Cancer Chemother Pharmacol (1995) 36: 506–512

ORIGINAL ARTICLE

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Etoposide protein binding in cancer patients

Received: 20 July 1994/Accepted: 31 January 1995

Abstract The protein binding of etoposide was studied in vivo in 36 cancer patients receiving etoposide therapy. Free etoposide was separated from plasma using an ultrafiltration method and the etoposide concentrations (free and total) were measured by high-performance liquid chromatography (HPLC). Considerable interpatient variation in the protein binding of etoposide was observed. The protein binding of etoposide varied from 80% to 97% (mean, 93%). Univariate analysis showed a significant inverse correlation between the free fraction of etoposide and serum albumin ($r = -0.74$), daily dose ($r = -0.37$) and age ($r = -0.34$). Multivariate analysis demonstrated that serum albumin and age were independent predictors of the etoposide free fraction. Serum bilirubin showed no correlation with etoposide protein binding. There is wide variation in etoposide protein binding in cancer patients, which is mostly dependent on serum albumin concentration.

Key words Etoposide · Protein binding · Free fraction

Introduction

Etoposide (VP 16 213) is a semisynthetic glycoside derivative of podophyllotoxin, originally an extract of the mandrake plant, and has been widely used in the treatment of a variety of malignancies [13]. Its activity at achievable serum levels derives from topoisomerase II inhibition [4], although at higher levels it also acts to inhibit microtubule assembly [8]. Etoposide is a highly schedule-dependent cytotoxic drug [7, 18, 19], which can be given both by intravenous infusion and by chronic low-dose oral administration [11]. Clinically, an important problem with oral administration is its extraordinary variability in bioavailability, with absorption varying between 30% and 80% [5]. Major dose-limiting toxicities include myelosuppression and oral mucositis. Detailed pharmacokinetics studies examining total plasma etoposide concentrations have failed to demonstrate a clear relationship between pharmacokinetic parameters and toxic pharmacodynamic endpoints [1–3, 17].

Drug protein binding is one of the most important factors affecting the pharmacokinetics and pharmacodynamics of individual drugs [15, 22]. Etoposide is heavily protein-bound, and protein binding has been reported to be 94%–98% in vitro as determined using both equilibrium dialysis [1] and ultrafiltration techniques [10]. Depending on clearance kinetics, small changes in the proportion of bound etoposide could have major implications for the unbound drug concentration and, therefore, for response and toxicity. Stewart et al. [20, 21] studied etoposide protein binding in cancer patients receiving etoposide in combination with platinum compounds and demonstrated important interpatient variations in the unbound fraction.

In this study we examined the extent of interpatient variation in etoposide protein binding in vivo in 36 cancer patients, 30 of whom received etoposide as a single agent, and explored the factors that may influence this variation.

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Patients and methods

Patients

A total of 36 adult patients (29 women and 7 men) with histologically proven cancer were included in this study. Their mean age was 58

years (range, 30–82 years). The distribution of cases by tumour type was as follows: 22 ovarian cancer, 8 small-cell lung cancer (SCLC), 2 breast cancer, 2 lymphoma, 1 hepatoma and 1 colonic cancer. In all, 25 patients with ovarian, breast and colonic cancer had failed standard chemotherapeutic regimens and were entered into a phase II study of single-agent etoposide carried out at the Birmingham Oncology Centre (Poole et al., manuscript in preparation). Five patients with SCLC received single-agent oral etoposide as first-line treatment. Six patients received etoposide as part of a combination chemotherapy regimen; three patients with SCLC received etoposide and cisplatin, two patients with relapsed high-grade non-Hodgkin's lymphoma received etoposide and cytosine arabinoside, and one patient with hepatoma was treated using etoposide with 5-FU and folinic acid [23].

Previous chemotherapy

Altogether, 30 patients had received previous chemotherapy for their cancer, with a range of 1–5 regimens being given per patient. In all, 23 patients had received prior cisplatin. Only six patients received etoposide as part of first-line chemotherapy, of whom five had SCLC and one had hepatoma.

