Treatment of Neoplastic Meningitis with Intrathecal Temozolomide


INTRODUCTION

Neoplastic meningitis (NM) results from leptomeningeal dissemination of cancers arising within the central nervous system or metastasizing to the leptomeninges from systemic neoplasms. The inability to produce therapeutic drug levels intrathecally (IT) with systemic administration and the minimal efficacy of chemotherapeutic agents currently available for direct IT use limit therapy.

Temozolomide [8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(1H)-one] is a novel methylaing agent with proven activity against intraparenchymal malignant gliomas (MGs). Insolubility of the standard formulation prevents its efficacious use as an IT agent, however. To overcome this obstacle, we have developed a unique microcrystalline formulation of temozolomide with greatly enhanced solubility. Treatment of athymic rats bearing subarachnoid MER-human MG xenografts with four doses of IT microcrystalline temozolomide over a 2-week period produced a 142% increase in median survival at individual doses of 2.2 μmol (P = 0.0073) and a >367% increase in median survival at individual doses of 6.8 μmol (P = 0.0015). At the higher dose tested, three of eight rats treated developed no neurological symptoms and had no evidence of residual tumor on histological examination after treatment. Use of this microcrystalline formulation in athymic rats bearing subarachnoid MER+ human MG xenografts increased median survival >132% (P < 0.0008) at both dose levels tested. Toxicity directly attributable to the IT administration of microcrystalline temozolomide was limited in the highest dose groups only and was limited to small patchy areas of focal demyelination involving <5% of spinal cord long tracks.

These results suggest that a dose range for both toxicity and activity has been defined for IT microcrystalline temozolomide in the treatment of NM in athymic rats, and a Phase I trial for the treatment of patients with NM using IT microcrystalline temozolomide should now be undertaken.

ABSTRACT

Neoplastic meningitis (NM) results from leptomeningeal dissemination of cancers arising within the central nervous system or metastasizing to the leptomeninges from systemic neoplasms. The inability to produce therapeutic drug levels intrathecally (IT) with systemic administration and the minimal efficacy of chemotherapeutic agents currently available for direct IT use limit therapy.

Temozolomide [8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(1H)-one] is a novel methylaing agent with proven activity against intraparenchymal malignant gliomas (MGs). Insolubility of the standard formulation prevents its efficacious use as an IT agent, however. To overcome this obstacle, we have developed a unique microcrystalline formulation of temozolomide with greatly enhanced solubility. Treatment of athymic rats bearing subarachnoid MER-human MG xenografts with four doses of IT microcrystalline temozolomide over a 2-week period produced a 142% increase in median survival at individual doses of 2.2 μmol (P = 0.0073) and a >367% increase in median survival at individual doses of 6.8 μmol (P = 0.0015). At the higher dose tested, three of eight rats treated developed no neurological symptoms and had no evidence of residual tumor on histological examination after treatment. Use of this microcrystalline formulation in athymic rats bearing subarachnoid MER+ human MG xenografts increased median survival >132% (P < 0.0008) at both dose levels tested. Toxicity directly attributable to the IT administration of microcrystalline temozolomide was limited in the highest dose groups only and was limited to small patchy areas of focal demyelination involving <5% of spinal cord long tracks.

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2 To whom requests for reprints should be addressed, at Department of Surgery, Division of Neurosurgery, Box 3807, Duke University Medical Center, Durham, NC 27710. Phone: (919) 684-9041; Fax: (919) 684-9045.
3 The abbreviations used are: NM, neoplastic meningitis; AGAT, O6-alkylguanine DNA alkyltransferase; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CNS, central nervous system; CSF, cerebrospinal fluid; FCC, final CSF concentration; IT, intrathecal; MG, malignant glioma; MTIC, 5-(3-methyl-1-triazen-1-yl)imidazole-4-carboxamide.
MATERIALS AND METHODS

