Cancer Chemother Pharmacol (2003) 52: 435–441
DOI 10.1007/s00280-003-0670-4

ORIGINAL ARTICLE

John C. Panetta · Mark N. Kirstein · Amar Gajjar
Geeta Nair · Maryam Fouladi · Richard L. Heideman
Mark Wilkinson · Clinton F. Stewart

Population pharmacokinetics of temozolomide and metabolites in infants and children with primary central nervous system tumors

Received: 27 January 2003 / Accepted: 14 May 2003 / Published online: 16 September 2003

Abstract *Purpose*: To construct a population pharmacokinetic model for temozolomide (TMZ), a novel imidazo-tetrazine methylating agent and its metabolites MTIC and AIC in infants and children with primary central nervous system tumors. *Methods*: We evaluated the pharmacokinetics of TMZ and MTIC in 39 children (20 boys and 19 girls) with 132 pharmacokinetic studies (109 in the training set and 23 in the validation set). The median age was 7.1 years (range 0.7 to 21.9 years). Children received oral TMZ dosages ranging from 145 to 200 mg/m^2 per day for 5 days in each course of therapy. Serial plasma samples were collected after the first and fifth doses of the first and third courses. Approximately eight plasma samples were collected up to 8 h after each dose, and assayed for TMZ, MTIC, and AIC by HPLC with UV detection. A one-compartment model was fitted to the TMZ and metabolite plasma concentrations using maximum likelihood estimation. Covariates, including demographics and biochemical data were tested for their effects on TMZ clearance (CL/F) and MTIC AUC utilizing a two-stage approach via linear mixed-effects modeling. *Results*: The population mean (inter- and intrapatient variability expressed as %CV) for the pharmacokinetic parameters (based on the training set) were as follows: TMZ CL/F 5.4 l/h (53.4, 17.5), Vc/F 14.0 l (48.5, 39.2), C_{max} 9.1 mg/l (20.8, 29.1), and MTIC AUC 1.0 μg/ml·h (13.9, 30.0). Covariate analysis showed that increasing age and body surface area (BSA) were associated with a significant increases in TMZ CL, Vc, and C_{max} ($P < 0.05$), and that increasing age was associated with significant decreases in TMZ and MTIC AUC. Indicators of liver and renal function were not significantly associated with TMZ pharmacokinetics or MTIC AUC. The final model with the significant covariates was validated using the remaining 23 pharmacokinetic studies. *Conclusions*: This study extends previous work done in adults, and identified BSA and age as covariates that account for variability in TMZ disposition in infants and children with primary CNS malignancies.

Keywords TMZ · MTIC · Children · Population pharmacokinetics · Covariates

This work was supported in part by USPHS awards CA 23099, Cancer Center CORE grant CA21765, the Schering Plough Institute, and by the American Lebanese Syrian Associated Charities (ALSAC).

J. C. Panetta · M. N. Kirstein · G. Nair · M. Wilkinson
C. F. Stewart (✉)
Department of Pharmaceutical Sciences,
St. Jude Children's Research Hospital,
332 N. Lauderdale, Memphis,
TN 38105, USA
Tel.: +1-901-4953665
Fax: +1-901-5256869

A. Gajjar · M. Fouladi · R. L. Heideman
Department of Hematology and Oncology,
St. Jude Children's Research Hospital,
Memphis, TN 38105, USA

C. F. Stewart
The Center for Pediatric Pharmacokinetics and Therapeutics,
University of Tennessee, Memphis, Tennessee, USA

Present address: M. N. Kirstein
College of Pharmacy and Cancer Center,
University of Minnesota, Minneapolis,
MN 55455, USA

Introduction

TMZ (3,4-dihydro-3-methyl-4-oxoimidazo-[5,1-d]-1,2,3,5-tetrazin-8-carboxamide) is a relatively novel anticancer methylating agent that is approved for treatment of refractory anaplastic astrocytoma in adults [3, 17]. This highly bioavailable compound undergoes spontaneous base-catalyzed hydrolysis to form the methyl triazene, MTIC (Fig. 1), which is the final active methylating species. MTIC is believed to exert its toxic effects through acid catalysis, forming the methyldiazonium ion, which ultimately leads to the formation of O^6-methylguanine in DNA plus the inactive metabolite, AIC [1, 16, 19].

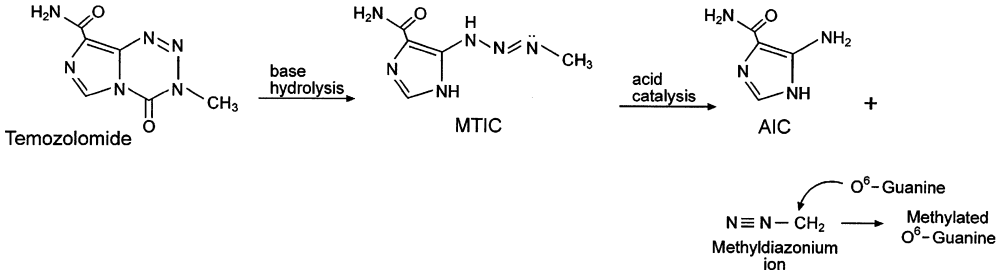

Fig. 1 Schematic of TMZ and pH-dependent conversion to metabolites, MTIC, and AIC, and formation of methylated DNA

