

The Neuropharmacokinetics of Temozolomide in Patients with Resectable Brain Tumors: Potential Implications for the Current Approach to Chemoradiation

Jana Portnow,¹ Behnam Badie,² Mike Chen,² An Liu,³ Suzette Blanchard,⁴ and Timothy W. Synold⁵

Abstract **Purpose:** Intracerebral microdialysis (ICMD) is an accepted method for monitoring changes in neurochemistry from acute brain injury. The goal of this pilot study was to determine the feasibility of using ICMD to examine the neuropharmacokinetics of temozolomide in brain interstitium following oral administration. **Experimental Design:** Patients with primary or metastatic brain tumors had a microdialysis catheter placed in peritumoral brain tissue at the time of surgical debulking. Computerized tomography scan confirmed the catheter location. Patients received a single oral dose of temozolomide (150 mg/m²) on the first postoperative day, serial plasma and ICMD samples were collected over 24 hours, and temozolomide concentrations were determined by tandem mass spectrometry. **Results:** Nine patients were enrolled. Dialysate and plasma samples were successfully collected from seven of the nine patients. The mean temozolomide areas under the concentration-time curve (AUC) in plasma and brain interstitium were 17.1 and 2.7 µg/mL × hour, with an average brain interstitium/plasma AUC ratio of 17.8%. The mean peak temozolomide concentration in the brain was 0.6 ± 0.3 µg/mL, and the mean time to reach peak level in brain was 2.0 ± 0.8 hours. **Conclusions:** The use of ICMD to measure the neuropharmacokinetics of systemically administered chemotherapy is safe and feasible. Concentrations of temozolomide in brain interstitium obtained by ICMD are consistent with published data obtained in a preclinical ICMD model, as well as from clinical studies of cerebrospinal fluid. However, the delayed time required to achieve maximum temozolomide concentrations in brain suggests that current chemoradiation regimens may be improved by administering temozolomide 2 to 3 hours before radiation. (Clin Cancer Res 2009;15(22):7092–8)

The treatment of malignant brain tumors continues to challenge clinicians and scientists alike. A major obstacle to successful pharmacologic management of central nervous system (CNS) tumors is the presence of the blood-brain barrier (BBB), which prevents most anticancer agents from entering the CNS. As a result of the ever-expanding list of new targeted

agents, and given the pharmacologic limitations associated with the BBB, there exists a significant need for tools that will allow assessment of a new drug's CNS biodistribution prior to applying that drug for brain tumor therapy.

Microdialysis is a technique for continuously sampling the concentration of a drug or biomolecule in the extracellular fluid (ECF) of body tissues, without significantly disturbing tissue function. This technique consists of implanting into a body tissue a catheter that contains a semipermeable membrane at the end. The dialysis membrane acts as an artificial capillary, so that when perfusion fluid is pumped through the microdialysis catheter, diffusion of molecules occurs down their concentration gradients as the ECF equilibrates with the perfusion fluid. The dialysate, or solution that exits the probe, is then collected at regular intervals for analysis. The fraction of drug that is recovered in the dialysate is an indirect measurement of the free drug concentration in the interstitium.

A microdialysis catheter (CMA Microdialysis) with a molecular weight cutoff membrane of 20,000 Da, has received Food and Drug Administration (510 k) clearance for intracerebral use in humans. This catheter is smaller in caliber than that typically used for doing ventriculostomies and monitoring

Authors' Affiliations: ¹Department of Medical Oncology and Experimental Therapeutics, ²Division of Neurosurgery, ³Departments of Radiation Physics, ⁴Information Sciences, and ⁵Clinical and Molecular Pharmacology, City of Hope/Beckman Research Institute, Duarte, California Received 5/27/09; revised 8/27/09; accepted 8/27/09; published OnlineFirst 10/27/09.

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Requests for reprints: Jana Portnow, Department of Medical Oncology and Experimental Therapeutics, 1500 East Duarte Road, Duarte, CA 91010. Phone: 626-256-4673; Fax: 626-301-8233; E-mail: jportnow@coh.org.

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Translational Relevance

Microdialysis is a technique for continuously sampling the concentration of a drug or biomolecule in a body tissue without significantly disturbing the function of the tissue. The fraction of drug that is recovered in the dialysate is an indirect measurement of the free drug concentration in the interstitium. We applied intracerebral microdialysis to study the neuropharmacokinetics of temozolomide. Our findings have potential implications for the timing of temozolomide administration relative to daily brain radiation treatment and show the value of intracerebral microdialysis as a research tool for use in the early development of appropriate new drugs to be tested in brain tumor clinical trials.

intracranial pressure. With the use of image guidance, the catheter can be safely and accurately placed in brain interstitium. A gold filament at the catheter tip is visible on computerized tomography (CT) scan, enabling confirmation of correct positioning of the catheter with a noncontrast CT scan of the brain. Collection of interstitial fluid samples can be done while patients are awake and mobile.

