Cancer Chemother Pharmacol (1982) 10:7–10

Cancer Chemotherapy and Pharmacology

Original Articles

Protein Binding of Anthraquinone Glycosides, with Special Reference to Adriamycin

Staffan Eksborg, Hans Ehrsson, and Britta Ekqvist

Karolinska Pharmacy, Box 60024, S-104 01 Stockholm, Sweden

Summary. *The binding of anthraquinone glycosides (adriamycin, adriamycinol, daunorubicin, daunorubicinol, and 4'epi-adriamycin) to human serum albumin and human plasma has been studied by equilibrium dialysis. About 62% of adriamycin was bound to human serum albumin (45 g/l). Only minor variations in the degree of binding were observed between the anthraquinone glycosides.*

The binding degree of adriamycin in plasma from cancer patients was not significantly different from that observed in healthy volunteers, the fraction of free adriamycin being 24.56% ± 4.51%, and 27.67% ± 2.78%, respectively. The plasma albumin concentration was significantly lower in cancer patients than in the healthy volunteers (26.90% ± 5.88% and 39.24% ± 1.74%, respectively). In cancer patients the fraction of free adriamycin decreased with increasing plasma albumin concentration.

Introduction

During the last decade anthraquinone glycosides have successfully been used as antineoplastic drugs. Daunorubicin and adriamycin (Fig. 1) are frequently used for the treatment of leukemia [3, 4]; adriamycin is moreover a very effective drug against solid tumours [4, 9]. It has been suggested that 4'epi-adriamycin (Fig. 1) has a broader spectrum of antitumour activity than adriamycin, as well as less acute toxicity [5]. Neoplastic diseases are often associated with a lowering of the serum albumin concentration [25, 30], which may result in a decreased degree of protein binding of drugs [22]. Since it is generally accepted that only the free fraction of the drug can pass through the capillary walls of blood vessels to exert biological activity at their specific sites of action [6, 22, 24], it is reasonable to assume that the antineoplastic effect of anticancer drugs will affected by differences in the degree of protein binding [18, 29]. A study of the interaction of human serum albumin with anticancer drugs in vitro supports this assumption [31].

Only limited information in the protein binding of anthraquinone glycosides is available. Adriamycin appears to be bound to about 50% to rabbit and human plasma according to ultracentrifugation studies [19].

In the present paper the binding of adriamycin and daunorubicin, their reduced metabolites adriamycinol and daunorubicinol, and 4'epi-adriamycin to human serum albumin and human plasma was studied by equilibrium dialysis.

Reprint requests should be addressed to S. Eksborg

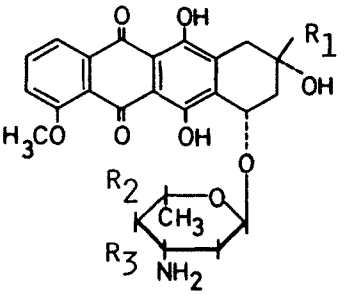

Fig. 1. Structural formulae:

Compound	R_1	R_2	R_3
Adriamycin	$-COCH_2OH$	$-H$	$-OH$
Adriamycinol	$-CH(OH)CH_2OH$	$-H$	$-OH$
Daunorubicin	$-COCH_3$	$-H$	$-OH$
Daunorubicinol	$-CH(OH)CH_3$	$-H$	$-OH$
4'epi–Adriamycin	$-COCH_2OH$	$-OH$	$-H$

The inter-individual variation in the fraction of free adriamycin in plasma from healthy volunteers and cancer patients was also investigated.

Methods

Human serum albumin, essentially fatty-acid-free (Sigma, St Louis, USA), was dissolved in isotonic phosphate buffer at pH 7.35 (concentration of NaCl: 0.095 *M*). Blood samples from cancer patients (Table 1) and healthy drug-free volunteers were collected in glass test tubes each containing 150 IU heparin in 30 µl sterile water. The plasma fractions were separated by centrifugation (4,000 *g*) for 10 min and stored at −80° C until use.

Bilirubin (Sigma, St Louis, Missouri, USA) was dissolved in 0.02 *M* NaOH. Plasma, 2 ml, was allowed to equilibrate with 100 µl of the bilirubin solution (3.3 g/l) at room temperature overnight before the dialysis experiments [8].

Adriamycin, adriamycinol, and 4'epi-adriamycin were kindly supplied by Farmitalia Carlo Erba AB, Täby, Sweden. Daunorubicin and daunorubicinol were obtained from Pharma Rhodia AB, Stockholm, Sweden.