Investigations

All patients had the following investigations prior to each cycle of chemotherapy: serum albumin, total bilirubin, alkaline phosphatase, creatinine and calculated creatinine clearance using the standard Cockcroft formula [6].

Etoposide schedules

A total of 29 patients received oral etoposide and 27 of these were treated with 2 different schedules as follows: schedule 1 (20 patients), etoposide given at 50 mg, every 12 h (50–68 mg/m^2 per day) for 3–14 days, with cycles being repeated every 21 days; and schedule 2 (7 patients), etoposide given at 50 mg with a 16-h dosing interval (50 mg/m^2 per day) for 10 days, with cycles being repeated every 21 days. In addition, one patient was treated with etoposide given at 50 mg daily for 5 days, with cycles being repeated every 28 days; and a further patient was given etoposide at 50 mg twice a day alternating with 50 mg daily for 21 days. The majority of patients who received oral etoposide had 10 days of treatment every 3 weeks. Seven patients received intravenous etoposide, six received a 24-h infusion (30–50 mg/m^2 per day) for 4–7 days, and one patient had a 30-min infusion corresponding to 120 mg/m^2 daily for 3 consecutive days.

Plasma sample collection

Blood samples were taken during the first and second courses of etoposide treatment. For analysis of steady-state plasma etoposide levels in patients receiving oral etoposide, blood samples were taken at or after 72 h from the start of treatment (after 10 x $t_{1/2}$ values) [5]. In patients who were receiving continuous-infusion etoposide, steady-state samples were taken at 48–120 h after the start of the infusion [3]. In seven patients, blood samples were obtained and analysed from each of two or three cycles of treatment. Blood samples were put into heparinized tubes and centrifuged at 2000 rpm and 4°C for 10 min, and plasma was separated and stored at − 20°C.

Etoposide analysis

Pure etoposide standard was kindly provided by Bristol Myers Squibb. All the solvents used were of analytical or high-performance liquid chromatography (HPLC) grade.

Analysis of total etoposide concentration

Total plasma concentrations of etoposide were determined by reverse-phase HPLC on a system equipped with a 229-nm absorbance detector. The assay was carried out according to the method of Harvey et al. [12]. Diphenylhydantoin was used as an internal standard. The recovery of etoposide from plasma was 90% (±5%), the lower limit of sensitivity was 0.2 μg/ml, and the intra-assay and inter-assay coefficients of variation were 5% and 7%, respectively.

Analysis of free etoposide concentration

Free etoposide was separated from plasma by an ultrafiltration technique using a Millipore Ultrafree-CI filter unit with a low-binding membrane (30,000 NMWL). The retention rate for albumin (mol. wt, 67,000 Da) is more than 98% on this filter membrane.

The etoposide concentration in the ultrafiltrate (unbound etoposide) was measured by reverse-phase HPLC with an electrochemical detection method developed in our laboratory. Calibration curves were obtained by analysing spiked protein-free water with various concentrations of etoposide in ethanolic solution. Altogether, 1ml of the plasma samples and 250 μl of the standards were centrifuged using a Hettich centrifuge for 8 h at 1700 *g* and 4°C. Of the ultrafiltrates, 150 μl was extracted with 2 × 5 ml of chloroform to remove any plasma components that might interfere with the HPLC detection and measurement of etoposide. After shaking and centrifugation (1500 g for 10 min), the organic layer was removed into a clean tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted with 150 μl of mobile phase, and 40 μl of this solution was injected into the HPLC system.

