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

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ABSTRACT

Purpose: Scarcely 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 (Vp), 30.3 liters; absorption constant rate, 5.8 h−1; elimination half-time, 2.1 h; transfer rate from plasma to CSF (Kplasma→CSF), 7.2 × 10−4 h−1 and the backwards rate, 0.76 h−1. Body surface area significantly influenced both clearance and Vp, and clearance was sex dependent. The AUCCSF corresponded to 20% of the AUCplasma. A trend toward an increased Kplasma→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 AUCCSF/AUCplasma 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 procarcbazine, 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 methylidiazonium, the methylating entity, and into an inactive metabolite 5-aminoimidazole-4-carboxamide (AIC), which is finally excreted by the kidney (13, 14). The cytoxicity 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.
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); chemonaive 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 in-phase II trials were also eligible for participation in this ancillary study. 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 prove 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 UV diode-array detector set at UV 330 nm. Separations were performed on a Nucleosil 100–5 C18 AB column (125 × 4 mm inside diameter; 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.

Pharmacokinetic Sampling and Processing. Plasma and CSF concentrations of TMZ were analyzed by reversed-phase high-performance liquid chromatography analysis using a slight modification of our method described previously (20), to improve the sensitivity 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.

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The plasma calibration standards (0.4, 2, 4, 10, and 20 μg/ml) and control samples (1, 10, and 18 μg/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 μg/ml) and controls (0.33, 0.5, and 1 μg/ml) were prepared with an artificial CSF solution (0.8
Plasma and CSF Pharmacokinetics of TMZ in Glioma Patients

The central compartment.

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 comediations (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 $\chi^2$ 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

<table>
<thead>
<tr>
<th></th>
<th>Pilot study</th>
<th>Recurrent study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 (36–65)</td>
<td>49 (30–79)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>72 (46–105)</td>
<td>72 (53–85)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 (150–188)</td>
<td>170 (151–181)</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.80 (1.46–2.11)</td>
<td>1.83 (1.59–2.06)</td>
</tr>
<tr>
<td>Gender</td>
<td>158 M/F</td>
<td>93 M/F</td>
</tr>
<tr>
<td>KPS (%)</td>
<td>90 (70–100)</td>
<td>70 (30–100)</td>
</tr>
<tr>
<td>ECOG</td>
<td>1 (1–2)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>Creatinine (µmol/liter)</td>
<td>84 (61–108)</td>
<td>84 (53–129)</td>
</tr>
<tr>
<td>GBM</td>
<td>23 (100%)</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>AA/AOA</td>
<td>0</td>
<td>4 (33%)</td>
</tr>
</tbody>
</table>

*BSA, body surface area; KPS, Karnofsky performance status; ECOG, eastern cooperative oncology group; GBM, glioblastoma multiforme; AA/AOA, anaplastic astrocytoma/anaplastic oligoastrocytoma.*
Pharmacodynamics. Posthoc Bayesian estimates of the pharmacokinetic parameters were obtained for all patients and used to calculate the area under the curve-time-concentration curve (AUC) in plasma (AUC<sub>plasma</sub>) and CSF (AUC<sub>CSF</sub>) according to the following relationships:

\[
\text{AUC}_{\text{plasma}} = \text{Dose}/\text{CL}
\]

\[
\text{AUC}_{\text{CSF}} = \frac{\text{Dose}}{\text{CL}} \cdot (V_D \cdot K_{23}/K_{32})/V_p
\]

The AUC<sub>plasma</sub> and AUC<sub>CSF</sub> 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<sub>plasma</sub> was assessed by logistic regression.

