Cancer Chemother Pharmacol (2005) 55: 433–438
DOI 10.1007/s00280-004-0896-9

ORIGINAL ARTICLE

**Mark N. Kirstein · John C. Panetta · Amar Gajjar
Geeta Nair · Lisa C. Iacono · Burgess B. Freeman III
Clinton F. Stewart**

Development of a pharmacokinetic limited sampling model for temozolomide and its active metabolite MTIC

Received: 25 February 2004 / Accepted: 1 July 2004 / Published online: 26 January 2005

Abstract *Purpose*: To develop a pharmacokinetic limited sampling model (LSM) for temozolomide and its metabolite MTIC in infants and children. *Methods*: LSMs consisting of either two or four samples were determined using a modification of the D-optimality algorithm. This accounted for prior distribution of temozolomide and MTIC pharmacokinetic parameters based on full pharmacokinetic sampling from 38 patients with 120 pharmacokinetic studies (dosage range 145–200 mg/m^2 per day orally). Accuracy and bias of each LSM were determined relative to the full sampling method. We also assessed the predictive performance of the LSMs using Monte-Carlo simulations. *Results*: The four strategies generated from the D-optimality algorithm were as follows: LSM 1 = 0.25, 1.25, and 3 h; LSM 2 = 0.25, 1.25, and 6 h; LSM 3 = 0.25, 0.5, 1.25, and 3 h; LSM 4 = 0.25, 0.5, 1.25, and 6 h. LSM 2 demonstrated the best combination of low bias [0.1% (−8.9%, 11%) and 11% (4.3%, 15%)] and high accuracy [−1.0% (−12%, 24%) and 14% (7.9%, 37%)] for temozolomide clearance and MTIC AUC, respectively. Furthermore, adding a fourth sample (e.g., LSM 4) did not substantially decrease the bias or increase the accuracy for temozolomide clearance or MTIC AUC. Results from Monte-Carlo simulations also revealed that LSM 2 had the best combination of lowest bias (0.1 ± 6.1% and −0.8 ± 6.5%), and the highest accuracy (4.5 ± 4.1% and 5.0 ± 4.3%) for temozolomide clearance and MTIC apparent clearance, respectively. *Conclusions*: Using data derived from our population analysis, the sampling times for a limited sample pharmacokinetic model for temozolomide and MTIC in children are prior to the temozolomide dose, and 15 min, 1.25 h and 6 h after the dose.

Keywords Temozolomide · MTIC · Children · D-optimal sampling · Monte-Carlo simulation

This work was presented at AACR 2002 Annual Meeting.

M. N. Kirstein · J. C. Panetta · A. Gajjar · G. Nair
L. C. Iacono · B. B. Freeman III · 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

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

A. Gajjar
Department of Hematology and Oncology,
St Jude Children's Research Hospital,
Memphis, TN 38105, USA

Introduction

Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo-[5,1-d]-1,2,3,5-tetrazin-8-carboxamide) is an anticancer methylating agent that is approved for treatment of refractory anaplastic astrocytoma in adults [1, 2]. This highly bioavailable compound undergoes spontaneous base-catalyzed hydrolysis to form the methyl triazene MTIC 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 O^6-methylguanine formation in DNA plus the inactive metabolite, AIC [3–5]. We are currently using temozolomide to treat infants and children with primary central nervous system (CNS) tumors.

While pharmacokinetic studies are frequently reported for adults, similar studies in small children pose many challenges. Numerous blood draws, coupled with volumes required to accurately measure drug concentrations are an ethical consideration in this population. Few published guidelines exist to guide investigators on the safety of maximal blood collection, particularly when separate blood collection is also required for daily clinical management of the patient. Meanwhile, pharmacokinetic disposition of anticancer agents can differ between children and adults, and even among children

of various ages and body sizes; so conducting these studies in children is important for gaining insight into maximizing drug administration in small children [6, 7].

