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Pharmacokinetic study of VM26 given as a prolonged IV infusion to ovarian cancer patients

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Summary. *Plasma levels of VM26 were assayed by HPLC in six ovarian cancer patients with normal renal and liver function who received the drugs as an initial 1-h IV infusion of 80 mg/m² followed by a 24-h IV infusion of 120 mg/m². These doses and infusion rates were chosen on the basis of mean VM26 clearance values found in a previous study, with the aim of reaching plasma steady-state levels of approximately 6 μg/ml in a short time.*

Plasma steady-state levels of 4–10 μg/ml, close to those predicted theoretically, were in fact attained at 4–9 h during the second, slower infusion. Mean half-lives and clearance values were 8.6 ± 1.1 h and 0.78 ± 0.08 l/h/m². Six percent of the VM26 dose was recovered as unchanged drug in the urines collected up to 24 h after the end of infusion. The glucuronide of VM26 aglycone (4′-demethylepipodophyllotoxin) was identified in the urine of all patients, in amounts corresponding to about 8% of the drug dose.

Introduction

4′-Demethylepipodophyllotoxin 9-[4,6-0-2-(thenylidene)-β-D-glucopyranoside] (VM26) is an anticancer agent with proven efficacy in several human malignancies [12, 17]. Although the drug's mode of action has been only partially elucidated, the timing of drug exposure is known to be crucial, as this agent is specifically cytotoxic against cells in the S and G_2 phases of the cell cycle [14]. The markedly time-dependent cytotoxicity of VM26 [11] probably explains the much greater antitumor activity of split than of single doses observed in both L1210 [20] and 3LL carcinoma of the mouse [3]. In the latter model it has been reported that split doses give better antitumor activity than single doses with even less bone marrow toxicity [7].

These experimental data provided a rationale for trying a prolonged infusion of VM26 to expose the cancer cells for a longer time to effective drug concentrations. In a previous study in which plasma VM26 levels were determined in patients given a 24-h continuous infusion the steady-state levels were not reached until the end of the infusion [6].

In this study, to achieve rapid attainment of a plasma steady-state level within the therapeutic range, and its maintenance throughout infusion, the total dose (200 mg/m²) was split into a bolus and an infusion given at a rate computed on the basis of the mean body clearance determined in a previous study [6]. Good agreement was found between predicted and observed steady-state plasma concentrations.

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In addition, we report the identification and quantitative determination of the glucuronide of VM26 aglycone (glucuronide of 4′-demethylepipodophyllotoxin), a new, previously unknown metabolite of VM26 found in the urine of all patients investigated.

Materials and methods

Patients. Six ovarian cancer patients, ranging in age from 32 to 57 years, entered this study, giving their agreement. They all had measurable progressive disease after conventional therapy, accompanied by a neoplastic ascitic effusion in two subjects (nos. 2 and 3). Previous treatment consisted of *cis*-platinum, adriamycin, and cyclophosphamide in combination in four patients (no. 1, 3, 4, and 6), and of *cis*-platinum and cyclophosphamide in two patients (no. 2 and 5). Two patients (no. 4 and 6) had received radiotherapy to the abdomen and pelvis. All patients had normal liver and renal functions.

Drug treatment. All patients received the loading dose of 80 mg/m², diluted in 350 ml physiological saline, as a 1-h infusion, followed by a continuous infusion of 120 mg/m² over approximately 24 h. The infusion bottles (containing 60 mg/m² drug in 500 ml physiological saline) were prepared every 12 h, just before administration. No detectable degradation of VM26 occurred in 12 h in these conditions, as checked by HPLC. The continuous infusion was maintained by a controlled-rate pump (infusion pump produced by DELTA, Milan).