Clinically, the technique of intracerebral microdialysis (ICMD) has mainly been applied to the study of head trauma (1–4), subarachnoid hemorrhage (5–7), and epilepsy (8). In these settings, microdialysis catheters are typically used to monitor changes in biochemical markers, such as glucose, lactate, and glutamate, to evaluate the effects or detect possible complications of a therapeutic intervention. ICMD is also a suitable method for conducting neuropharmacokinetic studies (9–11) of potential chemotherapy agents for the treatment of brain tumors, because microdialysis catheters can serially sample free drug concentrations in the peritumoral cerebral cortex or within the brain tumor itself (12, 13).

Temozolomide is an orally bioavailable alkylating agent that is converted to its active metabolite 5-(3-methyl triazen-1-yl)imidazole-4-carboxamide (MTIC) in a spontaneous process that does not require hepatic activation. Maximum plasma temozolomide concentrations occur within 30 to 90 minutes following an oral dose (14), and drug levels in the cerebrospinal fluid (CSF) have been reported to be approximately 20% of those measured in the systemic circulation (15). In addition to its activity as a single agent, temozolomide is a potent radiosensitizer and a key component of chemoradiation therapy for patients with newly diagnosed glioblastoma (16).

In this pilot feasibility study, we applied ICMD for determining the neuropharmacokinetics of temozolomide. Although the primary end point of this trial was safety and feasibility, a major secondary objective was to assess the difference in both the magnitude and time course of drug levels measured in brain interstitium versus systemic circulation.

Materials and Methods

Study subjects. To be eligible to participate in this pilot feasibility study, patients had to be at least 18 y of age and to have either a primary or metastatic brain tumor for which temozolomide would be

an appropriate treatment postoperatively. Patients had to be in need of a surgical debulking or a stereotactic brain biopsy for the purpose of diagnosis or differentiating between tumor progression versus treatment-induced effects following radiation therapy and/or chemotherapy. Other conditions required for study participation included: (a) Karnofsky performance status (KPS) $\geq 60\%$; (b) recovery from toxicity of any prior therapy; (c) adequate bone marrow function (absolute neutrophil count $\geq 1,500$ cells/mm³, platelet count $\geq 100,000$ cells/mm³); (d) adequate hepatic function (total bilirubin ≤ 2.0 mg/dL, serum levels of aspartate aminotransferase $\leq 4\times$ the institutional upper limit of normal); (e) adequate renal function (serum creatinine ≤ 1.5 mg/dL); and (f) a Mini Mental Status Exam score ≥ 15 . Patients were excluded from participating in this study if they (a) were receiving chemotherapy or radiation therapy or were enrolled in another clinical trial; (b) were allergic to temozolomide; (c) were pregnant or breastfeeding; or (d) had a serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to the protocol. All participating patients gave written informed consent. The clinical protocol and the informed consent document were approved by the City of Hope Institutional Review Board.

Study design. Eligible brain tumor patients were asked to participate in this nontherapeutic study at the time plans were being made for them to undergo a debulking craniotomy or stereotactic brain biopsy. If the frozen section indicated the presence of viable tumor, the study neurosurgeon inserted a CMA 70 Microdialysis Brain Catheter (membrane length, 10 mm; shaft length, 100 mm; ref. no. P000050, CMA) into residual tumor or peritumoral brain interstitium, within 5 mm of the resection cavity. After a postoperative noncontrast CT scan of the brain confirmed proper placement of the intracerebral catheter, the inlet tubing of the catheter was connected to a portable syringe pump (CMA 107 Microdialysis Pump, ref. no. P000127, CMA), which perfused the catheter with artificial CSF (Perfusion Fluid CNS, ref. no. P000151, CMA) at a rate of 1 μ L/min.

At least 24 h after the surgery, when patients were alert and tolerating oral intake, they were given a single dose of temozolomide (150 mg/m²) orally. To determine the neuropharmacokinetic profile of temozolomide, dialysate samples were collected continuously during the next 24 h. The microvial at the end of the catheter's outlet tubing was changed to a new one every 30 min during the first 2 h, and then every 60 min for the remainder of the 24-h collection period. In order to stabilize temozolomide, 6 μ L of acetic acid (HOAc) were added to each microvial prior to use. Blood samples for defining the plasma concentration-time profiles of temozolomide were obtained prior to the dose of temozolomide, then 30, 60, and 90 min, and 2, 3, 4, 8, and 24 h after the dose of temozolomide was taken by the patient. The samples of blood were collected in tubes containing 12 μ L of hydrochloric acid (HCl). After all of the dialysate samples were collected, the microdialysis catheter was removed at the bedside, and patients were discharged home when medically ready.