Only two pediatric clinical pharmacology studies of temozolomide have been published. In the first study, Estlin and colleagues described temozolomide disposition in 19 children [8], and they reported rapid oral absorption and elimination, with a linear relationship between temozolomide AUC and dosage. More recently, we reported the population pharmacokinetics for temozolomide, and its active metabolite MTIC, in children with primary CNS tumors [9]. The results of our studies showed that for children, both body surface area and age are important covariates that account for variability in temozolomide disposition. However, after accounting for these covariates, we still observed more than a five-fold interpatient variability for temozolomide clearance in our population. Moreover, in our analysis of MTIC disposition, we found that age was a minor though significant covariate [9].

Neutropenia and thrombocytopenia are the most common toxicities associated with temozolomide administration [8, 10]. In adults, dose-limiting myelosuppression is associated with a statistically greater temozolomide systemic exposure as measured by either C_{max} or AUC [11]. However, in a temozolomide population analysis, Jen and colleagues observed that neither temozolomide dosage nor temozolomide AUC was predictive of neutrophil or platelet nadir count [12]. While the pharmacodynamic effects of temozolomide and MTIC are still not well understood, pharmacokinetic variability may be important for antitumor effect as well as toxicity profile. To predict and improve our understanding of temozolomide-associated myelosuppression, we have recently developed a mathematical model that mechanistically describes the temporal dynamics of this myelosuppression [13]. Thus, studies that assess temozolomide and MTIC disposition in children are warranted to increase our understanding of myelosuppression. The objective of this study was to utilize our population pharmacokinetic data to develop a pharmacokinetic limited sampling model (LSM) to assess temozolomide and MTIC disposition in infants and children.

Patients 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 as described previously [9]. The St Jude Children's Research Hospital IRB reviewed and approved the study, and informed written consent was obtained from each parent/guardian or patient, as appropriate. All patients were evaluated for toxicity. The toxic effects of temozolomide were assessed weekly by NCI criteria (version 2.0). Complete blood counts with differentials and serum chemistries were obtained at least twice weekly.

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

Pharmacokinetic sampling and analysis

Pharmacokinetic studies for temozolomide and metabolites were conducted on days 1 and 5 of courses 1 and 3. Plasma samples were collected before and 0.25, 0.5, 1, 2, 2.5, 3, 6 and 8 h after the temozolomide dose. The median number of samples collected from each individual was eight. At each time point, 3 ml of whole blood was collected from a central venous catheter and placed into a lithium heparin tube. Immediately after collection, the blood sample was centrifuged in a refrigerated microcentrifuge for 3 min at 14,600 *g*. The resultant plasma was then divided into aliquots for processing to assay temozolomide, MTIC, or AIC as described previously [9].

Structural pharmacokinetic model

As described previously [9], we used a two-stage approach [14] to describe the population pharmacokinetics for temozolomide, MTIC, and AIC [14]. Briefly, a one-compartment model was fitted to the temozolomide and metabolite plasma concentrations using maximum-likelihood (ML) estimation as implemented in ADAPT II [15]. The structure of the error model (for the measured samples) used with the ML estimation method was: SD = absolute error + relative error×concentration, where absolute error represents an absolute amount of error in the concentration time point [e.g., related to the lower limit of quantification (LLOQ) of the assay] and the relative error represents the relative amount of error in the concentration–time point (e.g., assay error). For this study we assumed that the relative error was 10% (based on an assay error of $<10\%$) and the absolute error was either 0.1 (related to the LLOQ of TEM, 0.25 μg/ml) or 0.01 (related to the LLOQ of MTIC, 0.05 μg/ml) [9].

Model parameters estimated included the apparent volume of the temozolomide compartment (V/F) where F is the bioavailability, first-order elimination rate constants (k_t, k_m, and k_{aic} for temozolomide, MTIC, and AIC, respectively), and the absorption rate constant (k_a). Standard equations were used to calculate apparent systemic clearance (CL/F) from parameter estimates [16]. Due to a lack of parameter identifiability, the volume estimates for MTIC and AIC were fixed at V/F. The

