

Plasma and Cerebrospinal Fluid Population Pharmacokinetics of Temozolomide in Malignant Glioma Patients

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ABSTRACT

Purpose: Scarce information is available on the brain penetration of temozolomide (TMZ), although this novel methylating agent is mainly used for the treatment of malignant brain tumors. The purpose was to assess TMZ pharmacokinetics in plasma and cerebrospinal fluid (CSF) along with its inter-individual variability, to characterize covariates and to explore relationships between systemic or cerebral drug exposure and clinical outcomes.

Experimental Design: TMZ levels were measured by high-performance liquid chromatography in plasma and CSF samples from 35 patients with newly diagnosed or recurrent malignant gliomas. The population pharmacokinetic analysis was performed with nonlinear mixed-effect modeling software. Drug exposure, defined by the area under the concentration-time curve (AUC) in plasma and CSF, was estimated for each patient and correlated with toxicity, survival, and progression-free survival.

Results: A three-compartment model with first-order absorption and transfer rates between plasma and CSF described the data appropriately. Oral clearance was 10 liter/h; volume of distribution (V_D), 30.3 liters; absorption constant rate, 5.8 h^{-1} ; elimination half-time, 2.1 h; transfer rate from plasma to CSF ($K_{\text{plasma} \rightarrow \text{CSF}}$), $7.2 \times 10^{-4} \text{ h}^{-1}$ and the backwards rate, 0.76 h^{-1} . Body surface area significantly influenced both clearance and V_D , and clearance was sex dependent. The AUC_{CSF} corresponded to 20% of the $\text{AUC}_{\text{plasma}}$. A trend toward an increased $K_{\text{plasma} \rightarrow \text{CSF}}$ of 15% was observed in case of concomitant radiochemotherapy. No significant correlations between AUC in plasma

or CSF and toxicity, survival, or progression-free survival were apparent after deduction of dose-effect.

Conclusions: This is the first human pharmacokinetic study on TMZ to quantify CSF penetration. The $\text{AUC}_{\text{CSF}}/\text{AUC}_{\text{plasma}}$ ratio was 20%. Systemic or cerebral exposures are not better predictors than the cumulative dose alone for both efficacy and safety.

INTRODUCTION

Primary brain tumors represent only about 2% of all adult cancers but are associated with high morbidity and mortality. In adults, glioblastoma multiforme (WHO grade IV) and anaplastic astrocytoma (WHO grade III) are the most common types of malignant primary brain tumors, with an incidence of 6–7 per 100,000 individuals (1, 2). Despite surgery followed by standard fractionated radiotherapy with or without adjuvant chemotherapy (3, 4), these tumors almost invariably recur and lead rapidly to death. Many of the commonly used chemotherapy agents (e.g., nitrosoureas or the combination regimen procarbazine, lomustine, vincristine) have limited activity against glioma (5). Although the blood-brain barrier is partially disrupted in malignant gliomas, penetration into the brain of many chemotherapy agents appears to be insufficient. Furthermore, tumor-induced aberrant capillary growth may also limit the penetration of the agents into the brain (6).

Temozolomide (TMZ), a novel oral imidazotetrazinone methylating agent has demonstrated a schedule-dependent antitumor activity in various advanced cancers, including malignant gliomas (7–12). After oral administration, TMZ is rapidly absorbed with almost 100% bioavailability and undergoes spontaneous hydrolysis at physiological pH into its active metabolite, 5-(3-methyl triazen-1-yl)imidazole-4-carboxamide (MTIC) (13). This is then rapidly degraded into methyl diazonium, the methylating entity, and into an inactive metabolite 5-aminoimidazole-4-carboxamide (AIC), which is finally excreted by the kidney (13, 14). The cytotoxicity of MTIC is thought to be caused by methylation of the O⁶ position of guanine with additional alkylation at the N⁷ position (13, 15).

TMZ has demonstrated ubiquitous distribution into all tissues including the brain (7, 13). Its plasma pharmacokinetics is linear and predictable. However, there is only scarce information on the concentration profile of TMZ into the brain or cerebrospinal fluid (CSF; Refs. 16, 17). Although TMZ is mainly used in the treatment of malignant brain tumors, a complete pharmacokinetic profile in the CSF, commonly used as a surrogate marker for central nervous system penetration, has never been described for this agent.

Here we report the TMZ pharmacokinetics in plasma and CSF from patients enrolled in two clinical trials. Because repeated lumbar punctures are not feasible for ethical and practical reasons, a population pharmacokinetic approach using the nonlinear mixed-effect modeling (NONMEM) computer program was used.

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Note: L. A. Decosterd and R. Stupp designed the study and contributed equally to this work.

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The objectives of this study are to characterize the population pharmacokinetics of TMZ in plasma and CSF along with the inter- and intra-individual variability, to evaluate their dependence toward demographic and clinical covariates, and to further explore the relationships between TMZ systemic and cerebral exposure and clinical outcomes in glioma patients.