Analytical method for determining temozolomide concentrations in plasma and dialysate. High performance liquid chromatography grade acetonitrile (ACN) and methanol (MeOH) were purchased from Fisher Scientific. American Chemical Society grade formic acid (FA), HCl, and HOAc were purchased from J.T.Baker. Water was purified using the Millipore Milli-Q system (Milford). Caffeine (trimethyl-13C₃, 99%) was purchased from Cambridge Isotope Laboratories, Inc., and was used as an internal standard (IS). Temozolomide was kindly provided by Schering-Plough.

Concentrations of temozolomide were determined in plasma and dialysate. For plasma analysis, 50 μ L of 2.5 mol/L HCl were added per mL of plasma. Standard solutions containing temozolomide over the range of 0.1 to 10 μ g/mL were prepared in blank plasma with HCl. High and low controls were prepared at 7 μ g/mL and 0.2 μ g/mL, respectively. The IS was diluted in MeOH to 2 μ g/mL. A 20 μ L aliquot of plasma sample, standard, or control was added to 80 μ L MeOH with IS and vortex mixed for 10 s then centrifuged at 14,000 rpm for 5 min at 4°C. A 20 μ L aliquot of the supernatant was diluted with 300 μ L 0.5% HOAc.

The diluted supernatant was transferred to an assay vial, and 20 μ L were injected per assay. For dialysate analysis, 2 μ L of 6% HOAc were added per 10 μ L fluid. In order to measure concentrations of temozolomide over the range of 5 to 1,500 ng/mL, working standard solutions were prepared over the range of 0.5 to 150 ng/mL in 0.5% HOAc. High and low control solutions were prepared at 120 ng/mL and 1 ng/mL, respectively. The IS was prepared at a concentration of 500 ng/mL in MeOH/0.5% HOAc (50:50). A 12 μ L aliquot of blank perfusion fluid (or sample) with HOAc was mixed with 10 μ L IS solution and 100 μ L working standard or control solution (or 0.5% HOAc for sample) and transferred to the assay vial. Twenty microliters were injected per assay.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis was done using an Agilent Technologies LC 1100 series system interfaced with a Micromass Quattro Ultima Triple Quadrupole Mass Spectrometer (Micromass, Inc.). High performance liquid chromatography separation was achieved using a Prodigy 5 micron 250 \times 2.0 mm analytical column (Phenomenex). The auto-injector temperature was maintained at 5 $^{\circ}$ C and the column temperature at 25 $^{\circ}$ C. An isocratic mobile phase of 13.8% ACN, 0.1% FA in water was used to elute the analytes from the column at a flow rate of 0.2 mL/min. Total run time was 12 min. The electrospray ionization source of the mass spectrometer was operated in positive ion mode with a cone gas flow of 190 L/h and a desolvation gas flow of 550 L/h. The capillary voltage was set to 1 kV. The cone voltage was optimized at 10 V for both temozolomide and IS. The collision cell energy was 10 eV for temozolomide and 21 eV for IS. The source temperature was 125 $^{\circ}$ C and the desolvation temperature was 350 $^{\circ}$ C. A solvent delay program was used from 0 to 3 min and from 10 to 12 min to minimize the mobile phase flow to the source. MassLynx version 4.1 software was used for data acquisition and processing.

Positive electrospray ionization of temozolomide and IS produced abundant protonated molecular ions (MH⁺). The fragmentation of these compounds was induced under collision-induced dissociation conditions and acidic mobile phase. The precursor \rightarrow product ion combinations at m/z 194.89 \rightarrow 137.75 for temozolomide and 197.98 \rightarrow 139.82 for IS were used in multiple reaction monitoring mode to determine these compounds. The use of multiple reaction monitoring provided sufficient specificity and sensitivity. LC-MS/MS experimental conditions, such as collision energy and collision cell pressure, were optimized from continuous flow injection sample introduction of standard solutions. Under optimized assay conditions, the retention time was 4.8 min for temozolomide and 8.3 min for IS.

In vitro determination of the fractional recovery of temozolomide by the CMA 70 microdialysis catheter. In preparation for the clinical microdialysis study, an *in vitro* assessment was done to estimate the recovery of temozolomide at a given flow rate and length of catheter. Slower perfusion rates produce a higher fractional recovery (i.e., a concentration of drug in the dialysate that is close to the true interstitial drug concentration), but a longer sample collection interval is needed. The *in vitro* fractional recovery of temozolomide is used as a correction factor for the *in vivo* results later.