The HPLC system consisted of a high-pressure pump (SA 6410, Severn Analytical), an injector with a 20-μl loop (Model 7125 Pheodyne), a 5-μm ODS Hypersil column (100 × 4.6 mm) and an electrochemical detector (Model 4000, Princeton Applied Research) equipped with dual glassy carbon electrodes. The detection was performed using a 10-nA current range, two potentiostat (2 pstat) model (E1 = + 300 mV, E2 = + 800 mV) with 2 × gain. The difference (ΔI) between the cell currents from potentiostats 1 (E1) and 2 (E2) was traced on a pen-chart recorder (Tekaman) at a speed of 1 cm/min. Separation was achieved with an isocratic solvent system of methanol:water (50:50, v/v) at a flow rate of 1 ml/min. For each patient, total and free etoposide levels were measured and the free fraction (Ff) of etoposide was calculated (Ff = free etoposide concentration/total etoposide concentration).

Statistical methods

The data were analysed using the BMDP statistical software package. Exploratory data analysis was carried out to determine correlations inherent in the data. Simple linear regression was employed with Pearson's correlation coefficient (*r*) for continuous data to test for significant associations. Stepwise multiple regression was carried out to see which patient variables (serum albumin, bilirubin, alkaline phosphatase, creatinine, creatinine clearance, free level of etoposide concentration, liver function, renal function, age, sex, daily dose and total dose) were independently associated with the free fraction of etoposide.

Table 1 Recovery of etoposide in the Millipore Ultrafree-Cl filter

Concentration (μg/ml)	Volume	Recovery[a]	(SD)
10	1 ml	102%	(3.2%)
1	1 ml	104%	(2.3%)
0.1	1 ml	93.16%	(2.4%)
0.8	250 μl	104.75%	(3.9%)
0.4	250 μl	98.25%	(4.2%)
0.2	250 μl	102.8%	
0.1	250 μl	83.68%	(8.5%)
0.05	250 μl	80.77%	
10	200 μl	100%	
0.8	200 μl	91.43%	
0.1	200 μl	83.54%	
0.05	200 μl	75.36%	

[a] Recovery = drug concentration after filter/drug concentration before filter

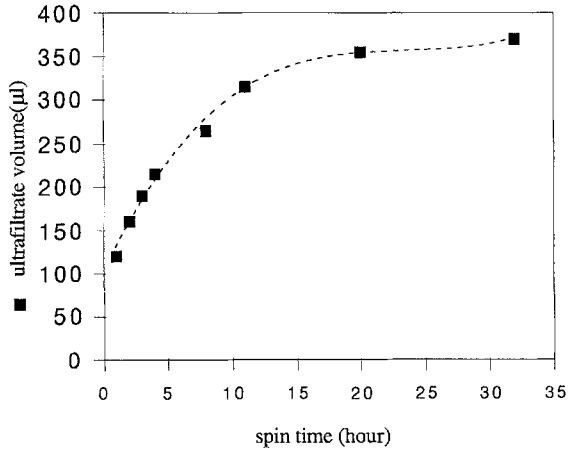

Fig. 1 The relationship between spin time and ultrafiltrate volume

Results

Assay of free etoposide concentration in plasma

Using different concentrations and volumes of etoposide aqueous solution, assessment was made of any binding to the filter membrane (Table 1). The results showed that the recovery of etoposide increased with etoposide concentration and sample volume. When the free etoposide concentration and filtered volume were 0.2 μg/ml and 250 μl, respectively, the recovery of etoposide approached 100%. Figure 1 shows the relationship between the volume of ultrafiltrate and the spin time as determined using 1 ml of plasma centrifuged at constant spin force (1700 g) and at controlled temperature (4°C). Figure 2 shows the relationship between the etoposide concentration in the ultrafiltrate and the spin time as determined using ultrafiltration of human plasma spiked with a 5-μg/ml concentration of etoposide. The results showed that both the ultrafiltrate volume and the etoposide concentration in the