We gave a fixed total dose (D) of 200 mg/m² over approximately 25 h, split into a fast (1-h) infusion followed by a slower infusion. We used the following equation to calculate the rate *(R)* of the slower infusion: $R = C_{ss} \cdot Cl$, where C_{ss} (steady-state concentration) was 6 μg/ml and *Cl* (clearance) was 0.84 l/h/m², corresponding to the mean values in eight patients who had received VM_{26} as a 24-h infusion [6]. Taking $R = 5$ mg/m²/h we gave the remaining dose ($D - 14 \cdot R = 80$ mg/m²) in the 1st h.

All patients received at least two courses of treatment, repeated every 3 or more weeks as WBC counts permitted.

Pharmacokinetic studies were performed after the first drug cycle.

Sample collection. An indwelling IV cannula was inserted in the arm not receiving the IV infusion and 5-ml blood samples were collected in heparinized tubes at various times during and

after the infusion and spun down at 2,000 rpm. Plasma samples were frozen immediately and stored (−20° C) until analysis. In general, samples were taken halfway through and at the end of the first 1-h infusion, at 15 and 30 min and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 18 h during the continuous infusion, and at the end and at 15 and 30 min and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18, 24 and 48 h after the end of the slow infusion.

Urine was collected throughout the infusion and in the first 24 h after the end of infusion. Urine samples (10 ml) were stored at −20° C until analyzed.

VM26 HPLC assay. The method used to determine VM26 in plasma and urine has been previously described by Evans et al. [8]. It can be summarized as follows: after addition of VP16 or 4′-demethylepipodophyllotoxin-9-(4,6-0-ethylidene-D-gluco-pyranoside), kindly supplied by International Bristol Myers, New York, USA, as internal standard, 1 ml plasma or urine was extracted with chloroform. The organic phase was dried under vacuum and the residue dissolved in 100 µl methanol; 10–25 µl was injected into a Perkin-Elmer HPLC equipped with a LC-75 spectrophotometric detector at 254 nm absorbance.

Separation was achieved with an isocratic solvent system of $CH_3CN : H_2O : CH_3COOH$ (35 : 64 : 1) at a flow rate of 1 ml/min with a µBondapak phenyl column 25 cm long purchased from Waters Assoc., Milford, Mass, USA. VM26 was found to be stable in plasma or urine kept for 24 h at 37° C.

Determination of VM26 aglycone glucuronide. For this determination urine samples were incubated with β-glucuronidase-arylsulfatase at 37° C for 24 h; to 1 ml urine, 1 ml 0.1 *M* acetate buffer pH 4.5 was added with 50 µl (corresponding to 250 mU) β-glucuronidase-arylsulfatase from *Helix pomatia* (Boehringer-Biochemia Robin, Milan, Italy). In parallel, identical samples were treated similarly except that no β-glucuronidasearylsulfatase was added. To establish whether the VM26 aglycone conjugate was a sulfate or a glucuronide we incubated urines with either β-glucuronidase-arylsulfatase or β-glucuronidase from *Helix pomatia.* The results were always superimposable, indicating that the conjugate was a glucuronide. All samples were then extracted and injected into an HPLC column as previously described for VM26, but using a mobile phase of $CH_3CN : H_2O : CH_3COOH$ (25 : 74 : 1) at a flow rate of 1.2 ml/min. For quantitative determination of VM26 aglycone glucuronide, VP16 was added as internal standard. For some samples a complete UV spectrum of the aglycone was taken, stopping the flow of mobile phase at its peak.

Pharmacokinetic analysis. Plasma concentration-time data for each subject can be divided into three phases: (a) during the first infusion; (b) during the second, slower infusion; (c) washout. The data in the three phases were fitted simultaneously using the following model [1]:

$$C_{(t)} = \frac{R}{V_C}\left[\frac{(e^{\delta\alpha} - 1)}{\alpha} \cdot C_1 e^{-\alpha t} + \frac{(e^{\delta\beta} - 1)}{\beta} C_2 e^{-\beta t}\right],$$

where:

t = time from the beginning of the infusion
R = infusion rate (80 mg/m^2/h or 120 mg/m^2/24 h)
V_C = volume of the central compartment
α, β = complex constants [9]
C_1, C_2 = dimensionless ($C_1 + C_2 = 1$)
$C_{(t)}$ = plasma concentration
δ = t, during infusion or after infusion at 1 h or 24 h.

