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Pharmacokinetics and Efficacy of i.v. and i.p. VM26 Chemotherapy in Mice Bearing Krebs II Ascitic Tumors

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Abstract—*The pharmacokinetics and the efficacy of VM26 are studied following i.p. or i.v. administration in mice bearing Krebs II ascitic tumors. The i.p. inoculation of 30.10^6 Krebs II cells in Swiss mice leads to the formation of ascites. The effects of VM26 were dependent upon the route of administration. A 2 mg/kg i.p. single dose induces an equivalent per cent increase of median survival time than a 20 mg/kg i.v. single dose. The survival advantage of i.p. VM26 was found to be related to the pharmacologic benefit of i.p. administration. If local toxicity does not prove to be a major problem, i.p. VM26 may constitute a safe and practical mode of therapy in patients with intraabdominal tumors.*

INTRODUCTION

THE UNSATISFACTORY results of conventional therapy [1] for advanced ovarian cancer have led to the investigation of newer or more novel approaches. As a result, systemic combined therapies and i.p. administration of cytotoxic drugs are currently under clinical investigation: studies of intraperitoneal methotrexate [2, 3], fluorouracil [4, 5], doxorubicin [6], melphalan [7, 8] and cisplatin [9–11] have provided substantial experimental data showing a pharmacologic advantage for intraperitoneal administration compared with the intravenous route.

VM26 (Teniposide) is a pure antimitotic agent used against ovarian cancer [12]; its pharmacokinetics in ascites after i.v. administration in humans is well established [13, 14] and shows low levels in peritoneum cavity. This work was undertaken to demonstrate the feasibility of intraperitoneal therapy of VM26. Pharmacokinetics and efficacy of i.v. and i.p. chemotherapy were compared in mice bearing Krebs II ascitic tumors.

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MATERIAL AND METHODS

Tumor line

Female Swiss mice (9.12 weeks old — body wt 25 ± 2 g) from Janvier Laboratoires, France were used for these studies. The tumor was maintained by serial i.p. transplantation of malignant ascites Krebs II cells (30×10^6 viable cells/0.3 ml/mouse) every week [15]. This i.p.inoculation leads to the rapid formation of ascites (7 ml 7 days after injection) rich in cells (cytocrit ≈ 30%).

Drug

VM26 was supplied by Sandoz labs, Switzerland. The drug was dissolved immediately before injection in 0.9% Na Cl solution so that injection volumes were 300 µl for i.v. administration and 1000 µl for i.p. injection.

Survival studies

For assessment of antitumor activity, mice (3 days after inoculation) were given a single dose i.v. (via the tail vein) or i.p. Four doses were experimented: 2, 8, 14, 20 mg/kg. Control animals received an injection of solution used to dissolve the drug. Lots of 40 animals were used and observed during 60 days. The per cent increase in median survival time (MST) was then calculated (MST of treated mice/MST of control mice × 100%). Statistical significance of differences between mean survival time for controls and treated groups was assessed by log rank test.

Pharmacokinetic studies

Mice bearing 7 days Krebs ascitic tumor were given VM26 in a single i.v. or i.p. dose of 2, 8, 14 or 20 mg/kg. Eight animals per time were killed 1, 5, 10, 15, 30 min, 1, 2, 3, 6, 10, 24 hr after i.v. injection and 1,30 min, 1, 2, 3, 4, 6, 10, 24 hr after i.p. administration. One millilitre of blood and at least 5 ml ascitic fluid (upper 7 ml) were removed; Krebs cells were separated from the exudate by centrifugation at 700 ***g*** (10 min) and were washed twice in 3 vol. of PBS. Serum, ascites liquid without cells and Krebs cells were frozen at −20°C until analysis.

Analytic assay

The method described in detail elsewhere [16] can be summarized as follows: after adding etoposide as internal standard, 0.2 ml serum, 1 ml ascites liquid and 1 ml sonicated cells were extracted with chloroform. The organic phase was dried and redissolved with 200 μl of mobile phase and 20 μl were injected into a Waters Model 440 HPLC equipped with an electrochemical detector (Bioanalytical systems). Separation was achieved with an isocratic solvent system (water, methanol, acetic acid 45/54/1 buffered by 250 mM ammonium acetate). For determining VM26 levels in Krebs cells, the cells were homogenated in 3 ml of PBS and sonicated. One millilitre of homogenate was treated as described above and the protein content was evaluated according to the method of Lowry [17].