Neutropenia and thrombocytopenia are reported as the most common toxicities associated with TMZ administration [8, 14]. In adults, dose-limiting myelosuppression is associated with a significantly greater TMZ systemic exposure as measured in terms of either C_{max} or AUC [12]. However, Jen et al. in a TMZ population analysis observed that neither TMZ dosage nor AUC is predictive of neutrophil or platelet nadir count [13]. While the pharmacodynamic effects of TMZ and MTIC are still not well understood, pharmacokinetic variability may be important for its antitumor effect as well as its toxicity profile. Normalizing the TMZ dose to body surface area (BSA) in adults has been shown to reduce interpatient variability in TMZ clearance [13]. Other covariates that may further reduce variability have not been identified. Moreover, covariates for MTIC and AIC disposition have not been reported.

The disposition of TMZ in children has been investigated in only one study [8]; however, these authors did not report covariate effects on TMZ disposition in their population. Moreover, the dispositions MTIC and AIC in children have not been reported. We conducted a clinical trial of multimodality therapy for children with high-grade glioma, which incorporated 6 months of TMZ. The primary objective of this study was to evaluate the population pharmacokinetics of TMZ and its active metabolite MTIC in children with primary central nervous (CNS) tumors. In this process, we assessed the significance of demographic and serum chemistry covariates on the population pharmacokinetics.

Materials and methods

Patients and treatment

Patients less than 22 years of age with histologically documented CNS tumors were evaluated in prospectively designed phase II studies. The St. Jude Children's Research Hospital IRB reviewed and approved the study, and informed written consent was obtained from the parent/guardian or patient, as appropriate. All patients were evaluated for toxicity. The toxic effects of TMZ were assessed weekly by NCI criteria (version 2.0). Complete blood counts with differentials and serum chemistries were obtained at least twice weekly.

TMZ was generously provided by Schering Plough Research Institute. TMZ was administered either 2 h before or 2 h after a meal at 145 to 200 mg/m^2 once daily for 5 days every 21 days. Premedication with ondansetron was provided before TMZ treatment. Treatment courses were planned for every 21 days after the first daily dose for each course for up to six courses of therapy.

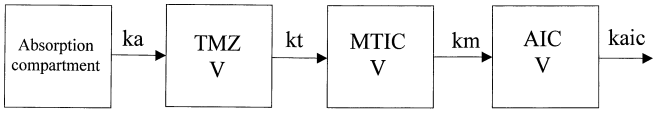

Fig. 2 One-compartment model describing TMZ, MTIC, and AIC. Included are TMZ absorption compartment, absorption rate constant (k_a), TMZ compartment, TMZ elimination rate constant (k_t), MTIC compartment, MTIC elimination rate constant (k_m), AIC compartment, and AIC elimination rate constant (k_{aic})

Pharmacokinetic sampling and analysis

Pharmacokinetic studies for TMZ and metabolites were conducted on days 1 and 5 of courses 1 and 3. Plasma samples were collected before and at 0.25, 0.5, 1, 2, 2.5, 3, 6, and 8 h after the TMZ dose. At each time-point, 3 ml whole blood was collected from a central venous catheter and placed into a lithium heparin tube. Immediately after collection, the blood samples were centrifuged in a refrigerated microcentrifuge for 3 min at 14,600 *g*. The resultant plasma was then divided into aliquots for processing to assay TMZ, MTIC, or AIC. TMZ plasma samples (800 µl) were treated with 80 µl 1.0 *N* HCl, and 200 µl of this acidified plasma was further diluted with 800 µl 0.1 *N* HCl. Ethazolastone was added as an internal standard prior to extraction with ethyl acetate. The organic phase was separated, isolated and dried under a stream of nitrogen. The dried pellet was resuspended in 800 µl mobile phase, 50 m*M* phosphoric acid and 10% MeOH, and 100 µl was injected onto an isocratic high-performance liquid chromatographic (HPLC) column with UV detection [16, 21].

Plasma samples for MTIC (400 µl) were treated with 800 µl cold methanol, stored on ice for 5 min, vortexed, and centrifuged at 7200 *g* for 3 min. A 50-µl aliquot was combined with 60 µl mobile phase consisting of methanol/50 m*M* ammonium phosphate, pH 6.5 (20:80 v/v) and analyzed by HPLC [19]. A third 500-µl aliquot, used for quantifying AIC, was combined with 1000 µl methanol, kept 5 min on wet ice, vortexed, and centrifuged at 14,600 *g* for 3 min. A 60-µl aliquot was combined with 60 µl mobile phase, which consisted of 10% methanol and 25 m*M* phosphoric acid and 5 m*M* 0.075% TEA at pH 2.5, and analyzed by HPLC [19]. These methods were determined to be precise (intraday and interday %CV, 3.9–1.2 for TMZ, 7.9–4.9 for MTIC, and 1.2–3.5 for AIC) at TMZ, MTIC, and AIC concentrations ranging from 0.25 to 40, 0.05 to 2, and 0.1 to 5 µg/ml, respectively.