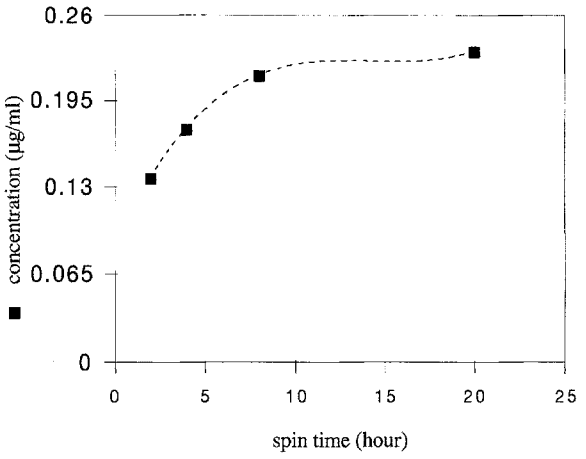

Fig. 2 The relationship between spin time and etoposide concentration in ultrafiltrate

ultrafiltrate approached constant levels after 8 h of centrifugation.

For free etoposide analysis, the limit of detection for etoposide was 20 ng/ml. The extraction efficiency of etoposide (at 0.05 μg/ml) from ultrafiltrate was approximately 95%. Coefficients of variation were less than 10% both within and between runs of plasma samples.

Protein binding of etoposide

The laboratory data for all patients are presented in Table 2. The mean serum albumin concentration was 34 g/l, the mean serum bilirubin value was 26 μmol/l, the mean serum alkaline phosphatase level was 439 IU/l, the mean serum creatinine concentration was 105 μmol/l, and the mean calculated creatinine clearance was 55 ml/min. Only eight patients had normal values for all parameters measured. The total and free steady-state plasma etoposide concentration is shown in Table 3, as is the calculated free fraction of etoposide in all 36 patients. The mean free fraction was 0.074 (SD, ±0.034; range, 0.033–0.195).

Univariate analysis

The correlation between both the free fraction and the free concentration of etoposide and serum albumin, bilirubin, alkaline phosphatase and serum creatinine values as well as the calculated creatinine clearance, daily dose (milligrams per square meter per day) and total dose per course was analysed. The results are shown in Table 4. The most significant correlation was found between the serum albumin concentration and the free fraction of etoposide ($r = -0.74$, $P < 0.0001$; Fig 3). There was also a significant negative correlation between age and free fraction ($r = -0.34$, $P = 0.04$) as

Table 2 Laboratory data and free fraction of etoposide for patients (*Alk. phos* Alkaline phosphatase, *S. Cr* serum creatinine, *Cr Cl* creatinine clearance, *chemo.* chemotherapy, *Ca* cancer, *Cis.* cisplatin, *Ara-c* arabinofuranosylcytosine, *Bleo* bleomyin, *5-FU* 5-fluorourocil, *Folin.* folinic)