Pharmacokinetic calculations

The pharmacokinetic parameters (A, B, α and β) of each dose were determined by a general non-linear fitting procedure using the unweighted least -squares criterion. Distribution and elimination half-lives (T 1/2 α, T 1/2 β) were calculated from the equation $t\,1/2 = \ln 2/k$ where k is the elimination rate constant given by the slope of ln serum concentration × time. AUC from time 0 to the final time determined was estimated by the trapezoidal method. The remaining AUC from t to ∞ was estimated from the equation AUC $(t = \infty) = Ct/k$ where Ct is the ascitic or serum concentration at t time. AUC values given for AUC $(0 = \infty)$ are obtained by adding AUC $(0 - > t)$ and AUC $(t - > \infty)$. The clearance (Cl) was calculated from the relation : total dose amount injected/AUC $(0 - > \infty)$.

RESULTS

Effects of VM26 on mice survival

Table 1 summarized the effects of VM26 on survival in Swiss mice bearing Krebs ascitic tumors. The effect of VM26 was dependent upon the route of administration since a same increase in the MST was obtained either with i.v. 20 mg/kg or i.p. 2 mg/kg. With high doses (14 or 20 mg/kg) injected by i.p. route, 20 mice (50%) were alive without ascites at the 60th day after Krebs cells inoculation. No overt sign of toxicity was noticed. The peritoneum did not look hemorragic and fibrin deposits were not observed. However, further microscopic studies should be performed. A MST i.p. dose relationship clearly appeared. In contrast increased doses by i.v. route did not result in MST benefit.

Pharmacokinetics of VM26 after i.v. injection

Figure 1 shows the plasma disappearance curves of VM26 after i.v. injection of the different doses in mice bearing Krebs ascitic tumors. At all doses, the rate of disappearance appeared to be biphasic with a rapid initial decline followed by a second slower phase. Table 2 summarizes the corresponding pharmacokinetic parameters. The α half-lives were similar for all treatments (ranged between 7.2. and

Table 1. *Effects of VM26 on mice bearing Krebs ascitic tumors*

Route	Dose	Mean suvival time (days)	Log-rank test	Median survival time MST (days)	Per cent increase
Control		13.7 ± 1.7		13.5	
i.v.	2 mg/kg	13.6 ± 1.5	NS	13.5	0
	8 mg/kg	13.5 ± 1.5	NS	13.5	0
	14 mg/kg	15.6 ± 2.6	$p < 0.01$	15.5	15
	20 mg/kg	16.0 ± 2.4	$p < 0.01$	16	18.5
i.p.	2 mg/kg	17.1 ± 2.2	$p < 0.001$	16.5	22
	8 mg/kg	26.7 ± 4.1	$p < 0.0001$	27.5	104
	14 mg/kg	> 60*		> 60[d]	
	20 mg/kg	> 60**		> 60[d]	

* 20 mice were dead with ascites at the 60th day.