Structural pharmacokinetic model

A one-compartment model was fitted to the TMZ and metabolite plasma concentrations (Fig. 2) using maximum-likelihood estimation as implemented in ADAPT II [5]. Model parameters that were estimated included the apparent volume of the TMZ compartment (V/F) where F is the bioavailability, elimination rate constants (k_t, k_m, and k_{aic} for TMZ, MTIC, and AIC, respectively), and the absorption rate constant (k_a). Because bioavailability was not identifiable, it was fixed at a value of one [1, 8]. Likewise, the volumes of the metabolite compartments were not identifiable, so

they were fixed to V/F. Standard equations were used to calculate apparent systemic clearance (CL/F) from parameter estimates [9]. The model parameters for each patient were used to simulate the plasma concentration-time profile, from which the area under the plasma concentration-time curve from time zero to infinity ($AUC_{0\rightarrow\infty}$) was calculated using the log-linear trapezoidal method.

Statistical analysis

The population pharmacokinetics were determined using the two-stage approach [22]. First, the pharmacokinetic parameters for each individual were estimated for each of the four courses using the above-described methods. Second, the population pharmacokinetics were analyzed using linear mixed-effects modeling as implemented in S-Plus (S-plus version 6.1; Insightful Corporation, Seattle, Wash.) using the following model:

$$\ln\left(X_{ij}\right) = \theta_1 + \sum_{k=2}^{n} \theta_k \cdot \text{covariate}_k + \eta_i + \varepsilon_{ij}$$

where X_{ij} is the pharmacokinetic parameter of interest (e.g., clearance, V, etc.) for patient i course j, θ_1 is the logarithm of the population mean parameter, θ_k are the coefficients that describe the effects for each covariate, and η and ϵ represent the interpatient and intrapatient (residual) variability, both of which are assumed to have a zero mean. By taking the logarithm of the pharmacokinetic parameter, we assume that the pharmacokinetic parameters are log-normally distributed. Covariate effects (demographics, serum chemistry, TMZ dose, and AUC) were investigated for their ability to significantly improve the model fit [as determined by a reduction of 3.84 ($P<0.05$) in the negative 2 log-likelihood, based on the *F*-test] and for the significance of their corresponding parameter estimates θ_k [as determined by θ_k differing from zero ($P<0.05$), based on the *t*-test].

Validation

The results of the final linear mixed-effects model were validated using the last 23 pharmacokinetic studies, which coincided with the available course 1 day 5, course 3 day 1, and course 3 day 5 studies for the last ten patients. Bias and accuracy were determined as follows [20]:

$$\%Bias = \frac{100\left(X_{actual} - X_{predict}\right)}{X_{actual}}$$

$$\%Accuracy = \frac{100\left|X_{actual} - X_{predict}\right|}{X_{actual}}$$

where X_{actual} represents the actual individual estimated pharmacokinetic parameter of interest and $X_{predict}$ represents the population model-predicted pharmacokinetic parameter of interest.

Results

Characteristics of patients

A total of 39 patients were evaluated for this study. All patients had normal age-adjusted levels of serum creatinine, total bilirubin, serum albumin, and liver function (SGPT and SGOT) (Table 1). The median age was 7.1 years with a range of 0.7 to 21.9 years. Tumor diagnoses were predominantly brainstem glioma ($n=12$), followed by other high-grade glioma ($n=11$), anaplastic astrocytoma ($n=8$), pontine glioma ($n=7$), and medulloblastoma ($n=1$). Most patients had received two courses of irinotecan therapy followed by irradiation therapy prior to TMZ therapy. Most patients were studied after receiving TMZ 200 mg/m^2 ($n=88$), and other dosages evaluated included 180–190 ($n=4$), 160 ($n=16$), 150 ($n=17$), 144–145 ($n=4$), and 140 mg/m^2 ($n=2$).

Table 1 Patient characteristics of the training set

	No. of patients	Median (range)
Gender (M/F)	20/19	
Race		
White	28	
African American	9	
Other	2	
Diagnosis		
Brainstem glioma	12	
Other high-grade glioma	11	
Pontine glioma	7	
Anaplastic astrocytoma	8	
Medulloblastoma	1	
Age (years)		7.1 (0.7–21.9)
BSA (m^2)		1.1 (0.45–2.1)
Baseline hematology		
Hemoglobin (g/dl)		11.7 (8.7–14.9)
WBC (10^9/mm^3)		4.4 (1.9–20.1)
ANC (/mm^3)		2860 (828–17,286)
Platelets (10^9/mm^3)		264 (60–612)
Blood chemistry		
Serum creatinine (mg/dl)		0.5 (0.2–1.0)
Total bilirubin (mg/dl)		0.4 (0.1–10)
Albumin (g/dl)		3.9 (3.1–4.5)
Aspartate amino transferase (U/l)		29.0 (11.0–228)
Alanine amino transferase (U/l)		20.0 (3.0–284)

