Cancer Chemother Pharmacol (1982) 7:151–156

Cancer Chemotherapy and Pharmacology

Combination Chemotherapy of the Epipodophyllotoxin Derivatives, Teniposide and Etoposide

A Pharmacodynamic Rationale?

Larry M. Allen, Francisco Tejada, Anthony D. Okonmah, and Staffan Nordqvist

Florida A and M University/VA Pharmacokinetic Laboratory, VA Medical Center;
Miami Cancer Institute, Inc., and Cedars of Lebanon Health Care Center, Miami, Florida, USA

Summary. *Previous studies in vitro on the influence of extracellular protein binding of Teniposide (VM26) and Etoposide (VP16-213) on subsequent cellular uptake by experimental murine tumor cells [Cancer Res 38:2549 (1978); Drug Metab Rev 8:119 (1978)] suggested that a timed-sequential combination of VM26 and VP16-213 may increase the bioavailability of VP16-213. This was studied clinically in six cancer patients with ascites (five ovarian, one rectal) whereby VM26 (20 mg/m^2) was given i.p. 2 h prior to VP16-213 (100 mg/m^2; i.p.). In some patients, this regimen was administered i.v. The i.v. regimen was found to be more toxic (myelosuppression, nausea, vomiting) than i.p. regimen at same doses of drugs. Several patients remained stable to disease during 1–2 courses of therapy (3 weeks per course), one patient had partial remission, and has been stable in her disease for more than 4 months.*

In two patients, plasma and ascites fluid was analyzed for VP16-213 and VM26 by a new reverse-phase high performance liquid chromatography method. Both VM26 and VP16-213 could be eluted isocratically (28% v/v acetonitrile in water) from a c_{18} column with retention times of 6.6 and 13.3 min, respectively. Subsequent pharmacokinetic analysis of one patient suggests that protein binding displacement of VP16-213 in plasma and perhaps ascites fluid increased the pharmacokinetic volume of distribution (28 l) and reduced the elimination half-life (12 h). The data suggests that VP16-213 is distributed more widely in the body and is represented by a single compartment pharmacokinetic model. Analysis of VM26 in ascites and plasma suggests that the so-called "deep pharmacokinetic compartment" represents ascites equivalent space and that the plasma concentration represents VM26 as free and protein-bound drug in kinetic distinguishable compartments.

Determinants of drug action are potentially composed of a multiplicity of physiological, biochemical, and other factors. The potential for manipulating the pharmacodynamic properties of drugs to achieve greater therapeutic potential needs further study.

Introduction

Multifactorial determinants of cancer chemotherapeutic drug action include favorable and unfavorable interaction which take place at the subcellular, cellular, and physiological level of the whole organism. The relationship between drug concentration and pharmacological effect is the basis for the clinical use of pharmacokinetics in improving drug therapy.

It is generally recognized that differences between tumor and normal host tissue are quantitative rather than qualitative. Therefore, tumors which are inherantly sensitive to neoplastic drug may become more susceptible to therapy by modulation of the pharmacodynamic properties of the agent. If the mechanism of action of a drug is precisely known, then modulation of drug action may possibly be achieved by biochemical means.

We have previously demonstrated the influence of extracellular protein binding of Teniposide (VM26) and Etoposide (VP16-213) on cellular uptake of these drugs using experimental murine tumor cells, Fig. 1 [2–5]. The binding affinity of VM26 for human serum albumin is about ten times that of VP16-213 [1]. We have shown that over a wide concentration range of drug that VM26 is more than 99% bound and VP16-213, 95% bound (Fig. 2). Since VM26 and VP16-213 are minor structural analogs of one another, we have devised a therapeutic regimen

Send offprint requests to L. M. Allen, 546/119, VA Medical Center, Miami, FL 33125, USA