A CMA 70 Microdialysis Brain Catheter was placed into a reservoir containing a 1 μ g/mL solution of temozolomide in artificial CSF. A CMA 107 Microdialysis Pump perfused the catheter with artificial CSF. Serial dialysate samples were collected at different flow rates (0.5–5 μ L/min).

Pharmacokinetic analysis. Pharmacokinetic parameters were determined using the measured plasma and microdialysis temozolomide concentration versus time data for each individual using noncompartmental methods. The collection times assigned to the concentration for the vials of dialysate from brain interstitium were determined by subtracting half the measurement time plus 5 min (allowing travel time through the tubing into the vial) from the time of ingestion of temozolomide. The maximum concentration (C_{max}) and time of maximum concentration (T_{max}) were determined directly from the measured data. The area under the concentration-time curve (AUC) was determined using the rule of linear trapezoids extrapolated to infinity using the

elimination rate constant (K_{el}) derived from the last four measured concentrations. Half-lives (t_{1/2}) were calculated from the elimination rate constant derived from the last four measured concentrations.

The primary objective of the study was to determine the feasibility of using the microdialysis technique to assess the distribution of an anti-cancer drug in the brain. The objective measures of feasibility included incidence rates of clinically symptomatic intracerebral hemorrhage, CNS infection, and catheter malfunction. All toxicities were recorded and summarized by incidence rates, severity, duration, and attribution. The secondary objective of this study was to determine the systemic and intracerebral pharmacokinetic profile of temozolomide using a microdialysis catheter.

Results

Feasibility/safety/tolerability. Nine patients were enrolled in this pilot feasibility study from June 2006 to August 2007. Table 1 summarizes the patients' characteristics. The majority of patients had high-grade gliomas. All patients underwent debulking craniotomies. Dexamethasone was administered to every patient perioperatively.

All microdialysis catheters were placed in peritumoral brain interstitium (nonenhancing brain tissue), as was determined by fusing images from postoperative noncontrast CT scans (where catheter tip was visible) and brain magnetic resonance imaging (MRI; Fig. 1).

Dialysate samples were obtained from seven of the nine patients. One patient declined temozolomide after catheter placement. Catheter occlusion occurred in one patient, resulting in no collection of dialysate samples for that patient.

All patients tolerated placement of the microdialysis catheter and collection of the dialysate samples well; however, one patient subsequently developed several grade 3 and 4 neurologic adverse events whose etiologies remain unclear. This 20-year-old man underwent resection of a recurrent right frontal lobe high-grade glioma. He did well in the immediate postoperative period. Administration of temozolomide, collection of dialysate samples, and removal of the microdialysis catheter proceeded uneventfully. He was discharged home on postoperative day 3, ambulating and without any neurologic complaints. The next day he began experiencing back pain and nausea and became obtunded. A CT scan of his brain done at an outside hospital showed no hemorrhage or significant edema. He was treated with a single dose of ceftriaxone for presumed meningitis and was transferred to our institution. A lumbar puncture revealed an elevated opening pressure of 55 mmHg. CSF total protein was elevated at 180 mg/dL (normal 15–44); CSF glucose was <10 mg/dL (normal 50–70).

Table 1. Patient characteristics

Patient	Age	Gender	KPS	Diagnosis
1	48	Female	100	Glioblastoma
2	71	Male	80	Non-small cell lung cancer
3	56	Male	90	Non-small cell lung cancer
4	57	Male	100	Glioblastoma
5	20	Male	80	Anaplastic oligodendroglioma
6	75	Female	60	Non-small cell lung cancer
7	54	Male	90	Glioblastoma
8	72	Male	90	Glioblastoma
9	56	Male	80	Anaplastic astrocytoma