MATERIALS AND METHODS

Patients and Treatments. Patients with histologically proven malignant glioma were enrolled in two parallel clinical phase II trials. Newly diagnosed glioblastoma multiforme patients were treated with radiotherapy and concomitant and adjuvant temozolomide (pilot study; Ref. 18); chemo-naïve patients with recurrent or progressive malignant gliomas (glioblastoma multiforme or anaplastic astrocytoma/anaplastic oligoastrocytoma) received TMZ as first-line chemotherapy (recurrent study; Ref. 19). All patients participating in one of these two phase II trials were also eligible for participation in this ancillary and nonmandatory pharmacokinetic study, and a separate informed consent was obtained for this part. Other eligibility criteria included absence of increased intracranial pressure documented by magnetic resonance imaging, age ≥ 18 years, eastern cooperative oncology group performance status ≤ 2 or Karnofsky performance status $\geq 70\%$, adequate hematological, renal, and hepatic functions, defined as absolute neutrophil count $\geq 1.5 \times 10^9$ /liter; platelet count $\geq 100 \times 10^9$ /liter; hemoglobin > 90 g/liters; serum creatinine and total serum bilirubin ≤ 1.5 times the upper limit of normal; aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase < 2.5 times the upper limit of normal. Exclusion criteria included any medical conditions that could interfere with the oral administration of TMZ or any previous or concurrent malignancies at other sites, with the exception of the cervix and nonmelanoma skin cancer. The clinical and pharmacokinetic protocols were reviewed and approved by the local ethics committee.

TMZ was administered on one of the two following schedules:

1. *Continuous schedule* (pilot study). 75 mg/m² TMZ daily for 42–49 days in a fasting state, 1 h before radiotherapy (2 Gy/fraction; 5 days/week, total dose, 60 Gy) or in the morning on weekends. After a 4-week interval, patients received up to 6 cycles of adjuvant TMZ at 200 mg/m²/day \times 5 days, every 4 weeks (18).
2. *Intermittent schedule*. Administration of TMZ at 150–200 mg/m²/day \times 5 days every 4 weeks, for up to 6 adjuvant treatment cycles (pilot study) or up to 17 cycles in recurrent disease (recurrent study), until occurrence of unacceptable toxicity, or until evidence of tumor progression (19).

Prophylactic antiemetics (4 mg/day ondansetron, 1 mg/day granisetron; 10–40 mg/day metoclopramide, 50 mg/day alizapride) were used only as required during concomitant radiotherapy plus TMZ therapy and routinely prescribed once a day before adjuvant or recurrent TMZ treatment. Anticonvulsants [cytochrome P-450 inducers (phenytoin, carbamazepine); cytochrome P-450 inhibitors (valproic acid); others (lamotrigine, gabapentin, clonazepam)], corticosteroids (dexamethasone, prednisone), antacids (omeprazole, ranitidine) or the cytoprotective agent, sucralfate, were administered as needed.

Pharmacokinetic Sampling and Processing. Plasma and CSF concentrations of TMZ were analyzed in sparse samples collected on different occasions over a 17 months period. Venous blood samples (5 ml) were drawn into cooled lithium-heparinized tubes at three different times between 0.2 and 8.4 h after oral TMZ administration on the first (day 1) and last day (day 42–49) of the concomitant radiotherapy plus TMZ therapy (*continuous schedule*) and on day 1 and day 5 during first cycle of adjuvant or recurrent TMZ treatment (*intermittent schedule*). The exact time of TMZ administration as well as the time of blood and CSF sampling were carefully recorded. After collection, samples were immediately centrifuged (2000 rpm during 10 min at 4°C), the plasma was then acidified with 0.1 ml 1 M HCl and stored at -20°C until analysis. CSF was collected on two occasions through a lumbar puncture between 1.3 and 6.6 h after oral TMZ intake. A lumbar puncture was performed on day 1 of the concomitant radiochemotherapy (*continuous schedule*) and on the first day of the adjuvant or recurrent chemotherapy (*intermittent schedule*). In one patient, the presence of an Ommaya reservoir, implanted previously because of the development of an occlusive hydrocephalus, allowed the collection of sequential CSF samples. Processing of the CSF samples was analogous to the blood samples but did not require centrifugation.

Blood and CSF Samples Analysis. Quantitation of TMZ in plasma and CSF was performed by reversed-phase high-performance liquid chromatography analysis using a slight modification of our method described previously (20), to improve the sensibility of the method for the CSF samples analysis, for which lower TMZ levels were expected. The clean-up procedure involved a solid-phase extraction of biological sample (100 μl) on a 100 mg C₁₈-endcapped cartridge (Chromabond, Macherey-Nagel, Düren, Germany). Matrix components were eliminated with 750 μl of 0.5% acetic acid. TMZ was subsequently eluted with 1250 μl of methanol. The resulting eluate was evaporated under nitrogen at room temperature, reconstituted in 100 and 200 μl of 0.5% acetic acid for CSF and plasma samples respectively, and analyzed by high-performance liquid chromatography.

The chromatographic system consisted of a HP 1050 isocratic/quaternary pump (Hewlett-Packard, Böblingen, Germany) connected to an HP 1050 autosampler and an HP 1050 UV diode-array detector set at UV 330 nm. Separations were performed on a Nucleosil 100–5 C₁₈ AB column (125 \times 4 mm inside diameter; Macherey-Nagel) equipped with an Nucleosil C₁₈ AB (8 \times 4 mm) guard column. The high-performance liquid chromatography mobile phase was methanol/0.5% acetic acid (7:93 v/v). The flow rate was 1 ml/min. The retention time for TMZ and ethazolastone were 2.7 and 6.8 min, respectively. A HP ChemStation A-O6-03 software loaded onto a Compaq Deskpro EP/SB was used for the data processing of the chromatograms.