0344-5704/82/0007/0151/$01.20

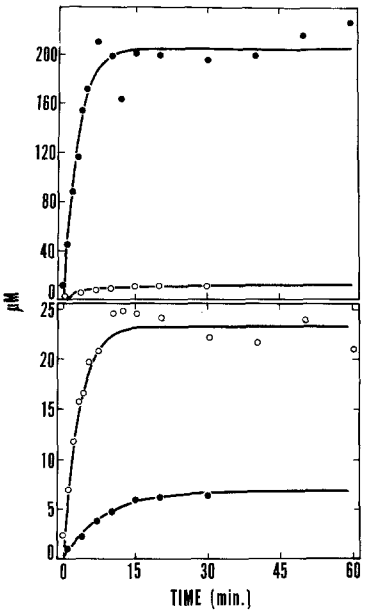

Fig. 1. Uptake by L1210 cells of VM26 and VP16 in presence and absence of human serum albumin top panel. VM26 (16 μM) in presence (○) or absence (●) of 1% human serum albumin [2–5]. *Lower panel:* VP16 (16 μM) in presence (○) or absence (●) of human serum albumin

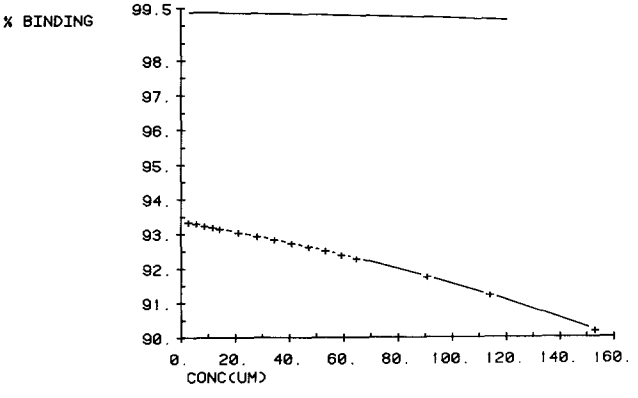

Fig. 2. Percent binding of VP16-213 and VM26 to human serum albumin. Binding of VM26 (–) and VP16-213 (+) to serum albumin as function of drug concentration, 100 μM is equivalent to 175 mg of VP16-213 or 187 mg of VM26 in 3 l of body fluid

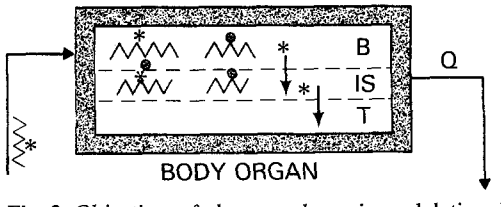

Fig. 3. Objectives of pharmacodynamic modulation. Modification of etoposide and teniposide disposition by protein binding displacement in blood and interstitial space. (∿∿∿) protein, (✲) drug VP16-213, (○) drug VM26; *B* = blood, *IS* = interstitial space, *T* = tissue, *Q* = blood or plasma flow

whereby VM26 is given 2 h prior to VP16-213 in order that VM26 become widely distributed in the body and bound to albumin in plasma and interstitial space. The systemic bioavailability of VP16-213 should be enhanced by the extent of its diminished protein binding.

The objectives of our new clinical regimen are shown in Fig. 3. If the critical biochemical interaction of VM26 and VP16-213 is at the subcellular level then non-specific intracellular and extracellular macromolecular binding of these agents can reduce free, unbound drug to below critical cytotoxic concentrations (Fig. 4). This is especially important since the extent of VM26 and VP16-213 uptake is proportional to extracellular drug concentration. Using initial velocity and steady-state cellular uptake experiments we have determined the intrinsic (absence of human serum albumin), KI, and effective (in the presence of human serum albumin), KE, partition coefficient of VM26 and VP16-213 for mouse leukemia L1210 cells [2–5]. The KE/KI ratios for VM26 and VP16-213 as a function of percent human serum albumin is shown in Fig. 5 (lowest curve of each panel). Each successive curve to the right of the lowest curve (14 μM) represents increasing concentration of VP16-213 or VM26 depending which drug is the protein binding displacing agent. This figure demonstrates the greater extent of VM26 displacement of VP16-213 protein binding than VP16-213 of VM26 binding. The consequence of protein binding displacement at the cellular level is greater cellular uptake and steady-state accumulation of drug. This in turn increases intracellular irreversible binding of VM26 or VP16-213 [2–5].