Chromatographic Equipment. The chromatographic equipment used was from LDC (Laboratory Data Control, Riviera Beach, Florida) and has been described in detail elsewhere [14].

0344-5704/82/0010/0007/$ 01.00

Stability of Anthraquinone Glycosides. Solutions containing an anthraquinone glycoside (1 μg/ml) in isotonic phosphate buffer at pH 7.35 were stored at 37.0 ± 0.5° C. At appropriate time intervals the concentration of remaining anthraquinone glycoside was determined by reversed-phase liquid chromatography [14].

Table 1. Patients participating in the study

Patient	Sex	Age (years)	Primary tumor	Metastases
1	M	77	Prostate	Bone
2	F	54	Unknown	Neck
3	M	56	Bladder	Bone, lymph nodes
4	M	60	Bladder	No
5	F	79	Ureteral	No
6	M	58	Penis	No
7	M	54	Bladder	Bone
8	F	74	Oesophagus	No
9	M	56	Prostate	Bone, brain, liver (?)
10	M	57	Oesophagus	Thyroid gland

Table 2. Stability of anthraquinone glycosides

Compound	$t_{1/2}$ (h)	n
Adriamycin	–[a]	
Adriamycinol	128 ± 24[b]	7
4'epi-Adriamycin	20.6 ± 0.7	14
Daunorubicin	–[a]	
Daunorubicinol	94 ± 11	14

Conditions: 1 μg/ml of anthraquinone glycoside in isotone phosphate buffer pH 7.35; t = 37.0° C
[a] The degradation was less than 5% in 48 h
[b] ± Standard error of the mean

Table 3. Adsorption losses during dialysis experiments

Compound	Yield (%)[a]
Adriamycin	77.3 ± 4.0[b]
Adriamycinol	50.1 ± 4.5
Daunorubicin	85.8 ± 3.6
Daunorubicinol	92.0 ± 3.7
4'epi-Adriamycin	64.2 ± 1.4

Initial concentration of anthraquinone glycoside: 1 μg/ml
Concentration of albumin: 45 g/l
[a] After correction for degradation (cf. Table 2)
[b] ± Standard deviation (n = 8)

Table 4. Initial drug concentration and degree of protein binding

Initial daunorubicin concentration μg/ml	Bound to albumin (%)[a]
0.10	54.9
0.50	60.1
1.0	63.1
2.5	59.7
10.0	63.2
50.0	59.1

Each value is the mean of two determinations
[a] Concentration of albumin: 45 g/l

Equilibrium Dialysis and Analytical Technique. The binding of the anthraquinone glycosides to human serum albumin and human plasma was studied by equilibrium dialysis, using an equilibrium time of 7 h. The anthraquinone glycosides were dissolved in 0.1 M H_3PO_4, and diluted 10 times with the dialysis buffer. For the dialysis experiments one part of the buffer solution was diluted with 100 parts of plasma or albumin solution. The dialysis cells used were similar to those described by Patel and Foss [26], except that cellophane membranes were used (Union Carbide Corp., Chicago, Ill.) The cells were rocked gently in a thermostated water bath (37.0 ± 0.5° C). The concentration of the anthraquinone glycosides was determined in both cell compartments.

The concentration of the drugs in the unbound form was determined by direct injection of the protein-free solutions into the liquid chromatograph after dilution with phosphoric acid.

The concentration of the anthraquinone glycosides in protein solutions was determined after extraction into an organic phase and re-extraction into diluted phosphoric acid according to principles given by Eksborg [14–16]. The recovery of the most hydrophilic compound, adriamycinol [15], was > 96%.

Results and Discussion

Conditions of Equilibrium Dialysis

Stability of the Anthraquinone Glycosides. The stability of the various anthraquinone glycosides varied widely in the isotonic phosphate buffer used in the dialysis experiments (Table 2). However, the compound with the shortest half-life, 4'epi-adriamycin, showed less than 20% decomposition during storage in the dialysis buffer for 7 h at 37° C. Under the same conditions only trace amounts of the other anthraquinone glycosides degraded.

Adsorption of Anthraquinone Glycosides. The recovery of the anthraquinone glycosides from the dialysis cells was not quantitative (Table 3). However, losses of drugs to cell membranes and cell walls should not cause erroneous results in the protein binding study, since the drug concentration in both cell chambers was measured [17]; the degree of binding to albumin was independent of the drug concentration in the actual concentration range, as proved to be valid for daunorubicin (Table 4). Due to the very similar chemical structures of the other anthraquinone glycosides studied it was assumed that the protein binding was unaffected by the drug concentration in their cases also.

