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The vascular compartment hampers accurate determination of teniposide penetration into brain tumor tissue

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Abstract After a pre-operative 1-h i.v infusion of 150 mg/m^2 of teniposide (Vumon; VM26), the drug levels were determined in resected brain tumor specimens from three patients with malignant glioma and from three patients with brain metastases. Tissue dissections were performed within 0–2.5 h after drug administration in three patients and after 24 h in the other three patients. Teniposide was quantified by high-performance liquid chromatography and the levels of albumin in the resected tissue samples were quantified by radial immunodiffusion. In addition, albumin levels were quantified in normal brain tissue, in malignant glioma and in metastatic brain tumor tissue obtained post mortem from deceased patients. The albumin levels indicated that a substantial fraction (range: 0.16–0.50) of the resected brain tumor specimens consisted of blood. As the plasma concentration of teniposide during the first hours after infusion is high, the major part of the drug measured in the tumor specimens collected within 2.5 h after drug administration originated from the blood compartment. At 24 h after drug administration, when the plasma level of teniposide had declined to approximately 0.20 μg/ml, we could discern a real tissue uptake of teniposide ranging from 0.15–0.27 μg/g wet tissue weight in the resected tumor. Although the number of patients in this study is small, this work clearly illustrates that an accurate determination of the tissue concentration of teniposide is hindered by the high concurrent plasma levels. It is therefore essential that future tissue distribution studies also include a suitable procedure that establishes the contribution of drug originating from the blood compartment.

Key words Teniposide · Brain · Metastasis · Malignant glioma

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Introduction

For decades, surgery and cranial irradiation have been the mainstay of treatment of malignant brain tumors. Owing to infiltration of tumor into adjacent brain tissue and to limited tolerance of the brain to radiation, these therapies are hardly ever curative, and the median survival of patients with primary or metastatic malignant brain tumors is still only a few months. More recent experience indicates that progress in the treatment of these tumors might be achieved by the use of systemic chemotherapy [2, 3, 7]. Formerly, chemotherapy was considered useless in the treatment of brain tumors, because the blood-brain barrier (BBB) was thought to prevent the penetration of cytostatic drugs into brain tumor tissue. The brain capillaries are the anatomic location of the BBB. The main features that define this barrier and signify the difference from capillaries outside the central nervous system (CNS) are the tight junctions between the endothelial cells, the paucity of transport vesicles in the endothelial cells and the presence of a drug efflux transport protein (MDR1 *P*-glycoprotein) [12]. Furthermore, tissue distribution depends on other physiologic factors, besides drug characteristics (e.g., lipophilicity, pK_a, protein binding), such as local blood flow, intercapillary distance and perivascular diffusion [5], and it has now become clear that the vascular morphology varies greatly within malignant brain tumors [9].

Chemotherapy still has little effect in most brain tumors. Pharmacologic research is essential for a better understanding of this lack of efficacy. Teniposide is one of the most commonly used drugs in both primary and

secondary malignant brain tumors, and its penetration in normal brain tissue and in brain tumor tissue has been examined widely. However, drug distribution studies in man have shown considerable variations in uptake, with drug concentrations in brain tumor tissues ranging from undetectable to similar to or higher than concurrent plasma levels [13, 16]. Presumably, these wide variations reflect differences in capillary permeability and blood flow in brain tumors [5]. Thus far, however, little attention has been paid to the possible role in this of drug in blood present in resected specimens. Importantly, one of the main characteristics of malignant brain tumors is the increased number and size of the tumor blood capillaries. The present study was undertaken to estimate the contribution of the plasma component to the teniposide concentrations measured in the resected brain tumor tissue.

Materials and methods

Drugs and reagents

Teniposide (Vumon) was obtained from Bristol Myers Squibb (Syracuse, N.Y.). All other reagents were purchased from E. Merck (Darmstadt, Germany) and were of analytical grade, except for methanol and acetonitrile, which were of gradient quality. Water was purified by the milli-Q plus system (Millipore, Milford, Ma.).

