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Pharmacokinetics and toxicity of the epipodophyllotoxin derivative etoposide (VP 16-213) in patients with gestational choriocarcinoma and malignant teratoma

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Summary. Serum levels of etoposide obtained 5 min after administration of 100 mg/m^2 were between 11 and 30 μg/ml. By 24 h after drug administration, serum levels had fallen to between 0.19 and 1.11 μg/ml. Interpatient variation of etoposide serum concentrations obtained 5 min after drug administration was low, whereas interpatient variation 24 h later was noticeably higher. A significant correlation was observed ($r = -0.698$) between the WBC nadir and the mean etoposide serum concentrations, measured 24 h after drug administration, in patients receiving etoposide in combination with cyclophosphamide and actinomycin D. However, a relationship was not observed in those patients receiving etoposide alone.

There was no observed difference in the efficacy or toxicity of 500 mg/m^2 etoposide when the dose was administered either as 100 mg/m^2 on each of 5 consecutive days or as 250 mg/m^2 on days 1 and 3. There was no significant difference between AUC values calculated from etoposide concentration versus time profiles in patients receiving the drug on days 1 and 3 and those values obtained with the 5-day schedule.

Patients resistant to a conventional dose of etoposide were given a higher dose of 1 g/m^2/24 h, but this schedule did not cause an increase in efficacy despite an increase in serum levels of the drug. CSF levels in two of these patients receiving high-dose etoposide were 1.28% and 2.09% of the serum concentrations.

Introduction

Etoposide is a semisynthetic podophyllotoxin derivative extensively used in the treatment of small cell carcinoma of bronchus, malignant lymphoma, gestational choriocarcinoma, and malignant teratoma [12, 18, 19, 25]. Animal studies have shown the drug to be schedule-dependent for optimum antitumour activity [8]. In clinical practice the drug is frequently given at a total dose of 500 mg/m^2 fractionated over 5 consecutive days. However, the optimum dosage and schedule for etoposide in patients are unknown, and it therefore seemed appropriate to compare the efficacy and serum pharmacokinetics of the drug with different schedules.

A number of authors [2, 6, 17, 22] suggest that the results of cancer treatment with cytotoxic drugs can be improved by adjusting the therapy after monitoring the individual patient's metabolism and excretion of the drug; methotrexate serum concentrations, for example, are measured after high-dose therapy [9, 13, 15].

In the study reported here an attempt was made to correlate serum concentrations of etoposide with its dose-limiting toxicity to facilitate the rational use of the drug in the clinical setting.

Etoposide is thought to enter cells by passive diffusion [1, 4]. On this basis, increasing the dose should increase the intracellular concentration, thereby potentially increasing its cytotoxicity in those patients with tumours resistant to a low-dose regimen. Since >95% of etoposide is bound to plasma proteins [5], higher serum concentrations may also result in a greater proportion of free drug owing to saturation of protein binding sites. Since only unbound drug enters the cell, more etoposide would be available to elicit its cytotoxic effect. Patients resistant to a low dose of etoposide were therefore given a higher dose of 1 g/m^2/24 h, and both the efficacy and the serum levels of the drug were monitored.

Material and methods

Assay of etoposide by HPLC. Etoposide was donated by Bristol-Myers Pharmaceuticals, Syracuse, USA. Organic solvents were obtained from BDH chemicals and were 'Analar' grade reagents.

Serum, urine, and CSF samples were stored at −20 °C until required for analysis. The assay was similar to that described by Farina et al. [11] and Strife and Jardine [24] and is briefly decribed below.

A volume of 1 ml serum, urine, or CSF was gently mixed in a glass-stoppered tube with 5 ml chloroform for 20 min. After centrifugation the organic layer was decanted and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was redissolved in 100 μl mobile phase and again centrifuged to remove particulate matter. The supernatant (5–40 μl) was injected onto the HPLC column.