The associations between both AUC<sub>plasma</sub> and AUC<sub>CSF</sub> 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 2 Co-medications during temozolomide treatment

<table>
<thead>
<tr>
<th>Comedications</th>
<th>Pilot study (n = 23)</th>
<th>Recurrent study (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticonvulsants CYP-450 &quot; inducers</td>
<td>17 (74)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>Anticonvulsants CYP-450 inhibitors</td>
<td>6 (26)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Others anticonvulsants</td>
<td>3 (13)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Antiemetics</td>
<td>18 (78)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>18 (78)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>Antacids</td>
<td>12 (52)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Cytoprotective agent (sucralfate)</td>
<td>2 (9)</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>

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

\[ ^{a} \text{CYP-450, cytochrome P450.} \]

### Table 3 Influence of patient covariates on TMZ " CL, \( V_D, K_{23}, K_{32} \) (selection)

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Model</th>
<th>( \theta_a )</th>
<th>( \theta_b )</th>
<th>( \theta_c )</th>
<th>( \Delta \text{OF} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does BW influence CL?</td>
<td>( \theta_a + \theta_b \cdot \text{BW} )</td>
<td>11.2</td>
<td>2.2</td>
<td></td>
<td>-5.5</td>
</tr>
<tr>
<td>Does BW influence ( V_D )?</td>
<td>( \theta_a + \theta_b \cdot \text{HT} )</td>
<td>29.9</td>
<td>4.2</td>
<td></td>
<td>-1.4</td>
</tr>
<tr>
<td>Does HT influence CL?</td>
<td>( \theta_a + \theta_b \cdot \text{HT} )</td>
<td>11.2</td>
<td>19.6</td>
<td></td>
<td>-27.8</td>
</tr>
<tr>
<td>Does HT influence ( V_D )?</td>
<td>( \theta_a + \theta_b \cdot \text{HT} )</td>
<td>29.3</td>
<td>&lt;0.1</td>
<td></td>
<td>-0.2</td>
</tr>
<tr>
<td>Does age influence CL?</td>
<td>( \theta_a \cdot (1 - \theta_b \cdot \text{age}) )</td>
<td>11.2</td>
<td>&lt;0.1</td>
<td></td>
<td>-0.0</td>
</tr>
<tr>
<td>Does age influence ( V_D )?</td>
<td>( \theta_a \cdot (1 - \theta_b \cdot \text{age}) )</td>
<td>29.3</td>
<td>1.8</td>
<td></td>
<td>-0.0</td>
</tr>
<tr>
<td>Does sex influence CL? (male = 1)</td>
<td>( \theta_a \cdot (1 + \theta_b \cdot \text{sex}) )</td>
<td>9.6</td>
<td>0.2</td>
<td></td>
<td>-28.2</td>
</tr>
<tr>
<td>Does sex influence ( V_D )?</td>
<td>( \theta_a \cdot (1 + \theta_b \cdot \text{sex}) )</td>
<td>29.3</td>
<td>&lt;0.1</td>
<td></td>
<td>-0.0</td>
</tr>
<tr>
<td>Does BSA influence CL?</td>
<td>( \theta_a + \theta_b \cdot \text{BSA} )</td>
<td>11.3</td>
<td>3.7</td>
<td></td>
<td>-19.2</td>
</tr>
<tr>
<td>Does BSA influence ( V_D )?</td>
<td>( \theta_a + \theta_b \cdot \text{BSA} )</td>
<td>29.8</td>
<td>19.0</td>
<td></td>
<td>-6.7</td>
</tr>
<tr>
<td>Concomitant medications on oral CL (inducers or inhibitors = 1 if present)</td>
<td>( \theta_a + \theta_b \cdot \text{Inducers} )</td>
<td>11.1</td>
<td>0.1</td>
<td></td>
<td>-0.2</td>
</tr>
<tr>
<td>CYP inducers</td>
<td>CL = ( \theta_a + \theta_b \cdot \text{Inducers} )</td>
<td>10.6</td>
<td>0.7</td>
<td></td>
<td>-2.2</td>
</tr>
<tr>
<td>Corticosteroids on ( K_{23} ) and ( K_{32} )</td>
<td>( K_{23} = \theta_a + \theta_b \cdot \text{Corticosteroids} )</td>
<td>6.6 ( ^{-a} )</td>
<td>0.0</td>
<td>-0.1</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>( K_{32} = \theta_a + \theta_b \cdot \text{Corticosteroids} )</td>
<td>0.8</td>
<td>0.0</td>
<td>-0.1</td>
<td></td>
</tr>
<tr>
<td>Concomitant radiotherapy on ( K_{23} )</td>
<td>( K_{23} = \theta_a + \theta_b \cdot \text{Radiotherapy} )</td>
<td>6.4 ( ^{-a} )</td>
<td>1.2</td>
<td>-2.6</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>( K_{23} = \theta_a + \theta_b \cdot \text{Radiotherapy} )</td>
<td>10.0</td>
<td>0.2</td>
<td>4.2</td>
<td>-31.8</td>
</tr>
<tr>
<td>Multi variate analysis</td>
<td>( V_D = \theta_a + \theta_b \cdot \text{BSA} )</td>
<td>30.3</td>
<td>19.9</td>
<td>-37.4</td>
<td></td>
</tr>
<tr>
<td>BSA and sex on CL</td>
<td>( \text{CL} = \theta_a \cdot (1 + \theta_b \cdot \text{sex}) + \theta_c \cdot \text{BSA} )</td>
<td></td>
<td></td>
<td>6.6</td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a} \text{TMZ, temozolomide; CL, oral clearance; } V_D, \text{ volume of distribution in the central compartment; } K_{23}, \text{ rate constant from plasma to cerebrospinal fluid; } K_{32}, \text{ rate constant from cerebrospinal fluid to plasma; } V_D, \text{ volume of distribution in the cerebrospinal fluid; CYP, cytochrome P-450; BW, body weight; HT, height; BSA, body surface area; RT, radiotherapy; OF, decrease in objective function.} \)

