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ORIGINAL ARTICLE

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Pharmacokinetics of temozolomide in association with fotemustine in malignant melanoma and malignant glioma patients: comparison of oral, intravenous, and hepatic intra-arterial administration

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Abstract *Purpose*: Depletion of the DNA repair enzyme O^6-alkylguanine-DNA alkyltransferase (AT) has been shown to increase tumor sensitivity to chloroethylnitrosoureas. Temozolomide (TMZ), an analogue of dacarbazine, can deplete AT, suggesting that it may be used to sensitize tumors to chloroethylnitrosoureas. However, the influence of nitrosoureas on the pharmacokinetics of TMZ is unknown, and a pilot study was performed to assess the pharmacokinetics of TMZ given via, various routes to 29 patients (27 malignant melanomas, 2 gliomas) with or without sequential administration of i.v. fotemustine. *Methods*: On day 1, TMZ was given intravenously (i.v.), orally (p.o.), or by intrahepatic arterial infusion (h.i.a.) at four ascending dose levels (150 to 350 mg/m^2 per day). On day 2 the same dose of TMZ was given by the same route (or by another route in six patients for determination of its bioavailability), followed 4 h later by fotemustine infusion at 100 mg/m^2. Plasma and urinary levels of TMZ were determined on days 1 and 2 by high-performance liquid chromatography after solid-phase extraction. *Results*: The pharmacokinetics of i.v. TMZ appeared linear, with the area under the curve (AUC) increasing in proportion to the dose expressed in milligrams per square meter ($r = 0.86$ and 0.91 for days 1 and 2, respectively). The clearance after i.v. administration was 220 ± 48 and 241 ± 39 ml/min on days 1 and 2, respectively. The apparent clearance after p.o. and h.i.a. administration was 290 ± 86 and 344 ± 77 ml/min, respectively. The volume of distribution of TMZ after i.v., p.o., and h.i.a. administration was 0.4, 0.6, and 0.6 l/kg on day 1 and 0.5, 0.5, and 0.6 l/kg on day 2, respectively. The absolute bioavailability of TMZ was 0.96 ± 0.1, regardless of the sequence of the i.v.-p.o. or p.o.-i.v. administration, confirming that TMZ is not subject to a marked first-pass effect. A comparison of TMZ pharmacokinetics after i.v. and h.i.a. treatment at the same infusion rate revealed little evidence of hepatic extraction of TMZ. However, the systemic exposure to TMZ (AUC) appeared to decrease at a lower infusion rate. TMZ excreted unchanged in the urine accounted for 5.9 ± 3.4% of the dose, with low within-patient and high interpatient variability. TMZ crosses the blood-brain barrier and the concentration detected in CSF amounted to 9%, 28%, and 29% of the corresponding plasma levels (three patients). The equilibrium between plasma and ascitic fluid was reached after 2 h (assessed in one patient). *Conclusion*: The sequential administration of fotemustine at 4 h after TMZ treatment had no clinically relevant influence on the pharmacokinetics of TMZ. The potential clinical effect of TMZ given by h.i.a. or by locoregional administration has yet to be established, as has the impact of the infusion duration on patients' tolerance and response rate.

Keywords Temozolomide · Pharmacokinetics · Melanoma · Glioma · Intrahepatic artery

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Introduction

Malignant glioma and metastatic malignant melanoma are generally resistant to available treatment modalities [1, 2]. Following standard treatment (surgery and

radiotherapy) the median survival of patients with glioblastoma does not exceed 9 months [1]. The efficiency of chemotherapy in recurrent gliomas is limited. Nitrosoureas, procarbazine, vincristine, and dacarbazine are cytotoxic agents known to possess some degree of activity against malignant gliomas. The response rate achieved by these agents is poor, however, because of limited blood-brain barrier penetration and tumor resistance.

Malignant ocular melanoma is characterized by a high incidence of hepatic metastases during the first years after treatment of the primary tumor. In 40–90% of cases the liver is the only metastatic site. The median survival of patients with hepatic metastases of ocular melanoma is less than 3 months. Hepatic intra-arterial treatment with the nitrosourea fotemustine has achieved a 40% response rate and a median survival of 14 months in such patients [3].

Temozolomide (TMZ) is an imidazotetrazinone derivative with methylating properties, which has shown activity in patients with primary brain tumors or malignant melanomas during preliminary trials. TMZ undergoes chemical degradation at physiologic pH to form MTIC, the active metabolite of dacarbazine (DTIC). However, TMZ and DTIC differ in the mechanism by which they give rise to MTIC [4]. Whereas DTIC is metabolized to MTIC in the liver via oxidative N-demethylation, TMZ undergoes spontaneous conversion to MTIC at physiologic pH. Since enzymatic activity and liver metastases may markedly influence the hepatic metabolism, TMZ may provide more predictable levels of the active species MTIC than does dacarbazine. TMZ seems to exert its cytotoxic action mainly through the methylation of the O^6 position of guanine on DNA strands. However, this O^6-methylguanine lesion can be repaired by cellular O^6-alkylguanine-DNA alkyltransferase (AT). Since AT is a ''suicide enzyme,'' the restoration of AT activity in cells requires new enzyme synthesis. The resistance of tumors to chloroethylnitrosoureas has been related to the AT level in tumor cells [5–8]. It has therefore been proposed that depletion of cellular AT by pretreatment with TMZ may extend the antitumor activity of alkylating agents such as chloroethylnitrosourea derivatives [9].