Etoposide was separated using an isocratic solvent system consisting of methanol (60%) and water (40%) for serum and CSF analysis and (50%) methanol, (49%) water, and (1%) acetic acid for the urine assay. The mobile phase flowed through a C_{18}, 5 μm, 8 mm Waters rad-pak column at 1 ml/min with the aid of a Waters 6000A pump. Extracted samples were injected onto the HPLC column us-

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ing a Waters automatic injector (WISP) with a precision error of less than 0.5%. Etoposide was detected by UV absorbance at a wavelength of 254 nm.

Drug quantitation was achieved by means of external standardisation. The coefficient of variation for repeated measurement was less than 10%. The calibration curve was linear in the concentration ranges $0.05-10$ μg/ml ($r>0.998$) and $10-100$ μg/ml ($r>0.999$).

Recovery of etoposide from serum and urine was $81\%\pm5\%$ and $79\%\pm6\%$ respectively. The limit of detection was 50 ng/ml.

The 95% confidence limits (T) were calculated from:

$$T = \frac{\textit{Standard deviation of mean value} \times 1.96}{\textit{Number of values}}$$

Pharmacokinetics. The serum etoposide concentration curves were fitted and the pharmacokinetic parameters estimated using the interactive computer program, STRIPE [16]. Etoposide concentration versus time profiles best fitted a two-compartment model. The terminal phase parameters were fitted to a straight line by the method of least squares linear regression analysis. The distribution phase parameters were estimated by curve stripping, and concentrations were calculated from the equation $C_t = A \cdot e^{-Bt}$.

Elimination half-life ($t½$) was calculated from the equation $t½ = \ln 2/k$ where k is the elimination rate constant given by the slope of ln serum concentration × time. AUC from time 0 to the final time t was estimated by the trapezoidal method. The remaining AUC from t to ∞ was estimated from the equation AUC $(t-\infty) = C_{t/k}$, where C_t is the blood concentration at t. AUC values given in the Results section for AUC $(0-\infty)$ are obtained by adding AUC $(0-t)$ and AUC $(t-\infty)$. The volume of distribution (Vd) and the clearance (CL) were calculated from Vd = Drug dose AUC $(0-\infty) \times k$ and CL = $k \times Vd$.

The AUC obtained for the 5-day schedule after a total dose of 500 mg/m^2 etoposide was calculated by multiplying the AUC obtained after a single administration by a factor of 5. This is theoretically correct, since no evidence of etoposide serum accumulation was observed over the 5 consecutive days either in our studies or those of D'Incalci et al [7].

Therapeutic drug monitoring. In all, 21 male patients with malignant teratoma and 13 female patients with gestational choriocarcinoma were studied. Patients received a total dose of 500 mg/m^2 etoposide given as a 100-mg/m^2 IV infusion over 30 min on each of 5 consecutive days (schedule 1). Those patients with malignant teratoma also received actinomycin D and cyclophosphamide [20].

Blood samples ($5-10$ ml) were taken 5 min after each infusion was complete and again 24 h later (immediately before the next administration of etoposide). Three or four serum samples were obtained 24 h after etoposide administration and the mean drug concentration was then calculated for each patient. In four patients, 24-h pooled urine samples were collected.

Schedule dependency. Four female patients with gestational choriocarcinoma were treated according to schedule 1. Six weeks later each patient again received a total dose of 500 mg/m^2, but in this case 250 mg/m^2 was infused over 3 h and repeated 48 h later (schedule 2).

Blood samples were taken at the following times; 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 12.0, and 24 h after drug administration according to schedule 1. Blood samples were taken from patients treated according to schedule 2 at the following times; 0, 1.5, 3.0, 3.5, 4.0, 6.0, 8.0, 12.0, 24, and 48 h after administration.

High-dose therapy. Three patients with gestational choriocarcinoma who were resistant to etoposide were given 1 g/m^2 drug as a 24-h infusion (schedule 3). Pharmacokinetic studies were performed up to 96 h after drug administration. In two patients, 1 ml CSF was also obtained by lumbar puncture 0.5 or 6.5 h following etoposide infusion.

