daunorubicin, epirubicin, pirarubicin, zorubicin, aclarubicin, and mitoxantrone to 600 μ*M* human serum albumin and 50 μ*M* alpha$_1$-acid glycoprotein was studied by ultrafiltration at 37 °C and pH 7.4. Anthracycline concentrations (total and free) were determined by high-performance liquid chromatography (HPLC) with fluorometric detection. Binding to albumin (600 μ*M*) varied from 61% (daunorubicin) to 94% (iododoxorubicin). The binding to alpha$_1$-acid glycoprotein (50 μ*M*) was more variable, ranging from 31% (epirubicin) to 64% (zorubicin), and was essentially related to the hydrophobicity of the derivatives. Simulations showed that the total serum binding varied over a broad range from 71% (doxorubicin) to 96% (iododoxorubicin).

Key words Anthracycline derivatives · Serum protein binding · Alpha$_1$-acid glycoprotein · Human serum albumin · Hydrophobicity

Introduction

Anthracycline derivatives are prescribed in a wide spectrum of malignancies, usually in combination with other drugs. Some studies have shown that anthracyclines are bound to human plasma proteins to an extent of 50–85% [3]. The drug plasma-unbound fraction is a parameter of drug distribution, and the free drug concentration is generally best related to pharmacological effects [7]. For example, systemic exposure to unbound etoposide has been correlated with hematological toxicity [8].

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We recently reported that the binding to lipoproteins of a series of eight anthracycline analogues could be ascribed to chemicophysical determinants of lipophilicity [2]. The present study was conducted to evaluate in vitro the contribution of albumin and alpha$_1$-acid glycoprotein to the total serum binding of these drugs.

Materials and methods

Drugs

The anthracycline pharmaceutical preparations (doxorubicin, iododoxorubicin, daunorubicin, epirubicin, pirarubicin, zorubicin, aclarubicin, and mitoxantrone) were obtained from the respective pharmaceutical companies. Stock solutions were prepared by dissolution of the anthracycline in distilled water/methanol (4:1, v/v) and were stored at –20 °C under nitrogen. Just prior to use, they were diluted in the protein solution at the desired concentration. The maximal methanol concentration did not exceed 1% in the protein samples at the maximal ligand concentration (10 μ*M*).

Proteins

Human serum albumin (HSA, Sigma A-1887, 99% pure) and alpha$_1$-acid glycoprotein (AAG, Behring, 99% pure) were dissolved in buffer (pH 7.4; 120 m*M* NaCl, 5 m*M* KCl, 2.5 m*M* $CaCl_2$, 1 m*M* $MgSO_4$, 20 m*M* TRIS) to obtain protein concentrations of 600 and 50 μ*M*, respectively (physiological serum concentrations).

Binding experiments

The protein solution (1 ml) containing the anthracycline (concentration range between 1 and 10 μ*M*) was incubated at 37 °C for 10 min, then centrifuged at 2,000 *g* and 37 °C in an Ultrafree-MC (ultrafiltration kit; Millipore, cutoff 10,000) for 7 min. The total and free drug concentrations were determined in duplicate from the sample before ultrafiltration and from the ultrafiltrate, respectively, by high-performance liquid chromatography (HPLC) with fluorometric detection (UV detection at 658 nm for mitoxantrone) [6]. The bound concentration was obtained by difference: total concentration minus free concentration.

Simulations

To appreciate the role of each protein in the serum binding, the ligand distribution on serum proteins was calculated as previously described in detail [9]. In brief, the contribution of a given protein is proportional

Table 1 Binding parameters (and standard deviations) of anthracycline analogues to HSA at 600 μ*M* and AAG at 50 μ*M*[a]

Ligand	HSA		AAG	
	nK	% Bound	*nK*	% Bound
Aclarubicin	4,700	75.8	12,000	37.4
	(330)	(2.8)	(875)	(2.8)
Daunorubicin	2,300	60.9	28,100	58,4
	(140)	(0.5)	(3,360)	(7.0)
Doxorubicin	2,600	62.2	9,400	32.0
	(75)	(0.5)	(1,190)	(4.1)
Epirubicin	4,700	78.4	9,000	31.1
	(420)	(3.7)	(375)	(1.3
Iododoxorubicin	24,300	93.7	34,200	62.3
	(2260)	(6.2)	(3,680)	(6.7)
Mitoxantrone	2,900	64.8	20,300	51.0
	(85)	(0.3)	(1,460)	(3.7)
Pirarubicin	6,300	78.7	17,000	46.0
	(255)	(1.2)	(850)	(2.3)
Zorubicin	7,700	82.2	35,300	63.8
	(200)	(0.5)	(3,700)	(6.7)

[a] Binding constants are expressed as nK in M^{-1}. Binding constants and average % Bound values were determined from 20–24 experimental points. Values in parentheses represent standard deviations

to the binding coefficient, $nK.P_t$ (dimensionless quantity), where nK is the binding constant (n, number of sites, multiplied by K, association constant) and P_t is the protein concentration.