Fotemustine is a chloroethylnitrosourea derivative that induces DNA interstrand cross-links and has demonstrated some clinical activity against hematologic malignancies and solid tumors, notably malignant melanoma and primary brain tumor [10–12]. As part of protocols based on the AT-depletion rationale, fotemustine has been used in combination with DTIC for the treatment of disseminated malignant melanoma [13]. After the combination chemotherapy with sequential administration of DTIC and fotemustine the overall response rate has varied from 27% to 33% and the complete response rate, from 24% to 50% in lung metastases [14, 15].

Though pharmacokinetics studies of TMZ used as a single agent have previously been reported [16–20, 22], little is known about its pharmacokinetics during coadministration of other drugs such as nitrosoureas. This may be of importance since the use of TMZ has been advocated in combination with such alkylating agents. Furthermore, as the liver is the main metastatatic site of ocular melanoma, the administration of TMZ and fotemustine through the hepatic artery may minimize systemic exposure to these alkylating agents. No report has thus far been published on the pharmacokinetics of TMZ after hepatic arterial administration. Finally, since temozolomide may be used as an antiglioma agent [17, 18], the determination of its concentration in cerebrospinal fluid (CSF) would enable assessment of the extent of the tumor-site exposure.

The aims of the present study were therefore (a) to determine the pharmacokinetics of TMZ given with or without subsequent administration of fotemustine at 4 h after TMZ treatment, (b) to compare TMZ pharmacokinetics according to the route of administration (p.o., i.v., h.i.a.), and (c) to measure TMZ in CSF to assess the degree of its blood-brain barrier penetration.

Patients and methods

Patients with histologically proven metastatic melanoma or recurrent high-grade glioma were eligible for the study. The inclusion criteria were an age over 18 years; a performance status of less than 3 according to the Eastern Cooperative Oncology Group (ECOG); and normal hematologic (white cell count $> 4{,}000/mm^3$, platelets $> 100{,}000/mm^3$), hepatic (bilirubin, alkaline phosphatase, and ASAT levels < 1.5 times the normal values), renal (serum creatinine < 120 μg/l or creatinine clearance > 60 ml/min), and pulmonary function tests. The patients had to give their written informed consent. Those who had received chemotherapy within 8 weeks or who had a history of lung irradiation were not included. The study protocol was approved by the ethics committee of the university hospital.

A total of 29 patients (M/F 17/12) were included in the study. The median age was 50 years (range 31–71years). The patients were suffering from either metastatic malignant melanoma (19 patients), ocular melanoma (8 patients), or recurrent malignant glioma (2 patients). Each patient had metastases localized mainly in the lung (50%), liver (40%), brain (25%), skin (25%) or lymphatic nodes (20%). In all, 7 patients had previously received chemotherapy, 3 patients had had radiotherapy, 3 patients had undergone immunotherapy and 2 patients undergone surgery, whereas 14 patients had received no prior treatment.

Before the beginning of the therapy a full history and physical examination; performance index; complete blood count; biochemistry profile (blood chemistry ASAT, ALAT, alkaline phosphatase, gamma-GT, bilirubin, LDH, creatinine, creatinine clearance); urinanalysis; pulmonary function test, including carbon monoxide diffusion; chest X-ray, and tumor evaluation (X-ray, ultrasonic scanning, computerized tomography, or magnetic resonance imaging scans) were performed. Patients were monitored (blood count, biochemistry profile, and clinical examination) on the 1st day of treatment and every week thereafter for at least 4 weeks.

Chemotherapy

TMZ was supplied by the Cancer Research Campaign (CRC, London, UK). It was available as 5-ml vials containing 150 mg TMZ in dimethylsulfoxide (DMSO) or as 5 g of bulk TMZ powder. Infusions were prepared by dilution of the vials with 500 ml

normal saline. Bulk powder of TMZ was formulated in 20- or 100-mg gelatin capsules at the Pharmacy of the Centre Hospitalier Universitaire Vaudois. The dose of TMZ in selected oral and i.v. formulations was analyzed by high-performance liquid chromatography (HPLC) using an adaptation of the method previously described [23], and its concentration was within ±5% of the nominal value. Fotemustine was supplied by I.R.I. Servier (Courbevoie, France) in 4-ml vials containing 200 mg freeze-dried product to be reconstituted with ethanol solvent. The solution was diluted with 250 ml isotonic glucose solution prior to infusion.

On day 1, TMZ was given intravenously (i.v.), orally (p.o.), or by intrahepatic arterial infusion (h.i.a.) at four ascending dose levels (150, 200, 250, and 350 mg/m^2 per day). On day 2 the same dose of TMZ was given, followed 4 h later by a 1-h infusion of fotemustine at 100 mg/m^2. The same route was used for TMZ on day 1 and day 2 except in six patients, who received TMZ p.o. on day 1 and i.v. on day 2 or on the opposite regimen for determination of its bioavailability. Ondansetron (8 mg i.v.) was given prophylactically as an antiemetic to all patients at 15 min before TMZ administration. TMZ capsules were given p.o. with mineral water at 20 min after a light meal (toasted bread with jam, butter, and tea). TMZ was given i.v. or h.i.a. as a 1-h constant-rate infusion unless stated otherwise.

