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Note

High-performance liquid chromatography determination of 4'-demethyl-epipodophyllotoxin-9-(4,6-O-ethylidene β-D-glucopyranoside) (VP 16-213) in human plasma

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VP 16-213 (VP16) or 4'-demethyl-epipodophyliotoxin-9-(4,6-O-ethylidene β-D-glucopyranoside) (NSC 141540) is a semisynthetic derivative of podophyllotoxin which has antitumour activity in several experimental and human malignancies [1,2]. In clinical use it is an effective drug in small cell lung cancer [3—5] and has also shown considerable activity in testicular teratomas [6,7], myeloid leukemia [8,9], teratocarcinoma of the ovary [6], choriocarcinoma [10] and in non small cell lung cancer [8,9].

The pharmacokinetics of VP16 have been studied in animals and man by radiochemical analytical assay using the tritium-labelled compound [11,12]. These procedures, however, lack specificity, among other reasons because the drug is metabolized in vivo [13]. In addition, they are not appropriate for clinical pharmacokinetic studies as they require the administration of radioactive material to patients. The need for knowledge of the pharmacokinetics of VP16 in animals and man prompted us to develop an analytical method with high-performance liquid chromatographic (HPLC) separation and UV absorbance detection which offer sufficient specificity, sensitivity and simplicity to be employed clinically.

EXPERIMENTAL

Patients

VP16 was assayed in plasma from two women, 28 and 35 years old, with choriocarcinoma who received the drug at a dose of 100 mg/m^2 as a 60-min intravenous infusion. They did not receive any other drugs in combination;

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both patients had normal kidney and liver function, as assessed by standard tests.

Blood was collected in heparinized tubes before the drug treatment, at the end of VP16 infusion and 5, 15, 30, 60, 120, 180, 240, 480, 720 and 1440 min after the end of the infusion. The blood was centrifuged at 400 *g* for 20 min and plasma was kept at −20°C until analyzed.

Standard and reagents

VP16 and 4-demethyl-β-epipodophyllotoxin-D-thenylidene glucoside (VM26) used as standards were generously provided by Dr. Lenaz of Bristol Myers (New York, NY, U.S.A.). VP16 and VM26 used as standards were dissolved in methanol (100 μg/ml) and stored at −20°C when not in use. VP16 administered to the patients was the commercial preparation for clinical use. The following reagents were used: diisopropyl ether, chloroform and methanol (Carlo Erba, Milan, Italy). Water and methanol were filtered through 0.40-μm nucleopore polycarbonate membranes (BDH, Milan, Italy) prior to use.

Extraction procedure

Heparin-treated plasma (1 ml) was washed with 5 ml diisopropyl ether, shaking for 5 min. VM 26 was then added as internal standard and 8 ml of chloroform were used for extraction. The test-tubes were mechanically shaken for 20 min, centrifuged at 600 *g* for 5 min, then the aqueous phase was discarded and the organic phase was transferred to a second test-tube and brought to dryness at room temperature in a rotary evaporator under vacuum. The drug residue was redissolved in 100 μl of water—methanol (45:55) and 5—20 μl of this solution were injected into the chromatograph.

High-performance liquid chromatography

HPLC separation was performed on a Waters Model 440 instrument equipped with a 254-nm absorbance detector. Separation was achieved with an isocratic solvent system of water—methanol (45:55) at a flow-rate of 1 ml/min using a LiChrosorb RP-8 (5 μm) column from Merck (Darmstadt, G.F.R.).

Stability of VP16 in plasma

The stability of VP16 in plasma (20 μg/ml) was determined at several incubation times (0, 1, 2, 3, 4, 5, 6, 12 and 24 h), during which samples were maintained at 37°C. All analyses were performed in triplicate.

Calibration curve

Pools of plasma containing 0.5, 1.0 and 5.0 μg/ml of VP16 were divided into 1-ml samples which were extracted for VP16 determination as described. Experiments were run in quadruplicate for each concentration and replicated three times. After chromatographic analysis the peak area ratios of VP16 to the internal standard were plotted for the linear regression analysis against the theoretical concentration in the samples.

Recovery

In order to estimate the recovery, various amounts of VP16 (0.5, 1.0, 5.0

and 10 μg) were added to 1 ml of human plasma and VM26 (50 μg) was added after extraction. The VP16:VM26 peak area values were compared with those obtained when injecting corresponding external samples at the same concentrations.

Pharmacokinetic calculations

The results were processed using a two-compartment open model after intravenous infusion described by the equation $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ where C_p is the plasma concentration at time t, A and B are the intercepts on the ordinate at zero time and α and β represent the slopes of the respective exponential segments.

The elimination half-life ($T_{½}$) as calculated by the "peeling" method [14] ($T_{½} = 0.693/\beta$) using a Hewlett-Packard Model 9810 computer.

RESULTS AND DISCUSSION

Typical HPLC chromatograms of extracts from human plasma before and after VP16 treatment are shown in Figs. 1 and 2. VM26 was used as internal standard for the quantitative determination of VP16 because of its structural similarity. The extraction recovery of VP16 was 79±3% and of VM26 81±2%.

