Cancer Chemother Pharmacol (1982) 7: 127–131

Cancer Chemotherapy and Pharmacology

Pharmacokinetics of VP16-213 in Lewis Lung Carcinoma Bearing Mice*

Tina Colombo, Massimo Broggini, Laura Torti, Eugenio Erba, and Maurizio D'Incalci

Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea, I-62-20157 Milan, Italy

Summary. *The pharmacokinetics of VP16 have been investigated in Lewis lung bearing mice after i.v. doses of 13 and 40 mg/kg. At both doses the plasma elimination half-life was around 30 min. The lowest VP16-213 levels were in brain and primary tumor. Drug concentrations were much higher in metastases than in primary tumor. The highest concentrations were in small intestine, liver and kidney. Drug levels in the liver were disproportionally higher after 40 mg/kg, the AUC value being approximately 12 times greater than after 13 mg/kg. Urinary excretion of VP16-213 as unchanged drug accounted for 20–30% of the administered dose in the 60 h after treatment. The concentration cytotoxicity curve was very steep and apparently similar for cells derived from primary tumor or metastases grown in vitro.*

Introduction

VP16-213 (VP16) or 4′-demethyl-epipodophyllotoxin-9-(4,6-0-)ethylidene-β-D-glucopiranoside (NSC 141540) is a semisynthetic podophyllotoxin derivative reported to be active in several experimental and human malignancies [6, 8–10]. The pharmacokinetics of this compound have been studied in rat and man using radioisotopic methods [1, 6]. No data are available in the mouse in spite of the fact that is the most commonly employed species for studies on the mechanism of action and activity of this compound.

This lack of information prompted us to investigate the disposition of VP16 in tumor bearing mice. We selected the Lewis Lung carcinoma (3LL) of the mouse in order to find out whether pharmacokinetic knowledge could provide some explanation of the preferential antimetastatic effect of VP16 described in this model [2].

Materials and Methods

Animals and Tumor. C57B1/6 male mice (20 ± 2 g body weight), obtained from Charles River Breeding Laboratories, Italy, were used for these experiments. The animals received an intramuscular (i.m) transplant of 10^5 viable cells of Lewis Lung carcinoma (3LL) maintained by i.m. passages in the same strain every 2 weeks.

Drug. VP16 was kindly supplied by Dr. G. Lenaz, Bristol Myers, NY, USA. The drug was dissolved in Tween 80 and saline (1 : 10) and injected i.v. at the doses of 13 and 40 mg/kg in mice bearing 25-day-old i.m. 3LL; 1, 5, 15, 30, 60, 120, 240, 360 min after the injection, four animals per point were killed and plasma and tissues (tumor, metastases, heart, liver, spleen, small intestine, kidneys, and brain) were removed and frozen at −20° C until use.

Drug Assay. The method, described in detail elsewhere [5], can be summarized as follows: after diisopropyl ether washing a plasma or tissue extraction was carried out with chloroform using VM26 as internal standard. After drying the organic phase under vacuum, the residue was re-dissolved with 100 µl of mobile phase and 5–20 µl of this solution were injected into a Waters model 440 HPLC equipped with a 254 nm absorbance detector.

Separation was achieved with an isocratic solvent system of water (45%) and methanol (55%) at a flow rate of 1 ml/min using a 25 cm long Lichrosorb RP-8 (5 µm) column from Merck, Darmstadt, FRG. Extraction recovery was 79 ± 3% and sensitivity was 0.1 µg/ml of plasma or 0.3 µg/g of tissue.

Pharmacokinetic Analysis. Pharmacokinetic parameters in plasma were calculated using the peeling method assuming a two-compartment open model. For tissues, the areas under the concentration versus time curves (AUC) were measured by trapezoidal integration.