Two patients with liver metastasis (patients 19 and 27) received a second cycle of treatment with TMZ h.i.a. over 1 h (i.e., at the same flow rate as the first TMZ i.v. administration) for assessment of the hepatic extraction of TMZ. Otherwise, all patients received subsequent treatment cycles with TMZ being replaced by 500 mg/m^2 DTIC i.v. The treatment was repeated at 4-week intervals until disease progression.

Blood-sampling procedure

Blood samples (2 ml) were drawn into heparinized tubes precooled to 4 °C at 0, 30, 60, 90, 120, 180, 240, 360, 720, and 1,440 min after the beginning of the infusion or at 0, 20, 40, 60, 90, 120, 180, 240, 480, and 1,440 min after oral administration. The blood samples were immediately centrifuged at 900 *g* for 10 min at 4 °C. A 1-ml aliquot of plasma was transferred into Eppendorf tubes containing 0.1 ml 1 *M* HCl, vortexed, and stored at −20 °C. Urine was collected by spontaneous voiding up to 48 h (days 1 and 2 of chemotherapy) and was processed immediately after voiding; an aliquot (10 ml) was acidified with 1 *M* HCl (1 ml) and stored at −20 °C. Processing of the CSF and ascites samples, when available, was performed as described above for plasma; known volumes of CSF and ascites were mixed with 1 *M* HCl (0.1 ml/ml biological fluid) and stored at −20 °C.

Drug assay

Quantitation of TMZ in biological fluids was performed by reversed-phase HPLC analysis as previously described [23]. In brief, the clean-up procedure involved a solid-phase extraction of biological sample (100 µl) on a 100-mg C_{18} end-capped cartridge (Chromabond, Macherey-Nagel, Düren, Germany). Matrix components were eliminated with 750 µl 0.5% acetic acid (AcOH). TMZ was subsequently eluted with 1,250 µl methanol (MeOH). The resulting eluate was evaporated under nitrogen at room temperature, reconstituted in 200 µl 0.5% AcOH, and analyzed by HPLC.

The chromatography system consisted of an HP 1050 isocratic/quaternary pump (Hewlett Packard, Germany) connected to an HP 1050 autosampler and an HP 1050 multiwavelength UV detector set at 330 nm. Separations were performed with an HP ODS-Hypersil 5-µm column (100 mm × 4.6 mm inside diameter) equipped with an HP ODS-Hypersil 5-µm (20 × 4.0 mm) guard column. The HPLC mobile phase was MeOH/0.5% AcOH (10:90, v/v). The flow rate was 1 ml/min. The retention times for TMZ and ethazolastone (internal standard) were 3.2 and 7.4 min, respectively. HPChemStation A-OO-33 software loaded onto an HP Vectra 486/33 N was used for the data processing of the chromatograms.

The preparation of calibration samples in human CSF is impeded by the restricted availability of a large volume of drug-free CSF. Nevertheless, the solid-phase extraction (SPE) step applied as preliminary cleanup tends to circumvent the influence of matrix composition [23]. Therefore, TMZ concentrations in CSF (and in ascitic fluid) were calculated with the calibration curve established for plasma in the same HPLC run. In this method the limit of quantitation of TMZ in plasma and urine was 0.2 and 2.0 µg/ml, respectively. The intra-assay precision of quality-control samples at 1, 10, and 18 µg/ml, in plasma and at 4, 70, and 130 µg/ml, in urine was 1.6–6% and 1.8–4.3%, respectively.

Pharmacokinetic analysis

Pharmacokinetic analysis was carried out for each patient using the computer program SIPHAR (4.0, SIMED, Créteil, France). Pharmacokinetic parameters were obtained by a non compartmental approach. The elimination rate constant was determined as the slope of the monoexponential curve fit of logarithmic plasma concentrations versus time. The area under the plasma concentration-time curve (AUC) was obtained by the linear trapezoidal method, and the residual area from the last data point to infinity was estimated as the plasma concentration at this time divided by the elimination rate constant. The clearance of TMZ was calculated as the dose divided by the AUC, and the volume of distribution was determined as the clearance divided by the elimination rate constant. The half-life was calculated as the natural logarithm of 2 divided by the terminal elimination rate constant. For the oral route a first-order absorption process with a time lag was assumed; however, due to the few data available for characterization of the absorption phase, the corresponding parameters are not reported herein.

Statistical analysis

Pharmacokinetic parameters were compared using the Kruskal-Wallis test. When paired data were compared the Wilcoxon signed-rank test was used instead. Correlations were calculated according to Pearson except when the data appeared clearly non-normal, in which case Spearman's approach was applied.

Safety and tolerance

The toxicity and the clinical response have been reported in detail elsewhere [Gander et al., submitted for publication]. In brief, follow-up consisted of a weekly blood cell count. Liver- and lung-function tests were repeated before the second cycle of treatment. Toxicity was graded according to WHO criteria [24]. The antitumor response was evaluated before the second cycle of treatment and then every two cycles according to WHO response criteria.