Drug protein binding displacement from drug-drug interaction is a well known phenomenon in clinical pharmacology. The therapeutic consequence from such pharmacodynamic interaction is often difficult to predict a priori. For highly protein bound lipophilic drugs, binding displacement may increase initial and stead-state volumes of distribution whereby reducing the blood or plasma drug concentration and increase to varying degrees the concentration of drug in other body space. In the presence of protein binding, drug concentration in peripheral body space may be found to decrease depending on initial distributional properties (body content) of the drug.

Figures 6 and 7 summarize the pharmacokinetic data for VP16-213 and VM26 as compartmental models. A generalized pharmacokinetic model that can be used to describe both drugs is illustrated in Fig. 8. For extensive protein binding, the central pharmacokinetic compartment is divided between bound and unbound drug (VM26). For less extensive

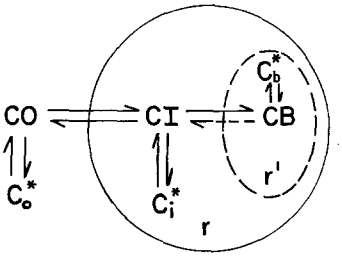

Fig. 4. Cellular transport model for VP16-213 and VM26. Co, free drug; C_0*, bound drug (extracellular); C_1, free drug, intracellular; C_0*, intracellular bound drug; C_b*, bound drug in nucleus, r, radius of nucleus

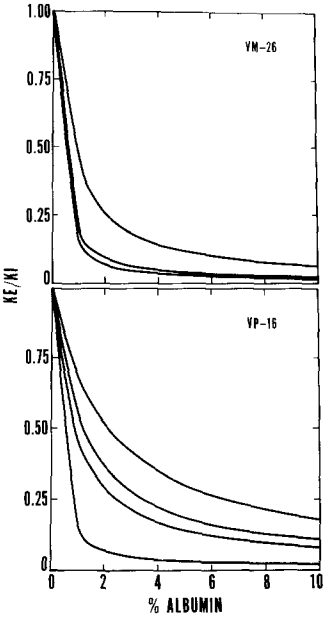

Fig. 5. Cellular transport partition ratio for VM26 and VP16-213 as function of albumin. Lowest curve of each panel is for VM26 (*top*) or VP16-213 (*bottom*), 14 μM, in the absence of competing drug. Each successive curve to right (*bottom panel*) is the partition ratio in the presence of 14, 20, 40 μM VM26. Each successive curve to right (*top panel*) is the partition ratio in the presence of 30 and 300 μM VP16-213

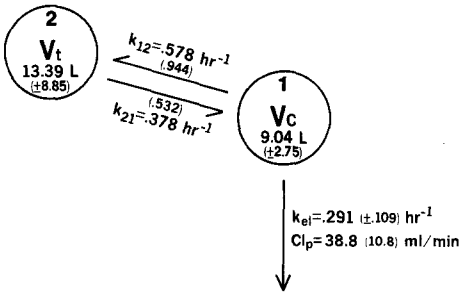

Fig. 6. Summary pharmacokinetic model for VP16-213. Data summarized [1]

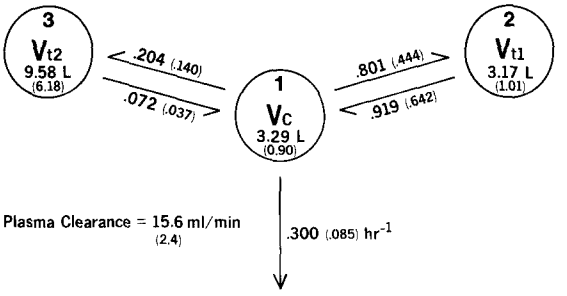

Fig. 7. Summary pharmacokinetic model for VM26. Data summarized [1]