** No death at the 60th day.

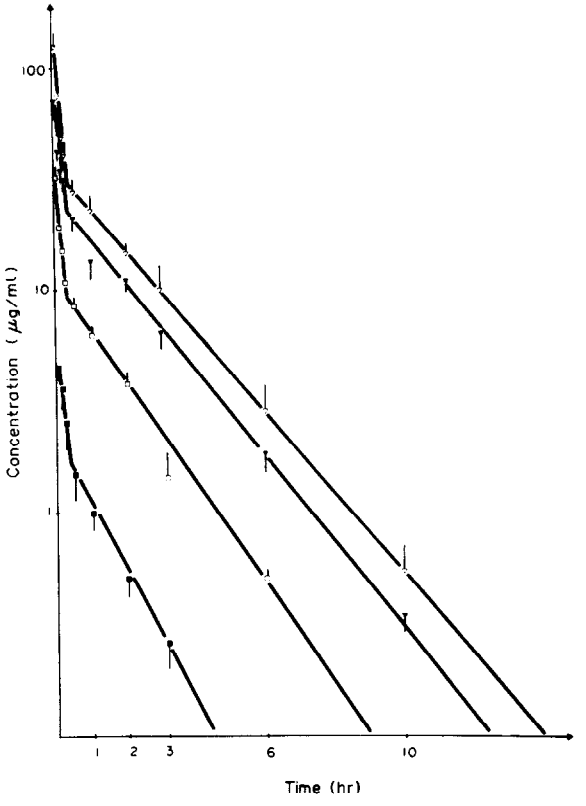

Fig. 1. Semilogarithmic plot of serum concentration (± S.D.) of VM26 vs time following i.v. administration in mice bearing Krebs II ascitic tumors. ■ 2 mg/kg) □ 8 mg/kg; ▼ 14 mg/kg; ▽ 20 mg/kg.

9.5 min), while the T 1/2 β were dependent upon the dose ($r = 0.931$). The zero time concentrations and the area under the curves values were proportional to the doses. Following i.v. injection, VM26 levels were detectable in ascites immediately. The concentration reached a peak 1 hr after injection except for 20 mg/kg (Fig. 2). The pharmacokinetic parameters of ascites were summarized in Table 3. Disappearance of VM26 in ascites was monoexponential, but the slope of elimination was slower at low doses. The area under curves values in ascites were proportional to the dose.

Pharmacokinetics of VM26 after i.p. injection

Figure 3 shows the ascites disappearance curves of VM26 following i.p. injection. The elimination was monoexponential but the concentrations declined more rapidly at high doses. The half-lives for all treatments decreased with doses ($r = 0.960$) (Table 4). The maximum concentrations and area under curves in ascites were proportional to the dose delivered. After i.p. administration, serum VM26 maximum concentrations were reached between 2 and 3 hr after injection (Fig. 4) and the half-lives for all treatments were similar (Table 5). However, the serum AUC and serum peak concentrations were not proportional to the doses. After i.v. or i.p. administration of VM26, the intracellular levels of the drug followed those of ascites (Fig. 5). In no case was any metabolite of VM26 detected in plasma or ascites or cells whatever the route of administration.

Comparison of pharmacokinetic parameters after i.v. or i.p. administration

After i.p. injection of VM26, the ratio of peak concentrations in ascites and in serum ranged from 44.1 for 2 mg/kg dose level to 15 for 20 mg/kg (Table 6). The ratio between peak concentrations in ascites after i.p. or i.v. injections was also dose dependent.

I.p. administration led to an higher exposure of the ascites to the drug (the ratio of ascitic AUC i.p./ascitic AUC i.v. varies between 15.9 and 5.94) and to a lower systemic exposure (ratio of serum AUC i.v./serum AUC i.p. comprised between 1.87 and 2.97 according to the dose). We noticed that ascitic AUC after i.p. 2 mg/kg dose level injection was equivalent to the ascitic AUC obtained after i.v. 20 mg/kg dose level. The peritoneal clearance of VM26 is about one tenth of the total body clearance.

DISCUSSION

Direct instillation of chemotherapic agents in the peritoneal cavity of patients with intraabdominal malignancies is intended to increase drug levels at the predominant sites of disease, thereby resulting in more cytotoxicity than can be achieved with systemic route [18] without exposition to systemic side effects of these drugs.

Table 2. Serum pharmacokinetic parameters after i.v. injection of VM26 in mice bearing Krebs ascitic tumors

Dose	A (μg/ml)	B (μg/ml)	T 1/2 α (min)	T 1/2 β (min)	A.U.C. (0– > ∞) (μg/ml min)	Cl (ml/min/kg)
2 mg/kg	5.706	2.238	7.2	55.6	234	8.55
8 mg/kg	32.2	9.96	9.5	79.6	1670	4.79
14 mg/kg	71.9	22.79	8.6	80	3596.4	3.89
20 mg/kg	112	35.082	8.6	92.9	5109.5	3.91