Results

The hematologic toxicities and mean pharmacokinetic parameters of TMZ according to the route and sequence (i.e., without or with fotemustine) of administration are presented in Tables 1 and 2, respectively. After oral administration, TMZ was absorbed with a mean lag time of 26 ± 8 and 18 ± 7 min for days 1 and 2, respectively, and the mean C_{max} value was achieved at 94 ± 58 min. The clearance, volume of distribution, and half-life were similar, irrespective of the day, for the same route of administration. However, mean apparent

Table 1 Characteristics, pharmacokinetics, and hematologic toxicities of the 29 patients (*MM* Malignant melanoma, *OM* Ocular melanoma, *MG* Malignant glioma, *PO* Oral administration, *IV* Intravenous infusion, *HIA* Hepatic intra-arterial infusion, *NE* Not evaluable)

TMZ dose (mg/m^2/day)	Patient number	Cancer site	Sex	Weight (kg)	Height (cm)	Dose/day (mg/day)	Route day 1	Route day 2	Infusion duration (min)		AUC (mg min l^{-1})		$t_{1/2}$ (min)		Toxicity grade (WHO)	
									Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	WBC	Platelets
150	17	OM	F	76	167	280	PO	PO			1061	1002	97	126	0	1
150	9	MM	M	74	191	300	PO	PO			1435	1453	70	116	0	0
150	16	OM	M	85	173	300	PO	PO			1124	868	100	92	0	0
150	21	MM	M	58	170	250	IV	IV	83	79	1036	1093	89	82	0	0
150	22	MM	M	88.7	178	300	IV	IV	74	79	966	1010	96	101	0	0
150	1	MM	M	107	174	300	IV	IV	59	58	1491	1255	94	87	0	0
150	3	MM	M	71	189	300	IV	IV	60	60	1256	1254	94	88	0	0
150	2	OM	F	65	163	250	HIA	HIA	60	58	711	818	89	86	0	0
200	29	MM	F	61	159	320	IV	PO	98		1774	1625	76	62	4	4
200	26	MM	F	68	168	360	IV	PO	60		1533	1711	112	99	4	4
200	24	MM	M	70	178	370	IV	PO	72		1639	1443	87	85	0	2
200	23	MM	F	76.8	160	360	IV	PO	91		1872	1927	99	104	0	0
200	25	MM	M	63	167	340	PO	IV		65	1219	1393	106	104	0	1
200	28	MM	M	80	178	400	PO	IV		59	1239	1359	98	84	0	0
250	10	MM	F	78	160	460	PO	PO			1033	907	139	122	4	4
250	18	MM	M	70	172	460	PO	PO			1249	1203	96	91	4	3
250	11	MG	M	65	162	420	PO	PO			1484	1809	131	121	0	1
250	14	MM	M	70	164	440	PO	PO			1482	1482	89	73	0	1
250	12	MG	M	80	170	460	PO	PO			1178	1332	92	81	0	NE
250	15	MM	F	47	160	360	PO	PO			1875	1952	69	81	0	0
250	5	MM	F	62.5	153	400	IV	IV	61	63	2556	2299	86	99	2	3
250	8	MM	F	55.8	165	400	IV	IV	88	68	2600	1880	75	125	2	1
250	6	OM	F	54	162	380	IV	IV	60	57	1846	1667	95	84	1	2
250	4	MM	F	38	157	340	IV	IV	56	60	1950	1706	85	69	0	0
250	27	OM	F	77	164	450	HIA	HIA	61	41	1764	1961	82	87	1	4
200				72.5		360	IV	IV	59	58	1576[a]	1541[b]	69	75	0	2
250	19	OM	M	90	180	500	HIA	HIA	67	65	1676	1618	94	92	0	0
250				86.5		500	IV		68		1602[a]		95		0	0
250	13	OM	M	75	170	450	HIA	HIA	82	87	1147	1100	86	83	0	0
250	20	OM	M	60	170	450	HIA	HIA	86	80	1054	972	78	79	NE	NE
350	7	MM	M	91	175	700	IV	IV	60	60	2819	2367	123	104	2	3

[a] Day 28
[b] Day 29

Table 2 Pharmacokinetic parameters of TMZ without (day 1) or with administration of fotemustine at 4 h after TMZ treatment (day 2). Data represent mean values ± SD

Route and sequence of administration	CL (ml/min)	$t_{1/2}$ (min)	V_d (l/kg)
IV day 1 (n = 15)	220 ± 48	92 ± 14	0.4 ± 0.1
IV day 2 (n = 12)	241 ± 39	92 ± 16	0.5 ± 0.1
HIA day 1 (n = 5)	345 ± 69[a]	86 ± 6	0.6 ± 0.2[b]
HIA day 2 (n = 5)	343 ± 93[a]	85 ± 5	0.6 ± 0.2[b]
PO day 1 (n = 11)	302 ± 76[a]	99 ± 21	0.6 ± 0.2[b]
PO day 2 (n = 13)	280 ± 95[a]	96 ± 20	0.5 ± 0.2[b]

[a] Apparent clearance (CL/F)
[b] Apparent V_d (V_d/F)

clearances and volumes of distribution measured in all patients after h.i.a. and p.o. administration were significantly higher (P = 0.0003 and 0.007, respectively) than those recorded for the i.v. route. The half-lives were similar among the three routes of administration (P = 0.13). Representative examples of concentration-time curves generated for the three routes tested are shown in Fig. 1.

The pharmacokinetics of TMZ appeared linear, with the AUC increasing proportionally to the dose (expressed in milligrams per square meter) after i.v. administration (r = 0.86 and 0.91 for day 1 and day 2, respectively). AUC and C_{max} values were more variable following p.o. or h.i.a. administration. The bioavailability of TMZ was assessed in six patients (patients 23–26, 28, and 29; see Table 1), of whom four received TMZ i.v. on day 1 and p.o. on day 2, whereas two were treated on the inverse sequence. The average bioavailability (F) was 0.96 ± 0.1 without noticeable influence of the administration sequence. The difference in clearance (dose/AUC) observed between day 1 and day 2 (Table 2) was accordingly not statistically significant (Kruskal-Wallis one-way nonparametric statistics). A comparison of the subgroup of patients who received TMZ by the same route on the 2 days of chemotherapy – without (day 1) or with (day 2) fotemustine – showed no difference for the p.o. route, whereas for the i.v. route the mean TMZ AUC was statistically significantly higher on day 1 (TMZ only) than on day 2 (TMZ i.v. + fotemustine i.v.; 1,836 versus 1,615 mg min l^{-1}, P = 0.02).