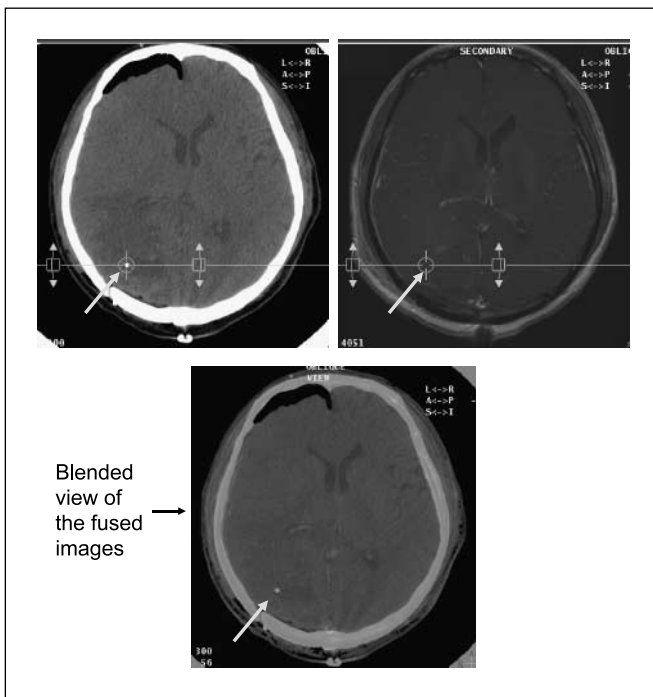


Fig. 1. Fused images of a noncontrast CT scan and a T1 postcontrast MRI of the brain of a 70-year-old man with non-small cell lung cancer are displayed side by side. The blended image is shown at the bottom. Images were autoregistered with mutual information matching algorithm using Varian Eclipse version 8.0 treatment planning system. The patient underwent a craniotomy for gross total resection of a right occipital lobe metastasis. The gold filament at the tip of the microdialysis catheter (arrows) is visible as bright signal on the CT scan shown inside the circle, but is not visible on the T1 postcontrast MRI. Fused CT and T1 postcontrast MRI show that the tip of the microdialysis catheter is placed in nonenhancing brain.

The WBC in the CSF was $18/\mu\text{L}$, with a differential of 3% segmented neutrophils, 6% lymphocytes, and 69% monocytes. The CSF RBC count was $10,000/\mu\text{L}$. Gram stains were negative for bacteria and no fungal elements were seen on direct exam. All bacterial, viral, and fungal cultures from the CSF were negative. His peripheral complete blood count was within normal range except for an elevated WBC of 14,200, with a differential

of 91% segmented neutrophils, 6% lymphocytes, and 3% monocytes. The patient was on a tapering dose of dexamethasone, which may explain his elevated WBC.

Mild subarachnoid hemorrhage in the left occipital, left frontal, and right parietal regions was seen on brain MRI. Diffusion-weighted images showed increased signal in the right frontal lobe deep to the postoperative changes, consistent with acute ischemia. An MR angiogram showed no vasospasm or arterial narrowing in the intracranial circulation. MRI of his thoracic and lumbar spine was negative for spinal cord compression or leptomeningeal enhancement. The patient was treated with broad-spectrum antibiotics and acyclovir. Because of persistently elevated intracranial pressure, a lumbar drain was placed for diversion of CSF. When the patient became more alert, it was discovered that he had cortical blindness, cranial neuropathies (left 6th and right 12th nerve palsies), hearing loss, an expressive aphasia, and bilateral lower extremity paralysis. All of his neurologic deficits subsequently improved, but did not resolve completely.

Neuropharmacokinetics of temozolomide. From the *in vitro* recovery experiments, it was determined that the highest fractional recovery of temozolomide was $87 \pm 5.5\%$ and was obtained using a flow rate of $1 \mu\text{L}/\text{min}$. Therefore, measured temozolomide concentrations in the brain interstitium were corrected for the fractional recovery determined *in vitro*. Temozolomide is a pro-drug that spontaneously converts to its active metabolite, MTIC, at physiologic pH. Attempts to recover MTIC by microdialysis were unsuccessful due to instability of the drug in the perfusion solution and possibly due to drug binding to the catheter.

The temozolomide plasma and brain interstitium pharmacokinetic data for each subject are summarized in Table 2. Peak temozolomide concentrations in plasma and brain ECF were 5.5 ± 3.2 and $0.6 \pm 0.3 \mu\text{g}/\text{mL}$, respectively. Mean T_{max} in plasma and brain were 1.8 ± 1.2 and 2.0 ± 0.8 hours, respectively. Temozolomide AUCs in plasma and brain ECF were 17.1 ± 6.8 and $2.7 \pm 1.0 \mu\text{g}/\text{mL} \times \text{hour}$. The ECF/plasma AUC ratio was $17.8 \pm 13.3\%$.

The temozolomide concentration versus time data show that temozolomide levels in brain interstitium rise more gradually over time and stay elevated slightly longer than those in plasma (Fig. 2). This is particularly evident in the inset, which is a plot of the average temozolomide concentrations in the brain

Table 2. Summary of temozolomide pharmacokinetics in plasma and brain interstitium