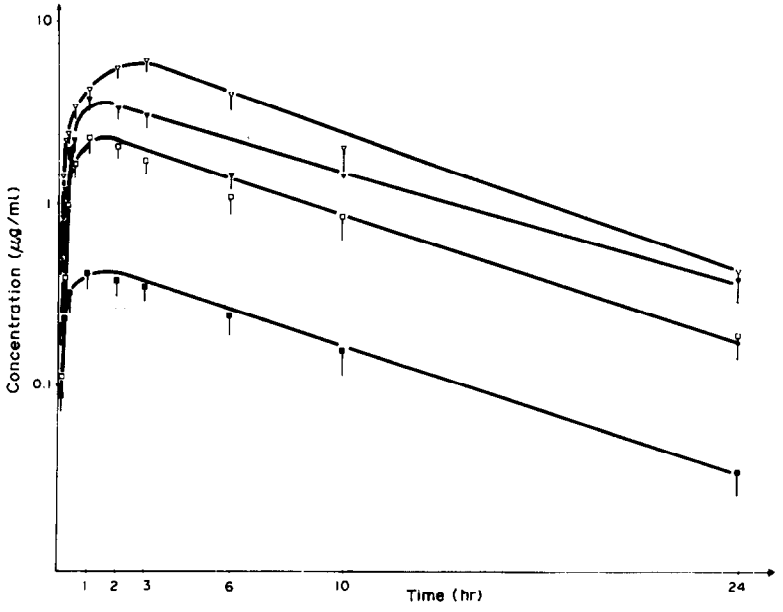

Fig. 2. Semilogarithmic plot of ascites concentration (± S.D.) of VM26 vs time following i.v. administration in mice bearing Krebs II ascitic tumors.
■ 2 mg/kg; □ 8 mg/kg; ▼ 14 mg/kg; ▽ 20 mg/kg.

Table 3. Ascitic pharmacokinetic parameters after i.v. injection of VM26 in mice bearing Krebs ascitic tumors

Dose	A (µg/ml)	T 1/2 α (min)	A.U.C. (µg/ml.min)
2 mg/kg	0.475	395	266.6
8 mg/kg	2.33	393	1282.8
14 mg/kg	3.79	390	2528
20 mg/kg	5.953	273	3259

The present study compared the pharmacokinetics of VM26 following i.v. and i.p. administration to mice bearing Krebs ascitic tumors. It demonstrated a pharmacologic advantage of intraperitoneal administration of VM26 which can be calculated according to the equation [3] defined by Collins [18]. It ranged from 28.7 at low dose (2 mg/kg) to 15.5 at high dose (20 mg/kg). Such a ratio is comparable to those obtained with methotrexate [3] or cisplatin [11]. The ratio of

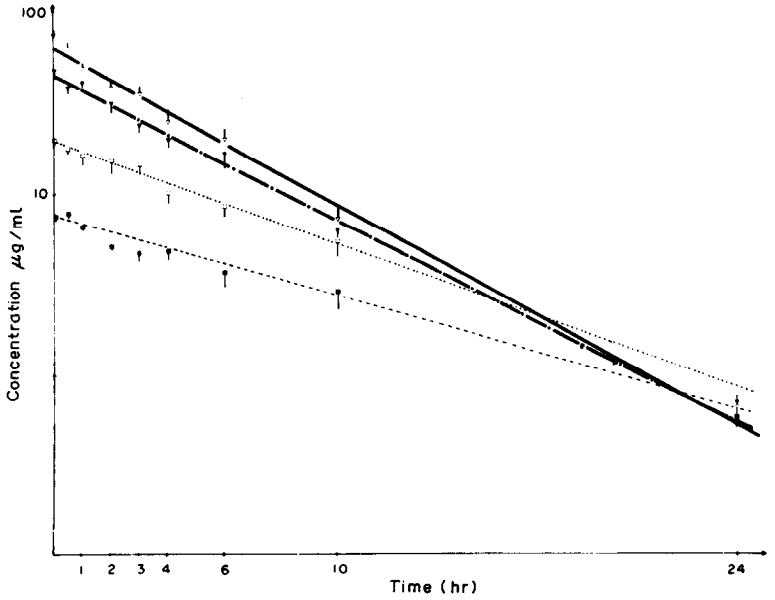

Fig. 3. Semilogarithmic plot of ascites concentration (± S.D.) of VM26 vs time following i.p. administration in mice bearing Krebs II ascitic tumors.
■ 2 mg/kg; □ 8 mg/kg; ▼ 14 mg/kg; ▽ 20 mg/kg.