The parent TMZ excreted unchanged in urine accounted for 6 ± 3% (day 1) and 6 ± 4% (day 2) of the dose, with low within-patient (day 1 versus day 2) and high interpatient variability, extreme values ranging from 0.4% to 15% of the dose excreted as the parent compound. In three patients, CSF samples were obtained; the concentration measured in CSF corresponded to 9%, 28%, and 29% of the plasma levels taken, respectively, at 90 min after a p.o. dose of 250 mg/m^2 TMZ in the first two patients and at 4 h after an i.v. dose of 200 mg/m^2 in the third. In one patient the ascites fluid was drained throughout the 2 days of chemotherapy. Ascites samples were taken before and at 60 and 120 min after the beginning of TMZ administration. The TMZ concentrations determined in ascitic fluid were

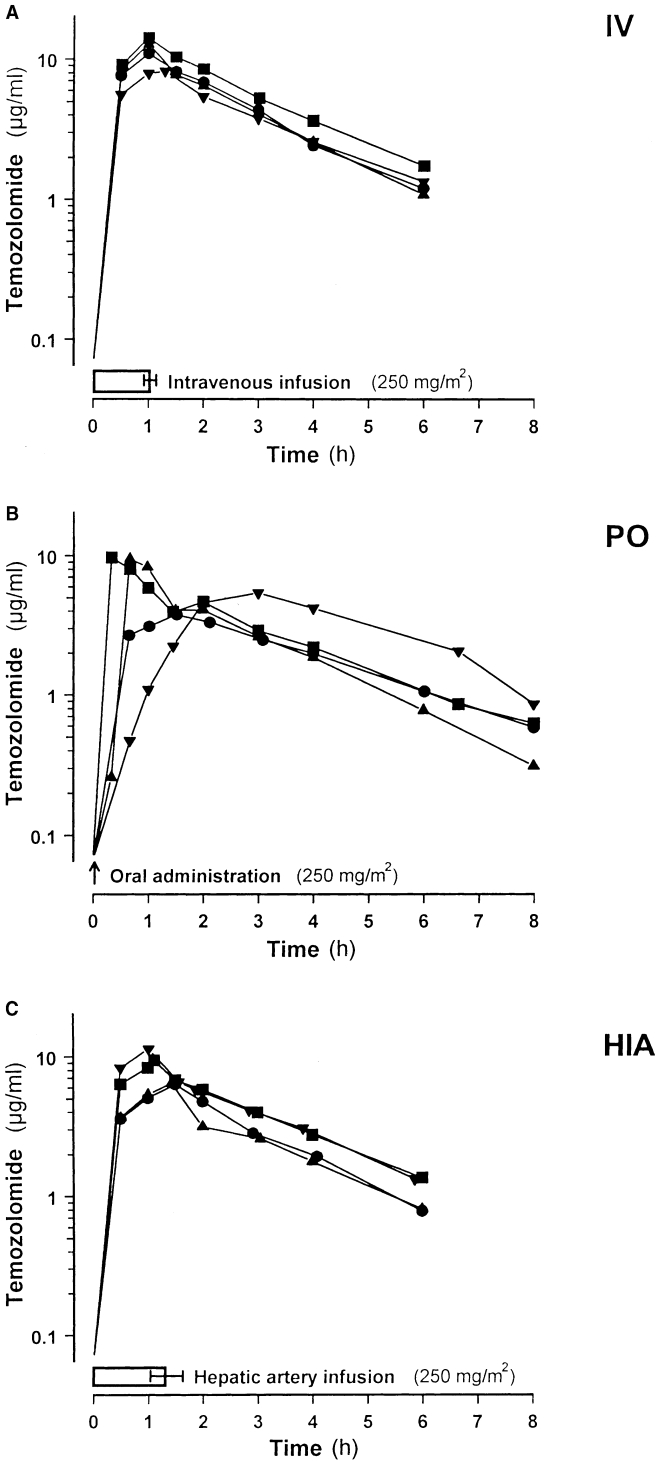

Fig. 1 Plasma concentration profiles of patients receiving temozolomide at a dose of 250 mg/m^2 via different routes on day 1: intravenous infusion (*top:* patients ● 4, ■ 5, ▲ 6, ▼ 19 second cycle), oral administration (*middle:* patients ● 10, ■ 11, ▲ 12, ▼ 14), and infusion through the hepatic artery (*bottom*: patients ● 13, ■ 19 first cycle, ▲ 20, ▼ 27). The *rectangles* represent the average duration of infusion and the *attached bars*, the range of individual durations

28% and 95%, respectively, of the corresponding plasma concentrations. Five patients with liver metastasis received TMZ h.i.a. either exclusively by this route (patients 2, 13, and 20) or in combination with the i.v. route (patients 19 and 27). A comparison of the TMZ systemic AUC according to the dose, the site (i.v. or h.i.a.), and the duration of infusion is shown in Table 1.