The plasma concentration-time data in phases b and c were computed numerically using the superposition principle [10]. The pharmacokinetic parameters (V_C, α, β, C_1) of each subject were determined by a general nonlinear fitting program [18], using the unweighted and weighted ($1/y^2$) [2] least-squares criterion, with the aid of a microcomputer. From the standardized residuals plots, the unweighted parameter estimations looked more appropriate to describe the data. Clearance and volume of distribution (V_B) were computed using the formula:

$$Cl = \frac{\alpha \cdot \beta}{\alpha C_2 + \beta C_1} \cdot V_C \qquad V_B = \frac{Cl}{\beta}.$$

Results

Figure 1 shows the theoretical curve calculated on the basis of previously described mean parameter values [6] and on the basis of the rates of infusion. Figure 2 shows the experimental plasma levels measured in six cancer patients. After the end of the 1-h initial infusion the plasma levels of VM26 declined, achieving steady-state values in 4–5 h during the second, slower infusion except in patient no. 1, in whom equilibrium was reached in 9 h. In spite of some variability in the steady-state levels (4–10 µg/ml) there was good agreement between the theoretical curve and the experimental data.

Table 1 shows the pharmacokinetic parameters of VM26 in each patient. The mean of $t_{1/2\beta}$, V_B, and Cl were 8.62 ± 1.11 h, 9.09 ± 0.83 l/m^2, and 12.96 ± 1.49 ml/min/m^2, respectively. Figure 3 shows a chromatogram of urine extract before (Fig. 3A) and after (Fig. 3B) β-glucuronidase-arylsulfatase incubation.

After incubation a new peak (I) was found with the same retention time (Fig. 3C) and with a UV spectrum similar to the aglycone.

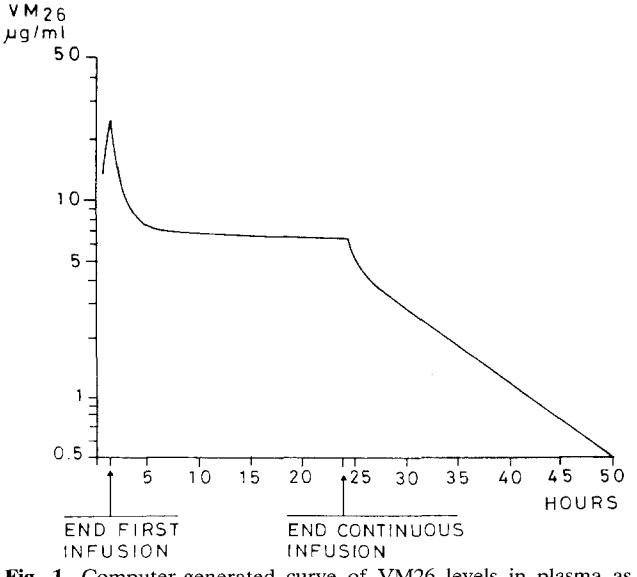

Fig. 1. Computer-generated curve of VM26 levels in plasma as a function of time using the mean values of α(1.15 h^{-1}), β(0.09 h^{-1}), C_1 (0.609 dimensionless), V_C (2.38 l/m^2) and on the basis of the infusion rates (see test)