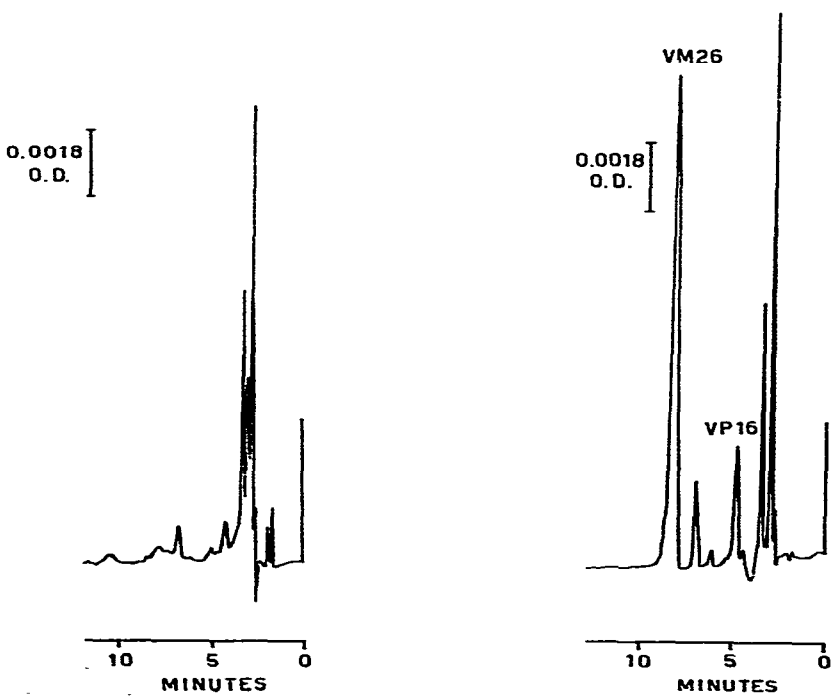

Fig. 1. Chromatogram of human plasma extract before VP16 administration. The equivalent of 100 μl of plasma was injected at 0.02 a.u.f.s.

Fig. 2. Chromatogram of human plasma showing VP16 concentration 24 h after intravenous administration of 100 mg/m². The final calculation indicates a concentration of 0.54 μg of VP16 per ml of plasma.

As can be seen in Table I, the calibration curve of VP16 is linear ($r = 0.99$) and the coefficient of variation (C.V.) is less than 10%. No interference from endogenous compounds was observed. VP16 appears to be stable in plasma at 37°C; in fact no significant degradation was found under these conditions even after 24 h of incubation. The suitability of the analytical procedure for clinical pharmacokinetic studies was tested by determining VP16 plasma levels in two cancer patients who had received a single dose of 100 mg/m^2 as a 60-min intravenous infusion. Fig. 3 shows the plasma disappearance of VP16 in the two

TABLE I

REPRODUCIBILITY OF VP16 ASSAY

$r = 0.99$ ($p < 0.05$) for a line with a slope of 0.29 and an intercept of —0.01, not significantly different from zero.

	Plasma concentration (μg/ml)		
	0.5	1.0	5.0
Peak area ratio	0.132	0.283	1.456
S.D.	0.0119	0.0232	0.1455
C.V.	9.0	8.2	9.9
n	12	12	12

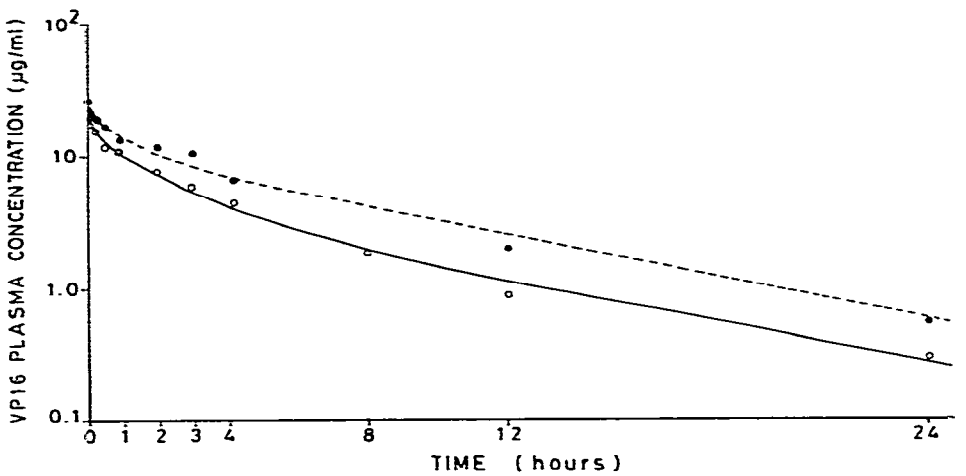

Fig. 3. Plasma concentration—time curves from two patients after an intravenous infusion dose of 100 mg/m^2 of VP16.

patients. Applying a two-compartment open model, the elimination half-lives ($T_{½}$) were 4 and 5 h, whereas previous studies, in which the ^{3}H-labelled compound was measured reported a half-life of 6—16 h. The slightly shorter half-life we found, might be explained by a lack of specificity of the radiochemical method which could not perhaps distinguish the drug from metabolites.

In conclusion the method described in the present study appears to possess a sufficient degree of specificity, sensitivity and reliability to be employed for experimental and clinical pharmacological studies.

The preliminary pharmacokinetic data obtained in the cancer patients studied show that the method is suitable to be routinely used in clinical situations. This method can also be applied for the determination of VM26 which is also clinically employed as an antitumour agent [2]. The optimal conditions of sample preparation before HPLC analysis of this drug are under investigation in our laboratory at the moment.

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