In two patients (patients 19 and 27) who had received TMZ both i.v. and h.i.a. at the same infusion rate the measured clearance values were similar (312 versus 304 and 231 versus 255 ml/min, respectively) and there was no evidence of marked hepatic extraction of TMZ. However, when all five patients who had received TMZ h.i.a. were considered, the systemic exposure to TMZ (i.e., plasma AUC) was significantly lower ($P < 0.01$, Kruskal-Wallis) when h.i.a. TMZ was infused at a slower rate (over 1.5 h, patients 13 and 20) and a relatively good correlation ($r = 0.88$) was observed between the systemic clearance (dose/AUC) and the h.i.a. infusion duration. In contrast, the infusion-duration dependency of the systemic clearance was not observed for i.v. administration, precluding any spurious findings due to the possible transformation of TMZ in the infusion bags over time.

The toxicity to white cells and platelets observed after the sequential administration of escalated dose of TMZ, i.v., p.o. or h.i.a. on days 1 and 2 followed by i.v. infusion of 100 mg/m^2 fotemustine on day 2 are shown in Table 1. The dose-limiting toxicity of the chemotherapy protocol was myelosuppression. At the first dose level (150 mg/m^2 per day), no patient presented WBC toxicity, but myelosuppression was more frequently seen at higher dose levels. The toxicity to platelets was similarly correlated with the dose. The nadir of leukocytes and thrombocytes appeared at 24 and 23 days, respectively, after administration of the combined chemotherapy. The association of TMZ and fotemustine produces hematologic toxicities at lower dose levels, with thrombocyte and leukocyte nadirs occurring earlier, as compared with TMZ monotherapy [17].

There was some correlation (Spearman rank test) between the TMZ AUC determined after i.v. administration with fotemustine and the degree of thrombocytopenia, neutropenia and leukopenia [percentage of decrease in counts at the nadir: $r = 0.75$ ($n = 12$, $P < 0.01$), $r = 0.66$ ($n = 10$, $P = 0.04$) and 0.70 ($n = 12$, $P = 0.012$), respectively]. After oral administration, however, the AUC of TMZ was not a predictive index of WBC toxicity.

Discussion

Preclinical and clinical studies have suggested that the benefit of TMZ may best be appreciated when the latter is used in combination with other cytotoxic drugs [22] and that different schedules of TMZ administration should be explored [20] . Sequential association of TMZ with nitrosoureas has shown promising antitumor activity in mice [25], although other authors [26] cautiously suggest that treatment with methylating agents such as the analogue DTIC may be too toxic as assessed by their in vitro studies. Nevertheless, further clinical studies of TMZ in association with other agents have been recommended and a thorough pharmacokinetic evaluation is therefore required for optimization of the dosage of agents with such a narrow therapeutic range and interaction potential.

To our knowledge, this is the first report of the pharmacokinetics of TMZ given sequentially with a nitrosourea. When we consider the mean pharmacokinetics measured in all patients (Table 2), fotemustine given at 4 h after TMZ treatment does not seem to have much of an effect on TMZ pharmacokinetics. However, exploratory statistical analysis of the subgroup of patients who had received TMZ exclusively by the i.v. route, showed a slight decrease in AUCs on day 2. Therefore, a small influence of either subsequent administration of fotemusine by the same route or some as yet not understood sequence effects observable only on i.v. administration cannot be totally excluded. However, the selection of subgroups for a posteriori statistical analysis is at high risk of revealing a fortuitous difference, which in our case is unlikely to have clinical significance (less than 15%).

At these doses, TMZ showed linear pharmacokinetics after i.v. infusion. The AUC observed after p.o. or h.i.a. administration was more variable and did not satisfactorily correlate with the dose. The pharmacokinetic analysis shows a mean temozolomide $t_{1/2}$ of 1.5 ± 0.1 h, in accordance with the values reported by Dhodapkar et al. [19], Newlands et al. [17], and Statkevitch et al. [27] of 1.7, 1.8 ± 0.4, and 1.8 ± 0.3 h, respectively. The slight differences in $t_{1/2}$ observed in our study among the three routes of administration (Table 2) were not statistically significant. The mean clearance values recorded after i.v. administration (220 ± 48 and 241 ± 39 ml/min for days 1 and 2, respectively) were in good agreement with previously reported values (196 ± 69 ml/min) [17] but were lower than those obtained by the p.o. or h.i.a. route (Table 2), suggesting incomplete bioavailability when the full set of data from all patients are considered. However, the spread of AUC values observed after p.o. administration indicates marked intersubject variability, possibly enhanced by the conditions of capsule administration (position of the patients), nausea (an adverse event that occurred in 7/29 patients, even with the use of i.v. ondransetron), and perturbed gastric transit (vomiting in 1/29 patients), which make standardization difficult in this patient population. However, on drug administration to the same patients ($n = 6$) under well-controlled conditions, AUC and clearance values obtained in the same patients were in good agreement after p.o. and i.v. administration, with an average bioavailability of close to 1, in accordance with previously reported values [17], regardless of the sequence of the i.v.-p.o. or p.o.-i.v. administration.