Patients

Six patients who were scheduled for surgical resection of a (metastatic) brain tumor gave their informed consent to participation in this study. The patient characteristics are shown in Table 1. Teniposide, diluted in 500 ml of saline, was given as a 1-h i.v. infusion at a dose of 150 mg/m^2. The lag time between the end of infusion and surgical resection ranged between 0 and 24 h. In one patient (patient 1) the exact time of tumor resection could not be specified more exactly than between 0 and 1 h after teniposide infusion. All patients had normal renal and hepatic function, and all received dexamethasone routinely before surgery. Resected tumor material that was not required for histopathological examination was used for the teniposide concentration study.

Additional samples of normal brain tissue, of malignant glioma and of metastatic brain tumor tissue (primary bladder cancer) were obtained directly post mortem from patients who had not received teniposide, and these served as 'blank-control' specimens.

Table 1 Characteristics of the patient population[a]

Patient	Age (years)	Gender	Diagnosis
1	55	F	Metastatic breast carcinoma
2	68	M	Glioblastoma
3	54	M	Glioblastoma
4	25	M	Metastatic melanoma
5	47	F	Metastatic breast carcinoma
6	48	F	Malignant oligodendroglioma

[a]No patient had received prior chemotherapy or radiotherapy. All patients received dexamethasone before surgery (as indicated in text), whereas osmotic agents were not used in any patient

Collection and handling of biological specimens

During neurosurgical resection of the brain tumor, samples of tumor tissue for histological examination and drug concentration studies were obtained with the use of forceps. The minimum diameter of the collected pieces of tumor tissue was 5 mm. Before tissue was frozen and stored excessive blood was removed by gently shaking the tissue sample. The dissected brain tumor specimens were stored on liquid nitrogen immediately after sampling. Before analysis, they were powdered under liquid nitrogen and freeze-dried overnight. An amount of the dried powder was accurately weighed and homogenized in 0.1 M phosphate buffer pH 7.0 and 0.154 M NaCl in water (PBS) to approximately 50–150 mg of tissue per ml.

A full pharmacokinetic curve was obtained in patients 1–3 by serial blood sampling over a 24-h period, whereas in patients 4–6 a blood sample was drawn only at the time point of the resection of the tumor. Plasma was separated by centrifugation (10 min, 2000 *g*) from blood collected in evacuated heparinized tubes and was stored at −20 °C. Blank human blood was obtained from healthy volunteers.

Drug extraction from biological samples

Volumes of 100–500 µl of plasma or tissue homogenate samples were pipetted into tubes containing 2000 ng etoposide (internal standard). A volume of 2 ml of 1,2-dichloroethane (DCE) was then added, and the samples were mixed thoroughly for 5 min. After centrifugation (5 min, 1500 *g*) the aqueous top layer was discarded. The organic layer was transferred into a clean tube and evaporated under nitrogen (37 °C). The residue was dissolved in 200 µl of the mobile phase. Aliquots of 20–100 µl of sample were subjected to chromatography. Calibration samples were prepared by spiking blank human plasma samples with teniposide in the range of 50–2000 ng/ml and the internal standard.

Instrumentation and chromatography

The HPLC equipment consisted of a Spectroflow SF400 pump (Kratos, Ramsey, N.J.) and an AMOR electrochemical detector provided with a standard glassy carbon electrode (Spark, Emmen, The Netherlands) and an MSI660 autosampler (Kontron, Basel, Switzerland). Peak integration was done using a SP4270 integrator coupled to a WINner/286 data station (Spectra Physics, San Jose, Calif.).