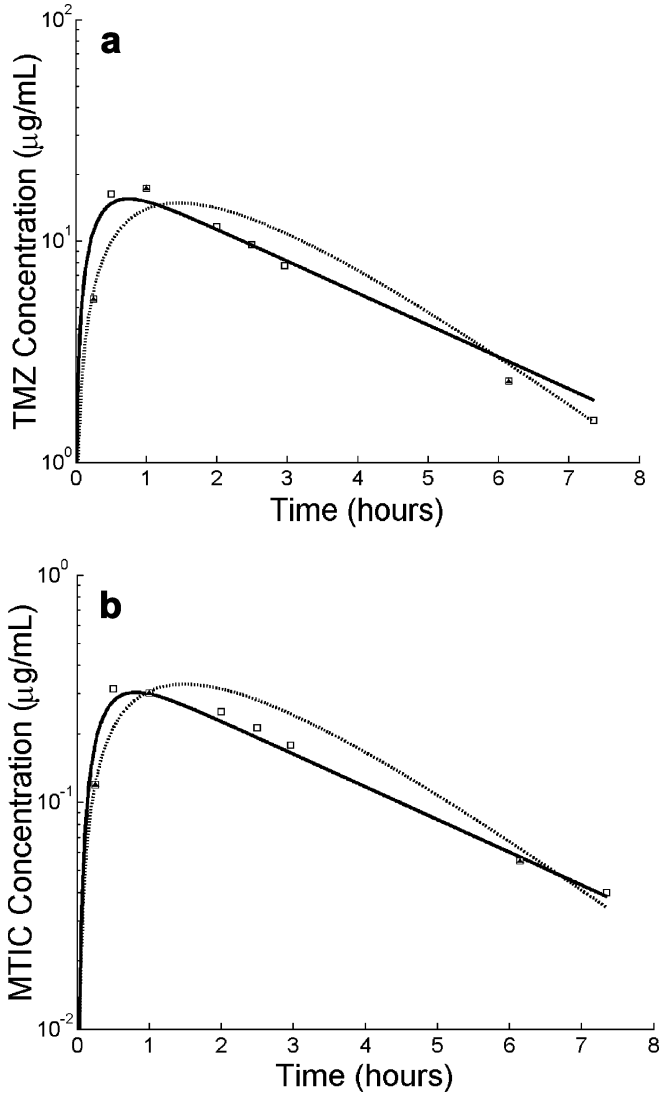

Fig. 1 Concentration vs time plots for temozolomide and MTIC for a representative patient. The *open squares* and *solid line* represent the extensive sampling time-points and best-fit line, respectively. The *filled squares* and *dashed line* represent the limited sampling time-points (LSM 2) and best-fit line, respectively

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 ($AUC_{0 \to \infty}$) was calculated using the log-linear trapezoidal method.

Selection and evaluation of LSMs

Sampling time points were selected using a variation of the D-optimality algorithm, which includes prior distribution of the pharmacokinetic parameters to assist in the determination of optimal sampling times [17, 18]. Since the observed half-life for temozolomide was approximately 2 h, and MTIC concentrations were near the LLOQ at 8 h, a maximum sample time constraint of 8 h after administration was used.

Two methods were used to evaluate the accuracy and bias for the LSMs: (1) comparison of results from the full sample set to LSMs using actual patient data, and (2) Monte-Carlo simulation. In the case of the clinical samples, the pharmacokinetic parameters determined using all available data were compared to the parameters determined using subsets of data most closely related to the LSM time-points. The limited sampling parameters were estimated using maximum a posteriori probability estimation (MAP) as implemented in ADAPT II.

For the Monte-Carlo simulation we generated 1000 data sets based on the distribution of the pharmacokinetic parameters derived from the full sampling scheme (eight samples) described in this paper. We estimated the pharmacokinetic parameters of these data using the maximum likelihood method in ADAPT II. This set of parameters is referred to as the "full" set (i.e., the fit that we compared to the various LSMs). Using the full set of simulated pharmacokinetic parameters, we then generated data sets for each of the LSMs described above and fitted each using MAP Bayesian with population priors from our previously published temozolomide pharmacokinetic study [9]. We then determined the bias and accuracy for each LSM using the following measures [19].

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

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

where X_{actual} represents the actual individual pharmacokinetic parameter of interest based on the full sampling scheme (or the simulated parameter in the case of the Monte-Carlo simulation) and X_{predict} represents the individual predicted pharmacokinetic parameter of interest based on the LSM.