Results

Figure 1 depicts VP16 plasma levels in the mouse after i.v. injection of 13 and 40 mg/kg. At both doses VP16 disappears biphasically with a rapid distribution

Send offprint requests to M. D'Incalci at the above address

* This work was partially supported by CNR Progetto Finalizzato No. 80.01593.96. The generous contribution of the Italian Association for Cancer Research, Milan, Italy, is gratefully acknowledged.

0344-5704/82/0007/0127/$01.00

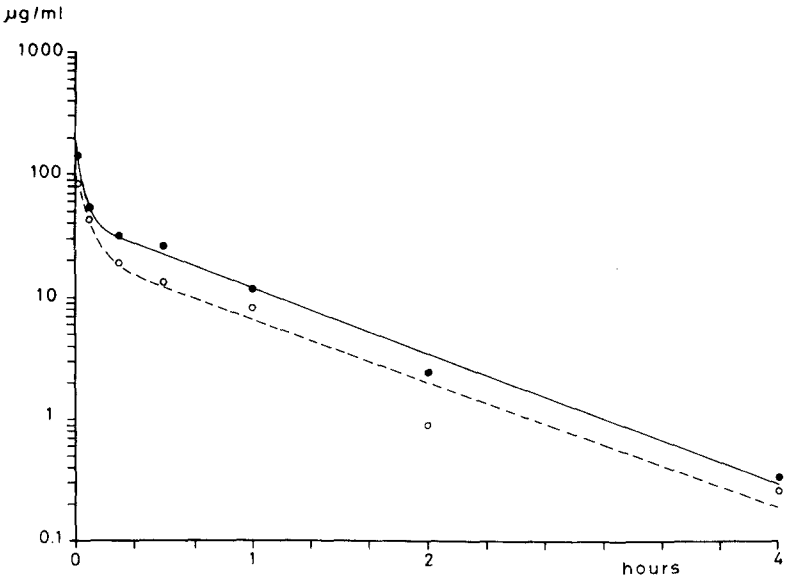


Fig. 1. Disappearance curve of VP16 in plasma of mice bearing i.m. 3LL (25 days old) after i.v. injection of 13 mg/kg (O---O) or 40 mg/kg (●——●)

Table 1. Plasma pharmacokinetic parameters of VP16 in mice bearing 25-days intramuscular Lewis lung carcinoma

	Dose (mg/kg i.v.)	
	13	40
Co (μg/ml)	104.7	203.8
$T_{1/2}$ (min) α	2 min 42 s	1 min 30 s
$T_{1/2}$ (min) β	34 min	33 min
V.d. (ml/kg)	455	819
Cl (ml/kg/min)	9.1	16.9
AUC (μg/ml × min)	1,437	2,366

Co, drug level extrapolated at time 0
$T_{1/2}$, half-life (α and β phase)
V.d., Volume of distribution β phase
Cl, body clearance
AUC, area under the concentration versus time curve (theoretical)

half-life of 1 and 2 min and an elimination half-life around 30 min.

Table 1 shows the plasma pharmacokinetic parameters. Comparison of the two dose levels shows that the Co level and the AUC were not proportional to the administered doses. The clearance values and the volume of distribution were consequently less after the lower dose.

As can be seen in Fig. 2, at both dose levels VP16 concentrations were much higher in metastases than in primary tumor where rather low drug levels were found. The highest levels were in small intestine, liver and kidney (Fig. 3). Concentrations even lower than in primary tumor were found in the brain.

As can be seen from Table 2, the AUC values of VP16 in some tissues were not proportional to the dose; in primary tumor and brain the levels after 40 mg/kg were disproportionately lower than after 13 mg/kg, whereas in the liver the levels after 40 mg/kg were more than 12 times higher than after 13 mg/kg.

Figure 4 shows that urinary excretion of VP16 as unchanged drug accounted for 20 and 30% of the doses in the 60 h after administration of 40 and 13 mg/kg.