Samples were chromatographed on a stainless steel column (30 cm × 4 mm ID) packed with µBondapak Phenyl material (Millipore, Milford, Mass.). The mobile phase comprised a mixture of methanol: 67 mM phosphate buffer, pH 6.6 (55:45; v/v), and was delivered at a flow rate of 1 ml/min. Detection was performed at +0.70 V [6]. The lower limit of detection (LOD) in plasma samples (500 µL) was 20 ng/ml. The LOD in the dried tissue specimens (100 mg dissolved in 1 ml of PBS) was approximately 0.2 µg/g

In vitro partitioning of teniposide over human blood cells and plasma

Fresh human blood was incubated with 0.1, 1 or 10 µg/ml teniposide at 37 °C. Aliquots were centrifuged (3 min, 4000 *g*) at serial time points over a 4-h period. The hematocrit (ht) was determined on a Model S-plus Coulter Counter (Coulter, Miami, Fla.). Plasma was stored at −20 °C until further processing within 2 weeks.

Determination of dry weights of blood and of brain tissue

A volume of 1000 µl of blood and a portion of normal post-mortem brain tissue were accurately weighed. The samples were frozen in

liquid nitrogen and the water was removed by freeze-drying overnight. Next, the samples were weighed again.

Determination of albumin levels in plasma and tissue homogenates

Albumin levels in plasma were determined by routine procedures on a Hitachi 704 discrete analyzer using the albumin (Bromocresol green method) test kit (Boehringer, Mannheim, Germany).

Albumin levels in the tissue homogenates in PBS were quantified by radial immune diffusion techniques on 1.5% (v/v) agarose gels containing 1.6% (v/v) of a precipitating anti-human albumin antiserum (Central Laboratory Blood Transfusion Service, Amsterdam, The Netherlands). Calibration standards were prepared by dilution of a 10% human serum albumin calibration standard (KabiVitrum, Stockholm, Sweden) in PBS in the range of 0.1–0.5 mg/ml.

Pharmacokinetic analysis

Pharmacokinetic modelling of the plasma concentration-time curves was performed with the MW/Pharm software package version 3.02 (MEDI\WARE, Groningen, The Netherlands) [10]. A two-compartment infusion model was used.

Results and discussion

In contrast to the pale pigmentation of the post-mortem specimens, the tumor specimens obtained by operation were reddish-brown in color, suggesting the presence of hemoglobin. To estimate the fraction of blood present in the resected brain tumor samples we analyzed the albumin contents in these specimens and in post-mortem samples from deceased patients. Albumin, which can be determined with high specificity by immunological techniques, is essentially a transport protein formed in the liver but not in peripheral tissues. It could not be detected (<1 mg/g tissue) in post-mortem normal brain tissue, and the albumin levels in control post-mortem brain tumor specimens were <1 mg/g (glioblastoma), 1.3 mg/g (oligodendroglioma) and 3.6 mg/g tissue (brain metastatic bladder carcinoma). These data are concordant with the finding that the permeability of the vessels in malignant brain tumors is higher, which may result in some extravasation of albumin into the brain tumor tissue [15]. Brain tumor specimens obtained by surgery from patients 1–6, however, contained at least 5-fold the amounts of albumin (Table 2), which can only be explained by the presence of a major part of blood in the biopsied specimen.

To obtain a good estimate of the fraction of blood in the dissected specimens from these observed albumin levels, relatively complex mathematics were required. The powdered specimens were lyophilized to minimize variations due to differences in water content between the samples. However, the ratios between wet and dry weight of blood and tissue are not equal. Lyophilization of 1000 µl (≈1.080 g) of human blood or 0.890 g of wet post-mortem tissue yielded 0.214 and 0.164 g of dried residue, respectively. In addition, our in vitro partitioning study of teniposide in blood indicated that

Table 2 Determination (stepwise calculations)[a] of the fraction of blood in the resected brain tumor samples (ht hematocrit)