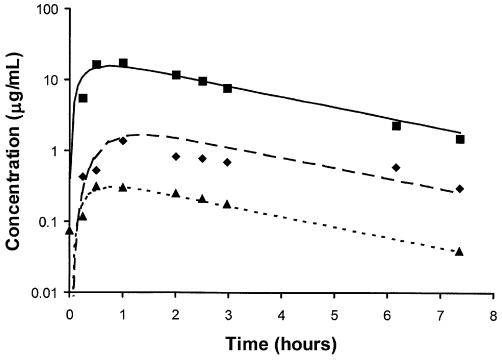

Fig. 3 Representative concentration-time plot depicting TMZ, MTIC, and AIC plasma concentrations for a patient who received TMZ at a dose of 200 mg/m^2. The lines represent the best-fit curves based on model-fit parameters. Individual data points and best-fit lines of the data are shown for TMZ (■), MTIC (▲), and AIC (◆)

Pharmacokinetic parameters

We performed 109 pharmacokinetic studies (approximately 4 per patient) for this population analysis. TMZ and metabolite plasma concentration-time data from a representative patient are depicted in Fig. 3. Table 2 summarizes the population pharmacokinetic parameters

Table 2 Population pharmacokinetics (V_c: TMZ volume of the central compartment, k_e: TMZ elimination rate constant, k_a: TMZ absorption rate constant, AUC: area under the concentration-time curve)

Parameter	Population mean (range)	Interpatient variability (%CV)	Intrapatient variability (%CV)
TMZ CL/F (l/h)	5.4 (1.7–18.7)	53.4	17.5
TMZ CL/F (l/h/m^2)	4.9 (1.6–10.8)	29.4	17.8
V_c/F (l)	14.0 (1.5–50.0)	48.5	39.2
V_c/F (l/m^2)	12.6 (1.5–29.3)	19.3	38.6
Cmax (mg/l)	9.1 (4.3–19.0)	20.8	29.1
Tmax (h)	1.2 (0.24–2.91)	8.8	52.1
k_e (1/h)	0.39 (0.06–2.9)	19.2	37.4
k_a (1/h)	2.4 (0.14–31.6)	1.0	125.0
AUC (μg/ml·h)			
TMZ	37.7 (18.6–120.3)	29.8	17.5
MTIC	1.0 (0.06–2.14)	13.9	30.0
AIC	6.2 (1.4–33.9)	18.1	40.0

(both non-normalized and normalized by BSA) derived from the mixed-effects model for all patients. TMZ was rapidly absorbed with the model-estimated T_{max} ranging from 0.24 to 2.91 h. TMZ C_{max} increased with dose ($P < 0.02$), TMZ AUC ($P < 0.001$), and MTIC AUC ($P < 0.003$). Somewhat unexpectedly, we noted a statistically significant ($P < 0.03$) relationship between TMZ CL/F and TMZ dosage. Reducing the TMZ dose from 200 to 150 mg/m^2 resulted in an average decrease of 17% in TMZ apparent systemic clearance. Among 11 patients whose TMZ doses were reduced from 200 to 150–160 mg/m^2 between courses 1 and 3, 4 patients showed clinically relevant decreases in CL/F (between 40% and 100%), while the rest had differences less than or equal to the population average of 17%. Finally, we investigated whether dose day or course number affected TMZ disposition; in all cases treatment schedule did not significantly affect the results.

Next, we investigated the effects of the demographics and serum chemistry covariates on the population pharmacokinetics. In particular, we tested each of the demographics and blood chemistries listed in Table 1. Of all these covariates tested, only BSA and age (both independently and in combination) significantly explained a portion of the variability of TMZ CL/F, V/F, and C_{max}, but not T_{max}. In particular, increases in both BSA and age were associated with increases in TMZ clearance and volume and decreases in TMZ C_{max} (Table 3). Figure 4 shows the relationship between TMZ clearance and BSA and age. Furthermore, increase in age was associated with decreases in both TMZ and MTIC AUC (Table 3). Figure 5 shows the relationship between actual TMZ clearance and the individual predicted clearance by the full population pharmacokinetic model, which included the covariates BSA and age along with the relationship between the residuals of the predicted clearance and the pharmacokinetic study number.

Model validation

The remaining 23 courses from course 1 day 5, course 3 day 1, and course 3 day 5 of the last ten patients, which where not used in the development of the model, were used to validate the covariate models described in Table 3. The demographics of the patients in this group are given in Table 4. Figure 6 shows the actual versus individual predicted TMZ clearance and AUC for both the training set and the validation set. For both sets of data, the results for TMZ clearance and AUC were not biased ($P > 0.1$, Wilcoxon Signed Rank's Test). The median (quartiles) accuracy for the training set was smaller than for the validation set for TMZ clearance, 9% (5%, 14%) vs 19% (11%, 43%) ($P < 0.001$, Mann Whitney U-test) and TMZ AUC, 9% (5%, 16%) vs 20% (10%, 27%) ($P < 0.002$, Mann Whitney U-test).