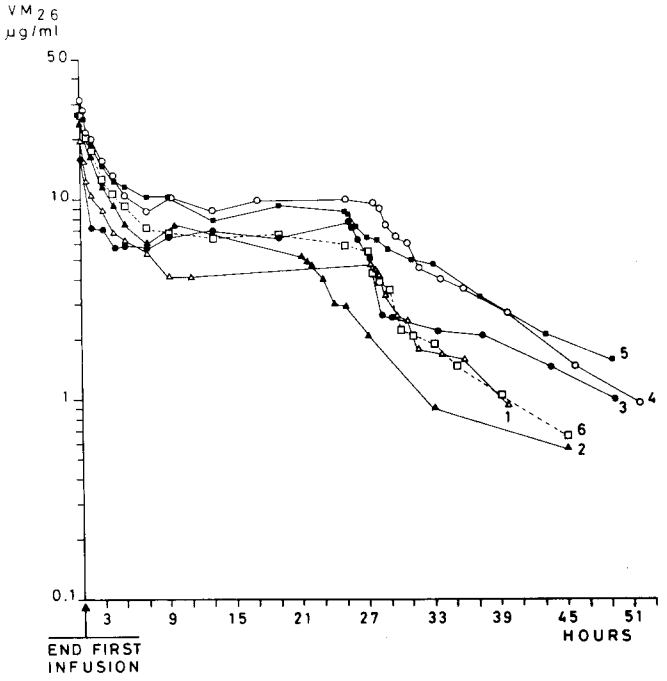

Fig. 2. VM26 plasma levels in six ovarian cancer patients, starting from the end of the initial 1-h infusion, during and after continuous infusion (the *lines* were drawn by hand to connect contiguous experimental *points*)

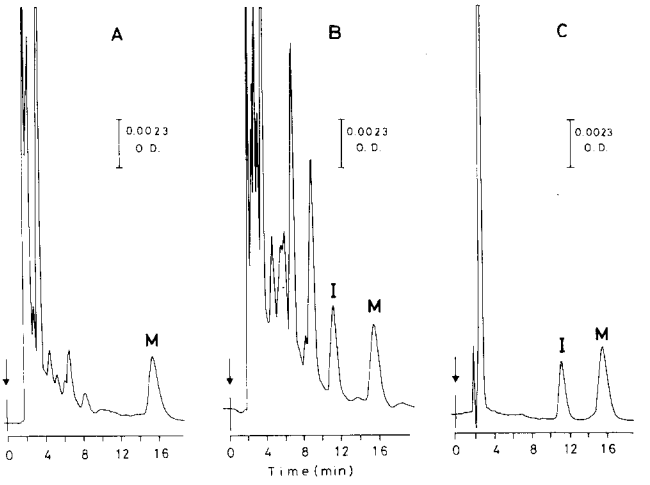

Fig. 3A–C. HPLC chromatograms of patients' urine samples treated by chloroform extraction, before **(A)** and after **(B)** enzymatic incubation. Retention time of peak I corresponds to the standard VM26 aglycone reported in **(C)**; *M* in the peak of VP16–23 used as internal marker. The final calculation indicates a concentration of 3.3 μg aglycone/ml urine

Table 1. Pharmacokinetic parameters of VM26 in ovarian cancer patients receiving a loading dose (80 mg/m^2) as a 1-h infusion, followed by 24-h continuous infusion (120 mg/m^2)

Patient no.	α (h^{-1})	β (h^{-1})	$t_{1/2}\beta$ (h)	V_C (l/m^2)	V_B (l/m^2)	Cl (l/h/m^2)
1	2.508	0.150	4.6	1.68	7.15	1.073
2	0.660	0.084	8.2	2.49	11.21	0.942
3	1.878	0.066	10.5	2.89	11.95	0.789
4	0.606	0.066	10.5	2.16	7.72	0.513
5	0.528	0.060	11.5	2.45	9.05	0.543
6	0.534	0.108	6.4	2.60	7.48	0.808
$\bar{X}\pm$SE	1.119 ±0.35	0.089 ±0.014	8.6 ±1.1	2.38 ±0.17	9.1 ±0.83	0.778 ±0.089

Table 2. Urinary excretion of VM26 and 4′demethylepipodophyllotoxin (aglycone of VM26) after IV VM26 (200 mg/m^2)