Patient	Albumin in freeze-dried sample (mg/g)	Albumin in plasma sample (mg/ml)	ht	Volume of plasma in freeze-dried sample (ml/g)	Volume of blood in freeze-dried sample (ml/g)	Fraction of blood in freeze-dried sample (g/g)	Fraction of tissue in freeze-dried sample (g/g)	Weight of blood in freeze-dried sample (g/g)	Weight of wet tissue in freeze-dried sample (g/g)	Fraction of blood in resected sample (g/g)	Fraction of wet tissue in resected sample (g/g)
1	21	40	0.42	0.53	0.91	0.19	0.81	0.84	4.38	0.16	0.84
2	26	45	0.45	0.58	1.05	0.22	0.78	0.97	4.21	0.19	0.81
3	56	38	0.44	1.47	2.63	0.56	0.44	2.44	2.37	0.51	0.49
4	44	41	0.44	1.07	1.92	0.41	0.59	1.77	3.21	0.36	0.64
5	30	36	0.37	0.83	1.32	0.28	0.72	1.22	3.90	0.24	0.76
6	28	34	0.36	0.82	1.29	0.28	0.72	1.19	3.94	0.23	0.77

[a]Calculations:
Specific gravity of blood (g/ml): 1.080 (1000 µl of blood weighs 1.080 g)
Dry weight of blood (g/ml): 0.214 (1000 µl of blood yielded 0.214 g of freeze-dried residue)
Dry weight of tissue (g/g): 0.184 (0.890 g of post-mortem tissue yielded 0.164 g of freeze-dried residue)
Volume of plasma in freeze-dried sample (ml/g): Albumin in freeze-dried sample (mg/g)/Albumin in plasma (mg/ml)
Volume of blood in freeze-dried sample (ml/g): Volume of plasma in freeze-dried sample (ml/g)/(1-ht)
Fraction of blood in freeze-dried sample (g/g): Blood in freeze-dried sample (ml/g) × Dry weight of blood (g/ml)
Fraction of tissue in freeze-dried sample (g/g): 1 – fraction of blood (g/g)
Weight of blood in freeze-dried sample (g/g): Blood in freeze-dried sample (ml/g)/Specific gravity of blood (g/ml)
Weight of wet tissue in freeze-dried sample (g/g): Fraction of tissue in freeze-dried sample (g/g)/Dry weight of tissue (g/g)
Fraction of blood in resected sample (g/g): Weight of blood in freeze-dried sample (g/g)/(Weight of blood in freeze-dried sample + Weight of wet tissue in freeze-dried sample)
Fraction of wet tissue in resected sample (g/g): 1–Fraction of blood in sample (g/g)

Table 3 Pharmacokinetic parameter of patient 1 to 3 given 150 mg/m^2 of teniposide by a 1-h intravenous infusion (C_{max} peak plasma level, $t^{1/2}(\alpha)$ distribution half-life, $t^{1/2}(\beta)$ elimination half-life, *AUC* area under the plasma concentration–time curve, *CL* clearance, V_d apparent volume of distribution)

Patient	Dose (mg)	C_{max} (mg/l)	$t^{1/2}(\alpha)$ (h)	$t^{1/2}(\beta)$ (h)	AUC (mg/l*h)	CL (l h^{-1}m^{-2})	V_d (l/m^2)
1	250	59.0	0.94	5.8	155	0.96	8.0
2	285	51.1	0.96	8.2	112	1.34	15.9
3	300	44.4	1.30	6.0	133	1.13	9.8

more than 95% of the drug was recovered in the plasma fraction. Consequently, we also needed to take into account the blood hematocrit value of each patient. The formulas are presented in the legend to Table 2. Based on all these data, a substantial fraction (viz 0.16 to 0.50) of the tumor specimens consisted of blood (Table 2).

In the cases of patients 1–3 sampling of serial blood specimens over a 24-h period allowed compilation of a full pharmacokinetic curve and calculation of pharmacokinetic parameters (Table 3). The derived pharmacokinetic parameters are very similar to those reported in previous studies [4], suggesting that the impact of the operation on the pharmacokinetics of teniposide is limited. Patients 4–6 had blood samples taken only at the time of tumor resection. The pharmacokinetic behavior of teniposide is dominated by the fact that only a very small fraction (i.e., less than 1% [1, 8]) represents non-protein-bound drug. In spite of the highly lipophilic character of this compound, which should allow easy diffusion through cellular membranes, its volume of distribution (V_d) is very low (typically 10 l/m^2), indicating poor tissue penetration and binding. This is in accordance with the results obtained in our in vitro partitioning experiment of teniposide in blood, in which more than 95% was recovered in the plasma fraction.