Patient #	Plasma temozolomide					Brain interstitial temozolomide					Brain/plasma ratio	
	C_{max} ($\mu\text{g}/\text{mL}$)	T_{max} (h)	K_{el} (h^{-1})	$t_{1/2}$ (h)	AUC 0-inf ($\mu\text{g}/\text{mL} \times \text{h}$)	C_{max} ($\mu\text{g}/\text{mL}$)	T_{max} (h)	K_{el} (h^{-1})	$t_{1/2}$ (h)	AUC 0-inf ($\mu\text{g}/\text{mL} \times \text{h}$)	C_{max} (%)	AUC 0-inf (%)
1	5.2	1.0	0.32	2.2	20.9	0.9	1.2	0.33	2.1	3.4	17.0	16.4
2	11.8	0.5	0.47	1.5	18.9	0.2	1.7	0.11	6.3	2.9	1.9	15.5
3	6.4	1.0	0.41	1.7	20.6	0.4	2.4	0.21	3.3	1.7	5.8	8.1
4	6.8	1.5	0.43	1.6	21.0	0.6	1.2	0.21	3.3	2.2	8.5	10.6
5	6.5	3.0	0.41	1.7	27.0	0.4	2.4	0.34	2.0	1.5	6.4	5.7
7	1.4	4.0	0.14	5.0	11.4	0.3	3.4	0.39	1.8	2.6	24.6	23.3
8	2.5	1.5	0.46	1.5	7.8	—	—	—	—	—	—	—
9	3.3	1.5	0.42	1.7	9.5	1.1	1.4	0.4	1.7	4.3	33.2	44.9
Avg	5.5	1.8	0.38	2.1	17.1	0.6	2.0	0.28	2.9	2.7	13.9	17.8
SD	3.2	1.2	0.11	1.2	6.8	0.3	0.8	0.11	1.6	1.0	11.5	13.3
%CV	59.2	66.6	28.2	56.1	39.5	56.6	42.7	38.3	55.4	36.3	82.4	74.8

Abbreviations: AUC 0-inf, area under the concentration-time curve extrapolated to infinity; Avg, average; %CV, percent coefficient of variation.

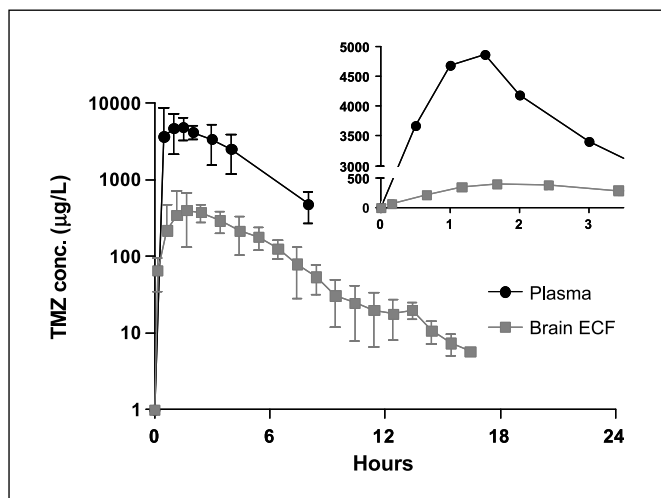


Fig. 2. Concentrations of temozolomide in plasma (triangles) and brain interstitium (squares) obtained by intracerebral microdialysis. Inset, expanded view of the initial 3.5-h interval following the dose. Symbols, mean; bars, SD.

interstitium and plasma during the first 4 hours using a linear rather than a log scale. The linear scaling makes it easier to appreciate the difference in the rate of rise and decline in temozolomide levels in plasma and brain interstitium between 2 and 4 hours. Furthermore, the rate of rise in temozolomide levels in the brain interstitium is more rapid than the rate of fall.

Trellis plots (temozolomide concentration versus time) for collected dialysate samples show that C_{max} values occur later than 1 hour (range, 1.2-3.4 hours) in every subject (Fig. 3). The mean T_{max} value of 2 hours is significantly different from the fixed value of 1 hour with a *P* value of 0.02 using a two-sided single sample *t*-test. The light grey bars highlight the portion of the concentration time curve from 2 to 3 hours, which represents a high temozolomide concentration period in every subject.

The temozolomide concentrations in brain interstitium at 1 hour and at C_{max} for each individual are compared in Table 3. The C_{max} levels are as high or higher than the levels at 1 hour in every patient, with a median fold difference of 1.8 (range, 1.0-7.0).

Discussion

The results of this study show that ICMD is both a safe and feasible technique for measuring concentrations of chemotherapy in the brain. ICMD is a promising research tool that can be incorporated into phase I studies of potential new anticancer therapies for the treatment of brain tumors. As described above, one study patient did develop multiple severe adverse events approximately 48 hours after the microdialysis catheter was removed. The cause of his significant neurologic deficits remains unclear. Possible explanations include bacterial or chemical meningitis, or diffuse microvasospasm precipitated by subarachnoid hemorrhage; nonetheless, it is unlikely that the microdialysis catheter was related to either of these potential etiologies. If the patient's neurologic sequelae were due to infection from the catheter, one would have expected to see enhancement on the MRI along the tract of the catheter. Simi-

larly, if the catheter was the cause of his subarachnoid hemorrhage, one would have expected to see a focus of blood in the region of the catheter tip on the MRI. Neither of these findings was present on any of his serial brain MRIs. Furthermore, ICMD catheters have been safely placed in hundreds of patients with acute brain injuries. There are no reported cases of catheter-associated infections. In the largest published study (17) of ICMD catheter use in neurointensive care patients, 4 of 97 patients (3%) were found to have a small (≤ 1 mL) collection of blood around the catheter on follow-up CT scans. None of these bleeds were clinically significant.