Discussion

We report here the first simultaneous pharmacokinetic evaluation of TMZ and its metabolites MTIC and AIC in children with cancer. Using a two-stage approach via linear mixed-effects modeling, we determined the population mean, and interpatient and intrapatient variability for the pharmacokinetic parameters along with TMZ, MTIC, and AIC AUC. Then, we evaluated covariate effects on these parameters. For the demographic and biochemical data tested, we found that age and BSA significantly explained TMZ clearance, volume, and C_{max}, and age significantly explained TMZ and MTIC AUC.

TMZ clearance in our patient population (4.9 l/h/m^2) was similar to that found in the only other published pediatric TMZ pharmacokinetic study (i.e., 4.3 l/h/m^2) [8]. The TMZ clearance values found in adult studies are between 25% and 41% greater then those found in this

Table 3 Covariate analysis

Covariate model[a]	$-\Delta 2LL$[b]	Δ interpatient CV%
LN[TMZ CL/F (l/h)] = 0.42 + 1.08*BSA ($P < 0.0001$)	−45.0 ($P < 0.0001$)	−27.6
LN[TMZ CL/F (l/h/m^2)[c]] = 1.38 + 0.024*Age ($P < 0.02$)	−6.0 ($P < 0.02$)	−3.0
LN[TMZ Vc (l)] = 1.34 + 1.09*BSA ($P < 0.0001$)	−51.8 ($P < 0.0001$)	−33.9
LN[TMZ Vc (l/m^2)][c] = 2.36 + 0.019*Age ($P < 0.05$)	−4.0 ($P < 0.05$)	−0.8
LN[TMZ Cmax (mg/l)] = 2.49−0.24*BSA ($P < 0.02$)	−5.4 ($P < 0.02$)	−3.4
LN[TMZ Cmax (mg/l)] = 2.44−0.026*Age ($P < 0.002$)	−9.8 ($P < 0.002$)	−5.1
LN[TMZ AUC (μg/ml·h)] = 3.9−0.026*Age ($P < 0.007$)	−7.2 ($P < 0.008$)	−3.3
MTIC AUC (μg/ml·h) = 1.12−0.014*Age ($P = 0.05$)	−4.4 ($P < 0.05$)	−1.3

[a] value describes the significance of the covariate parameter
[b] value describes the significance of the change in the negative 2 log-likelihood function from the base model
[c] TMZ PK parameter is normalized to BSA in this case

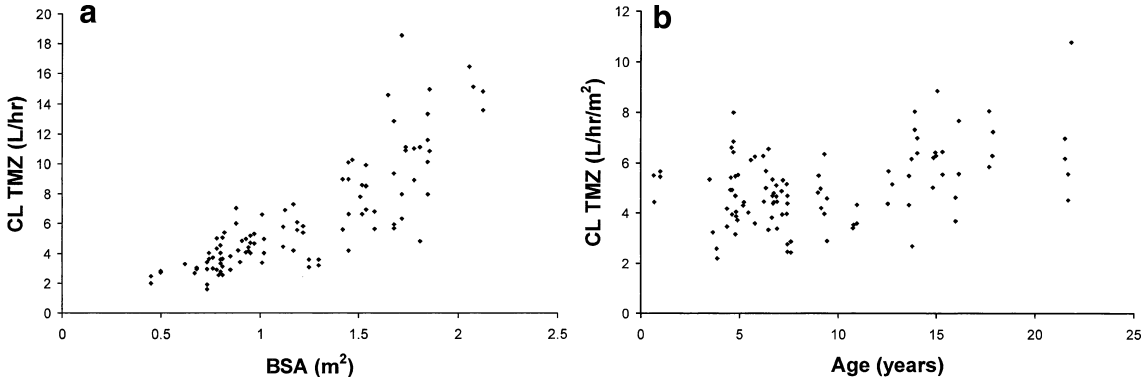

Fig. 4a, b Scatter plots of pharmacokinetic studies depicting (**a**) relationship between BSA and absolute TMZ clearance, and (**b**) relationship between age and absolute TMZ clearance

study [1, 4, 7, 12]. The results of our analysis showed that TMZ clearance increased with age. In particular, in our results the population average increase in TMZ CL from a patient of median age 7 years to maximum age of 21 years was 37%. Therefore, the TMZ clearance observed in the adult studies is remarkably consistent with our results. Baker et al. [1] reported AUC values for MTIC and AIC approximately 40% lower in adults than values observed with similar TMZ dosages in our pediatric population. Our results showed a 20% decrease in the population average MTIC AUC from the median age of 7 years to the maximum age of 21 years, consistent with the observed results in the adult population. When normalized to BSA, our other estimated pharmacokinetic parameters are comparable to those found in adult studies.

These population studies extend previous published work in adults, in which BSA has also been observed as a significant covariate for TMZ clearance [13]. By normalizing TMZ clearance to BSA, the observed interpatient variability was reduced by approximately 45%. Furthermore, owing to the broad age range of our population (range 6 months to 21 years), we were able to demonstrate that age further reduced the interpatient variability by 10%. None of the other covariates which we analyzed was associated with trends in pharmacokinetic parameters. Since this agent undergoes nonenzymatic hydrolysis to form the active and inactive metabolites, it is not surprising that measures of liver function or renal function would not be associated with measures of TMZ or MTIC disposition [1, 12, 19].