Owing to the arterial pressure, the well-vascularized resected tumor samples contain a large amount of blood. In addition, surgical resection of a lesion in the brain is inevitably accompanied by bleeding. A consequence of high concurrent teniposide plasma levels during brain tumor resection will be that adhering blood may cause an overestimation of the teniposide levels in the tumor tissue. So far, this effect has not been addressed in brain tumor distribution studies with teniposide. The uncorrected teniposide levels in freeze-dried tissues obtained within 2.5 h after drug administration ranged from 8.2 to 18.7 μg/g, corresponding to approximately 1.6–3.7 μg/g wet tissue weight, respectively. These values are similar to those observed in earlier studies [13, 16], but our other findings also indicate that most of the drug measured in the specimens taken at this early time point after drug administration, originates from plasma in the samples (Table 4). In patient 3 the calculated plasma contribution was even higher than the actual tissue level. This might indicate that during the drug distribution phase, the drug level in the arm vein may not be an accurate reflection of the concentration in the arterioles of the vascular bed in the brain. The very marked difference in regional tissue perfusion that has been observed in experimental brain tumors may contribute to this finding [14]. Another complicating factor, which limits the accuracy of the calculation of the plasma contribution of teniposide is the rapid decline of the drug plasma level during the distribution phase

Table 4 Real concentrations of teniposide in brain tumor tissue

Patient	Post-infusion time (h)	Teniposide concentration		Ratio teniposide:albumin in plasma[a] (μg/mg)	Plasma teniposide in freeze-dried sample[a] (μg/g)	Real teniposide level in tumor tissue[a] (μg/g)
		In freeze-dried sample (μg/g)	In concurrent plasma sample (μg/ml)			
1	0–1[b]	18.7	59∼25	1.48∼0.625	31.0∼13.1	−2.67∼1.21
2	2	8.2	12.4	.276	7.2	0.23
3	2.5	9.5	12.0	.316	17.7	−3.05
4	24	0.71	0.18	0.0044	0.19	0.15
5	24	1.31	0.22	0.0061	0.18	0.27
6	24	0.97	0.17	0.0050	0.14	0.20

[a]Calculations:
Ratio of teniposide to albumin in plasma (μg/mg): teniposide level in plasma (μg/ml)[c] / albumin level in plasma (mg/ml)[c]
Plasma teniposide in freeze-dried sample (μg/g): Albumin in freeze-dried sample (mg/g)[c] × ratio teniposide:albumin in plasma (μg/mg)
Real teniposide level in tumor tissue: (Teniposide concentration in freeze-dried sample − Plasma teniposide level in freeze-dried sample) / Fraction of tissue in sample × 0.184[c]
[b]The post-infusion time interval in patient 1 was not exactly recorded, but ranges between 0 and 1 h
[c]See Table 2

($t^{1/2}(\alpha) \approx 1$ h). This is clearly illustrated by the results in patient 1, whose lesion was removed within 1 h after the end of infusion. However, because the time was not exactly documented, the plasma contribution was estimated by using the teniposide concentration determined in the two nearest plasma samples (i.e., collected immediately after and 1 h after the end of infusion). Owing to the more than two fold decrease of the teniposide concentration the calculated contribution varied between 13.1 and 31.0 μg/g, being 70% and 160% of the actual tissue level.

At 24 h after drug administration the results were less scattered. After the plasma level had dropped to about 0.20 μg/ml a further decline occurred more slowly [$t^{1/2}(\beta) \approx 6$ h], and at 24 h more time had been available to reach an equilibrium between the plasma and the tissue compartments. In these three patients the real concentration in the tumor tissue ranged from 0.14 to 0.28 μg/g. Although these concentrations may appear low at first sight, such levels may be sufficient for cytotoxic activity; it has been shown by in vitro cell culture assays that a 24-h exposure to a concentration of 0.05 μg/ml of teniposide can produce a substantial cell kill [11].