Xenografts. Well-characterized human MG xenografts maintained in our laboratory were used for all experiments. Tumors were excised and minced, then dissociated with 0.5% collagenase at room temperature in a trypsinization flask for 2 h. Viable cells were separated on a Ficoll density gradient, washed twice with Dulbecco’s PBS, and resuspended. D54-MG is the Duke University subline of A-172 established by Giard et al. (11). D54-MG shows no AGAT activity and is considered MER- (12). D456-MG was derived in our laboratory from a childhood glioblastoma multiforme, as described previously (13, 14). D456-MG has been shown to have measurable AGAT levels (17.7 ± 3.6 fmol/mg protein) in prior studies and is considered MER+ (12). D456 has also been shown to be resistant to BCNU (15).

Temozolomide Preparations. Standard formulation temozolomide (MW 194) was provided by Schering-Plough Research Institute (Kenilworth, NJ). Saturated dosing solutions of this formulation were prepared by dissolving 3.1 mg/ml of the solid drug into sterile normal saline at room temperature and neutral pH, followed by filter sterilization. The microcrystalline formulation of temozolomide provided by Sparta Pharmaceuticals, Inc. (Horsham, PA) was prepared by a proprietary process using the phospholipid excipients 1,2-dilauroyl-sn-glycero-3-phosphocholine and 1,2-dimyristoyl-sn-glycero-3-phosphocholine. Saturated dosing solutions of this formulation were prepared by dissolving 33 mg/ml of the sterile microcrystalline drug into sterile water at room temperature and neutral pH.

Athymic Rat Model of NM. Subarachnoid catheters were implanted into female athymic rats [B6C3F1(CB6F1×NIMR)nu (SPF); 190–240 g], as described previously (16). These rats were maintained in the Duke University Cancer Center Isolation Facility according to institutional policy. Before catheter placement, the rats were anesthetized by an i.p. injection of a mixture of ketamine (55 mg/ml) and xylazine (9 mg/ml) at a dose of 1 ml/kg. The rats were then placed in a stereotactic frame (David Kopf Instruments, Tujunga, CA) with their necks flexed at a 90° angle using a tilt adapter. A midline sagittal incision was made from the inion to the laminar arch of C1. The atlanto-occipital membrane was exposed and incised (using the tip of a 20-gauge needle), as was the underlying dura mater over the cisterna magna. A PE-10 catheter (Intramedic; Franklin Lakes, NJ) with a 5-0 stainless steel wire stylet was then inserted into the subarachnoid space to the lumbar region (8.5 cm) by passing it along the posterior aspect of the spinal cord. The stylet was removed, a loose knot was tied in the catheter just above the opening of the dura mater, and the knot was secured in place with dental epoxy (Lang Dental Manufacturing Co., Chicago, IL). The exposed catheter end was then passed through the skin lateral to the incision. The incision was closed in three layers using 6-0 ethilon (Ethicon, Somerville, NJ), and the catheter was temporarily occluded with a small piece of 2-0 stainless steel wire. The rats were allowed to recover for 7–10 days, and only rats showing normal weight, motor, and sensory function were used in these experiments.

Tumor cells were injected through the indwelling subarachnoid catheter to initiate NM. The rats were anesthetized by light halothane anesthesia. The 2-0 wire stylet was removed, and the appropriate number of dissociated cells derived from the human MG xenografts described above were injected in a volume of 40 μl using a 1000-μl Hamilton syringe and injector. The catheter was then flushed with 20 μl of normal saline and was reoccluded with 2-0 wire. Treatment was initiated at a time corresponding to one-third of the median survival time, as estimated from past experiments with each xenograft in this model. At the time of tumor treatment, three rats selected at random from the pool of rats injected with tumor were killed and examined histologically. In each experiment, tumor presence was confirmed in all of the rats selected for pathological examination prior to therapy.