Administration to patient with renal failure. One patient (DC) received 100 or 160 mg etoposide prior to haemodialysis. Serum levels of etoposide were measured before and after dialysis.

Administration to patient with pineal choriocarcinoma. One patient (MS) with a pineal choriocarcinoma received 360 mg etoposide. At 14 h after drug administration a blood sample and a CSF sample were taken.

Results

Serum concentrations of etoposide obtained for patients receiving 100 mg/m^2 drug on each of 5 consecutive days are shown in Fig. 1. The 95% confidence limits for serum concentrations of etoposide calculated from mean values over 5 days are shown in Table 1. In patients with either choriocarcinoma (Fig. 1B) or teratoma (Fig. 1A) the etoposide serum concentrations obtained 5 min after administration did not reveal marked interpatient variation over the 5-day period. There was no significant increase in etoposide serum levels shown in Fig. 1, indicating a lack of drug accumulation over the 5-day course. The combination of actinomycin D and cyclophosphamide did not appear to have an effect on serum levels 5 min after infusion, since drug concentrations shown in Fig. 1 are virtually identical (see Table 1).

The mean amount of unchanged etoposide excreted in the urine within 24 h after the first administration was 38% of the dose administered in the four patients studied. Following the fourth consecutive administration of 100 mg/m^2 etoposide the amount of unchanged drug excreted had increased to 43% of the dose administered. However, for 4 days of the 5-day course there was no significant change in urinary excretion of etoposide. Approximately $30\%-40\%$ of etoposide was excreted unchanged within 24 h of administration, a similar value to those reported by other authors [3, 23]. Renal handling of this drug is relevant, since in one patient with renal failure serum concentrations were markedly higher $21-31.5$ h after receiving 100 mg drug than in patients receiving 100 mg/m^2 (Tables 1 and 2). It appears that since etoposide is protein-bound it is not efficiently dialysed.

Serum levels of etoposide obtained 24 h after drug administration had fallen to $0.24-0.94$ μg/ml and $0.19-1.11$ μg/ml in patients with teratoma and choriocarcinoma, respectively (Table 1). There was marked interpatient variation in serum levels over the 5-day course. However, variation in serum concentrations in individual patients was small. Also, AUC values calculated from etopo-