Table 1 Bias and accuracy for clinical samples expressed as medians (quartiles)

	Temozolomide CL (l/h/m^2)		MTIC AUC (μg/ml h)	
	Bias (%)	Accuracy (%)	Bias (%)	Accuracy (%)
LSM 1	−1.0 (−23, 11)	15 (6.6, 32)	6.6 (−11, 64)	19 (12, 68)
LSM 2	0.1 (−8.9, 11)	11 (4.3, 15)	−1.0 (−12, 24)	14 (7.9, 37)
LSM 3	−8.9 (−25, 1.9)	15 (7.0, 28)	10 (−3.5, 96)	22 (13, 96)
LSM 4	−0.3 (−9.4, 7.2)	9.1 (4.7, 15)	1.6 (−18, 21)	18 (8.8, 37)

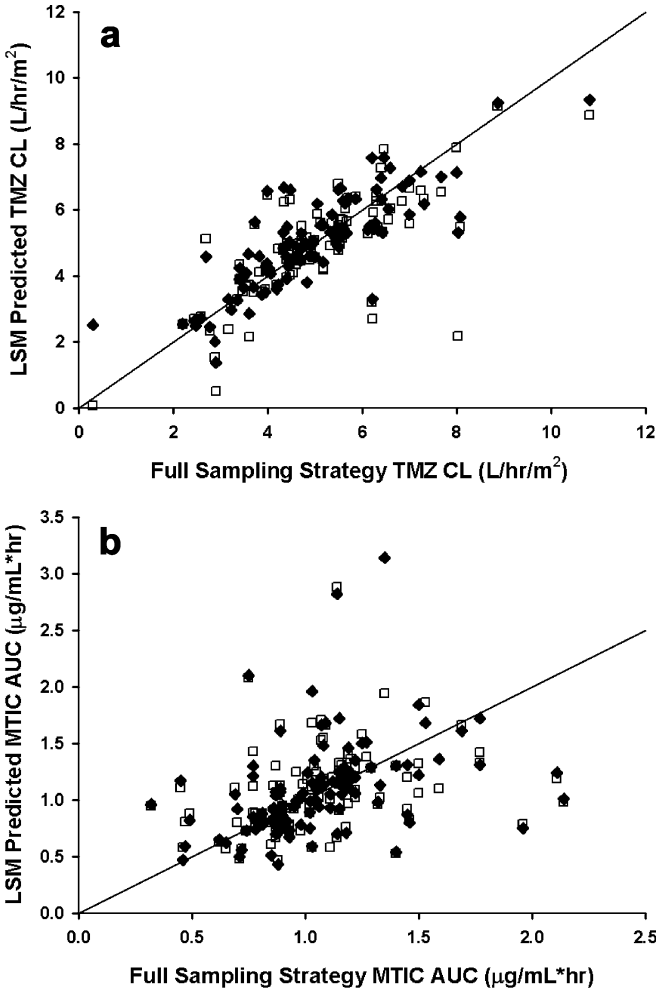

Fig. 2 Scatter plots of extensive plasma sampling versus LSM 2 (*filled diamonds*) and LSM 4 (*open squares*) depicting (**a**) temozolomide clearance and (**b**) MTIC AUC along the line of identity

Results

The pharmacokinetics of temozolomide and MTIC were evaluated in 38 children with 120 pharmacokinetic studies using the full sampling scheme. The distribution of the pharmacokinetic parameters from this population was used in the D-optimality algorithm to generate four LSMs, two with three samples and two with four samples, for further consideration. These LSMs were: LSM 1 = 0.25, 1.25, and 3 h; LSM 2 = 0.25, 1.25, and 6 h; LSM 3 = 0.25, 0.5, 1.25, and 3 h; and LSM 4 = 0.25, 0.5, 1.25, and 6 h. LSM 1 and LSM 3 assumed a larger absolute error (0.1), thus the last plasma sample was at 6 h to prevent sampling below the LLOQ of temozolomide or MTIC. LSM 2 and LSM 4 assumed a smaller absolute error (0.01), which allowed for later sampling where temozolomide was still above its LLOQ, but MTIC was not. In particular, with the larger absolute error model, all of the temozolomide samples and 97% of the MTIC samples were above their respective LLOQ at the end of sampling (3 h). However, with the smaller absolute error model all of the temozolomide samples and 42% of the MTIC samples were above their respective LLOQ at the end of sampling (6 h).