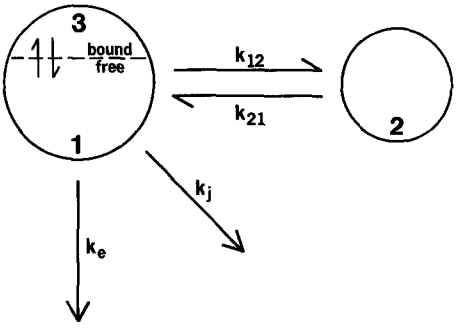

Fig. 8. Generalized clinical pharmacokinetic description of VM26 and VP16-213. Compartments 1 and 3 represent free and bound drug, respectively. k_{12}, k_{21}, represent first-order transfer constants; kj, all elimination pathways (metabolism) other than renal; ke, represents renal excretion pathway

protein binding (VP16-213), the central pharmacokinetic compartment may be expanded (3–9 l). Further reduction in protein binding may expand the central compartment until a one-compartment model would suffice to describe the pharmacokinetics of the drug. Therefore, the minimum toxic dose may be related in part to total dose and central compartment volume. This would suggest that in the face of protein binding displacement, a larger dose may be required to sustain therapeutic activity for a drug delivered to its site of action solely by drug in plasma equivalent space.

Materials and Method

All patients received an institutionally approved clinical protocol with informed consent (Cedars of Lebanon Hospital, Miami, FL, USA) prior to entry onto study. The patient characteristics are shown in Table 1. For the pharmacology study, ascites fluid was drained (2–4 l using a jelco plastic needle) prior to administering the drugs. VM26 (20 mg/m^2) was given in 0.5 l of 5%

Table 1. Clinical characteristics of ovarian carcinoma patients with ascites

Patient	Age (years)	Previous therapy
1	60	Cytoxan, adriamycin, platinum
2	70	L-Phenylalanine mustard
3	80	L-Phenylalanine mustard
4[a]	58	Surgery
5	57	Cytoxan, adriamycin, platinum methotrexate
6	31	Cytoxan, adriamycin, platinum cytosine arabinoside

[a] Adenocarcinoma of rectum

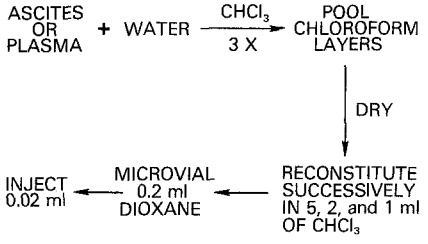

Fig. 9. Schematic representation of VM26 and VP16-213 extraction methods. Extraction method for etoposide and teniposide

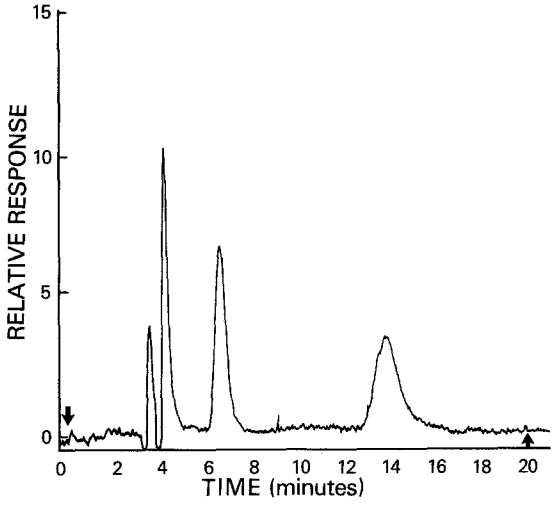

Fig. 10. High performance liquid chromatography profile of VP16-213 and VM26. Arrows indicate start (↓) and end (↑) of chromatogram. VP16-213 is at 6.6 min and VM26 at 13.3 min. Conditions outlined in Table 2

dextrose-water over a half hour period. Two hours from beginning of VM26 infusion, VP16-213 (100 mg/m^2) in 0.5 l of 5% dextrose water was administered by gravity within 30 min. Blood and ascites fluid for the analytical pharmacology were collected in heparinized tubes prior to treatment and at several intervals after drug administration. Multiple courses were given every 3 weeks as described above. Some patients received the combination regimen i.v.