Toxicity and Efficacy Studies. For toxicity and efficacy studies, athymic rats were injected with 40 μl of temozolomide solution twice a week for 2 weeks. For the standard temozolomide formulation, a saturated 16 mM solution was used so that each dose of 40 μl contained 0.64 μmol of temozolomide, resulting in an estimated maximal FCC of 1.6 mM after each dose. For the microcrystalline formulation of temozolomide, a 56-mM, 110-mM, or saturated 170-mM solution was used so that each dose of 40 μl contained 2.2 μmol, 4.5 μmol, or 6.8 μmol of temozolomide, respectively. This resulted in estimated maximal FCCs of 5.6 mM, 11 mM, or 17 mM, respectively. Estimated FCC is based on the reported rat CSF volume of 400 μl (17).

Rat weight, neurological status, survival, and histological examination of the neuraxis were compared with a control group treated with normal saline for all experiments. Clinical neurological function included testing of the stepping and placing reflex and the ability to climb a 60-degree incline ramp. These functions have been reported to correlate with subarachnoid tumor growth in other animal models of NM (18). Histological examination was conducted on six representative cross-sections of the CNS, including forebrain at level of lateral ventricles, hindbrain at the level of the occipital lobe, and four equally spaced spinal sections, including the cauda equina. These sections were evaluated microscopically for hemorrhage, necrosis, edema, demyelination, and arachnoid fibrosis. All experiments were performed in accordance with a Duke University Animal Use Committee-approved protocol.

Statistical Analysis. Survival estimates and median survivals were determined using the method of Kaplan and Meier (19), and survival data were compared using the nonparametric log rank test.

RESULTS

Lack of Efficacy of Standard Formulation Temozolomide against Subarachnoid Human MG Xenografts. Volumes >40 μl cannot be injected IT into rats in the model used in these experiments without producing deaths in control groups; therefore, injection of 40 μl of a saturated solution of the standard formulation of temozolomide containing 0.64 μmol of drug into rats twice weekly for 2 weeks via indwelling subarachnoid catheters was evaluated for efficacy and toxicity. With this regimen, which is based on our standard clinical dosing schedule, no toxicity on clinical or histological examination was evident. Therefore, this regimen of standard formulation of temozolomide was evaluated for efficacy.
Treatment of rats bearing subarachnoid D54-MG with this regimen was initiated 6 days after implantation of $5 \times 10^6$ tumor cells. It was elected to treat these rats 6 days after tumor challenge because, based on our previous experience with D54-MG in this model (16, 20), treating 6 days after implantation would correspond to one-third to one-half of the usual median survival. Standard formulation temozolomide, using the regimen described above, failed to increase median survival in this experiment.

Similarly, using the same regimen, standard formulation temozolomide was evaluated in rats bearing subarachnoid D456-MG with the same regimen. Treatment was initiated 8 days after implantation of $5 \times 10^6$ tumor cells. It was elected to treat these rats 8 days after tumor challenge because, based on our previous experience with D456-MG in this model (21), treating 8 days after implantation would correspond to one-third to one-half of the usual median survival. In this experiment, standard formulation temozolomide increased median survival by only 16.7% from 21 days in rats treated with saline to only 24.5 days in rats treated with temozolomide. This increase was not statistically significant.

**Toxicity of Microcrystalline Formulation Temozolomide.** To determine whether the efficacy of IT temozolomide could be enhanced by increasing its solubility, a microcrystalline preparation of temozolomide with greatly enhanced solubility was developed. The increased solubility of this compound allowed up to 6.8 $\mu$mol of drug to be delivered with each 40 $\mu$l dose. The toxicity of this new preparation was evaluated in rats without tumor at individual doses of 6.8 $\mu$mol, 4.5 $\mu$mol, and 2.2 $\mu$mol given twice weekly for 2 weeks. Only one death was observed in nine rats treated at the highest temozolomide dose group (11%), and no deaths or neurological signs were evident in any of the other rats treated with temozolomide at the lower doses (Table 1).