To compare our LSM with the extensive sampling data sets, we replaced the 1.25 h sample with the 1 h sample, since one was not drawn at 1.25 h. Figure 1 shows temozolomide and MTIC concentration vs time plots for a representative patient. The two concentration–time plots (temozolomide and MITC) contain both the full sampling strategy and present LSM 2 time-points, together with the respective model fit to these data. Table 1 shows the ability of each model to predict the pharmacokinetic parameters based on the extensive data set. Using the 6-h sample (i.e., LSM 2 and 4) as opposed to the 3-h sample (LSM 1 and 3) decreased the bias and increased the accuracy for predicting temozolomide clearance and MTIC AUC. Furthermore, at best, minor differences were observed when a fourth sample was added to LSM 2 (i.e., LSM 4). Figure 2 shows a plot of predicted vs full sampling strategy for temozolomide clearance and MTIC AUC, using LSM 2 and LSM 4. Visual inspection of these plots shows that removal of the 30-min sample from LSM 4 did not affect the outcome predicted with LSM 2. Thus, LSM 2 was the most appropriate of these four models tested.

The predictive performance of the LSMs was also assessed by Monte-Carlo simulations ($n = 1000$) based on the pharmacokinetic parameter distribution from full sampling in the studied patients [9]. Bias and accuracy are given in Table 2. Figure 3 shows the predicted (i.e., from LSM) versus actual (i.e., from full sampling) temozolomide and MTIC apparent CL from the Monte-Carlo simulation. As with the clinical samples, this simulation showed that LSM 2 and 4, which included the 6-h sample are more accurate than LSM 1 and 3. This simulation also showed that adding the fourth sample (LSM 4) added little to the accuracy and bias compared to the method with just three samples (LSM 2). Thus, the results support our preceding conclusion that LSM 2 was the most appropriate pharmacokinetic sampling method of the four models tested.

Next we tested the effects of small changes in the sampling scheme on the bias and accuracy of the results. In particular, although all the LSM suggested a 1.25-h sample, our full sampling data did not include this time-point, so we instead used the 1-h sample for comparison purposes. To determine the effects of this difference, we

Table 2 Monte-Carlo simulation bias and accuracy mean (SD) percent for temozolomide and MTIC apparent CL

	Bias		Accuracy	
	Temozolomide CL (l/h/m^2)	MTIC CL (l/h/m^2)	Temozolomide CL (l/h/m^2)	MTIC CL (l/h/m^2)
LSM 1	−0.4 (8.9)	−0.5 (8.0)	6.7 (5.9)	5.9 (5.4)
LSM 2	−0.3 (6.1)	−0.8 (6.5)	4.5 (4.1)	5.0 (4.3)
LSM 3	−0.4 (7.5)	−0.4 (6.3)	5.8 (4.8)	4.8 (4.2)
LSM 4	−0.6 (5.5)	−0.9 (5.8)	4.0 (3.9)	4.2 (4.0)