Our finding of a relationship between TMZ clearance and BSA supports the practice of dosing TMZ based upon BSA. The practice of dosing anticancer agents based on BSA is intended to reduce interpatient variability in anticancer drug systemic exposure, and presumably variability in pharmacologic response, either antitumor effect or toxicity [10]. While this approach has

Fig. 5a, b Plots depicting (**a**) relationship between measured TMZ absolute clearance and the individual predicted TMZ clearance, based upon the equation derived for the covariates, and the line of identity intersects the origin, and (**b**) plot of the residual error in TMZ absolute clearance vs pharmacokinetic study number

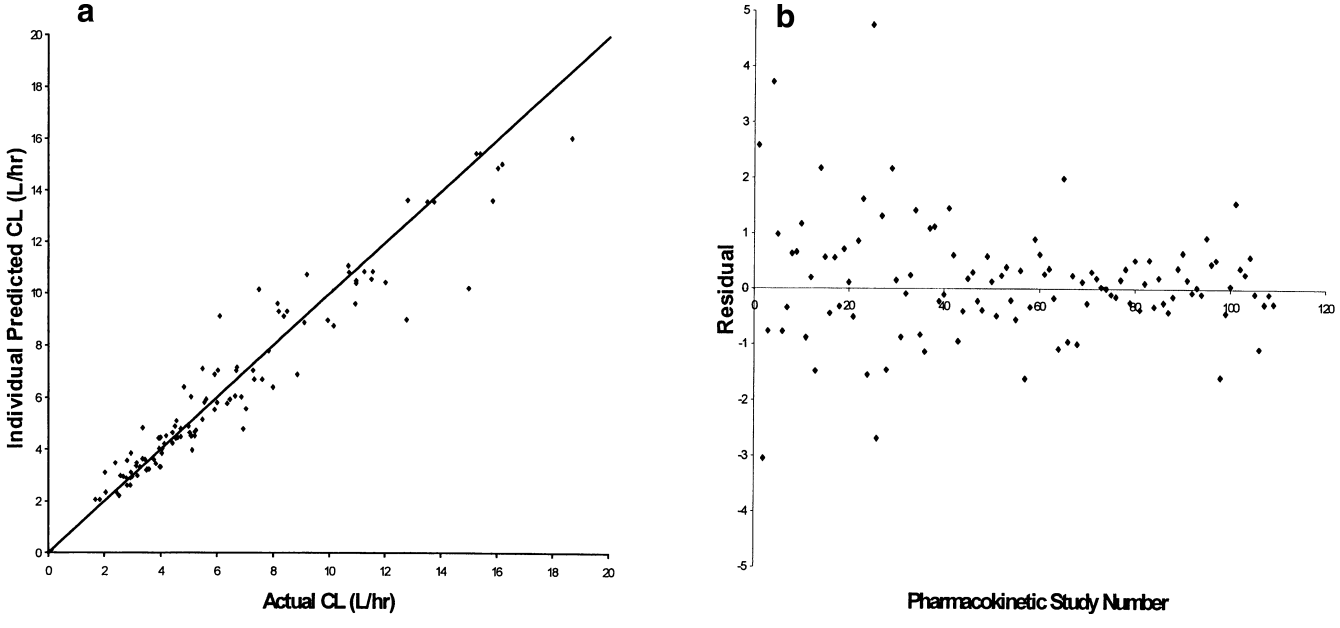

Table 4 Patient characteristics of the validation set

Gender (M/F)	6/4
Age (years)	
Median	6.9
Range	3.5–12.6
Race	
Whites	8
African American	2
Other	0
BSA (m^2)	
Median	1.0
Range	0.6–1.52