The plasma calibration standards (0.4, 2, 4, 10, and 20 $\mu\text{g/ml}$) and control samples (1, 10, and 18 $\mu\text{g/ml}$) were prepared with 5 ml of blank human plasma, added to TMZ stock solution (prepared in 0.1 M HCl), acidified with 250 μl of 2 M HCl and completed ad 5.5 ml with 0.1 M HCl. The CSF calibration standards (0.1, 0.2, 0.5, 1, and 2 $\mu\text{g/ml}$) and controls (0.33, 0.5, and 1 $\mu\text{g/ml}$) were prepared with an artificial CSF solution [0.8

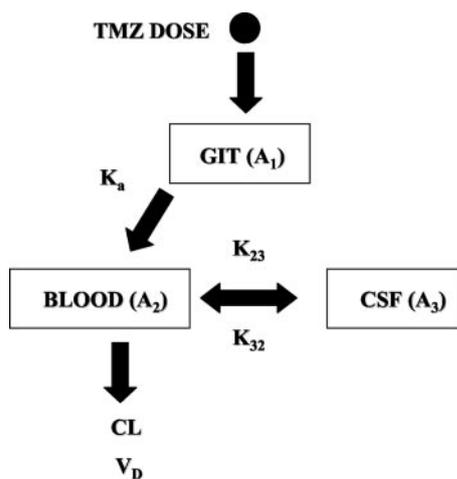


Fig. 1 Basic structural pharmacokinetics model for temozolomide (TMZ). A_1 - A_3 , amounts in the absorption, plasma, and cerebrospinal fluid (CSF) compartments; GIT, gastrointestinal tract; K_a , absorption rate constant; K_{23} , rate constant from plasma to CSF; K_{32} , rate constant from CSF to plasma; CL, oral clearance; V_D , volume of distribution in the central compartment.

g/liter glucose, 0.2 g/liter albumin, 7.3 g/liter NaCl, and 1.9 g/liter NaHCO_3 (adjusted to pH 7.5 with phosphate buffer)], added to various volumes of TMZ stock solution and acidified similarly to the plasma samples.

A linear response was observed for all calibration curves between 0.4 and 20 $\mu\text{g/ml}$ and 0.1 to 2 $\mu\text{g/ml}$ in plasma and CSF, respectively ($r > 0.999$). This method had a limit of quantitation of 0.2 $\mu\text{g/ml}$ and 0.1 $\mu\text{g/ml}$ in plasma and CSF, respectively, as well as an inter-assay variability and bias of $<15\%$, in accordance with the recommendations of the Conference Report on Bioanalytical Method Validation (21).

Model-Based Pharmacokinetic Analysis. Population analysis was performed using the NONMEM computer program (version V with NM-TRAN version II) developed by Beal and Sheiner (22). NONMEM uses mixed (fixed and random) effects regression to estimate the population mean and the variability of pharmacokinetic parameters and to identify factors that may influence them. A three-compartment model with first order absorption from the gastrointestinal tract (Fig. 1) was used to model TMZ in plasma and in CSF according to:

$$\frac{dA_1}{dt} = -K_a \cdot A_1$$

$$\frac{dA_2}{dt} = K_a \cdot A_1 - \frac{CL}{V_D} \cdot A_2 - k_{23} \cdot A_2$$

$$\frac{dA_3}{dt} = k_{23} \cdot A_2 - k_{32} \cdot A_3$$

$$C_{\text{plasma}} = \frac{A_2}{V_D} \quad \text{and} \quad C_{\text{CSF}} = \frac{A_3}{V_p}$$

where A_1 , A_2 , and A_3 are the amount in the absorption, plasma, and CSF compartments, respectively; K_a is the absorption rate constant; CL indicates the oral clearance; V_D is the volume of

distribution of the central compartment; K_{23} and K_{32} are the two transfer rate constants from plasma to CSF (K_{23}) and from CSF to plasma (K_{32}). V_p , the volume of distribution in the CSF, was fixed to 140 ml according to the literature (23, 24) because this parameter could not be identified simultaneously with K_{23} and K_{32} . C_{plasma} and C_{CSF} are the TMZ concentrations in plasma and CSF.

A bioavailability of 1, assumed in absence of i.v. drug administration, was in good accordance with its known high bioavailability of 97% (13). A proportional error distribution was used for the description of both the inter- and intra-individual variability of the pharmacokinetic parameters. The influence of each of the recorded population factors (covariates) on the pharmacokinetic parameters was tested sequentially. They included the demographic variables age, gender, body weight, height, BSA (according to the Dubois and Dubois formula; Ref. 25); a renal function surrogate (serum creatinine); the common comedication (anticonvulsants, antiemetics, antacids, cytoprotective agent, and corticosteroids); and concomitant radiotherapy to TMZ administration. Only those variables considered as potential covariates for developing the population pharmacokinetic model were tested. At the end of the analysis, all covariates that showed an influence on the parameters were evaluated again by comparison of the full model (with all factors included) with a model from which each of the factors was deleted sequentially.

The model was fitted to the data by using the first order method with the subroutine ADVAN5. NONMEM performs linearized maximum likelihood estimation by use of an objective function of minus twice the logarithm of the likelihood of the model. To determine the statistical significance between two models, we used the log-likelihood ratio test obtained by computing the difference in the minimum value of the objective function (the difference in the minimum value of the NONMEM objective function $[-2\Delta LL]$ for nested models is approximately χ^2 distributed); Model selection criteria were a decrease of the objective function (OF) of 3.8 points for one additional parameter and 5.9 points for two additional parameters ($P < 0.05$) as well as comparison of regression diagnostic plots. Figures were generated with Prism (version 4.0; GraphPad Software Inc., San Diego, CA).

Table 1 Patient demographics and baseline disease

	Pilot study <i>n</i> or mean (range)	Recurrent study <i>n</i> or mean (range)
Number of patients	23	12
Age (years)	50 (36–65)	49 (30–79)
Body weight (kg)	72 (46–105)	72 (53–85)
Height (cm)	169 (150–188)	170 (151–181)
BSA (m^2) ^a	1.80 (1.46–2.11)	1.83 (1.59–2.06)
Gender	15/8 M/F	9/3 M/F
KPS (%)	90 (70–100)	70 (30–100)
ECOG	1 (1–2)	1 (0–3)
Creatinine ($\mu\text{mol/liter}$)	84 (61–108)	84 (53–129)
GBM	23 (100%)	8 (67%)
AA/AOA	0	4 (33%)

^a BSA, body surface area; KPS, Karnofsky performance status; ECOG, eastern cooperative oncology group; GBM, glioblastoma multiforme; AA/AOA, anaplastic astrocytoma/anaplastic oligoastrocytoma.