Plasma AUC and systemic clearance (dose/AUC) seem to be correlated with the infusion duration on h.i.a. administration. Patients 13 and 20, who received TMZ h.i.a. on days 1 and 2 over a prolonged period (80–87 min versus ~60 min in most patients), clearly have a reduced plasma AUC and an increased systemic clearance. This observation suggests that lowering of the h.i.a. infusion rate of TMZ may increase the drug's diffusion into hepatic tissues and its presystemic extraction and could reduce the systemic exposure. A similar observation has been reported for other anticancer drugs after h.i.a. administration. For example, the AUC of 5-fluorouracil (5-FU) given h.i.a. depends on the h.i.a. infusion duration and tends to be lower than the value obtained after i.v. administration when the h.i.a. infusion rate is reduced [28]. Similarly, administration of fotemustine over 4 h by h.i.a. produces a lower AUC than does dosing by the i.v. route over 1 h [29].

The average 24-h urinary recovery of TMZ measured in the 29 patients was 5.9 ± 3.4%, in good correspondence with the 5.2% value obtained by Dhodapkar et al. [19] after administration of TMZ in monotherapy. The percentage of TMZ eliminated unchanged in the urine was stable within patients on days 1 and 2 but was highly variable between patients. Since TMZ spontaneously decomposes at a pH value around neutrality, the residence time of urine in the bladder at pH 5.5–7, which is not acidic enough to guarantee TMZ stability, may influence the percentage of the parent TMZ recovered in urine. Indeed, we observed that patients who had frequent spontaneous voidings were those associated with the higher recovery of TMZ in urine. The clinical relevance of the generation of reactive species in the bladder remains to be determined.

Preclinical studies have demonstrated that TMZ penetrates central nervous tissues in mice [22]. In humans, CSF TMZ concentrations represent 9%, 28%, and 29% of those measured simultaneously in plasma, values that are quite similar to the CSF/plasma ratio of 29 ± 8% measured in a nonhuman primate model [30]. This is consistent with the activity of TMZ in brain tumors shown in early clinical trials [17, 18, 21] TMZ concentrations measured in ascites indicate that the distribution in this compartment is slow and that an equilibrium seems to be reached only after approximately 2 h. However, since TMZ, unlike DTIC, does not require hepatic metabolism to generate the cytotoxic species MTIC, it may represent a good candidate for locoregional administration when high concentrations of drug are required – as in peritoneal metastases or in isolated limb perfusion (e.g., in melanoma patients).

The activity of TMZ against tumors highly resistant to current chemotherapeutic treatments needs to be explored further in additional phase II trials. Combination of TMZ with other drugs such as nitrososureas may be addressed. Plowman et al. [25] demonstrated in a preclinical study on mice, however, that the dramatic synergistic effect observed for a combination of TMZ with 1,3,-bis(2-chloroethyl)-1-nitrosourea (BCNU) was more prononced when TMZ was given 2 h after the nitrosourea. The reverse sequence was more toxic, but at a lower dose a synergistic effect was nonetheless observed [25]. These data therefore provide a frame for the administration of TMZ on an alternate – reversed (i.e., TMZ after the nitrosourea) – treatment schedule. It was indeed recognized very early that the pharmacodynamics of TMZ [31] as well as its cytotoxicity and clinical activity ([22] and literature cited) were highly schedule-dependent. Several phase I trials comparing various modes and schedules of administration are therefore currently under way. All these studies should address both pharmacodynamics (i.e., AT inhibition and toxicities [31, Gander et al., submitted for publication]) and pharmacokinetic issues, since the chronological sequence and the mode of administration of drugs given in association may reciprocally influence their pharmacokinetics. The observation that the plasma AUC and, hence, the systemic exposure could be infusion-duration-dependent after h.i.a. administration has to be taken into account in the consideration of this locoregional administration. However, the potential clinical benefit of h.i.a. administration has yet to be established, as has the impact of the infusion duration on the hepatic and systemic tolerance and on the response rate.

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References

1. Fine HA, Keith BG, Loefflers JS, Black P, Canellos GP (1993) Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. Cancer 71: 2585–2597
2. Lejeune F, Bauer J, Leyvraz S, Lienard D (1993) Disseminated melanoma: preclinical therapeutic studies, clinical trial, and patient treatment. Curr Opin Oncol 5: 390–396
3. Leyvraz S, Spataro V, Bauer J, Pampallona S, Salmon R, Dorval T, Meuli R, Gillet M, Lejeune F, Zografos L (1997) Treatment of ocular metastases to the liver by hepatic arterial chemotherapy. J Clin Oncol 15: 2589–2595
4. Tsang LLH, Quaterman CP, Gescher A, Slack JA (1991) Comparison of the cytotoxicity in vitro of temozolomide and dacarbazine, prodrugs of 3-methyl-(triazen-1-yl)imidazole-4-carboxamide. Cancer Chemother Pharmacol 27: 342–346
5. Pegg AE (1990) Mammalian O^6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. Cancer Res 50: 6119–6129
6. D'Incalci M, Citti L, Taverna P, Catapano CV (1988) Importance of the DNA repair enzyme O^6 - alkylguanine alkyltransferase (AT) in cancer chemotherapy. Cancer Treat Rev 15: 279–292
7. Catapano CV, Broggini M, Erba E, Ponti M, Mariani L, Citti L, D'Incalci M (1987) In vitro and in vivo methazolastone induced DNA damage and repair in L-1210 leukemia sensitive and resistant to chloroethylnitrosoureas. Cancer Res 47: 4884–4889
8. D'Incalci M, Taverna P, Erba E, Filippeschi S, Potenza D, Mariani L, Citti L, Catapano CV (1991) O^6-methylguanine and