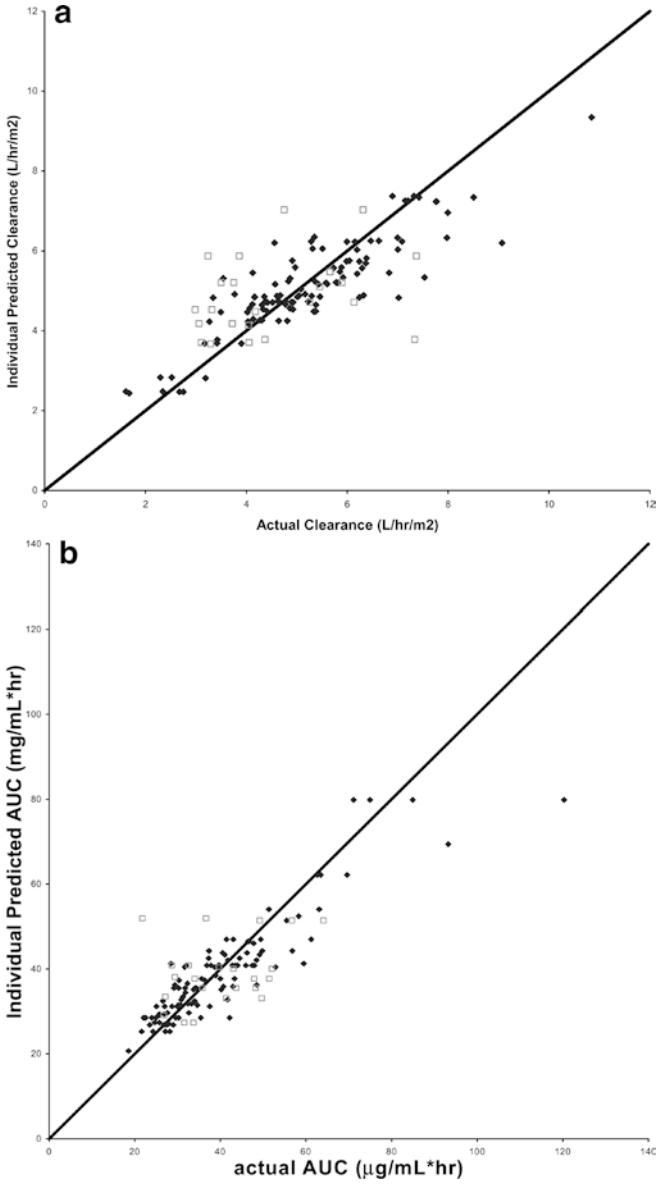

Fig. 6 **a** Relationship between measured TMZ clearance and the individual predicted TMZ clearance based upon the equation derived for the covariates (*solid line* line of identity, ◆ training set, □ validation set). **b** Relationship between measured TMZ AUC and the individual predicted TMZ AUC based upon the equation derived for the covariates (*solid line* line of identity, ◆ training set, □ validation set)

been useful for converting dosages administered to small laboratory animals to those administered to patients enrolled in phase I clinical trials, the overall use of BSA dosing of anticancer drugs for patients has recently undergone scrutiny in the literature [10, 11, 18]. Several recently reported studies in adult patients have demonstrated a lack of rationale for individualized dosing based upon BSA [2, 6, 15]. Based on TMZ clearance values, BSA-based dosing has been reported to reduce variability in TMZ clearance in adult patients [12, 13], and thus these authors recommended individualizing TMZ dosages to patient BSA. Our results for TMZ clearance in children support this approach.

We observed that patients whose TMZ dosages were decreased from 200 to 150 mg/m^2 had approximately a 17% decrease in TMZ systemic clearance; however, this resulted in a clinically relevant decrease in systemic clearance (40% to 100% decrease) in only four patients. We were unable to identify specific patient-related characteristics that would allow us to prospectively identify these patients. This observation of decreased clearance with decreased dosage has potential clinical significance, in that patients with TMZ-associated myelosuppression who also respond to therapy may require a dosage reduction in order to continue on therapy. However, if this reduction does not result in a proportional decrease in systemic exposure to TMZ or MTIC then the toxicities may persist even with the reduced dosage. Therefore, accurate knowledge of the relationship between dosage and clearance can be helpful in determining appropriate dosage reductions to prevent toxicity.

The model was validated using a subset of data that included the last ten patients' latter pharmacokinetic studies not used in the development of the model. This design permitted assessment of model robustness in predicting individual pharmacokinetic parameters (as opposed to population pharmacokinetic parameters). The ability to predict an individual patient's future TMZ systemic exposure is relevant to both toxicity and efficacy studies. Therefore, once the TMZ systemic exposure-effect relationship is defined, a clinician could alter an individual patient's TMZ dosage to presumably increase efficacy or decrease toxicity. Our validation results indicate that, based on the population pharmacokinetic model with BSA and age as covariates, TMZ systemic clearance could be determined without significant bias and a median accuracy of 19%. Thus, this model provides clinicians with a method to a priori estimate TMZ systemic exposure.

In summary, we described the pharmacokinetics for TMZ in infants and children with primary CNS tumors. We analyzed for the effects of covariates, including demographics and serum chemistry on TMZ and metabolite disposition utilizing a two-stage approach via linear mixed-effects modeling. Covariate analysis revealed that increases in age and BSA were associated with significant increases in TMZ clearance, volume and C_{max}. Furthermore, increases in age were also associated

with significant decreases in TMZ and MTIC AUC. The relationship between BSA, age, and TMZ clearance has been observed in other studies of TMZ disposition in adults as well, and has led to the common practice of individualizing TMZ dosages according to BSA.

Acknowledgements The authors would like to thank Lisa Walters, Terri Kuehner, Paula Condy, Margaret Edwards, and Sheri Ring for assistance in obtaining plasma samples, Suzan Hanna for her invaluable technical support in the laboratory, and Kathy Probst for her support with data management.