Table 4. *Ascitic pharmacokinetic parameters of VM26 after i.p. injection in mice bearing Krebs ascitic tumors*

Dose	A (μg/ml)	*T* 1/2 (min)	A.U.C. (μg/ml.min)	Cl (ml/min/kg)
2 mg/kg	7.473	396	4236	0.472
8 mg/kg	19.75	321	9162	0.873
14 mg/kg	45.4	224	14885	0.942
20 mg/kg	61.5	216	19346	1.03

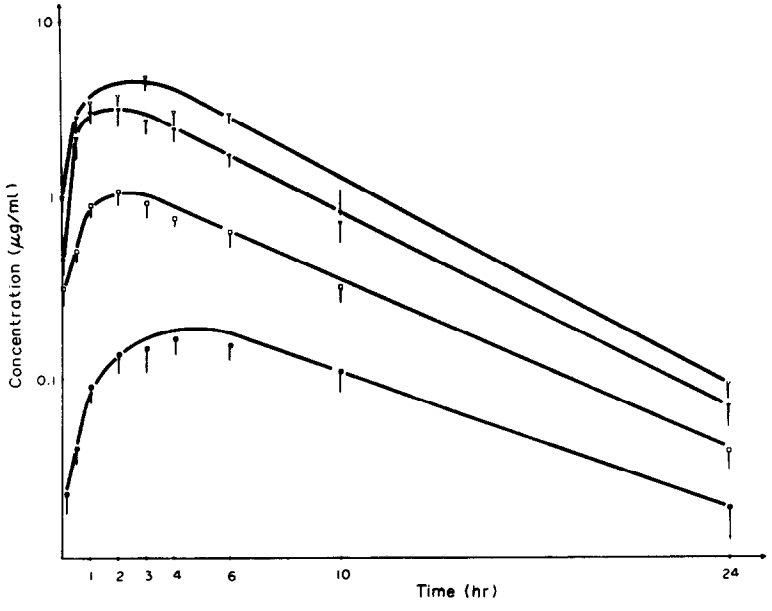

Fig. 4. *Semilogarithmic plot of serum concentration* (± S.D.) *of VM26 vs time following i.p. administration in mice bearing Krebs* II *ascitic tumors.*
■ 2 mg/kg; □ 8 mg/kg; ▼ 14 mg/kg; ▽ 20 mg/kg.

Table 5. *Serum pharmacokinetic parameters after i.p. administration of VM26 in mice bearing Krebs ascitic tumors*

Dose	A (μg/ml)	*T* 1/2 α (min)	A.U.C. (μg/ml.min)
2 mg/kg	0.400	303	124.97
8 mg/kg	1.14	312	621.3
14 mg/kg	3.43	317	1212.4
20 mg/kg	4.9	225	2095.2

total body clearance of VM26 to its regional exchange ratio is about 10-fold. According to Dedrick *et al.* [20], such a drug administered i.p. in large volumes is expected to maintain a significantly greater concentration in the peritoneal space than in plasma.

High levels of VM26 in peritoneal cavity were associated with low levels in systemic circulation and the systemic exposure to the drug (serum AUC) was diminished by a factor of 3 after i.p. administration. However, the considered murine model did not allow us to determine the local toxicity of VM26 on the mesothelium. It was demonstrated that teniposide, at low i.v. doses, had no effect on survival of mice bearing Krebs ascitic tumors whereas a single 2 mg/kg i.p. dose induced an equivalent per cent increase of MST than a single 20 mg/kg i.v. dose. On the other hand, mice treated with a single 20 mg/kg i.p. dose could be cured: there were markedly higher concentrations of drug in tumor cells and ascites fluid following i.p. administration. Similar results have been observed by Ozols *et al.* [21, 22] with adriamycin on murine ovarian cancer.