**Efficacy of Microcrystalline Formulation Temozolomide against Subarachnoid MER–D54-MG Human Xenograft.** To determine whether the microcrystalline formulation of temozolomide would be efficacious against NM, athymic rats bearing established subarachnoid human MG xenografts were treated with the formulation described above. Treatment of MER–D54-MG human xenograft NM with this regimen beginning 6 days after implantation of $5 \times 10^6$ cells increased median survival by 142% from 12 days in the group treated with saline to 29 days ($P = 0.0073$) in the group treated with individual doses of 2.2 $\mu$mol of microcrystalline temozolomide and was increased $>367\%$ to $>56$ days ($P = 0.0015$) in the group treated with individual doses of 6.8 $\mu$mol of microcrystalline temozolomide.

**Toxicological analysis of a multiple-dose regimen of IT microcrystalline temozolomide on athymic rats without tumor**

<table>
<thead>
<tr>
<th>Dose of microcrystalline temozolomide injected</th>
<th>Estimated FCC of temozolomide</th>
<th>Survival</th>
<th>Neurological symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2 $\mu$mol</td>
<td>5.6 mM</td>
<td>8/8</td>
<td>0/8</td>
</tr>
<tr>
<td>4.5 $\mu$mol</td>
<td>11 mM</td>
<td>9/9</td>
<td>0/9</td>
</tr>
<tr>
<td>6.8 $\mu$mol</td>
<td>17 mM</td>
<td>8/9</td>
<td>0/9</td>
</tr>
</tbody>
</table>

*a* Doses indicated were given twice weekly for 2 weeks in 40 $\mu$l.

Fig. 1 IT treatment of the MER–D54-MG human glioma xenograft NM in an athymic rat model with a microcrystalline formulation of temozolomide. The dose schedule was 40 $\mu$l of temozolomide solution containing the amount of drug indicated twice/week for 2 weeks. NM was induced by injection of $5 \times 10^6$ cells into the subarachnoid space via an indwelling catheter. Treatment was initiated 6 days later. Median survival was increased $>142\%$ from 12 days in the group treated with saline to 29 days ($P = 0.0073$) in the group treated with individual doses of 2.2 $\mu$mol of microcrystalline temozolomide and was increased $>367\%$ to $>56$ days ($P = 0.0015$) in the group treated with individual doses of 6.8 $\mu$mol of microcrystalline temozolomide.
Table 2  Histopathological effects of a multiple-dose regimen of IT microcrystalline temozolomide on athymic rats with D54-MG human glioma xenograft NM

<table>
<thead>
<tr>
<th>Dose of microcrystalline temozolomide injected</th>
<th>Estimated FCC of temozolomide</th>
<th>Survival</th>
<th>Neurological symptoms</th>
<th>Histological evidence of tumor</th>
<th>Hemorrhage</th>
<th>Necrosis</th>
<th>Demyelination</th>
<th>Arachnoid fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0 mm</td>
<td>0/7</td>
<td>7/7</td>
<td>7/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>2.2 μmol</td>
<td>5.6 mm</td>
<td>0/6</td>
<td>6/6</td>
<td>5/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>6.8 μmol</td>
<td>17 mm</td>
<td>4/8</td>
<td>5/8</td>
<td>5/8</td>
<td>0/8</td>
<td>0/8</td>
<td>3/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Doses indicated were given twice weekly for 2 weeks in 40 μL.<br><sup>b</sup> Small focal areas of demyelination in a patchy distribution located in the long tracts of the spinal cord, but not involving >5% of visible fibers.<br><sup>c</sup> One rat died secondary to ventriculitis and meningitis early in the experiment.