Response was characterized as stable disease if during a 4 week period patient conditions, body weight and abdominal circumferance, remain unchanged. Any increase or deterioration in these paremeters was considered progression. Partial response was determined by complete absence of symptoms, for example, absence of clinically detectable ascites and dry paracenthesis.

Table 2. Conditions high performance liquid chromatography for simultaneous analysis of VM26 and VP16-213

Parameters	Characteristics
Column	Partisil-10 ODS-2 (Whatman) 250 × 3 mm i.d.
Mobile phase	Acetonitrile/water (28 : 72 v/v)
Flow rate	1 ml/min (750 psi) ambient
Wavelength	252 nm
Injection volume	10 µl
Aufs	0.003
Sensitivity	0.1 µg
Retention time:	
vp	6.6 ± 0.2 (SD) min n = 21
vm	13.3 ± 0.5 (SD) min n = 23
Quantitation	External peak-height comparison R^2 = 0.991 (vp); 0.987 (vm)
Chart speed	1 cm/min
Extraction efficiency	95.7 ± 0.6%

VP16-213 and VM26 were extracted from biological fluids essentially as described previously [6]. These steps are summarized in Fig. 9. Aliquots of 0.01 ml were injected into a Varian Model 8500 high performance liquid chromatograph (HPLC). The mobile phase was acetonitrile/water (28 : 72 v/v) at ambient temperature. The stationary phase was a Whatman Partisil-10 ODS-2 reverse phase column (250 × 3 mm). A typical HPLC profile for VP16-213 and VM26 is shown in Fig. 10. The HPLC conditions are summarized in Table 2.

Pharmacokinetic studies and hypothesis testing of VP16-213 and VM26 disposition with intravenous or intraperitoneal administration were carried out with aid of the National Institutes of Health PROPHET Computer System [8].

Results and Discussion

To date six patients have been studied, of which two were able to have concentrations of VP16-213 and VM26 analyzed in their plasma and ascites fluid. A summary of therapeutic outcome is shown in Table 3. It should be noted that in those patients which received the drug regimen i.v., they became more toxic and leukopenic than those patients who received VP16-213 and VM26 i.p. Some transient stability of disease was observed in three patients with partial response in one followed by prolonged stability (greater than 4 months).

The concentration of VP16-213 and VM26 in ascites fluid fell below detectable levels (less than 0.1 µg/ml) in less than 8 h. The concentration in plasma remained at greater than 0.5 µg/ml beyond 18–26 h (see Fig. 11). In plasma, the concentration of

Table 3. Summary of patients with ovarian cancer and ascites

Patient	Regimen (vm-vp)		Numbers of courses	Toxicity		Response
	i.v.	i.p.		Gastro-int.	Myelos.	
1		+	1	–	–	Progress
2		+	1	–	–	Progress
3		+	1	–	–	Progress
4[a]		+	2	+(N, V)	–	Stab. 4 weeks
5	+		1	++(N)	700 WBC 55,000 PLT	Stable
		+	1	–	–	Progress
6		+	3	–	–	Part. response
	+		1	–	2,000 WBC 90,000 PLT	Stab. (−ASC)
		+	3	–	–	Stab. (4+MO)

[a] Adenocarcinoma of rectum with ascites

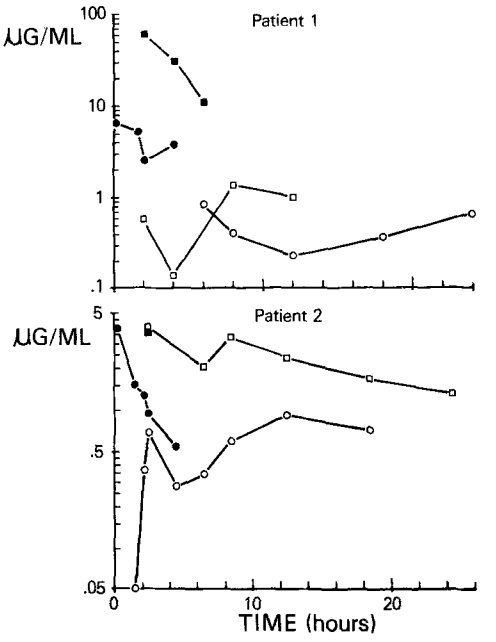

Fig. 11. Concentration of VP16-213 and VM26 in Plasma and ascites fluid. (■) VP16-213 ascites; (●) VM26 ascites; (□) VP16-213 plasma; (○) VM26 ascites