Equilibrium Dialysis Time Course. Studies on the influence of the dialysis time showed that equilibrium was reached after 7 h.

Binding of Anthraquinone Glycosides to Human Serum Albumin and Plasma

About 60% of the anthraquinone glycosides were bound to human serum albumin (45 mg/ml) (Table 5), with only minor differences between the compounds. Similar small variations in the degree of protein binding of the various compounds were also observed in human plasma (Table 6). Although albumin is considered as the principle drug-binding protein in plasma [21, 32], the results in Tables 5 and 6 indicate that adriamycin might also be bound to other macromolecules present in plasma.

Table 5. Binding of anthraquinone glycosides to human serum albumin

Compound	Bound to albumin (%)
Adriamycin	62.1 ± 1.6[a]
Adriamycinol	55.8 ± 2.5
Daunorubicin	63.1 ± 2.9
Daunorubicinol	63.6 ± 3.0
4'epi-Adriamycin	56.9 ± 2.0

Initial concentration of anthraquinone glycosides: 1 μg/ml
Concentration of albumin: 45 g/l
[a] ± Standard deviation (n = 8)

Table 6. Binding of anthraquinone glycosides in human plasma

Compound	Bound to plasma (%)
Adriamycin	71.0 ± 0.9[a]
Adriamycinol	75.5 ± 0.8
4'epi-Adriamycin	74.5 ± 2.1

Concentration of plasma albumin: 38 g/l
Concentration of anthraquinone glycoside: 1 μg/ml
Plasma from one of the volunteers was used
[a] ± Standard deviation (n = 10)

Binding of Adriamycin to Plasma from Healthy Volunteers and Cancer Patients

The inter-individual variation of the binding of adriamycin was studied in plasma from healthy volunteers as well as from cancer patients (Fig. 2). The plasma albumin concentration was higher in the healthy volunteers than in the cancer patients (39.24 ± 1.74 g/l, n = 10 and 26.90 ± 5.99 g/l, n = 10, respectively; $P < 0.001$), but no statistical difference in the fraction of unbound adriamycin could be observed (27.67% ± 2.78%, n = 10 and 24.56% ± 4.51%, n = 10, respectively; $P > 0.1$). The binding of adriamycin in plasma and the plasma albumin concentration exhibited wider inter-individual variation in the cancer patients than in the healthy volunteers. The free concentration of adriamycin decreased with increasing plasma albumin concentration in the cancer patients ($r = -0.670$, $P < 0.03$).

Since it is bound to the same extent in plasma from cancer patients and from healthy volunteers despite the differences in plasma albumin concentration, adriamycin seems to be bound to other components in plasma which are elevated in cancer patients.

In recent years it has been recognized that in addition to albumin, the serum protein alpha$_1$-acid glycoprotein (α_1-AG) plays a significant role in the binding of drugs, especially those which are cationic at physiologic pH [7, 23, 27, 28]. Plasma levels of α_1-AG increase in cancer [11, 20]. Therefore it is not unlikely that the wide inter-individual variation of the fraction of free adriamycin, partly cationic at physiologic pH [15], that is observed in cancer patients may be partially due to differences in α_1-AG levels in combination with differences in plasma albumin levels (Fig. 3). The plasma level of α_1-AG increased with decreasing concentration of plasma albumin, with wide inter-individual variations ($r = -0.633$), $P < 0.05$). The fraction of free adriamycin decreased with increasing plasma α_1-AG level in patients 1, 3, and 10 (Fig. 4), who had very similar plasma albumin concentrations (24–26 g/l), suggesting that α_1-AG may contribute to the binding of adriamycin in cancer patients. Multiple linear regression

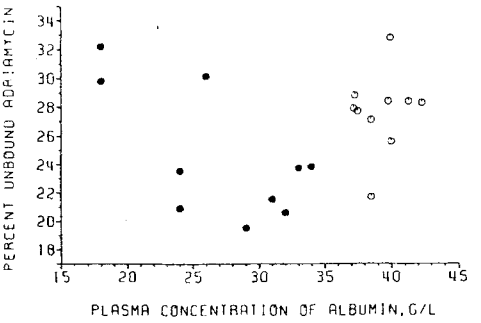

Fig. 2. Plasma albumin concentration and percent unbound adriamycin. *Open symbols.* Plasma samples from healthy volunteers; *solid symbols,* plasma samples from cancer patients