temozolomide can reverse the resistance to chloroethylnitrosoureas of a mouse L-1210 leukemia. Anticancer Res 11: 115–122
9. Reference deleted
10. Kleeberg UR, Engel E, Israels P, Brocker EB, Tilgen W, Kennes C, Gerard B, Lejeune F, Glabbeke MV, Lentz MA (1995) Palliative therapy of melanoma patients with fotemustine. Inverse relationship between tumor load and treatment effectiveness. A multicentre phase II trial of the EORTC-Melanoma Cooperative Group (MCG). Melanoma Res 5: 195–200
11. Khayat D, Giroux B, Berille J, Cour V, Gerard B, Sarkany M, Bertrand P, Bizzari JP (1994) Fotemustine in the treatment of brain primary tumors and metastases. Cancer Invest 12: 414–420
12. Lee SM, Margison GP, Woodcock AA, Thatcher N (1993) Sequential administration of varying doses of dacarbazine and fotemustine in advanced malignant melanoma. Br J Cancer 67: 1356–1360
13. Aamdal S, Gerard B, Bohman T, D'Incalci M (1992) Sequential administration of dacarbazine and fotemustine in patients with disseminated malignant melanoma – an effective combination with unexpected toxicity. Eur J Cancer 28: 447–450.
14. Gerard B, Aamdal S, Lee SM, Leyvraz S, Lucas C, D'Incalci M, Bizzari JP (1993) Activity and unexpected lung toxicity of the sequential administration of two alkylating agents – dacarbazine and fotemustine – in patients with melanoma. Eur J Cancer 5: 711-719
15. Tsang LLH, Farmer PB, Gescher A, Slack JA (1990) Characterisation of urinary metabolites of temozolomide in humans and mice and evaluation of their cytotoxicity. Cancer Chemother Pharmacol 26: 429–436
16. Newlands ES, Blackledge GRP, Slack JA, Rustin CSS, Smith DB, Stuart NSA, Quaterman CP, Hoffman R, Stevens MFG, Brompton MH, Gibson AC (1992) Phase I trial of temozolomide (CCRG 81045: M&B 39831: NSC 362856). Br J Cancer 65: 287–291
17. O'Reilly SM, Newlands ES, Glaser MG, Brampton M, Rice-Edwards JM, Illingworth RD, Richards PG, Kennard C, Colquhoun IR, Lewis P, Stevens MFG (1993) Temozolomide: a new oral cytotoxic chemotherapeutic agent with promising activity against primary brain tumors. Eur J Cancer 29A: 940–942
18. Dhodapkar M, Reid J, Ames J, Buckner MM, Burch JC, Rubin PA (1994) Phase I clinical trial and pharmacokinetics of temozolomide (NSC 362856). Proc Am Assoc Cancer Res 35: 463
19. Bleehen NM, Newlands ES, Lee SM, Thatcher N, Selby P, Calvert AH, Rustin GJS, Brampton M, Stevens MFG (1995) Cancer Research Campaign phase II trial of temozolomide in metastatic melanoma. J Clin Oncol 13: 910–913
20. Newlands ES, O'Reilly SM, Glaser MG, Bower M, Evans H, Brock C, Brampton MH, Colquhoun I, Lewis P, Rice-Edwards JM, Illingworth RD, Richards PG (1996) The Charing Cross Hospital experience with temozolomide in patients with gliomas. Eur J Cancer 32A: 2236–2241
21. Newlands ES, Stevens MFG, Wedge SR, Wheelhouse RT, Brock C (1997) Temozolomide: a review of its discovery, chemical properties, pre-clinical development and clinical trials. Cancer Treat Rev 23: 35–61
22. Shen F, Decosterd LA, Gander M, Leyvraz S, Biollaz J, Lejeune FJ (1995) Determination of temozolomide in human plasma and urine by high performance liquid chromatography after solid phase extraction. J Chromatogr [B] 665: 291–300
23. WHO (1979) Handbook for reporting results of cancer treatment. Offset publication 48, WHO, Geneva
24. Plowman J, Waud WR, Koutsoukos AD, Rubinstein LV, Moore TD, Grever MR (1994) Preclinical antitumor activity of temozolomide in mice: efficacy against human brain tumour xenographs and synergism with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 54: 3793–3799
25. Mitchell RB, Dolan ME (1993) Effect of temozolomide and dacarbazine on O^6-alkylguanine-DNA alkyltransferase activity and sensitivity of human tumor cells and xenografts to 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Chemother Pharmacol 32: 59–63
26. Statkevich P, Judson I, Cutler D, Dugan M, Salfi M, Batra V, Reidenberg P (1997) Effect of ranitidine on the pharmacokinetics of temozolomide. Clin Pharmacol Ther 61: 39, 147
27. Kerr DJ, Los G (1993) Pharmacokinetic principles of locoregional chemotherapy. Cancer Surv 17: 105–119
28. Fety R, Lucas C, Solere P, Cour V, Vignoud J (1992) Hepatic intra-arterial infusion of fotemustine: pharmacokinetics. Cancer Chemother Pharmacol 31: 118–122
29. Patel M, McCully C, Godwin K, Balis F (1995) Plasma and cerebrospinal fluid pharmacokinetics of temozolomide. Proc Am Soc Clin Oncol 14: 461
30. Lee SM, Thatcher N, Growther D, Margison GP (1994) Inactivation of O^6-alkylguanine-DNA alkyltransferase in human peripheral blood mononuclear cells by temozolomide. Br J Cancer 69: 452–456