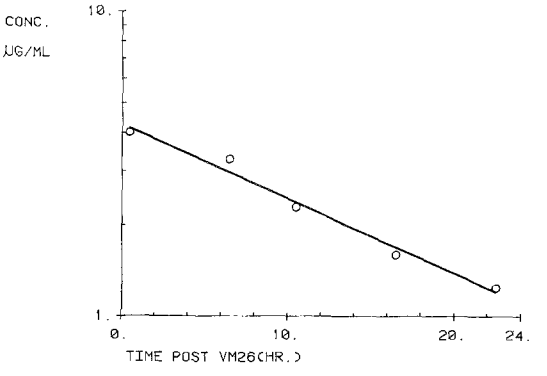

Fig. 12. First-order plasma decay curve for VP16-213. Non-linear least squares analysis of plasma decay curve of VP16-213 administered i.p. 2 h after VM26 (i.p.)

VP16-213 exceeded that of VM26. This would be expected based upon differences in dose and distributional properties of the drugs. The substantially lower concentration of VP16-213 in plasma after i.p. administration as contrasted to i.v. administration was in part anticipated if protein binding displacement by VM26 increased VP16-213 distribution volume.

The prolonged plasma concentration of VP16-213 and VM26 was unexpected and may indicate that more specific rather than non-specific protein binding has taken place, competition for metabolism has occurred, or renal clearance has reduced the overall elimination rate. Alternatively, more free drug may have penetrated circulating cells.

The plasma curve of VP16-213 (i.p.) in one patient was determined by weighted non-linear least-squares regression analysis to decrease monoexponentially, Fig. 12, ($A = 4.26 \pm 0.22$ μg/ml; $k_{el} = 0.056 \pm 0.005\ h^{-1}$) with a half-life of 12.4 h and a volume of distribution of 28 l. This finding would be consistent with our previous pharmacokinetic model of VP16-213 [1, 7] if protein binding largely accounted for the kinetic distinguishable compartments. Drug protein binding displacement by VM26 may cause the previous two compartment pharmacokinetic model of VP16-213 to collapse to a single compartment model with combined volumes (22 l). The single available determination of VP16-213 in ascites fluid (3.6 μg/ml) would agree with this conjecture. Comparison of the areas under the curve for drug concentration or amount for the two-compartment model with drug initially in C1, C2, or for the single compartment model is shown in Table 4. A similar area analysis was done for VM26 (Table 4).

The results of compartmental analysis for VM26 in ascites fluid and plasma using differential equations and curve fitting is shown in Fig. 13. The data for the concentration of VM26 in ascites fluid as determined experimentally is well represented in the pharmacokinetic model (Fig. 13) with VM26 (i.p.) as being in the "deep peripheral" compartment (C3) and the plasma concentration of VM26 being represented as free (C1) and bound (C2) drug, refer to Fig. 7.

Table 4. Assessment of VP16-213 and VM26 in various pharmacokinetic compartments from different hypothetical routes of drug administration

Model[a] for VP16-213 (or VM26)	Pharmacokinetic compartments[b]					
	c1 (μg/ml × h)	x1 (mg × h)	c2 (μg/ml × h)	x2 (mg × h)	c3 (μg/ml × h)	x3 (mg × h)
I, i.v. (VM26)	47.6 (21.2)	367 (75.3)	50.2 (14.4)	486 (50.2)	(12.8)	(130)
II, i.p. (VM26)	45.1 (13.7)	348 (48.8)	80.81 (9.6)	782 (33.3)	(38.8)	(395)
III	13.6	380	–	–	–	–