Table 2 Co-mediations during temozolomide treatment

Comedications	Pilot study (n = 23) n (%)	Recurrent study (n = 12) n (%)
Anticonvulsants CYP-450 ^a inducers	17 (74)	8 (67)
Anticonvulsants CYP-450 inhibitors	6 (26)	4 (33)
Others anticonvulsants	3 (13)	0 (0)
Antiemetics	18 (78)	11 (92)
Corticosteroids	18 (78)	9 (75)
Antacids	12 (52)	4 (33)
Cytoprotective agent (sucralfate)	2 (9)	1 (8)

NOTE. Three patients from the pilot study and three from the recurrent study had received more than one anticonvulsants. One patient from the recurrent study has received an antacid and sucralfate.

^a CYP-450, cytochrome P450.

Pharmacodynamics. Posthoc Bayesian estimates of the pharmacokinetic parameters were obtained for all patients and used to calculate the area under the curve concentration-time curve (AUC) in plasma (AUC_{plasma}) and CSF (AUC_{CSF}) according to the following relationships:

$$AUC_{\text{plasma}} = \text{Dose}/CL$$

$$AUC_{\text{CSF}} = \frac{\text{Dose}}{CL} \cdot (V_D \cdot K_{23}/K_{32})/V_p$$

The AUC_{plasma} and AUC_{CSF} were first calculated with cumulative dose of each patient (total TMZ dose received during pilot

or recurrent study), to evaluate the relationship between systemic or cerebral drug exposure and safety and efficacy outcomes (26), *i.e.* hematological toxicity, survival, and progression-free survival (PFS; calculated from the first administration of TMZ up to the date of disease progression).

Hematological toxicity, commonly defined by the percentage of decrease in platelet and absolute neutrophil count (ANC) from baseline to nadir (27, 28), was observed during the first two cycles of the adjuvant or recurrent treatments (*intermittent schedule*). Percent decrease was calculated as follows, using neutrophil as example:

$$\% \text{ Decrease} = \left(\frac{\text{Baseline ANC} - \text{Nadir ANC}}{\text{Baseline ANC}} \right) \times 100$$

and the relationship with AUC_{plasma} was assessed by logistic regression.

The associations between both AUC_{plasma} and AUC_{CSF} and the two efficacy outcomes, survival and PFS observed at the time of this analysis, were assessed by the Spearman rank correlation test and by logistic regression. For this statistical analysis, a model was fitted to the clinical data, initially expressed in months, which was transformed into binary response, as follows: (a) 0 = alive; 1 = dead, in terms of survival after 9, 12, and 24 months and (b) 0 = progression-free; and 1 = disease progression for PFS after 6, 9, and 12 months.

The results were considered statistically significant at $P < 0.05$, whereas $P < 0.1$ was regarded to be indicative of possible trends. All statistical tests were performed with the Statistix

Table 3 Influence of patient covariates on TMZ ^a CL, V_D, K₂₃, K₃₂ (selection)

Hypothesis	Model	θ _a ^b	θ _b	θ _c	ΔOF ^c
Demographic characteristics					
Does BW influence CL?	θ _a + θ _b · BW	11.2	2.2		-5.5
Does BW influence V _D ?		29.9	4.2		-1.4
Does HT influence CL?	θ _a + θ _b · HT	11.2	19.6		-27.8
Does HT influence V _D ?		29.3	<0.1		-0.2
Does age influence CL?	θ _a · (1 - θ _b · age)	11.2	<0.1		-0.0
Does age influence V _D ?		29.3	1.8		-0.0
Does sex influence CL? (male = 1)	θ _a · (1 + θ _b · sex)	9.6	0.2		-28.2
Does sex influence V _D ?		29.3	<0.1		-0.0
Does BSA influence CL?	θ _a + θ _b · BSA	11.3	3.7		-19.2
Does BSA influence V _D ?		29.8	19.0		-6.7
Concomitant medications on oral CL (inducers or inhibitors = 1 if present)					
CYP inducers	CL = θ _a + θ _b ^{Inducers}	11.1	0.1		-0.2
CYP inhibitors	CL = θ _a - θ _b ^{Inhibitors}	10.6	0.7		-2.2
Corticosteroids on K ₂₃ and K ₃₂					
Corticosteroids	K ₂₃ = θ _a + θ _b ^{Corticosteroids}	6.6 ⁻⁴	0.0		-0.1
	K ₃₂ = θ _a + θ _b ^{Corticosteroids}	0.8	0.0		-0.1
Concomitant radiotherapy on K ₂₃					
Radiotherapy	K ₂₃ = θ _a + θ _b ^{RTH}	6.4 ⁻⁴	1.2		-2.6
Multivariate analysis					
BSA ^d and sex on CL	CL = θ _a · (1 + θ _b · sex) + θ _c · BSA	10.0	0.2	4.2	-31.8
BSA on V _D	VD = θ _a + θ _b · BSA	30.3	19.9		-37.4

^a TMZ, temozolomide; CL, oral clearance; V_D, volume of distribution in the central compartment; K₂₃, rate constant from plasma to cerebrospinal fluid; K₃₂, rate constant from cerebrospinal fluid to plasma; V_p volume of distribution in the cerebrospinal fluid; CYP, cytochrome P-450; BW, body weight; HT, height; BSA, body surface area; RTH, radiotherapy; ΔOF, decrease in objective function.