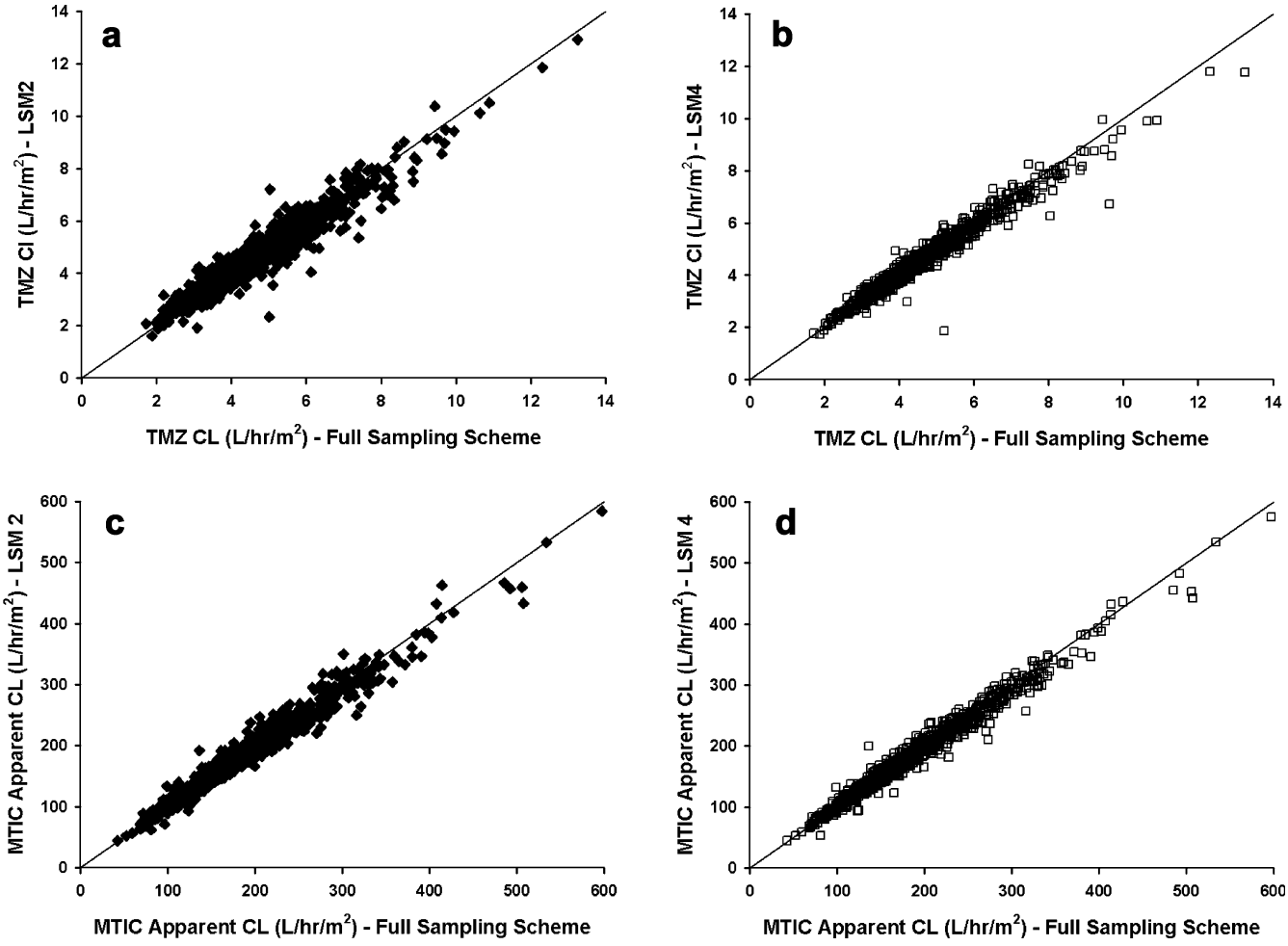

Fig. 3 Monte-Carlo simulation scatter plots of full vs limited sampling schemes for (**a**) temozolomide clearance with LSM 2, (**b**) temozolomide clearance with LSM 4, (**c**) MTIC apparent clearance with LSM 2, and (**d**) MTIC apparent clearance with LSM 4

simulated the concentration at 1.25 h in each patient course (based on each patient's pharmacokinetics from full sampling for that course), combined the result at this time-point with the results from 0.25 h to 6 h, estimated the parameters, and compared them with the results of the sampling which included the 1-h sample. In a similar manner we compared the effects of sampling at 5 h instead of 6 h. In both cases the change in the median accuracy and bias was not significant ($\leq$ 1% difference).

Discussion

This report describes the first LSM for pharmacokinetic evaluation of temozolomide and MTIC in children with cancer. We have previously reported population pharmacokinetic parameters for temozolomide and its metabolites [9], and we now describe an approach for selecting optimal sampling time-points using the D-optimality algorithm modified to account for the prior distribution of the parameters [7]. From the different sampling models evaluated, we found that plasma sampling prior to the temozolomide dose, and at 15 min, 1 h and 6 h yielded an accurate description of temozolomide and MTIC disposition in children. Although using an LSM with a 3-h time-point might be preferable from a logistical perspective, our analysis showed that an LSM with a 6-h time-point was less biased and more accurate than one with a 3-h time-point (see Table 1).