Although an initial goal of the study was to insert microdialysis catheters into residual tumor tissue, we found this task to be more complex for several reasons. First, intraoperative visualization of residual invasive gliomas at the resection margin can be difficult with current surgical techniques. Second, brain shift during and after surgery can significantly diminish the accuracy of the intraoperative navigation system and result in postoperative displacement of the catheter tip. Finally, the length and flexibility of the catheters can limit their precise placement into deeper targets. To more accurately study drug neuropharmacokinetics, future ICMD studies could be done

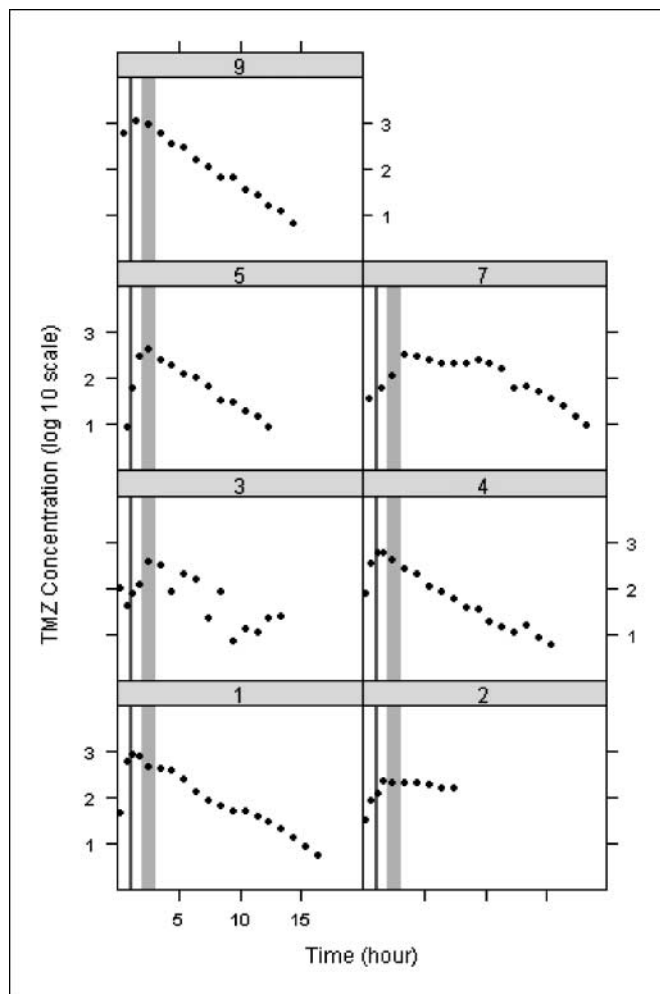


Fig. 3. Trellis plot of log₁₀ temozolomide concentrations in the brain interstitium as a function of time for each subject (black circles). Dark grey bar, 1-h time point; light grey bar, 2- to 3-h time span.

Table 3. Comparison of brain temozolomide levels at 1 hour with levels at Cmax

Patient #	C 1 h ($\mu\text{g/mL}$)	Cmax ($\mu\text{g/mL}$)	Fold difference
1	0.9	0.9	1
2	0.1	0.2	1.8
3	0.1	0.4	4.8
4	0.6	0.6	1
5	0.1	0.4	7.0
7	0.1	0.3	5.3
8	—	—	—
9	1.1	1.1	1

in patients undergoing stereotactic biopsies to improve the precision of catheter placement into tumor tissue.

Although it is desirable to determine drug concentrations in tumor, it is arguably just as important to know whether a drug is penetrating into nonenhancing peritumoral brain tissue, where the advancing edge of the tumor lies, to assess whether the drug is reaching areas of growth that “hide behind” intact BBB. Placement of more than one microdialysis catheter at the same time is well tolerated (12, 18–20). Although in this pilot feasibility study only one catheter was placed per patient, to carry out more comprehensive neuropharmacokinetic studies of potential drugs for the treatment of brain tumors, the design of future protocols could include simultaneous placement of catheters in tumor, peritumoral tissue, and/or normal brain.