^b θ_a = tested pharmacokinetic parameter; θ_b and θ_c = covariate estimates.

^c ΔOF = difference in the OF compared to the basic structural model including no covariates and estimates of CL = 11.2 liter/h, V_D = 29.3 liter, K_a = 5.2 h⁻¹, K₂₃ = 6.78 × 10⁻⁴, K₃₂ = 0.72, and V_p = 0.14 liter. Each individual dose has been implemented by nonlinear mixed-effect modeling.

^d BSA is expressed as the relative deviation of the individual body weight, height, age from the population mean.

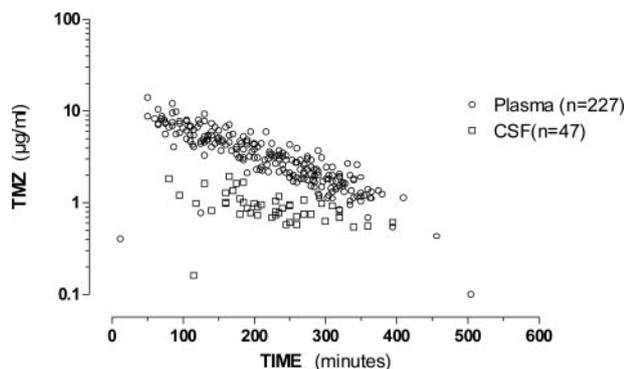


Fig. 2 Plasma (○) and cerebrospinal fluid (CSF; □) concentration-time profiles of temozolomide (TMZ). Peak plasma (○) level was achieved 1.5 h after TMZ administration and then gradually declined over the remaining dosing interval. Levels in CSF (□) remained essentially flat, suggesting that the equilibrium was reached.

program (version 7.0, 2000, Analytical software; Statistix, Tallahassee, FL).

RESULTS

Demographics. Data from 35 patients (23 from the pilot study; 12 from the recurrent study) with a total of 227 plasma and 47 CSF samples were collected for the population pharmacokinetics analysis. Patient demographics and baseline disease characteristics are listed in Table 1, and the different comedications are summarized in Table 2.

TMZ Concentrations in Plasma and CSF. Because TMZ has a linear pharmacokinetics over the studied range of doses (8, 16), the concentrations obtained with the low-dose *continuous schedule* (75 mg/m²/day) during concomitant radiochemotherapy were normalized to those obtained with the 200 mg/m²/day dose (proportional factor: 2.67; Ref. 29). TMZ levels were ranged from 0.10 to 13.99 µg/ml in the plasma and from 0.16 to 1.93 µg/ml in the CSF. The observed concentration-time profile of TMZ in plasma and CSF are displayed in Fig. 2. Because plasma concentrations decreased more quickly than in the CSF, the CSF/plasma ratio increased across the dosing interval (median, 30.1%; range, 2.1–65.2%). The CSF curve was essentially flat at approximately 1 µg/ml, likely attributable to slow influx and efflux rates from the CSF compartment (30).

Population Pharmacokinetic Analysis. A three-compartment model with first-order absorption from the gastrointestinal tract was found to describe the data appropriately. The rate of absorption was estimated with poor precision because few concentration data points were collected during the first hours after TMZ intake. An inter-patient variability was assumed on CL, assignment of a variability on both K_a and K_{23} significantly improved the fits [decrease in objective function (ΔOF) = -36.4], but no inter-patient variability on V_D and K_{32} could be detected. The model building steps for the covariate analysis are shown in Table 3.

Among the tested demographic covariates in the multivariate analysis, body weight, height, BSA, and gender influenced TMZ clearance to a statistically significant extent ($\Delta OF >$

-5.5) and V_D is influenced to a small but significant extent by BSA only ($\Delta OF > -6.7$).

Because body weight and height influenced TMZ pharmacokinetics, we retained BSA, which reflects both these values, as a covariate on CL and V_D in the model. All covariates (*i.e.*, BSA on V_D and CL, and gender on CL) remained statistically significant in the multivariate analysis.

Considering that corticosteroids could partially restore the integrity of the blood-brain barrier, thereby limiting drug delivery to the tumor, and on the other hand also reduce intracranial pressure by decreasing peritumoral vasogenic edema resulting in a increased drug delivery (6, 31–34), a relationship between corticotherapy and the two transfer rate constants, K_{23} and K_{32} , were examined. No significant influence could be observed ($\Delta OF = -0.1$).

No other demographic covariates (age, creatinine clearance) showed any statistical influence on TMZ disposition. Coadministration of drugs, such as anticonvulsants, antiemetics, antacids, cytoprotective agents, or again corticosteroids, did not influence any pharmacokinetic parameters to a statistically relevant extent. However, a trend toward a 15% increase of the rate constant of CSF entry, K_{23} , was observed in case of concomitant radiotherapy to TMZ ($\Delta OF = -2.6$).