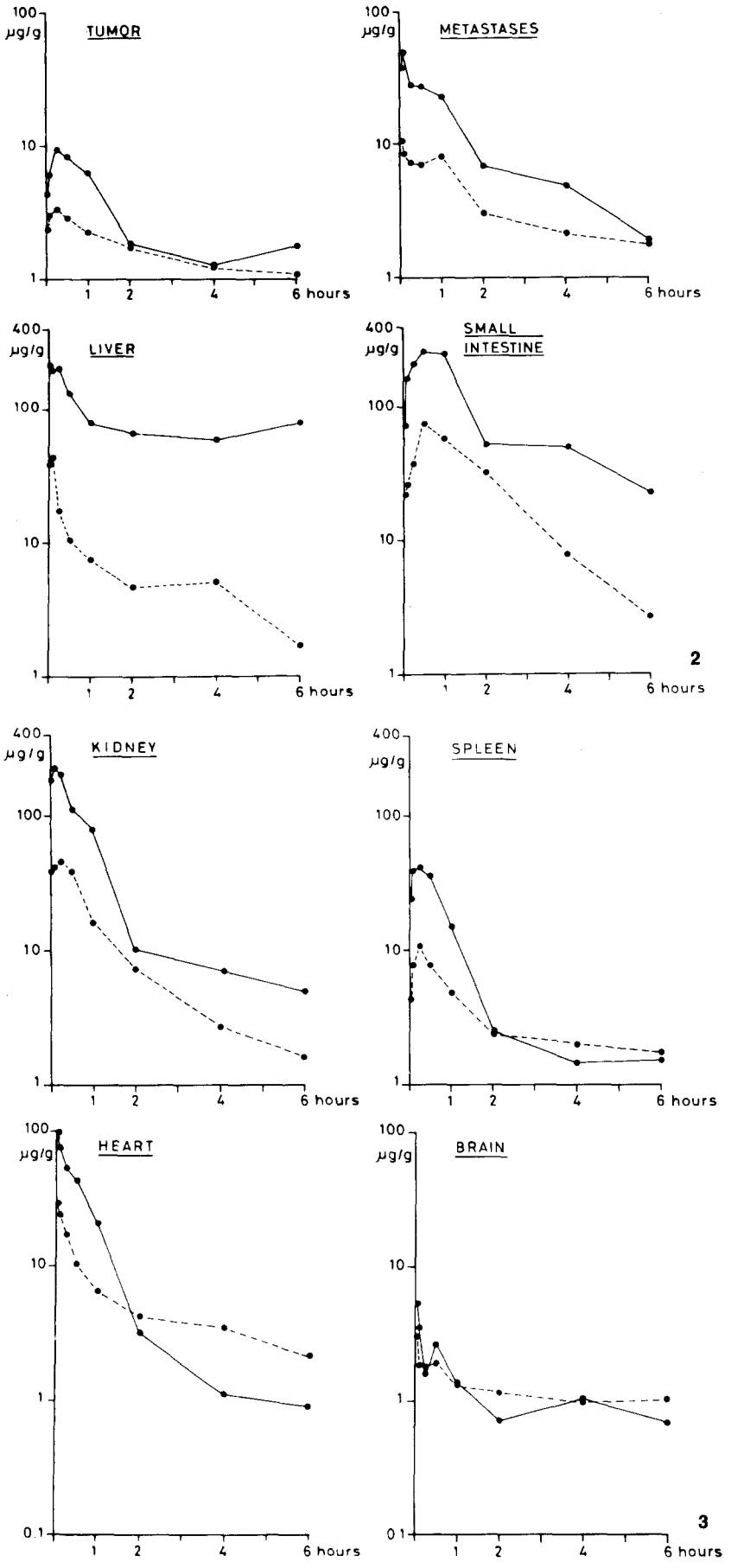
Figure 5 shows the cytotoxic effect of VP16 on cells from primary tumor and metastases of 3LL. Both the cell survival and the ^{3}H-TdR uptake studies show a steep concentration response curve in the range from 0.1–10 μg/ml. This effect was not reversed after 72 h of treatment.