It is possible that the *in vivo* recovery of temozolomide may be lower than predicted from our *in vitro* recovery experiments, resulting in an underestimation of the true concentration of temozolomide in the brain. Use of *in vitro* recovery to calibrate the catheter has its limits because it cannot take into account conditions in individual patients that may affect *in vivo* recovery, such as tortuosity of the tissue and blood flow (21). *In vivo* calibration using the retrodialysis method is ideally preferable. With retrodialysis, a small amount of the drug is first perfused through the catheter, and the loss of drug is used to determine its relative recovery. After a washout period, the drug is administered systemically and microdialysis is done. Temozolomide is only available in an oral formulation, which is unsterile, making it infeasible to do retrodialysis for catheter calibration in this study.

The concentrations of temozolomide in human brain tissue determined here are similar to reported CNS levels of temozolomide in rats using the technique of microdialysis (22). Zhou and colleagues measured temozolomide concentrations in the brains of rats via ICMD to gather data for development of a predictive pharmacokinetic model of drug concentrations in tumor. The model derived from their preclinical data predicted a mean temozolomide brain to plasma AUC ratio in humans of approximately 20%, which corresponds to our findings in patients. Therefore, our data confirm that the physiologically based predictive model proposed by Zhou and colleagues may indeed be very useful for developing clinical dosing regimens, as well as providing guidance for the appropriateness of testing new agents for the treatment of brain tumors.

More recently, Rosso and colleagues reported their results using a positron emission tomography imaging–based approach for the prediction of temozolomide concentrations in CNS tis-

ues of patients with brain tumors (23). By combining positron emission tomography imaging data following a single dose of [methyl- ^{11}C]-temozolomide with individual plasma pharmacokinetic data, we were able to develop a mathematical model to predict temozolomide concentrations in normal brain and brain tumor over time and noninvasively. Following doses of 75 to 200 mg/m^2 , predicted peak temozolomide concentrations ranged from 1.8 to 3.7 $\mu\text{g/mL}$ in normal brain, which are 3- to 6-fold higher than the levels measured using ICMD in the current study. This large difference is primarily due to the difference in the physiologic compartment being sampled. The positron emission tomography imaging approach measures temozolomide both in the vasculature and interstitial spaces, whereas ICMD samples the interstitium only. Indeed, the peak concentrations in the brain reported by Rosso and colleagues are more similar to the plasma concentrations measured in the current study. Therefore, although the ideal approach to studying neuropharmacokinetics in patients would be minimally invasive, such as the one proposed by Rosso and colleagues, ICMD still provides important additional information that can be used to further refine the model and allow discrimination between drug concentrations in the blood from those in the tissue microenvironment.

One criticism of the technique of ICMD is that it may produce falsely elevated drug levels due to disruption of the BBB that inevitably occurs to some degree with insertion of the catheter into brain interstitium. When doing ICMD studies in patients with high-grade gliomas and brain metastases, where the BBB is already disrupted (as evidenced by the presence of contrast enhancement on brain MRI), this theoretical concern is less of a consideration. Moreover, the temozolomide concentrations in brain that we determined with ICMD are similar to levels of temozolomide measured in the CSF via lumbar puncture (15), indicating that it is unlikely that ICMD significantly overestimated the concentration of temozolomide in brain interstitium.

Ultimately, determining absolute drug concentrations in the brain may not be as clinically important as being able to detect changes in intracerebral concentrations of a particular drug when given in combination with another chemotherapy agent or having the ability to know when the peak concentration of a drug occurs in the brain. Taking temozolomide as an example, it can act as a radiosensitizer (24–27) and is given concurrently with focal brain radiation therapy as part of standard treatment for newly diagnosed glioblastoma (16). When used as a radiosensitizer, low-dose daily temozolomide is typically taken 1 hour prior to radiation therapy based on data from phase I studies of temozolomide (28, 29), which determined that the peak plasma concentration of temozolomide occurs 1 hour after oral administration. Our neuropharmacokinetic data obtained using the technique of ICMD show that peak levels of temozolomide in brain interstitium occur between 1.2 and 3.4 hours after an oral dose, and the 2- to 3-hour period represents a time of high temozolomide concentration. Furthermore, temozolomide concentrations measured in the brain interstitium at 1 hour were as much as 7-fold lower than the maximum levels achieved between 2 and 3 hours. These findings suggest that the current recommendation for the schedule of chemoradiation for glioma may be suboptimal. Although the data presented here are hypothesis-generating in nature, and the true optimal schedule for chemoradiation will have to be determined by prospective investigation, the current study shows the utility of ICMD for the assessment of neuropharmacokinetics and

may lead to further refinement of therapeutic regimens for glioma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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