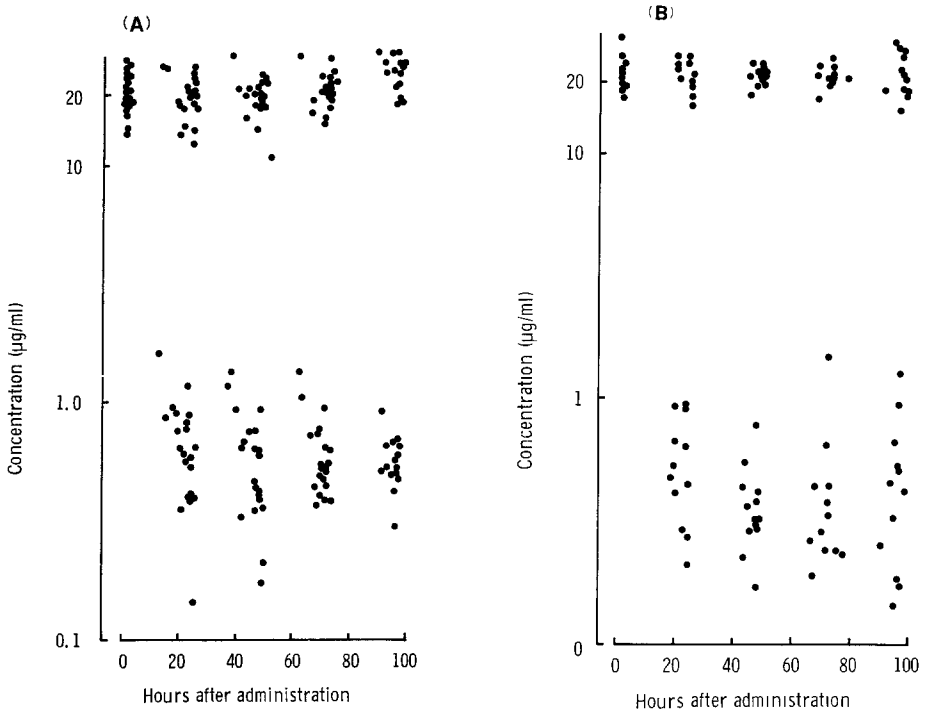

Fig. 1 A, B. Serum concentrations of etoposide in patiens with malignant teratoma **(A)** and gestational choriocarcinoma **(B)** who received 100 mg/m^2 of the drug. High drug levels were obtained from blood samples taken 5 min after infusion of etoposide and low levels from samples taken immediately before drug administration

Table 1. VP16 serum concentration[a] (μg/ml)

	Teratoma (n=21)	Choriocarcinoma (n=13)
5 min after administration	20.87 ± 1.30	19.88 ± 1.46
Concentration range	(14.30 – 26.59)	16.4 – 24.78)
24 h after infusion	0.56 ± 0.17	0.46 ± 0.13
Concentration range	(0.24 – 0.94)	(0.19 – 1.11)

[a] Figures not in parentheses express mean ±95% confidence limits

side concentration versus time curves were found to be directly proportionate to 24-h etoposide serum concentrations (r>0.99) after administration of 100 mg/m^2 of the drug to five patients with gestational choriocarcinoma. These observations raised the possibility of a correlation between mean etoposide serum levels obtained 24 h after administration and the degree of myelosuppression.

The dose-limiting toxicity of etoposide is myelosuppression and the WBC nadir usually occurs between 5 and 15 days after drug administration according to the 5-day schedule. Figure 2A shows a significant correlation (r= −0.698) between WBC nadir and mean etoposide concentrations taken 24 h after drug administration to patients with malignant teratoma (when actinomycin D and cyclophosphamide are included in the course). However, this correlation was not observed in those patients with gestational choriocarcinoma who received etoposide alone (Fig. 2B). In both tumour types a relationship between platelet count nadir and etoposide serum levels was not observed. There was also no significant correlation between WBC nadir and the dose (mg/kg).

The AUCs calculated from concentration versus time profiles after administration of 500 mg/m^2 etoposide according to schedule 2 were 30.8 – 89.4% higher (mean 48.7%) than the values obtained with schedule 1 (Fig. 3). However, this difference was not statistically significant.

The four patients with gestational choriocarcinoma who were resistant to the 5-day course showed no evidence of response when the dose was increased to 1 g/m^2 over 24 h. Although etoposide serum levels rose to 16 – 42 μg/ml in the three patients studied, increased efficacy of the drug was not obtained. In two of these patients treated according to schedule 3, the CSF levels of the drug measured were 1.28% and 2.09% of the serum etoposide levels (Table 3), whereas the drug was not measurable in the CSF of patients treated according to schedule 1. However, 0.21 μg/ml etoposide was found in the CSF in one patient (MS) with a pineal chorioncarcinoma who received 360 mg drug. This CSF concentration of etoposide represented 15.6% of the serum drug level.

Table 2. Etoposide serum levels before and after haemodialysis in one patient (DC) with renal failure

Dose (mg)	Time post-treatment (h)	Predialysis Serum concentration (μg/ml)	Time post treatment (h)	Postdialysis Serum concentration (μg/ml)
100	21.0	2.1	27.0	1.6
160	31.5	3.5	37.5	1.8