Our results showed that teniposide, no more than methotrexate [2, 3], fluorouracil [4, 5], doxorubicin [6], melphalan [7, 8], or cisplatin [9–11] does possess all properties proposed by Myers [23] in defining the ideal drug for i.p. use. However it comes close enough to be of some clinical interest.

Recognizing that drug-induced peritonitis may be a potential problem, a clinical phase I trial might be undertaken to determine whether VM26 can be safely given using the i.p. route to patients suffering from refractory intraabdominal diseases.

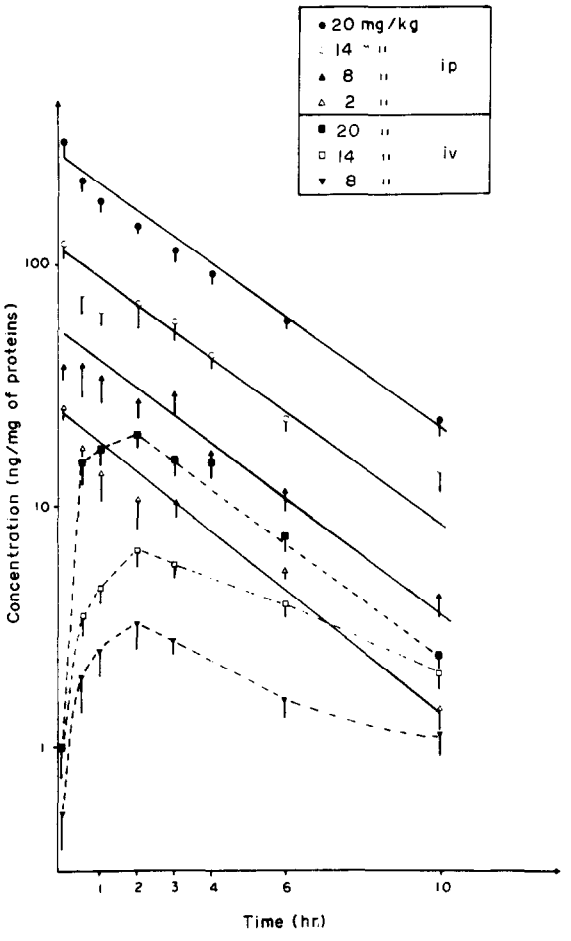

Fig. 5. Semilogarithmic plot of Krebs II cells concentration (± S.D.) of VM26 vs time following i.v. or i.p. administration in mice bearing Krebs II ascitic tumors.

Table 6. Comparison of pharmacokinetic parameters after i.v. or i.p. administration of VM26 in mice bearing Krebs ascitic tumors

Dose	2 mg/kg		8 mg/kg		14 mg/kg		20 mg/kg	
Route	i.v.	i.p.	i.v.	i.p.	i.v.	i.p.	i.v.	i.p.
Serum Cm	5.7	0.173	32.2	1.14	71.9	3.15	112	4.9
Ascites Cm	0.424	7.48	2.33	19.75	3.79	45.4	6.174	61.5
Ascites Cm i.p. / Serum Cm i.p.	44.1		18.1		16.1		15	
Ascites Cm i.p. / Ascites Cm i.v.	17.6		8.47		11.9		9.96	
Serum AUC	234	125	1670	621.3	3596.5	1212.4	5109.5	2095.2
Serum AUC i.v. / Serum AUC i.p.	1.87		2.69		2.97		2.44	
Ascites AUC	266.6	4236.3	1282.8	9162	2528	14855	3259	19346
Ascites AUC i.p. / Ascites AUC i.v.	15.9		7.14		5.88		5.94	
Total body Clearance (TBC)	8.55		4.79		3.89		3.91	
Peritoneal clearance (PC)	0.472		0.873		0.942		1.03	
TBC / PC	18.1		5.5		4.13		3.80	

If the survival and pharmacological advantage of i.p. VM26 in the considered murine model can be translated to a safe and practical mode of therapy in patients with intraabdominal tumors, and if local toxicity does not prove to be a major adverse effect, then a potentially useful agent will have been added to the known panel of local active drugs against persistent peritoneal neoplastic diseases.

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