In conclusion, teniposide does penetrate into brain tumor tissue, although the real drug level achieved in this tissue is much lower than reported previously. Future studies of brain tumor concentrations of teniposide, but also of other (cytostatic) drugs should include a procedure to measure the contribution of drug originating from the central (plasma) compartment, unless the concurrent plasma levels are low compared with the concentration in tissue.

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References

1. Allen LM, Creaven PJ (1975) Comparison of the human pharmacokinetics of VM-26 and VP-16, two antineoplastic epipodophyllotixin glucopyranoside derivatives. Eur J Cancer 11: 697–707
2. Cairncross G, MacDonald D, Ludwin S, Lee D, Cascino T, Buckner J, Fulton D, Dropcho E, Stewart D, Schold C (1994) Chemotherapy for anaplastic oligodendroglioma. J Clin Oncol 12: 2013–2021
3. DeAngelis LM, Yahaloma J, Thaler HT, Kher U (1992) Combined modality therapy for primary CNS lymphoma. J Clin Oncol 10: 635–643
4. D'Incalci M, Rossi C, Sessa C, Urso R, Zucchetti M, Farina P, Mangioni C (1985) Pharmacokinetics of teniposide in patients with ovarian cancer. Cancer Treat Rep 69: 73–77
5. Greig NH (1989) Brain tumors and the blood-tumor barrier. In: Neuwelt EA (ed) Implications of the blood-brain barrier and its manipulation, vol 2. Plenum, New York, pp 77–106
6. Holthuis JJM, Romkens FMGM, Pinedo HM, Van Oort WJ (1983) Plasma assay for the antineoplastic agent VP16-213 (etoposide) using high-performance liquid chromatography with electrochemical detection. J Pharm Biomed Anal 1: 89–97
7. Kristensen CA, Kristjansen PE, Hansen HH (1992) Systemic chemotherapy of brain metastases from small-cell lung cancer: a review. J Clin Oncol 10: 1498–1502
8. Petros WP, Rodman JH, Relling MV, Christensen M, Pui CH, Rivera GK, Evans WE (1992) Variability in teniposide plasma protein binding is correlated with serum albumin concentrations. Pharmacotherapy 12: 273–277
9. Plate KH, Mennel HD (1995) Vascular morphology and angiogenesis in glial tumors. Exp Toxicol Pathol 47: 89–94
10. Proost JH, Meijer DKF (1992) MW/PHARM, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. Comput Biol Med 22: 155–160
11. Roed H, Vindelov LL, Christensen IJ, Spang-Thomsen M, Hansen HH (1987) The effect of the two epipodophyllotoxin derivatives etoposide (VP-16) and teniposide (VM-26) on cell lines established from patients with small cell carcinoma of the lung. Cancer Chemother Pharmacol 19: 16–20
12. Schinkel AH, Smit JJ, Van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HPJ, Berns AJM, Borst P (1994) Disruption of the mouse mdrla P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 77: 491–502
13. Stewart DJ, Richard MT, Hugenholtz H, Dennery J, Nundy D, Prior J, Montpetit V, Hopkins HS (1984) Penetration of teniposide (VM-26) into human intracerebral tumors, preliminary observations on the effect of tumor type, rate of drug infusion and prior treatment with amphotericin b or oral glycerol. J Neurooncol 2: 315–324
14. Vaupel P (1994) Blood flow and metabolic microenvironment of brain tumors. J Neurooncol 22: 261–267
15. Yuan F, Salehi HA, Boucher Y, Vasthare US, Tuma RF, Jain RK (1994) Vascular permeability and microcirculation of gliomas and mammary carcinomas transplanted in rat and mouse cranial windows. Cancer Res 54: 4564–4568
16. Zucchetti M, Rossi C, Knerich R, Donelli MG, Butti G, Silvani V, Gaetani P, D'Incalci M (1991) Concentrations of VP16 and VM26 in human brain tumors. Ann Oncol 2: 63–66