Patient number	Age/Sex (years)	Diagnosis	Free fraction	Albumin (g/l)	Bilirubin (μmol/l)	Alk. phos (IU/l)	S. Cr (μmol/l)	Cr Cl (ml/min)	Other chemo.
1	54 /M	SCLC	0.045	37	10	364	82	75	Cis.
2	70 /F	SCLC	0.035	36	7	246	80	50	No
3	82 /M	SCLC	0.057	37	7	152	107	61	No
4	67 /M	SCLC	0.132	26	104	557	98	61	Cis.
5	62 /F	SCLC	0.067	37	23	1510	77	60	No
6	63 /F	SCLC	0.044	41	176	1203	66	50	Cis.
7	59 /M	SCLC	0.101	29	10	175	73	98	No
8	60 /F	SCLC	0.116	28	19	262	83	60	No
9	66 /M	Colonic Ca	0.056	32	10	123	117	45	No
10	67 /M	Lymphoma	0.082	29	161	1367	80	72	Ara-C/Bleo
11	57 /M	Hepatoma	0.093	25	150	268	87	85	5-FU/Folin. acid
12	45 /F	Breast Ca	0.099	33	10	134	74	85	No
13	55 /F	Breast Ca	0.093	29	18	295	71	70	No
14	57 /F	Ovarian Ca	0.138	22	4	229	67	65	No
15	30 /F	Ovarian Ca	0.064	35	4	134	82	66	No
16	58 /F	Ovarian Ca	0.105	29	10	137	275	19	No
17	42 /F	Ovarian Ca	0.07	39	6	459	136	41	No
18	72 /F	Ovarian Ca	0.033	44	7	139	127	42	No
19	40 /F	Ovarian Ca	0.093	41	12	155	137	42	No
20	58 /F	Ovarian Ca	0.068	45	7	190	100	46	No
21	63 /F	Ovarian Ca	0.042	34	5	247	90	52	No
22	61 /F	Ovarian Ca	0.036	39	9	2280	108	44	No
23	74 /F	Ovarian Ca	0.054	43	27	173	77	47	No
24	57 /F	Ovarian Ca	0.067	37	18	210	168	29	No
25	57 /F	Ovarian Ca	0.063	40	13	247	123	37	No
26	69 /F	Ovarian Ca	0.064	39	8	170	133	31	No
27	38 /F	Ovarian Ca	0.05	41	12	244	85	84	No
28	62 /F	Ovarian Ca	0.074	27	3	546	115	44	No
29	52 /F	Ovarian Ca	0.072	38	13	150	101	55	No
30	56 /F	Ovarian Ca	0.071	31	7	317	132	39	No
31	39 /F	Ovarian Ca	0.195	21	34	1649	102	58	No
32	67 /F	Ovarian Ca	0.036	38	4	189	80	49	No
33	55 /F	Ovarian Ca	0.096	24	12	317	73	78	No
34	68 /F	Ovarian Ca	0.041	39	6	272	99	45	No
35	50 /F	Ovarian Ca	0.048	39	8	470	206	33	No
36	68 /F	Lymphoma	0.073	23	10	220	81	105	Ara-C/Bleo
Mean	58		0.074	34	26	439	105	55	
SD	11		0.034	6.7	45	505	42	18	
Range	30–82		0.033–0.195	21–45	3–176	123–2280	66–275	19–105	

Table 3 Plasma etoposide concentration during steady-state

Route	Etoposide concentration (mg/ml) Mean (range)	Number of patients	Number of courses
Oral	Total: 3.14 (1.52–4.88)	27	36
	Free: 0.21 (0.09–0.68)	27	36
Intravenous	Total: 2.28 (1.42–3.48)	6	7
	Free: 0.31 (0.12–0.68)	6	7

well as daily dose (milligrams per square meter per day) and free fraction ($r = -0.37$, $P = 0.03$). There was no significant correlation between the free fraction and the serum bilirubin, alkaline phosphatase or creatinine value, creatinine clearance, or total dose. In analysis of the free etoposide concentration as opposed to the etoposide free fraction, the only significant inverse correlation was with serum albumin values ($r = -0.35$, $P = 0.04$), and there was no significant correlation with the other variables.

Table 4 Correlation coefficients for the free fraction of etoposide versus demographic and biochemical variables (*Alk. phos.* Alkaline phosphatase)

	Free fraction		Free concentration	
	r	*P* value	*r*	*P* value
Age	− 0.34	0.04*	− 0.03	0.86
Total dose (mg)	− 0.27	0.12	− 0.03	0.87
Dose/day	− 0.37	0.03*	− 0.15	0.38
Albumin	− 0.74	< 0.0001*	− 0.35	0.04*
Bilirubin	0.14	0.41	− 0.03	0.86
Alk. phos	0.13	0.47	0.24	0.16
Serum creatinine	− 0.02	0.91	− 0.02	0.91
Creatinine clearance	0.25	0.14	− 0.02	0.92

* Statistically significant ($P < 0.05$)