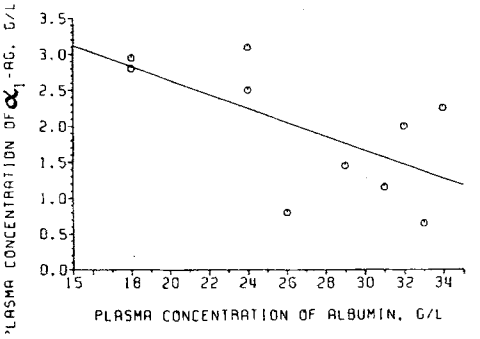

Fig. 3. Plasma concentrations of albumin and α_1-AG in cancer patients. The line is given by the linear regression equation $y = 4.58 - 9.702 \cdot 10^{-2}$ X; $r = -0.633$

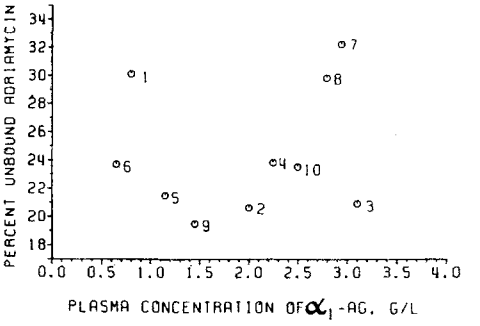

Fig. 4. Plasma α_1-AG concentration and percent unbound adriamycin. *Numbers* in the figure refer to the patients listed in Table 1

analysis of the data from the binding of adriamycin in plasma from cancer patients, where the plasma concentrations of both albumin and α_1-AG were taken into consideration, did not give a better fit of the experimental values for the fraction of free adriamycin than the model including only the plasma albumin concentration (Fig. 2). There was no correlation between the free adriamycin concentration and the plasma concentration of α_1-AG ($r = 0.18$, $P > 0.6$) (Fig. 4). Since only the free fraction of drugs exerts biological activity [6, 22, 24], it is likely that the wide inter-individual variation of the protein binding of adriamycin observed in cancer patients in combination with the reported wide inter-individual variation of the pharmacokinetics [13] contributes to the variation in therapeutic efficiency and in the side-effects observed with adriamycin therapy.

Enhanced adriamycin toxicity has frequently been reported in patients with liver metastases, being most pronounced in patients with elevated serum bilirubin levels [1, 2].

Recent reports state that the pharmacokinetic pattern of adriamycin is not usually altered in these patients [10, 12]. It may be that the fraction of free adriamycin is increased due to displacement from binding sites by bilirubin [8]. The fraction of free adriamycin in human plasma was 31.3% ± 1.4% (SD, n = 5) after the addition of 270 μmol bilirubin/l, a concentration only rarely exceeded in patients undergoing adriamycin therapy. In the same plasma sample without added bilirubin, 32.0% ± 2.2% (SD, n = 5) of adriamycin was unbound, showing that bilirubin does not affect the fraction of unbound adriamycin.