Table 3  Effects of a multiple-dose regimen of IT microcrystalline temozolomide on athymic rats with D456-MG human glioma xenograft NM

<table>
<thead>
<tr>
<th>Dose of microcrystalline temozolomide injected</th>
<th>Estimated FCC of temozolomide</th>
<th>Median survival</th>
<th>Number without neurological deficits and without evidence of tumor on histological examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0 mm</td>
<td>26 days</td>
<td>0/10</td>
</tr>
<tr>
<td>2.2 μmol</td>
<td>5.6 mm</td>
<td>&gt;60 days&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5/8</td>
</tr>
<tr>
<td>4.5 μmol</td>
<td>11 mm</td>
<td>&gt;60 days</td>
<td>9/10</td>
</tr>
<tr>
<td>6.8 μmol</td>
<td>17 mm</td>
<td>&gt;60 days</td>
<td>8/9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Doses indicated were given twice weekly for 2 weeks in 40 μL.<br><sup>b</sup> Experiment terminated at 60 days.

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experiment. In the rats treated with microcrystalline temozolomide at the low (2.2 μmol) and intermediate (4.5 μmol) doses, 5 of 8 and 9 of 10 rats, respectively, survived >60 days, and all survivors had no clinical evidence of neurological symptoms or histological evidence of tumor. In the group treated with the highest temozolomide dose (6.8 μmol), eight of nine rats survived, and all survivors had no clinical evidence of neurological symptoms or histological evidence of tumor.

DISCUSSION

NM represents a lethal final common pathway for a spectrum of malignancies originating in the CNS or in extraneural sites that commonly metastasize to the CNS (1–5). The deficiencies of current therapeutic interventions underlie the rapid death of patients after the diagnosis of leptomeningeal tumor spread. Temozolomide, the 3-methyl derivative of mitozolomide, is a second generation imidazo-tetrazine with activity against a spectrum of murine tumors (7, 12, 22). Despite severe and unpredictable thrombocytopenia observed with mitozolomide in clinical trials (23), temozolomide was advanced to clinical trial, in part due to the observation of its spontaneous chemical conversion to MTIC without the need for metabolic activation. Initial Phase I trials of single-dose i.v. and, subsequently, oral temozolomide demonstrated dose-limiting myelosuppression and trivial antineoplastic activity. However, Phase I and II trials that used a 5-day regimen revealed dose-limiting myelosuppression, but intriguing antineoplastic activity, including responses in patients with high-grade glioma (8, 9). Previous clinical trials have supported the activity of other DNA methylating agents, including procarbazine, against brain tumors, with results comparable with adjuvant BCNU (24–28). However, no other DNA methylating agents have undergone extensive evaluation in clinical trials for patients with CNS tumors, although streptozotocin was evaluated in a brain tumor study group trial. Specifically, no DNA methylating agents are currently available for IT use.

The attraction to the oncologist of regional therapy of NM is the potential for achieving very high drug concentrations in the subarachnoid space while minimizing systemic exposure and, hence, toxicity. The dose-limiting stem cell toxicity produced by temozolomide has limited the use of this agent, although stem cell reconstitution does broaden its applications. IT delivery in the model described in this study provided a maximal estimated FCC of temozolomide of 3300 mg/l after only a single dose. This compares favorably with peak serum concentrations after oral therapy of only ~10 mg/l (8). In addition, based on CSF to plasma ratios for the area under the plasma concentration time curve from zero to infinity, penetration of systemic temozolomide into the CSF ranges from 41–44% in the rat and averaged 30% in Rhesus monkeys (data on file at Schering-Plough International). Thus, IT delivery of microcrystalline temozolomide could potentially provide a >750-fold drug delivery advantage.

The recent generation of a panel of xenografts derived from childhood and adult high-grade gliomas (11–13) and the development of an athymic rat model of NM (16) provided the opportunity for preclinical definition of the activity of novel antineoplastic agents against tumors commonly progressing to NM. Previous studies demonstrated the activity of temozolomide against a series of human CNS xenografts growing s.c. in athymic nude mice (12) and provided the rationale for development of an IT approach. The marked insolubility of temozolomide, however, has previously precluded regional use of this methylative alkylator.