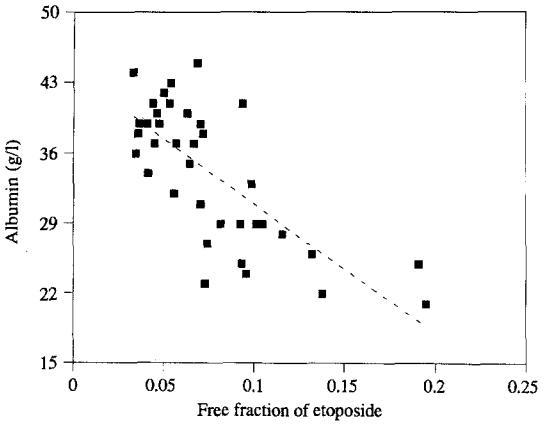

Fig. 3 Scattergram of the free fraction of etoposide in relation to the serum albumin concentration ($r = -0.744$, $P < 0.0001$)

Multiple regression analysis

A multiple stepwise regression analysis was performed using age; daily dose; total dose/course; serum albumin, bilirubin, alkaline phosphatase and creatinine levels; and creatinine clearance to see which factors might be independent predictors of the free fraction of etoposide. Table 5 shows that serum albumin and age are significant independent predictors of the free fraction of etoposide. The regression equation for the free fraction of etoposide is: $Ff = 0.256 - 0.001(age) - 0.004(albumin)$.

Discussion

This study of etoposide protein binding in 36 cancer patients shows that there is considerable interpatient variation in drug protein binding at steady state. The protein binding of etoposide varied between 80.5% and 96.7%, with the mean value being 92.6% (SD, ±3.4%). The percentage of free etoposide therefore varies between 3.3% and 19.5%, and this may have important implications for the pharmacodynamics of the drug. Studies of protein binding that have been carried out in vitro show etoposide protein binding of 94–98%. Stewart et al. [20], who also studied cancer patients, found results similar to our own, recording a mean protein binding of 86.1% (range, 62.3%–94%; SD, ±9%). Schwinghammer et al. [16] studied 16 patients undergoing autologous bone marrow transplantation for advanced lymphoma after receiving high-dose etoposide as a single intravenous infusion, and found etoposide protein binding of 84% (SD, ±5%). This group also found that the mean percentage of protein binding detected at the end of the high-dose infusion was higher than that found at the lowest measured concentration (21% and 13%, respectively; $P = 0.017$), suggesting that some concentration-dependent variation in binding may occur at high etoposide doses.

Why should there be this variation of binding in cancer patients and why should etoposide be less well protein-bound than is suggested by the in vitro studies? Our study demonstrates that serum albumin concentration has a strong negative correlation with etoposide protein binding ($r = -0.74$, $P < 0.0001$) such that at low levels of serum albumin there are higher percentages of free etoposide, which is likely to produce more activity for the same delivered dose and the same total serum level. Serum albumin was also the most important single independent predictive factor for etoposide protein binding in the multivariate analysis carried out in this study. The variation in the percentage of binding with protein concentration and drug concentration is non-linear and complex. Drugs that are weakly protein-bound (affinity constant, 10^4) show changes in binding that are somewhat proportional to the protein concentration, particularly over the physiological range of protein concentration (10^{-5}–10^{-3} *M*). The binding is only slightly different at different levels of total drug concentration [14]. In 1975 Allen and Creaven published the first paper dealing with etoposide protein binding, and reported an affinity constant for etoposide of 3.54×10^4 for serum albumin as determined by in vitro equilibrium dialysis, whereas a more

Table 5 Stepwise multiple regression analysis for the free fraction of etoposide

Dependent variable	Factors included	Coefficient	F-to remove	p-value
Free fraction	Albumin	− 0.004	51.20	< 0.0001
	Age	− 0.001	9.48	0.001
			Constant = 0.256	

recent paper by Fleming et al. [9] reports an affinity constant of 2.88×10^4. This would support our present findings that for etoposide the majority of the effect on protein binding may be caused by the variations in serum albumin concentration.

Endogenous substances such as bilirubin and fatty acids, and concomitant administration of other drugs, including chemotherapeutic agents, will interfere with etoposide protein binding both by competing for binding sites on serum albumin and by altering the conformation of albumin and thereby altering drug binding. In this study the majority of patients received etoposide as a single agent, and our results show a higher protein etoposide binding than was reported by Stewart et al. [10]. In that study, 88% of patients received etoposide in combination with platinum compounds, which may interfere with etoposide protein binding.