[a] I = 2-compartment, VP16-213 or 3-compartment, VM26; drug in cl as bolus (Figs. 6, 7)
II = 2-compartment, VP16-213 or 3-compartment, VM26; drug in c2 or c3 (VM26)
III = single pharmacokinetic compartment (VP16-213)
[b] C = concentration; X = amount (X × Vol = C)

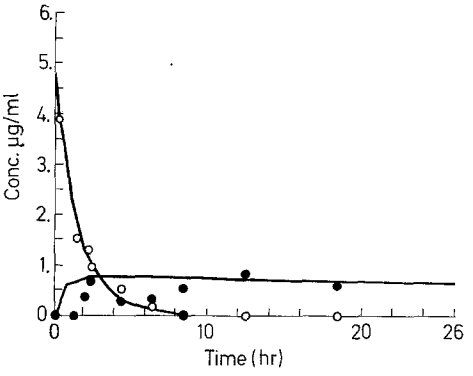

Fig. 13. Multicompartment model for VM26 administered by intraperitoneal route. (○) Data for VM26 in ascites fluid, (●) data for VM26 in plasma. Solid line is computer generated curve from parameters [1]. Ascites fluid comprises compartment 3 of Model. Plasma concentration is represented by compartments 1 (free drug) and compartment 2 (bound drug)

The fact that the i.p. regimen as reported here is essentially non-toxic despite prolonged cytotoxic concentrations of both VP16-213 and VM26, may suggest that high, initial concentrations of drug as delivered to the ascites tumor may circumvent systemic toxicity whereas the same regimen and dosage given i.v. delivers high concentrations of drug to dose limiting tissue (gastrointestinal and bone marrow), Table 4. This explanation would be in keeping with our previous findings that these drugs distribute into cells by passive diffusion which is in part concentration dependent [2–5].

It remains to be determined in the future if the same or better therapeutic outcome can be achieved when either drug alone is given by the i.p. route. Our observations need to be extended to other cancers with pleural effusions (i.e., lung cancer). Favorable therapeutic enhancement by pharmacodynamic manipulation may be achieved in the future from a better understanding of the human pharmacodynamic nature of these useful and effective anticancer agents.

Acknowledgement. The help and cooperation of the nursing staff of Cedars of Lebanon Health Care Center is greatly appreciated. Special gratitude is extended to the staff of Cedars of Lebanon Health Care Center for Assisting with the production of this manuscript.

References

1. Allen LM, Creaven PJ (1975) Comparison of the human pharmacokinetics of VM26 and VP16-213, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11: 697
2. Allen LM (1978a) Comparison of uptake and binding of two epipodophyllotoxin glucopyranosides, 4′-demethyl epipodophyllotoxin thenylidene-β-D-glucoside and 4′-demethyl epipodophyllotoxin ethylidene-β-D-glucoside, in the L1210 Leukemia cell. Cancer Res 38: 2549
3. Allen LM (1978b) Cellular pharmacodynamics of VM26 (VM) and VP16-213 (VP) uptake in the presence and absence of human serum albumin (HSA) (Abstract). Am Assoc Cancer Res Washington, DC
4. Allen LM (1978c) A model system for studying protein-binding displacement by other drugs of two antineoplastic epipohylotoxin glycosides, VM26 and VP16-213 (Abstract). 7th Int Congress Pharmacol, Paris
5. Allen LM (1978d) The role of drug disposition kinetics on cellular transport of the antineoplastic agent VM26. Drug Metab Rev 8: 119
6. Allen LM (1980) Analysis of 4′-demethylepipodophyllotoxin-9-(4,6-0-ethylidene-β-D-gluceopyranoside) by high-pressure liquid chromatography. J Pharm Sci 69: 1440
7. Pelsor F, Allen LM, Creaven PJ (1978) Multicompartment pharmacokinetic model of 4′-demethylepipodophyllotoxin-9-(4,6-0-ethylidene-β-D-glucopyranoside in humans. J Pharm Sci 67: 1106
8. Raub WF (1974) The Prophet system and resource-sharing. Fed Proc 33: 2390

Accepted July, 1981