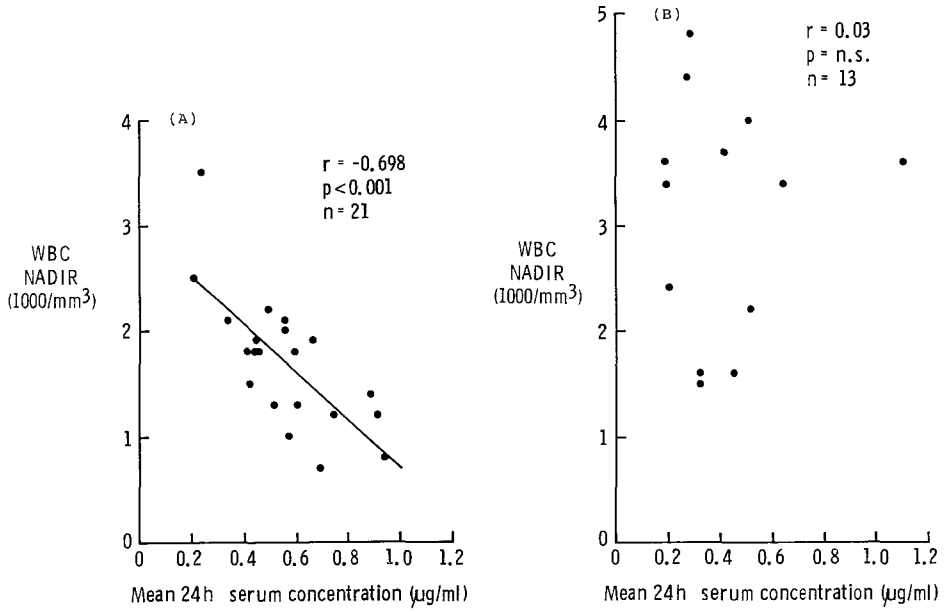

Fig. 2 A, B. Relationship between WBC nadir and etoposide serum concentration in patients with malignant teratoma **(A)** and gestational choriocarcinoma **(B)**

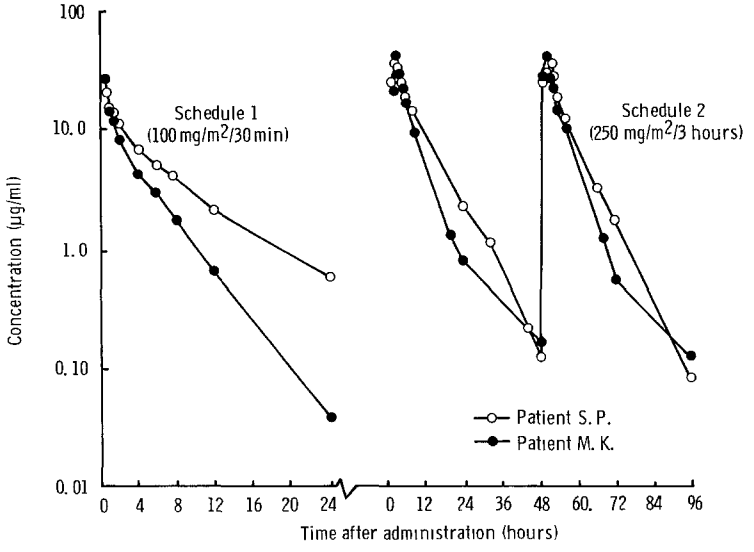

Fig. 3. Comparison of etoposide concentration versus time profiles obtained from two patients with gestational choriocarcinoma who received the drug by different schedules. AUC values calculated for a total dose of 500 mg/m^2 in the four patients studied are shown below:

Patient	AUC 0 – ∞ (mg·h/l)	
	Schedule 1	Schedule 2
S. P	484.28	633.27
M. K	298.55	424.22
Y. P	291.19	551.47
D. B	575.51	763.57

Table 3. CSF/serum ratio of etoposide

Patient	Dose (mg/m^2)	Time post infusion (h)	Drug serum level (μg/ml)	Drug CSF level (μg/ml)	% serum level
KW	1,000/24 h	6.5	11.0	0.23	2.09
IS	1,000/24 h	0.5	18.0	0.23	1.28