In univariate analysis, age showed a significant negative correlation with the free fraction of etoposide ($r = -0.34$, $P = 0.04$), and this was also an independent predictive factor in multivariate analysis. Thus, the older the patient, the lower the free fraction of etoposide. This is an unexpected finding since older patients demonstrate more toxicity to chemotherapy in general and to etoposide in particular. These findings would suggest that the increased toxicity in older patients is dependent not on changes in the free fraction of etoposide but, presumably, on other factors such as decreased drug clearance. In univariate analysis a negative correlation was found between the daily dose of etoposide (milligrams per square meter) and the free fraction of drug ($r = -0.37$, $P = 0.03$). In our multivariate analysis this was not found to be an independent predictive factor, and it is therefore difficult to know whether it is of any major significance.

We did not confirm any relationship between the free fraction of etoposide and the serum bilirubin concentration in this study. However, Stewart et al. [20] described an important positive correlation between the free fraction of etoposide and the serum bilirubin level in 17 cancer patients. Albumin has six recognised binding regions, and bilirubin binds to region three [24]. There is no available information on the binding site of etoposide, but Stewart et al.'s results might suggest either that bilirubin and etoposide share the same binding site or that bilirubin interferes with etoposide binding by producing conformational changes in the albumin molecule. Our own results, however, would not support this possibility and suggest that bilirubin does not interfere significantly with etoposide protein binding. Other serum factors that showed no relationship to etoposide protein binding in this study included serum alkaline phosphatase and serum creatinine levels and calculated creatinine clearance. This suggests that hepatic and renal function do not significantly influence the protein binding of etoposide.

There is wide variation in etoposide protein binding in this group of patients with cancer, with low levels of binding occurring in a significant number of cases. Whilst the majority of this effect may be due to lower serum albumin concentrations, there may also be other mechanisms in operation. There are many endogenous substances that normally bind to albumin and may interfere with the binding of etoposide. Endogenous substances that have higher affinity constants than etoposide ($3.54 \times 10^4 M^{-1}$) for albumin include haemin, palmitate, linoleate, oleate, L-thyroxine, progesterone, estradiol and lysolecithin [24], and these may be implicated in competing for albumin binding sites with etoposide and in contributing to the variation in protein binding that we found in our cancer patients.

In conclusion, we found considerable interpatient variation in etoposide protein binding in this group of 36 adult patients with cancer. Protein binding showed a significant correlation with serum albumin, age and daily dose in univariate analysis, and both serum albumin and age were independent predictive factors for etoposide protein binding in a multivariate analysis.

Acknowledgements. The authors would like to acknowledge Bristol Myers Squibb for financial support; St. Chad's Unit Trust Fund for the research grant awarded to Dr. Bo Liu; and Dr. Andy Barnes, Pharmacy, City Hospital Trust, for support and expert advice.

References

1. Allen LM, Creaven PJ (1975) Comparison of the human pharmacokinetics of VM-26 and VP-16, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11:697
2. Brindley CJ, Antoniw P, Newlands ES, Bagshawe KD (1985) Pharmacokinetics and toxicity of the epipodophyllotoxin derivative etoposide (VP16-213) in patients with gestational choriocarcinoma and malignant teratoma. Cancer Chemother Pharmacol 15:66