The final population estimate of oral CL was 10.0 liter/h and increased to 11.9 liter/h in male patients and oral V_D was 30.3 liters. These two parameters increased, respectively, to 14.2 liter/h and 50.2 liters on doubling BSA. K_a was 5.8 h⁻¹, K_{23} was 7.2 × 10⁻⁴ h⁻¹, and K_{32} was 0.76 h⁻¹. The derived elimination half-life was 2.1 h, whereas the absorption half-life was very rapid (7 min). The inter-individual variability (CV%) in CL (4.7%) and K_{23} (16.6%) were relatively small compared with

Table 4 Population pharmacokinetic parameters estimates of TMZ^a

Parameter	Population mean		Inter-individual variability ^b	
	Estimate	s.e. ^c	Estimate	s.e. ^c
CL (liter/h)	10.0	3.0%	4.7%	80.5% ^c
CL _{sex} (liter/h) ^f	0.19	24.9%		
CL _{BSA} (liter/h) ^g	4.2	72.6%		
V_D (liter)	30.3	4.8%		
$V_{D,BSA}$ (liter) ^g	19.9	39%		
K_a (h ⁻¹)	5.8	23.4%	111%	68.8% ^d
K_{23} (h ⁻¹)	7.2 · 10 ⁻⁴	12.4%	16.6%	65.5% ^d
K_{32} (h ⁻¹)	0.76	8.7%		
V_p (liter)	0.14			
σ (CV%) ^e	21.4%	42.8% ^d		

^a TMZ, temozolomide; CL, oral clearance; BSA, body surface area; V_D , volume of distribution in the central compartment; K_{23} , rate constant from plasma to CSF; K_{32} , rate constant from cerebrospinal fluid to plasma; V_p , volume of distribution in the cerebrospinal fluid; CV%, coefficient of variation.

^b Estimates of variability expressed as CV%.

^c s.e. = SE of the estimates, expressed as CV%.

^d s.e. = SE of the variance components, taken as $\sqrt{\text{s.e.}_{\text{estimate}}/\text{estimate}}$, expressed as a percentage.

^e Residual intra-individual variability of the plasma concentration, expressed as CV%.

^f Relative increase in CL in male compared to female patients.

^g Relative increase in CL or V_D according to BSA, expressed as the relative deviation from the population mean.

the high inter-individual variability in K_a (111%). A residual intra-individual variability of 21.4% was observed. The final population parameter estimates are presented in Table 4.

The comparison of individual and population-predicted (“typical subject”) values, obtained with the three-compartment model, *versus* observed concentrations in plasma and CSF are provided on Fig. 3. The individual and population predictions were symmetrically distributed around the line of identity, suggesting adequate model predictions, without any troublesome pattern in the residuals, as seen on Fig. 4.

AUC_{plasma} is generally considered as an indicator of systemic drug exposure whereas drug penetration into the central nervous system is reflected by AUC in the CSF. Thus, the AUC_{plasma} and AUC_{CSF} (mg/liter/h) were derived for each individual based on their final posthoc parameter estimates, yielding the respective mean values and $AUC_{\text{CSF}}/AUC_{\text{plasma}}$ ratio shown in Table 5. For both types of schedules (*continuous or intermittent*), the AUC_{CSF} represented ~20% of the AUC in plasma. The mean AUC_{plasma} after a daily dose of 75 or 200 mg/m²/day was 12.1 ± 1.1 and 30.1 ± 6.1 mg/liter/h, respectively. In the CSF, the corresponding values were 2.5 ± 0.3 and 6.1 ± 1.2 mg/liter/h.

Pharmacodynamic Relationships. The relationship between population-based AUC_{plasma} and AUC_{CSF} of TMZ and efficacy outcomes, such as survival and PFS, was explored among the homogeneous pilot study population. The statistical evaluations performed with the Spearman rank correlations test revealed that both survival and PFS were significantly associated with either the cumulative dose ($P = 0.0005$ and $P = 0.0009$, respectively), the AUC_{plasma} ($P = 0.0056$ and $P = 0.0065$), or the AUC_{CSF} ($P = 0.0005$ and $P = 0.0003$). However, all predictors were highly inter-correlated ($r > 0.86$) so neither AUC_{plasma} nor AUC_{CSF} improved the prediction based on cumulative dose alone.

The statistical analysis was then repeated with the AUC_{plasma} and AUC_{CSF} calculated with the daily dose (*i.e.*, TMZ 75 or 200 mg/m²/day) and the dose per cycle (continuous schedule, 75 mg/m²/day x 42–49day; intermittent schedule, 200 mg/m²/day x 5 days). It failed to demonstrate any significant correlation between both AUC_{plasma} or AUC_{CSF} and survival or PFS, with $P > 0.1$ in all cases.

Finally, no significant correlations could be observed by logistic regression between AUC_{plasma} or AUC_{CSF} and both survival and PFS censored at 6, 9, 12, or 24 months ($P > 0.19$).