Another important aspect of the proposed LSM is that it was robust to small changes in the sampling times. In particular, if either the last sample was collected at 5 h instead of 6 h, or the middle sample was collected at 1 h instead of 1.25 h, no significant differences were observed in the median bias or accuracy (results differed by 1% or less).

Even though we set a time constraint of 8 h, we found that 6 h was the latest time point that allowed accurate assessment of temozolomide and MTIC disposition. Frequently, the 8-h time-point in the full sampling scheme resulted in measurements for MTIC concentrations below our LLOQ. Thus, sampling earlier allowed us to measure both temozolomide and MTIC. Accurate measurement of these compounds also requires immediate processing of the patient samples, since the drug and metabolites are unstable in unprocessed plasma, even when stored at −80°C. Since measurement of each

compound requires a separate assay, each plasma sample must be divided before storage. Consequently, collection and processing of six samples within the first 2–3 h can place a significant burden on technical support to prepare the samples in a correct and timely manner, especially when studying more than one patient at a time. Reduction of time-points decreases the likelihood for technical error, and increases the possibility of a successful pharmacokinetic study.

In summary, we developed a pharmacokinetic LSM for estimating temozolomide and MTIC plasma pharmacokinetics in children with primary CNS tumors. The present LSM allows the calculation of temozolomide K_a, V/F, clearance, and MTIC AUC by using a reduced number of plasma samples with respect to the previously described extensive sampling approach. We are using this LSM in our ongoing studies of temozolomide disposition in children with cancer to better define the relationship between drug effect (e.g., antitumor efficacy and toxicity) and temozolomide and MTIC exposure. Moreover, the relationship between temozolomide and MTIC exposure and associated molecular effects (e.g., MGMT inhibition, mismatch repair status) are being examined in ongoing studies. Application of a pharmacokinetic LSM for temozolomide and MTIC such as the one presented here will enhance the conduct of those studies.

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. This work was supported in part by USPHS awards CA23099 and Cancer Center CORE grant CA21765, the Schering Plough Institute, and the American Lebanese Syrian Associated Charities (ALSAC).

References

1. Brandes AA, Pasetto LM, Monfardini S (2000) New drugs in recurrent high grade gliomas. Anticancer Res 20:1913
2. Prados MD (2000) Future directions in the treatment of malignant gliomas with temozolomide. Semin Oncol 27:41
3. 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
4. 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
5. 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
6. Reed MD (1999) Optimal sampling theory: an overview of its application to pharmacokinetic studies in infants and children. Pediatrics 104:627
7. Panetta JC, Iacono LC, Adamson PC, Stewart CF (2003) The importance of pharmacokinetic limited sampling models for childhood cancer drug development. Clin Cancer Res 9:5068
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. Panetta JC, Kirstein MN, Gajjar A, Nair G, Fouladi M, Heideman RL, Wilkinson M, Stewart CF (2003) Population pharmacokinetics of temozolomide and metabolites in infants and children with primary central nervous system tumors. Cancer Chemother Pharmacol 52:435
10. 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
11. 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
12. 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
13. Panetta JC, Kirstein MN, Gajjar AJ, Nair G, Fouladi M, Stewart CF (2003) A mechanistic mathematical model of temozolomide myelosuppression in children with high-grade gliomas. Math Biosci 186:29
14. 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
15. D'Argenio DZ, Schumitzky A (1990) ADAPT II user's guide. Biomedical Simulation Resource, University of Southern California, Los Angeles
16. Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Dekker, New York
17. D'Argenio DZ (1981) Optimal sampling times for pharmacokinetic experiments. J Pharmacokinet Biopharm 9:739
18. Panetta JC, Wilkinson M, Pui C-H, Relling MV (2002) Limited and optimal sampling strategies for etoposide and etoposide catechol in children with leukemia. J Pharmacokinet Pharmacodyn 29:171
19. Sheiner LB, Beal SL (1981) Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 9:503