References

1. Anderson JE, Hunt JM, Smith IE (1981) Prevention of doxorubicin-induced alopecia by scalp cooling in patients with advanced breast cancer. Br Med J 282: 423
2. Benjamin RS, Wiernik PH, Bachur NR (1974) Adriamycin chemotherapy – efficacy, safety, and pharmacological basis of an intermittent single high-dosage schedule. Cancer 33: 19
3. Bernard J, Paul R, Boiron M, Jacquillat C, Maral R (eds) (1969) Rubidomycin. Recent Results Cancer Res 20 (Special Issue)
4. Blum RH, Carter SK (1974) Adriamycin. A new anticancer drug with significant clinical activity. Ann Intern Med 80: 249
5. Bonfante V, Bonadonna G, Villani F, Martini A (1980) Preliminary clinical experience with 4'-epi-doxorubicin in advanced human neoplasia. Recent Results Cancer Res 74: 202
6. Borgå O, Hamberger B, Malmfors T, Sjöqvist F (1970) The role of plasma protein binding in the inhibitory effect of nortriptyline on the neuronal uptake of norepinephrine. Clin Pharmacol Ther 11: 581
7. Borgå O, Piafsky KM, Nilsen OG (1977) Plasma protein binding of basic drugs. 1. Selective displacement from α_1-acid glycoprotein by tris(2-butoxyethyl)phosphate. Clin Pharmacol Ther 22: 539
8. Brodersen R (1974) Competitive binding of bilirubin and drugs to human serum albumin studied by enzymatic oxidation. J Clin Invest 54: 1353
9. Carter SK (1975) Adriamycin – A review. J Natl Cancer Inst 55: 1265
10. Chan KK, Chlebowski RT, Tong M, Chen H-SG, Gross JF, Bateman JR (1980) Clinical pharmacokinetics of adriamycin in hepatoma patients with cirrhosis. Cancer Res 40: 1263
11. Chio L-F, Oon C-J (1979) Changes in serum alpha$_1$-antitrypsin, alpha$_1$-acid glycoprotein and beta$_2$-glycoprotein I in patients with malignant hepatocellular carcinoma. Cancer 43: 596
12. Chlebowski RT, Chan KK, Tong MJ, Weiner JM, Ryden VMJ, Bateman JR (1981) Adriamycin and methyl-CCNU combination therapy in hepatocellular carcinoma: clinical and pharmacokinetic aspects. Cancer 48: 1088
13. Ehninger G, Stocker H-J, Proksch B, Wilms K (1980) Die Pharmakokinetik von Adriamycin-Metaboliten. Klin Wochenschr 58: 927
14. Eksborg S (1978a) Extraction properties and liquid chromatographic separation of adriamycin and daunorubicin and their hydroxyl metabolites. Application to bioanalysis. In: Clinical pharmacology of anti-neoplastic drugs. Elsevier/North-Holland Biomedical Press, Amsterdam, p 193
15. Eksborg S (1978b) Extraction of daunorubicin and doxorubicin and their hydroxyl metabolites: Self-association in aqueous solution. J Pharm Sci 67: 782
16. Eksborg S (1978c) Reversed-phase liquid chromatography of adriamycin and daunorubicin and their hydroxyl metabolites adriamycinol and daunorubicinol. J Chromatogr 149: 225
17. Elofsson R (1975) In vitro studies on the binding of drugs to human serum proteins, mainly human serum albumin. Acta Universitatis Upsaliensis, no. 7 (Abstracts of Uppsala Dissertations from the Faculty of Pharmacy)
18. Esposito M, Rosso R, Santi L (1974) Effects of antineoplastic drugs on plasma protein binding of methotrexate in rats and humans. IRCS 2: 1181
19. Harris PA, Gross JF (1975) Preliminary pharmacokinetic model for adriamycin (NSC-123127). Cancer Chemother Rep [1] 59: 819
20. Hollinshead AC, Chuang Ç-Y, Cooper EH, Catalona WJ (1977) Interrelationship of prealbumin and α-1 acid glycoprotein in cancer patient sera. Cancer 40: 2993
21. Jusko WJ (1976) Pharmacokinetics in disease states changing protein binding. In: Benet LZ (ed) The effect of disease states on drug pharmacokinetics. American Pharmaceutical Association, Washington, p 99
22. Koch-Weser J, Sellers EM (1976) Drug therapy. Binding of drugs to serum albumin. N Engl J Med 294: 311
23. Kornguth ML, Hutchins LG, Eichelman BS (1981) Binding of psychotropic drugs to isolated α_1-acid glycoprotein. Biochem Pharmacol 30: 2435
24. Kunin CM (1966) Clinical pharmacology of the new penicillins. I. The importance of serum protein binding in determining antimicrobial activity and concentration in serum. Clin Pharmacol Ther 7: 166
25. Mider GB, Alling EL, Morton JJ (1950) The effect of neoplastic and allied diseases on the concentrations of the plasma proteins. Cancer 3: 56
26. Patel NK, Foss NE (1964) Interaction of some pharmaceuticals with macromolecules. I. Effect of temperature on the binding of parabens and phenols by polysorbate 80 and polyethylene glycol 4000. J Pharm Sci 53: 94
27. Piafsky KM, Borgå O (1977) Plasma protein binding of basic drugs. II. Importance of α_1-acid glycoprotein for interindividual variation. Clin Pharmacol Ther 22: 545
28. Piafsky KM, Borgå O, Odar-Cederlöf I, Johansson C, Sjöqvist F (1978) Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma α_1 acid glycoprotein. N Engl J Med 299: 1435
29. Steele WH, Lawrence JR, Stuart JFB, McNeill CA (1981) The protein binding of methotrexate in the serum of patients with neoplastic disease. Cancer Chemother Pharmacol 7: 61
30. Steinfeld JL (1960) I^{131} Albumin degradation in patients with neoplastic disease. Cancer 13: 974
31. Takahashi I, Ohnuma T, Kavy S, Bhardwaj S, Holland JF (1980) Interaction of human serum albumin with anticancer agents in vitro. Br J Cancer 41: 602
32. Vallner JJ (1977) Binding of drugs by albumin and plasma protein. J Pharm Sci 66: 447

Received May 25/Accepted September 27, 1982