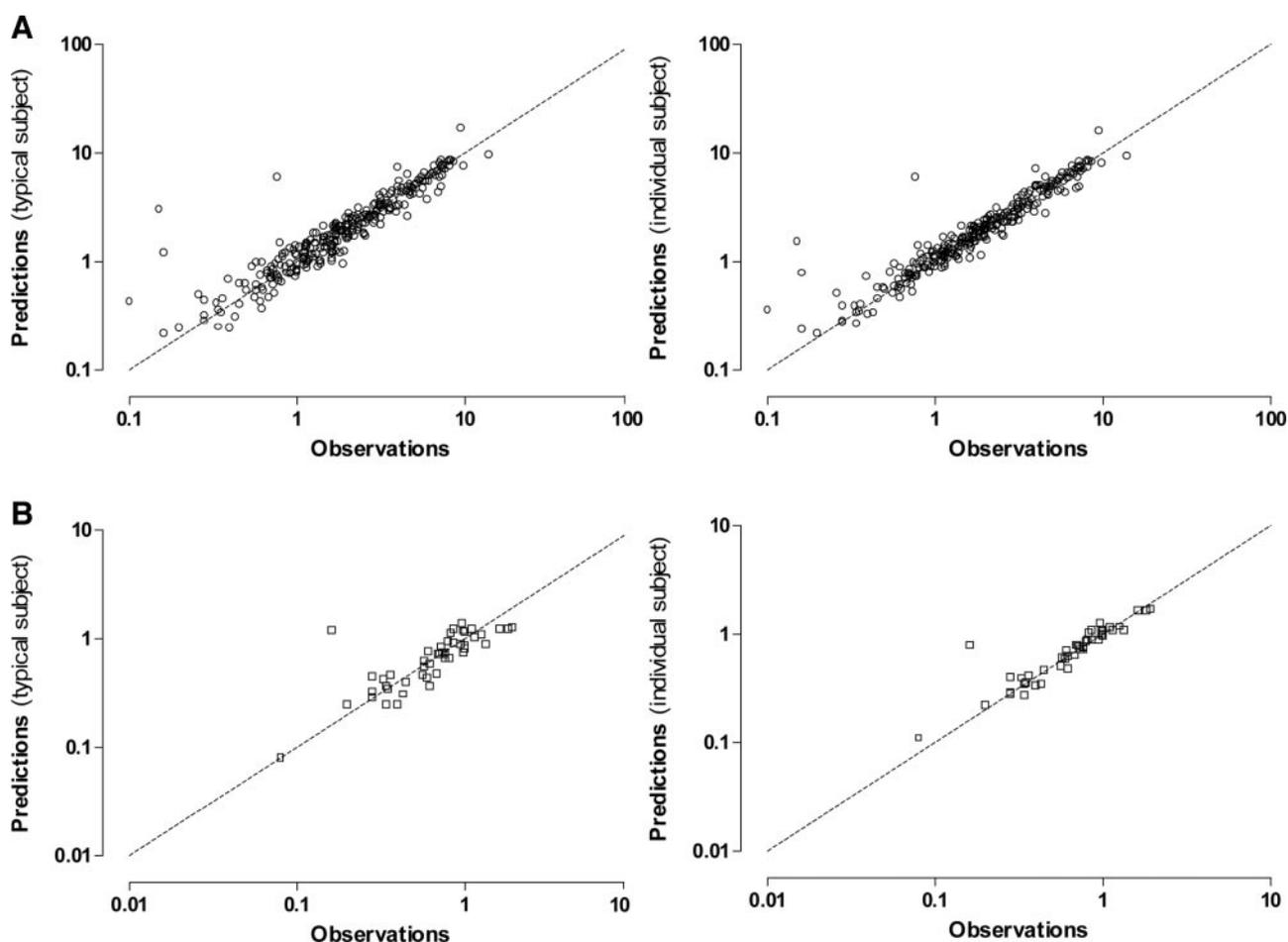


Fig. 3 Population and individual predictions *versus* observed concentrations of temozolomide in plasma (A) and cerebrospinal fluid (B) in microgram per milliliter. The line in the panel represents the line of identity (logarithmic scale).

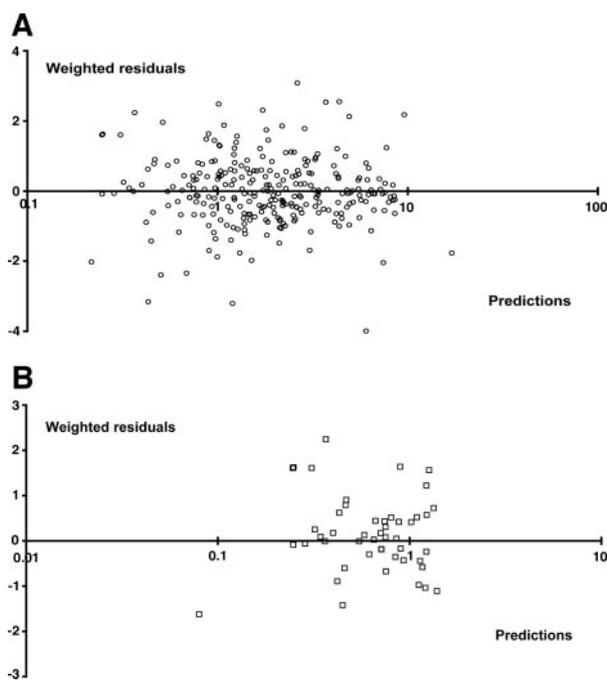


Fig. 4 Plots of weighted residuals versus final model-predicted plasma (A) and CSF (B) concentrations of temozolomide in micrograms per milliliter.

Myelosuppression, in particular delayed thrombocytopenia, is the primary dose-limiting toxicity of TMZ. Hematological toxicity observed in this study population (2–3% grade III/IV neutropenia, 5–6% grade III/IV thrombocytopenia) was in agreement with data reported previously (9–13). The mean decrement in absolute neutrophil count between baseline to nadir was 49% (range, 0–93%) and 47% (range, 4–97%) for platelets. It was then examined whether AUC_{plasma} was a predictor of hematological toxicity. No significant correlation between systemic TMZ exposure during the first two cycles of adjuvant or recurrent treatments (*intermittent schedule*) and the percentage of decrease in platelet ($P = 0.812$) nor absolute neutrophil count ($P = 0.599$) was observed.

DISCUSSION

Population pharmacokinetic approaches such as NONMEM are increasingly applied to the characterization of classical anticancer drugs (28, 35–40) or newer agents, such as capecitabine (41) or E7070 (42).

They enable the description of complex pharmacokinetic behavior, the generation of individual estimates of pharmacokinetic parameters (*e.g.*, CL , V_D) or systemic exposure (AUC_{plasma}), the assessment of inter-individual variability, and the identification of influent patients characteristics on drug disposition.

Very few studies evaluated the penetration of cytotoxic agents into the CSF. Except for anecdotic reports, no data are available today on the penetration of TMZ into the CSF in brain tumor patients. However, for agents with a presumed activity in the central nervous system, sufficient drug concentrations in the clinical situation are a prerequisite.