3. Chatelut E, Chevreau C, Blancy E, Lequellec A, Canal P, Roche H, Houin G, Bugat R (1990) Pharmacokinetics and toxicity of two modalities of etoposide infusion in metastatic non-small-cell lung carcinoma. Cancer Chemother Pharmacol 26:365
4. Chen GL, Yang L, Rowe TC, Halligan BD, Tewey KM, Liu LF (1984) Nonintercalative antitumor drugs interfere with the breakage reunion reaction of mammalian DNA topoisomerase II. J Biol Chem 259:3560
5. Clark PI, Slevin ML (1987) The clinical pharmacology of etoposide and teniposide. Clin Pharmacokinet 12:223
6. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31
7. Dombernowsky P, Nissen NI (1973) Schedule dependency of the antileukemic activity of the podophyllotoxin-derivative VP-16-213 in L1210 leukemia. Acta Pathol Micobiol Scand Immunobiol 81:715
8. Donehower RC, Rowinsky EK (1993) Anticancer drugs derived from plants. In: DeVita VT, Hellman JS, Rosenberg SA (eds) Cancer: principles and practice of oncology, 4th edn. J.B. Lippincott, Philadelphia, p 412
9. Fleming RA, Evans WE, Arbuck SG, Stewart CF (1992) Factors affecting the in vitro protein binding in humans. J Pharm Sci 81:259
10. Gouyette A, Deniel A, Pico JL, Droz JP, Baume D, Ostronoff M, Bail N, Hayat M (1987) Clinical pharmacology of high-dose etoposide associated with cisplatin: pharmacokinetics and metabolic studies. Eur J Cancer Clin Oncol 23:1627
11. Hainsworth JD, Johnson DH, Frazier SR, Greco FA (1989) Chronic daily administration of oral etoposide—a phase I trial. J Clin Oncol 7:396
12. Harvey VJ, Joel SP, Johnston A, Slevin ML (1985) High-performance liquid chromatography of etoposide in plasma and urine. J Chromatogr 339:419
13. Henwood JM, Brogden RN (1990) Etoposide–a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in combination chemotherapy of cancer. Drugs 39:438
14. Jusko WJ, Gretch M (1976) Plasma and tissue protein binding of durg in pharmacokinetics. Drug Metab Rev 5:43
15. Lin JH, Cocchetto DM, Duggan DE (1987) Protein binding as a primary determinant of the clinical pharmacokinetic properties of non-steroidal anti-inflammatory drugs. Clin Pharmacokinet 12:402
16. Schwinghammer TL, Fleming RA, Rosenfeld CS, Przepiorka D, Shadduck RK, Bloom EJ, Stewart CF (1993) Disposition of total and unbound etoposide following high-dose therapy. Cancer Chemother Pharmacol 32:273
17. Sinkule J, Hutson P, Hayes FA, Etcubanas E, Evans W (1984) Pharmacokinetics of etoposide (VP16) in children and adolescents with refractory solid tumours. Cancer Res 44:3109
18. Slevin ML, Clark PI, Joel SP, Thompson P, Talbot D (1989) A randomized trial to examine the effect of more extended scheduling of etoposide administration in small cell lung cancer. Proc Am Soc Clin Oncol 8:236
19. Slevin ML, Clark PI, Joel SP, Malik S, Osborne RJ, Gregory WM, Lowe DG, Reznek RH, Wrigley PF (1989) A randomized trial to evaluate the effect of schedule on the activity of etoposide in small cell lung cancer. J Clin Oncol 7:1333
20. Stewart CF, Pieper JA, Arbuck SG, Evans WE (1989) Altered protein binding of etoposide in patients with cancer. Clin Pharmacol Ther 45:49
21. Stewart CF, Fleming RA, Arbuck SG, Evans WE (1990) Prospective evaluation of a model for predicting etoposide plasma protein binding in cancer patients. Cancer Res 50:6854
22. Svensson CK, Woodruff MN, Baxter JG, Lalka D (1986) Free drug concentration monitoring in clinical practice: rationale and current status. Clin Pharmacokinet 11:450
23. Taal BG, Teller FGM, Huinink WWT, Boot H, Beijnen JH, Dubbelman R (1994) Etoposide, leucovorin, 5-fluorouracil (ELF) combination chemotherapy for advanced gastric cancer: experience with two treatment schedules incorporating intravenous or oral etoposide. Ann Oncol 5:90
24. Ulrich KH (1981) Molecular aspects of ligand binding to serum albumin. Pharmacol Rev 33:17