Discussion

In the mouse VP16 appears to be eliminated from plasma fast, with a half-life of approximately 30 min. Distribution was not homogeneous and varied at the two dose levels investigated. The highest levels were found in organs involved in the elimination of the drug such as liver, intestine, and kidney. The high VP16 concentration found in small intestine is likely to be due to the biliary excretion, reported to be the main route of drug elimination in the rat [6].

In the liver VP16 levels after injection of 40 mg/kg were approximately 12 times higher than after the dose of 13 mg/kg. This suggests a dose-dependent accumulation of VP16 in the liver, but the mechanism involved is still unclear and requires further studies.

The kidney is another important organ of drug elimination as demonstrated by the appreciable



Figs. 2 and 3. Disappearance curve of VP16 in tissues of mice bearing i.m. 3LL (25 days old) after i.v. injection of 13 mg/kg (●– – –●) or 40 mg/kg (●——●)

Table 2. Tissue levels of VP16 after i.v. injection in mice bearing 25-days intramuscular Lewis lung carcinoma

Tissue	13 mg/kg		40 mg/kg	
	Peak level (μg/g)	AUC 0→6 h (μg/g × min)	Peak level (μg/g)	AUC 0→6 h (μg/g × min)
Tumor	3.3 ± 0.5	606 ± 207	9.4 ± 1.2	1,049 ± 296
Metastases	10.9 ± 0.6	1,338 ± 318	51.2 ± 9.2	3,794 ± 449
Liver	41.9 ± 5.9	2,293 ± 307	212.0 ± 25.1	28,464 ± 4,835
Small intestine	75.5 ± 12.8	9,830 ± 3,441	262.8 ± 37.5	33,516 ± 9,236
Kidney	45.9 ± 11.7	3,633 ± 1,064	227.3 ± 21.1	12,025 ± 2,111
Spleen	11.0 ± 0.8	1,158 ± 143	41.2 ± 5.7	2,841 ± 597
Heart	29.4 ± 1.7	1,946 ± 323	99.1 ± 2.9	3,818 ± 664
Brain	3.0 ± 0.5	431 ± 46	5.4 ± 0.8	404 ± 79

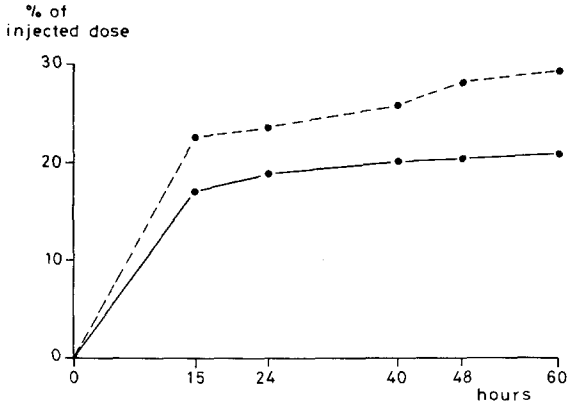


Fig. 4. Cumulative urinary excretion of VP16 after i.v. injection in i.m. 3LL bearing mice. 13 mg/kg (●---●); 40 mg/kg (●——●)

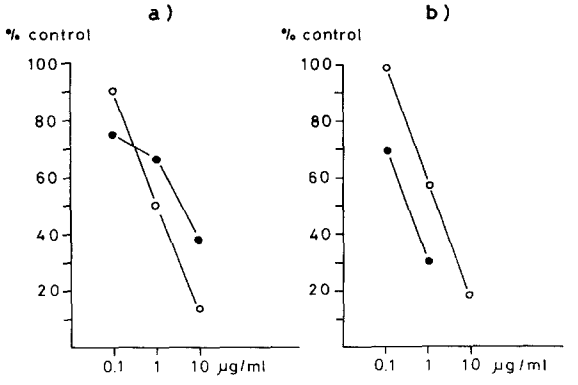


Fig. 5. Activity of VP16 on 3LL cells in vitro after 24 h of exposure. (**a**) primary tumor; (**b**) lung metastases. (●——●) cell count; (○——○) [^{3}H]TdR uptake

concentrations found in the renal parenchyma and by the urinary excretion which accounted for 20–30% of the administered dose.