\( ^{b} \text{\( \theta_a \) and \( \theta_b \) are covariate estimates.} \)

\( ^{c} \text{\( \Delta \text{OF} \) is difference in the } \text{OF} \text{ compared to the basic structural model including no covariates and estimates of } CL = 11.2 \text{ liter/h, } V_D = 29.3 \text{ liter, } K_{23} = 5.2 \text{ liter/h, } K_{32} = 6.78 \times 10^{-4}, \text{ and } V_p = 0.14 \text{ liter. Each individual dose has been implemented by nonlinear mixed-effect modeling.} \)

\( ^{d} \text{BSA is expressed as the relative deviation of the individual body weight, height, age from the population mean.} \)
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 comedinations 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. Levels in CSF (ṙ) remained essentially flat, suggesting that the equilibrium was reached.

Among the tested demographic covariates in the multivariate analysis were age, creatinine clearance, sex, body weight, height, body surface area, and BSA, which significantly improved the fits [increase in objective function (ΔOF) = −36.4], but no inter-patient variability on V̇ was detected. The model building steps for the covariate analysis are shown in Table 3.

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 K2 and K13 significantly improved the fits [increase in objective function (ΔOF) = −5.5] and V̇ was influenced to a small but significant extent by BSA only (ΔOF > −6.7).

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

Because 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, K2 and K13, were examined. No significant influence could be observed (ΔOF = −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, K2, was observed in case of concomitant radiotherapy to TMZ (ΔOF = −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 V̇ was 30.3 liters. These two parameters increased, respectively, to 14.2 liter/h and 50.2 liters on doubling BSA. K2 was 5.8 h⁻¹, K23 was 7.2 × 10⁻⁴ h⁻¹, and K13 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 K23 (16.6%) were relatively small compared with...
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.

$AUC_{\text{plasma}}$ 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 $AUC_{\text{plasma}}$ and $AUC_{\text{CSF}}$ (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 $AUC_{\text{CSF}}$ represented 20% of the $AUC$ in plasma. The mean $AUC_{\text{plasma}}$ after a daily dose of 75 or 200 mg/m$^2$/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 $AUC_{\text{plasma}}$ and $AUC_{\text{CSF}}$ 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 $AUC_{\text{plasma}}$ ($P = 0.0056$ and $P = 0.0065$), or the $AUC_{\text{CSF}}$ ($P = 0.0005$ and $P = 0.0003$). However, all predictors were highly inter-correlated ($r > 0.86$) so neither $AUC_{\text{plasma}}$ nor $AUC_{\text{CSF}}$ improved the prediction based on cumulative dose alone.