Our study provides the first description of the influence of population factors on TMZ pharmacokinetics in the plasma and the CSF. We demonstrate that TMZ oral clearance is only slightly influenced by BSA and gender and is associated with a remarkably small inter-patient variability (4.7%). The volume of distribution is influenced by BSA only. These results are in good agreement with Jen *et al.* (43) reporting an oral clearance of 11.2 liter/h with a 15% inter-patient variability and a similar influence of BSA and gender. In our study, neither individual determinants such as genetic background or organ failure nor comedications commonly used in gliomas patients (anticonvulsants, antiemetics, corticosteroids, antacids, and cytoprotective agent) altered TMZ oral clearance.

The AUC values estimated in our analysis revealed that CSF values corresponding to $20\% \pm 5\%$ of those observed in plasma. These results are comparable to the limited information reported in preclinical studies with TMZ and the camptothecins, irinotecan and topotecan. The $AUC_{\text{CSF}}/AUC_{\text{plasma}}$ ratio observed in nonhuman primate models were $33\% \pm 6\%$ for TMZ, $14\% \pm 3\%$ for irinotecan, and $32\% \pm 3\%$ for topotecan (17, 44, 45). Irinotecan and other camptothecins are currently in clinical trials as new treatments for malignant gliomas (46).

This $AUC_{\text{CSF}}/AUC_{\text{plasma}}$ ratio of $20\% \pm 5\%$ is somewhat lower than the average CSF/plasma concentration ratio (mean, $29.4\% \pm 15\%$; increasing up to 40% until 4 h after TMZ intake) reported previously on the same study population (29). This difference is constructed as $AUC_{\text{CSF}}/AUC_{\text{plasma}}$ ratio integrates the entire dosing interval. The ratio of the AUCs estimates more accurately the penetration into the central nervous system than only a CSF/plasma ratio obtained with plasma and CSF concentrations (30). A penetration of at least 20% of the AUC_{plasma} into the CSF may be used as a benchmark for comparison with other agents with a presumed activity in the CSF.

The lack of a significant relationship between AUC_{plasma} or AUC_{CSF} beyond the cumulative dose and efficacy outcomes is likely to be explained by the very low inter-patient variability in TMZ pharmacokinetics, thus minimizing the predictive value added by AUC over the cumulative dose alone.

Similarly, AUC_{plasma} was not a predictor of decrease of platelets or neutrophils. Detection of decrease in blood counts is obscured by the large normal fluctuation of leukocyte and platelet counts. Furthermore, severe hematological toxicity with the treatment of TMZ is rare and observed in <10% of patients only. Decrease in blood counts is also dependent on O^6 -methylguanine-DNA methyltransferase (MGMT) expression in hematopoietic stem cells (47). This ubiquitous DNA repair enzyme, which removes methylating and chloroethylating lesions at the O^6 position of guanine, is to a large extent variably expressed in peripheral blood mononuclear cells (48) and thus seems to be a stronger predictor for toxicity than systemic drug exposure.

It must be recognized that TMZ by itself is not the biologically active agent, but merely a prodrug which undergoes a spontaneous hydrolysis to generate the methylating agent MTIC. The determination of this compound in plasma and CSF could have a better chance to predict efficacy or safety outcomes and will be assessed in an additional investigation. Moreover, the biotransformation of TMZ may have a significant variability between organ, tissue, or cell compartments. This possibly lim-

Table 5 Average AUC_{plasma}^a and AUC_{CSF} and ratio (%) of TMZ

TMZ schedules	n	Dose (g) (range)	AUC _{Plasma}	AUC _{CSF}	AUC _{CSF} / AUC _{plasma}
Cumulative dose					
Pilot study	23	5.4–19.1	1126 ± 418	229 ± 83	20.6 ± 2.0
Recurrent study	12	3.6–33.4	1432 ± 1890	297 ± 430	19.0 ± 4.1
Dose per cycle					
Continuous ^b	23	3.8–7.1	516.4 ± 62.2	106 ± 14.2	20.6 ± 2.0
Intermittent ^c	32	1.0–2.1	150.4 ± 30.4	30.4 ± 6.3	20.3 ± 1.9
Daily dose					
75 mg/m ^{2b}	23	0.1–0.2	12.1 ± 1.1	2.5 ± 0.3	20.6 ± 2.0
200 mg/m ^{2c}	32	0.2–0.4	30.1 ± 6.1	6.1 ± 1.2	20.3 ± 1.9

^a AUC, area under the concentration-time curve; CSF, cerebrospinal fluid; TMZ, temozolomide.

^b Continuous schedule, 75 mg/m²/day × 42–49 days. All patients included in the pilot study (n = 23).

^c Intermittent schedule, 200 mg/m²/day × 5 days. All patients included in the recurrent study (n = 12) and all patients received adjuvant therapy (n = 20).

its the ability of blood or CSF concentrations to adequately reflect drug levels at target tissue sites.

Whether concomitant radiotherapy improves cerebral penetration of some chemotherapeutic agents by radiation-induced opening of the blood-brain barrier (49–52) still deserves additional confirmation (6, 53).

Finally, TMZ appears to be one of the few anticancer agents for which dose individualization entirely based on BSA may be actually justified. This finding was also confirmed by Baker *et al.* (54) in a retrospective study on the role of BSA for the dosing of new anticancer agents. The AUC in plasma and CSF have shown a low inter-individual variability and could not be correlated with efficacy nor toxicity outcomes. The AUC in the CSF, considered as the only ethically accepted surrogate of the brain penetration, corresponds to approximately 20% of the systemic TMZ exposure.

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