Patient no.	Total dose of VM26		Urinary excretion of VM26		Urinary excretion of VM26 aglycone	
	mg	mmol	mmol	% of dose	mmol	% of dose
1	350	0.5335	0.0244	4.6	0.0401	7.5
2	350	0.5335	0.0105	2.0	0.0552	8.3
3	330	0.5030	0.0366	7.3	0.0626	12.5
4	320	0.4878	0.0405	8.3	0.0221	4.5
5	285	0.4344	0.0466	10.7	0.0364	8.4
6	300	0.4573	0.0161	3.5	0.0321	7.0
$\bar{X}\pm$SE			0.0291 ±0.0058	6.1 ±1.3	0.0396 ±0.0055	8.0 ±1.0

Table 2 shows the amount of VM26 and of aglycone glucuronide found in the urine of patients collected throughout the infusion and for 24 h after the end of infusion. About 6% of the VM26 dose was eliminated as unchanged drug and about 8% as aglycone glucuronide.

Discussion

When VM26 was infused at an initial rate of 80 mg/m^2/h for 1 h then at about 5 mg/m^2/h for approximately 24 h, the steady-state levels were achieved rapidly in patients' plasma, as predicted theoretically on the basis of previously calculated parameters [6]. The steady-state levels were higher than 4 μg/ml in all cases. Considering that concentrations of 0.1–2 μg/ml show cytotoxic activity in vitro in various murine and human cancer cell types (Erba et al., unpublished data) [11, 19], the plasma levels achieved in these patients could be high enough to exert a cytotoxic effect on cells potentially sensitive to the drug action.

We could not prove the efficacy of this treatment as none of the six ovarian cancer patients responded, but this may be due to the advanced state of their disease.

In spite of the heavy chemotherapy received before VM26 by all patients, treatment was relatively well tolerated with moderate leukopenia; only in one case did the WBC count drop under 2,000/μl (1,800/μl), but it rapidly recovered.

We therefore feel this method of administration may be recommended for further investigations in patients with tumors sensitive to VM26.

A new VM26 metabolite, the glucuronide of 4′-demethyl-epipodophyllotoxin, was identified for the first time in this study; it amounted to about 8% of the VM26 dose. Studies are

in progress in this laboratory to see whether this metabolite plays any role in the cytotoxic effect of VM26.

It is known that the VM26 aglycone can exert a cytotoxic effect by inhibiting microtubule assembly, a mechanism similar to that of podophyllotoxin, but different from that of VM26 itself [16]. Tumors rich in β-glucuronidase [4] could therefore form the aglycone, which may contribute to the cytotoxicity of VM26. In addition, the fact that this glucuronide may be cytotoxic cannot be excluded, as glucuronic acid does not necessarily react with the 4′-OH group of the E ring that seems crucial for VM26 activity [15]; it might simply replace the β-glucopyranoside moiety reacting with the OH group of the *C* ring.

One point still to be clarified is the means by which about 85% of the VM26 dose is cleared from the body. We found that only about 6% was eliminated in the urine of patients as unchanged VM26. Even though we collected urine for only 24 h after the end of infusion we know that most VM26 is actually excreted during the infusion and only very small amounts in the subsequent 18 h (data not presented, obtained by collecting urine every 6 h). Therefore it is unlikely that the low recovery was due to the short collection time.

The glucuronide of VM26 aglycone accounts only for another 8% of the dose. Other possible metabolites are picro-VM26 and 4′-demethylepipodophyllic acid-9-(4,6,0-2-tenylhidine-β-D-glucopyranoside) (VM26 OH), identified by Evans et al. in the plasma of children receiving VM26 [8]; these authors, however, did not give many quantitative details. The HPLC method we used was suitable for determining picro-VM26 and VM26OH, but we did not detect peaks of these two compounds in the plasma or urine of these patients when the sensitivity was 0.5 μg/ml. Therefore, even if these metabolites are formed and were not detected because the assay was not sensitive enough, these inactive [13] metabolites could not account for more than 1% of the VM26 dose. One possibility is that VM26, in contrast to its analog VP16 [5], is excreted as unchanged drug or as metabolites in the bile. Other possibilities are that for VM26 there are different metabolic pathways than have been investigated so far.

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