Results

For all anthracycline analogues, the binding to HSA and AAG was nonsaturable under our experimental conditions; therefore, only a binding constant, nK (product of the number of binding sites times the association constant), relating the bound (B) to the free (F) concentration ($B = nK.F.P_t$) could be derived from these data [1]. There was a wide variation in the nK values, both between HSA and AAG for the same ligand and between the ligands for a given protein (Table 1). All ligands bound to albumin with similar binding constants (in the 10^3 M^{-1} range), except for iododoxorubicin, which displayed a 10-fold higher binding constant. The binding to AAG was stronger than that to albumin for all ligands and roughly involved three groups: doxorubicin, epirubicin, and aclarubicin in the 10^4 M^{-1} range, pirarubicin and mitoxantrone in the 2.10^4 M^{-1} range, and, finally, daunorubicin, iododoxorubicin, and zorubicin in the 3.10^4 M^{-1} range. There was no correlation between the binding to HSA and that to AAG.

Given the binding constants of the each analogue and the serum protein concentrations in cancer disease (biological syndrome of inflammation), the serum distribution and total binding were computed (Table 2). All the drugs were more than 70% serum-bound, the binding ranging from 72% (doxorubicin) to 96% (iododoxorubicin). Although the affinity of the drugs was higher for AAG than for HSA, the fraction bound to HSA was greater than that associated with AAG because albumin is 15–25 times more concentrated in serum than is AAG. The binding to lipoproteins amounted to 20–50% of the total serum concentration of drug.

Table 2 Computed distribution of bound anthracycline derivatives on serum proteins

Drug	% Drug associated with			
	Albumin	AAG	Lipoproteins	Serum[a]
Daunorubicin	22.91	15.27	43.67	81.88
Doxorubicin	42.05	8.29	20.23	70.59
Mitoxantrone	32.69	12.29	34.50	79.50
Aclarubicin	35.40	4.93	45.95	86.30
Epirubicin	50.29	5.25	24.98	80.54
Pirarubicin	45.37	6.67	34.84	86.90
Zorubicin	40.29	10.07	40.10	90.48
Iododoxorubicin	56.19	4.31	35.26	95.79

[a] The serum distribution was simulated given the serum protein concentrations observed in cancer disease for albumin (550 μ*M*) and AAG (30 μ*M*) and for lipoproteins high-density lipoproteins 8 μ*M* or 3.6 g/l, low-density lipoproteins 0.8 μ*M* or 2.5 g/l, very-low-density lipoproteins 0.05 μ*M* or 0.05 g/l)

Discussion

The protein binding of anthracycline derivatives was similar to that of basic drugs or neutral drugs [7], i. e., the binding involved HSA, AAG, and also lipoproteins as previously reported [2]. Because we used physiological serum protein concentrations and low ligand concentrations, the binding was nonsaturable and the total binding constant, nK, was determined by simple linear regression of bound versus free ligand concentrations.

The simulations showed that albumin and lipoproteins were the main serum-transport proteins of anthracyclines and that the total serum binding varied over a wide range from 71% (doxorubicin) to 96% (iodorubicin). The results of simulations were further supported by previous observations: the serum bindings of doxorubicin and epirubicin were measured to $71.0 \pm 0.9\%$ and $74.5 \pm 2.1\%$ [3], analogous to our simulated values of 70.6% and 80.5%, respectively. Mitoxantrone has been reported to be 78% plasma bound, similar to our value of 79.5%.

The relationship between the hydrophobicity of ligands and protein binding has been documented. The binding of vinca alkaloid analogues to AAG or HSA was related to the hydrophobicity of a substituent group [4]. In our series the difference in the albumin binding was particularly obvious between iododoxorubicin and the other analogues. The iodine substitution in the sugar residue reduces the basicity of the neighboring amino group, and at pH 7.4 this compound is more than 95% unprotonated and much more hydrophobic than doxorubicin [5]. As compared with albumin, the binding constants of the anthracycline analogues to AAG varied in a broader range. Doxorubicin and epirubicin, which do not differ in their substituents, had comparable affinity. Zorubicin displayed the highest affi-

nity for AAG, probably because of the very hydrophobic substituent in the tetracyclic moiety.

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