The concentrations of VP16 in the tumor were low in comparison with other tissues. It should be noted, however, that at the dose of 13 mg/kg VP16 levels were relatively higher than after 40 mg/kg; this suggests that splitting the dose should result in a higher exposure. In fact, recent studies in our laboratory indicate that with a dosage schedule of 13 mg/kg for 3 days a significant effect can be achieved even on primary tumor, whereas no effect was observed after a single dose of 40 mg/kg.

As already described for other drugs [4] VP16 concentrations in lung metastases are considerably higher than in primary tumor, probably because of better blood supply of the metastatic lesions. Considering that the steepness of the curve of VP16 concentration vs cytotoxicity and the lack of apparent differences in sensitivity shown by our in vitro studies between cells derived from primary and secondary neoplastic lesions, it can be assumed that at least one of the reasons for the much greater sensitivity of metastases is greater drug accumulation at this site.

The lowest concentrations of VP16 were found in the brain; this is in agreement with the very low activity found against intracerebrally implanted L1210 leukemia [7] which, when injected i.p. is one of the most susceptible mouse tumors to VP16. Further, the low brain concentrations are in accordance with the reportedly undetectable levels of VP16 in CSF of patients who had received the drug [3].

References

1. Allen LM, Creaven PJ (1975) Comparison of human pharmacokinetics of VM26 and VP16, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11: 697
2. Cattan A, Pourny C, Carpentier Y, Cattan E, Barouh M (1976) Antimetastasis effect of two derivatives of epipodophyllotoxin in mice. Eur J Cancer 12: 797
3. D'Incalci M, Farina P, Fasoli M, Marsoni S (1981) VP16 plasma levels after i.v. and two methods of oral administration to choriocarcinoma patients. Proc Am Soc Clin Oncol 22: 357

4. Donelli MG, Colombo T, Broggini M, Garattini S (1977) Differential distribution of antitumoral agents in primary and secondary tumors. Cancer Treat Rep 61: 1319
5. Farina P, Marzillo G, D'Incalci M (1981) High-performance liquid chromatography determination of 4′-demethylepipodophyllotoxin-9-(4,6-o-ethylidene-β-D-glucopyranoside) (VP16-213) human plasma. J Chromatogr 222: 141
6. Issell BF, Crooke ST (1979) Etoposide (VP16-213). Cancer Treat Rev 6: 107
7. Nissen NI, Larsen V, Pedersen H, Thomsen K (1972) Phase I clinical trial of a new antitumor agent, 4′-demethylepipodophyllotoxin-9-(4,6-o-ethylidene-β-D-glucopyranoside) (NSC-141540; VP16-213). Cancer Chemother Rep 56: 769
8. Rivera G, Avery T, Pratt C (1975) 4′-Demethylepipodophyllotoxin-9-(4,6-o-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM26) and 4′-demethylepipodophyllotoxin-9-(4,6-o-ethylidene-β-D-glucopyranoside) (NSC-141540; VP16-213) in childhood cancer: Preliminary observations. Cancer Chemother Rep 59: 743
9. Rozencweig M, Van Hoff DD, Henney JE, Muggia FM (1977) VM26 and VP16-213: A comparative analysis. Cancer 40: 334
10. Stähelin H (1973) Activity of a new glycosidic lignan derivative (VP16-213) related to podophyllotoxin in experimental tumors. Eur J Cancer 9: 215

Accepted July, 1981