Discussion

A linear relationship was found between WBC nadir and the concentration of etoposide in the serum of patients with malignant teratoma who also received actinomycin D and cyclophosphamide. However, other studies are required to confirm these findings before any clinical applications can be proposed. No relationship was found between WBC nadir and etoposide serum concentrations in those patients with gestational choriocarcinoma who received etoposide alone. We suggest three possible explanations for this difference. First, those patients with malignant teratoma also received actinomycin D and cyclophosphamide together with etoposide, and a drug interaction effect may have altered the late disposition of etoposide from the serum. The second possible explanation is greater myelosuppression owing to prior chemotherapy; that is to say, a higher serum concentration of etoposide in patients

with teratoma may have had a more intense effect on the already low WBC levels compared with the WBC levels in patients with choriocarcinoma. Thirdly, Sinkule et al. [23] have shown that clearance of etoposide was lower in patients receiving prior cisplatin. Our patients with malignant teratoma also received cisplatin prior to etoposide, and this may be an additional factor in the resultant myelosuppression.

Whether 500 mg/m^2 etoposide was administered according to schedule 1 or 2, did not cause any apparent difference in efficacy since gestational choriocarcinoma is so sensitive to this drug. That the AUCs obtained with schedule 2 were not significantly different from those seen with schedule 1 suggests that etoposide obeys linear (dose-related) pharmacokinetics (Fig. 3). Indeed, a linear relationship ($r=0.999$) was observed between AUC values obtained with schedules 1, 2, and 3 and the dose administered (Table 4). Evidence to support this hypothesis is given by other authors [3, 14].

Increasing the dose of etoposide to 1 g/m^2/24 h in patients resistant to a lower dose of the drug was not therapeutically effective. However, some penetration into the CSF was found in patients receiving high-dose etoposide (Table 3). This observation has also been reported recently elsewhere [21], and the finding may be relevant in the treatment of brain metastases. It is interesting that in the patient with a pineal choriocarcinoma, where the blood–brain barrier was not intact, appreciable concentrations of etoposide (0.21 μg/ml) were found in the CSF after administration of a relatively low dose of drug (360 mg).

Renal failure in one patient resulted in higher serum levels of etoposide after administration of a dose of either 100 or 160 mg drug. Haemodialysis failed to reduce circulating drug levels to any marked degree. Presumably the protein-bound fraction was unable to penetrate the dialysis membrane. These observations support the use of drug level monitoring with dose modification in those patients with renal dysfunction to reduce exposure to etoposide. Indeed, it has been shown previously that the systemic and renal clearance of etoposide is lower in those patients who have received prior cisplatin [23], and this effect on etoposide pharmacokinetics may be related to renal demage. Additional studies are required to establish guidelines for dosage modification.

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Table 4. Pharmacokinetic parameters of etoposide in patients with gestational choriocarcinoma

Patient	Age (years)	Dose (mg/m^2)	Weight (kg)	$t_{1/2}\alpha$[a] (h)	$t_{1/2}\beta$[a] (h)	AUC (0 – ∞) (mg·h/l)	CL (l/h)	Vd (l)
SP	23	100	46.6	0.838	5.545	100.56	1.39	11.1
MK	35	100	55.9	0.230	2.961	59.69	2.60	11.1
YP	37	100	71.1	0.744	4.156	58.60	3.07	18.4
DB	25	100	66.0	0.704	5.496	120.71	1.41	11.2
SP	23	250	47.0	0.515	5.774	335.68	1.04	8.3
MK	35	250	53.9	0.712	6.456	213.50	1.82	17.0
YF	37	250	70.8	0.880	6.458	250.25	1.78	16.6
DB	25	250	67.2	0.482	6.444	442.53	0.96	8.9
KW	33	1.000	54.6			1,180.08	1.27	
KW	33	1.000	58.1			945.30	1.59	
IS	46	1.000	44.5			859.50	1.75	
IS	46	1.000	46.3			1,248.18	1.20	
YH	44	1.000	59.2			421.49	3.80	

[a] $t_{1/2}\alpha$, distribution half-life; $t_{1/2}\beta$, elimination half-life

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