References

1. Baker SD, Wirth M, Statkevich P, Reidenberg P, Alton K, Sartorius SE, Dugan M, Cutler D, Batra V, Grochow LB, Donehower RC, Rowinsky EK (1999) Absorption, metabolism, and excretion of ^{14}C-temozolomide following oral administration to patients with advanced cancer. Clin Cancer Res 5:309
2. Baker SD, Verweij J, Rowinsky EK, Donehower RC, Schellens JH, Grochow LB, Sparreboom A (2002) Role of body surface area in dosing of investigational anticancer agents in adults, 1991–2001. J Natl Cancer Inst 94:1883
3. Brandes AA, Pasetto LM, Monfardini S (2000) New drugs in recurrent high grade gliomas. Anticancer Res 20:1913
4. Britten CD, Rowinsky EK, Baker SD, Agarwala SS, Eckardt JR, Barrington R, Diab SG, Hammond LA, Johnson T, Villalona-Calero M, Fraass U, Statkevich P, Von Hoff DD, Eckhardt SG (1999) A phase I and pharmacokinetic study of temozolomide and cisplatin in patients with advanced solid malignancies. Clin Cancer Res 5:1629
5. D'Argenio DZ, Schumitzky A (1990) ADAPT II user's guide. Biomedical Simulations Resource, USC, Los Angeles
6. de Jongh FE, Verweij J, Loos WJ, de Wit R, de Jonge MJ, Planting AS, Nooter K, Stoter G, Sparreboom A (2001) Body-surface area-based dosing does not increase accuracy of predicting cisplatin exposure. J Clin Oncol 19:3733
7. Dhodapkar M, Rubin J, Reid JM, Burch PA, Pitot HC, Buckner JC, Ames MM, Suman VJ (1997) Phase I trial of temozolomide (NSC 362856) in patients with advanced cancer. Clin Cancer Res 3:1093
8. Estlin EJ, Lashford L, Ablett S, Price L, Gowing R, Gholkar A, Kohler J, Lewis IJ, Morland B, Pinkerton CR, Stevens MC, Mott M, Stevens R, Newell DR, Walker D, Dicks-Mireaux C, McDowell H, Reidenberg P, Statkevich P, Marco A, Batra V, Dugan M, Pearson AD (1998) Phase I study of temozolomide in paediatric patients with advanced cancer. United Kingdom Children's Cancer Study Group. Br J Cancer 78:652
9. Gibaldi M, Perrier D (1982) Pharmacokinetics. Marcel Dekker, New York
10. Gurney H (1996) Dose calculation of anticancer drugs: a review of the current practice and introduction of an alternative. J Clin Oncol 14:2590
11. Gurney H (2002) How to calculate the dose of chemotherapy. Br J Cancer 86:1297
12. Hammond LA, Eckardt JR, Baker SD, Eckhardt SG, Dugan M, Forral K, Reidenberg P, Statkevich P, Weiss GR, Rinaldi DA, Von Hoff DD, Rowinsky EK (1999) Phase I and pharmacokinetic study of temozolomide on a daily-for-5-days schedule in patients with advanced solid malignancies. J Clin Oncol 17:2604
13. Jen JF, Cutler DL, Pai SM, Batra VK, Affrime MB, Zambas DN, Heft S, Hajian G (2000) Population pharmacokinetics of temozolomide in cancer patients. Pharm Res 17:1284
14. Lashford LS, Thiesse P, Jouvet A, Jaspan T, Couanet D, Griffiths PD, Doz F, Ironside J, Robson K, Hobson R, Dugan M, Pearson AD, Vassal G, Frappaz D (2002) Temozolomide in malignant gliomas of childhood: a United Kingdom Children's Cancer Study Group and French Society for Pediatric Oncology Intergroup study. J Clin Oncol 20:4684
15. Mathijssen RH, Verweij J, de Jonge MJ, Nooter K, Stoter G, Sparreboom A (2002) Impact of body-size measures on irinotecan clearance: alternative dosing recommendations. J Clin Oncol 20:81
16. Newlands ES, Blackledge GRP, Slack JA, Rustin GJS, Smith DB, Stuart NSA, Quarterman CP, Hofman R, Stevens MFG, Brampton MH, Gibson AC (1992) Phase I trial of temozolomide (CCRG 81045: M&B 39831: NSC 362856). Br J Cancer 65:287
17. Prados MD (2000) Future directions in the treatment of malignant gliomas with temozolomide. Semin Oncol 27:41
18. Ratain MJ (1998) Body-surface area as a basis for dosing of anticancer agents: science, myth, or habit? J Clin Oncol 16:2297
19. Reid JM, Stevens DC, Rubin J, Ames MM (1997) Pharmacokinetics of 3-methyl-(triazen-1-yl)imidazole-4-carboximide following the administration of temozolomide to patients with advanced cancer. Clin Cancer Res 3:2393
20. Sheiner LB, Beal SL (1981) Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 9:503
21. Shen F, Decosterd LA, Gander M, Leyvraz S, Biollaz J, Lejeune F (1995) Determination of temozolomide in human plasma and urine by high-performance liquid chromatography after solid-phase extraction. J Liq Chromatogr B 667:291
22. Steimer JL, Mallet A, Golmard JL, Boisvieux JF (1984) Alternative approaches to estimation of population pharmacokinetic parameters: comparison with the nonlinear mixed-effect model. Drug Metab Rev 15:265