The statistical analysis was then repeated with the $AUC_{\text{plasma}}$ and $AUC_{\text{CSF}}$ calculated with the daily dose (i.e., TMZ 75 or 200 mg/m$^2$/day) and the dose per cycle (continuous schedule, 75 mg/m$^2$/day x 42–49 day; intermittent schedule, 200 mg/m$^2$/day x 5 days). It failed to demonstrate any significant correlation between both $AUC_{\text{plasma}}$ or $AUC_{\text{CSF}}$ and survival or PFS, with $P > 0.1$ in all cases.

Finally, no significant correlations could be observed by logistic regression between $AUC_{\text{plasma}}$ or $AUC_{\text{CSF}}$ and both survival and PFS censored at 6, 9, 12, or 24 months ($P > 0.19$).
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 combinations 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% ± 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 AUCCSF/AUCplasma ratio observed in nonhuman primate models were 33% ± 6% for TMZ, 14% ± 3% for irinotecan, and 32% ± 3% for topotecan (17, 44, 45). Irinotecan and other camptothecins are currently in clinical trials as new treatments for malignant gliomas (46).

This AUCCSF/AUCplasma ratio of 20% ± 5% is somewhat lower than the average CSF/plasma concentration ratio (mean, 29.4% ± 15%; increasing up to 40% until 4 h after TMZ intake) reported previously in the same study population (29). This difference is constructed as AUCCSF/AUCplasma 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 AUCplasma 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 AUCplasma or AUCCSF 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, AUCplasma 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 O6-methylguanine-DNA methyltransferase (MGMT) expression in hematopoietic stem cells (47). This ubiquitous DNA repair enzyme, which removes methylating and chloroethylating lesions at the O6 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-
its the ability of blood or CSF concentrations to adequately reflect drug levels at target 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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### Table 5 Average AUC<sub>Plasma</sub> and AUC<sub>CSF</sub> ratio (% of TMZ

<table>
<thead>
<tr>
<th>TMZ schedules</th>
<th>n</th>
<th>Dose (g) (range)</th>
<th>AUC&lt;sub&gt;Plasma&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;CSF&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;CSF&lt;/sub&gt;/AUC&lt;sub&gt;Plasma&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilot study</td>
<td>23</td>
<td>5.4–19.1</td>
<td>1126 ± 418</td>
<td>229 ± 83</td>
<td>20.6 ± 2.0</td>
</tr>
<tr>
<td>Recurrent study</td>
<td>12</td>
<td>3.6–33.4</td>
<td>1432 ± 1890</td>
<td>297 ± 430</td>
<td>19.0 ± 4.1</td>
</tr>
<tr>
<td>Dosage per cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23</td>
<td>3.8–7.1</td>
<td>516.4 ± 62.2</td>
<td>106 ± 14.2</td>
<td>20.6 ± 2.0</td>
</tr>
<tr>
<td>Intermittent&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32</td>
<td>1.0–2.1</td>
<td>150.4 ± 30.4</td>
<td>30.4 ± 6.3</td>
<td>20.3 ± 1.9</td>
</tr>
<tr>
<td>Daily dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 mg/m&lt;sup&gt;2&lt;/sup&gt;d</td>
<td>23</td>
<td>0.1–0.2</td>
<td>12.1 ± 1.1</td>
<td>2.5 ± 0.3</td>
<td>20.6 ± 2.0</td>
</tr>
<tr>
<td>200 mg/m&lt;sup&gt;2&lt;/sup&gt;d</td>
<td>32</td>
<td>0.2–0.4</td>
<td>30.1 ± 6.1</td>
<td>6.1 ± 1.2</td>
<td>20.3 ± 1.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> AUC, area under the concentration-time curve; CSF, cerebrospinal fluid; TMZ, temozolomide.<n
<sup>b</sup> Continuous study, 75 mg/m<sup>2</sup>/day × 42–49 days. All patients included in the pilot study (n = 23).
<sup>c</sup> Intermittent schedule, 200 mg/m<sup>2</sup>/day × 5 days. All patients included in the recurrent study (n = 12) and all patients received adjuvant therapy (n = 20).
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Sandrine Ostermann, Chantal Csajka, Thierry Buclin, et al.


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