In this study, we report the successful preparation of a microcrystalline formulation of temozolomide with enhanced solubility. The data presented provide evidence supporting the efficacy of this novel preparation of temozolomide for IT use in...
a well-characterized model of NM in the athymic rat. Although NM usually arises secondary to intraparenchymal disease and is not seen in isolation as recapitulated in this model, very few animal models of NM have been established, and none induce NM secondary to parenchymal disease (18, 29, 30). The model used in this study was developed in our laboratory, recapitulates the clinical neurological findings in NM (16), and has allowed the selection of drugs that have subsequently produced responses in humans (31).4

Although only minimal efficacy was obtained using the standard formulation of temozolomide, the enhanced solubility of the microcrystalline formulation, by allowing increased drug delivery, produced substantial antineoplastic activity. In the experiments reported here, this enhancement in drug delivery at all temozolomide doses tested provided a significant increase in median survival when used to treat a BCNU-sensitive, MER-human MG xenograft growing in the subarachnoid space of athymic rats. IT microcrystalline temozolomide was also effective against a BCNU-resistant, MER+ human MG xenograft in the same model. This suggests that IT microcrystalline temozolomide may be active against a spectrum of malignancies metastatic to the subarachnoid space. As a DNA methylating agent, temozolomide has a unique mechanism of action among chemotherapeutic agents approved for IT use. This may be particularly useful against NM secondary to glial neoplasms because of the high incidence of alkylator drug resistance found with such neoplasms.

Activity of IT microcrystalline temozolomide in our experiments was seen at doses without significant systemic or CNS toxicity. In rats without tumor, only one death in nine rats was observed at the highest dose of microcrystalline temozolomide deliverable IT, and the remainder of the rats exposed to drug demonstrated no weight loss or neurological symptoms. At the highest deliverable dose of microcrystalline temozolomide used in the experiments described here, a cumulative dose of only 27.2 µmol (5.28 mg) of temozolomide was injected IT. Although in these experiments there was no evidence of systemic toxicity, as evidenced by weight loss or pathologically abnormal findings at autopsy, this was not unexpected because much higher doses of standard formulation temozolomide (~100 µmol) have been given intra-arterially to rats without evidence of systemic toxicity (32). This suggests that the CNS and not systemic toxicity may be dose-limiting for IT microcrystalline temozolomide.

Although there was no correlation with clinical symptoms or signs, three of eight rats in the high-dose group did demonstrate some evidence of patchy demyelination involving <5% of the spinal cord long tracks. Thus, the highest dose of microcrystalline temozolomide used in this study could be considered toxic. This would warrant calculating a starting dose for the Phase I human clinical trial based on the 4.5 µmol dose also shown to have efficacy in our model. Given that the measured CSF space in humans, which ranges from 90–200 ml (33), is >225-fold larger than the estimated 400 µl CSF space in rats (17), a reasonable estimate of a potentially maximum tolerated dose in a human clinical trial might be four doses of 1000 µmol (225 × 4.5 µmol) microcrystalline temozolomide each in 9 ml (225 × 40 µl) over 2 weeks. Therefore, 10% of this dose, four doses of 100 µmol of microcrystalline temozolomide over 2 weeks, would be a reasonable suggested starting dose for a Phase I dose-escalation study of IT microcrystalline temozolomide. This would provide an estimated maximal FCC after each individual dose of between 0.5 mm and 1.1 mm. On the basis of preclinical data, temozolomide is effective in vitro at 0.1 mm (34), and therapeutic concentrations in the brain after systemic administration peak at between 0.041 mmol/Kg and 0.056 mmol/Kg (data on file at Schering-Plough International). Therefore, even at the proposed starting doses, IT microcrystalline temozolomide might be expected to demonstrate efficacy.

These studies suggest that IT microcrystalline temozolomide warrants evaluation in patients with NM. Similar results with IT melphalan (20) led to a current Phase I trial of this alkylator, and activity has been seen in patients with leptomeningeal medulloblastoma and pineoblastoma.5 A novel microcrystalline preparation of busulfan that demonstrated efficacy in this NM model is now being evaluated in a Phase I clinical trial (21). On the basis of the data presented in this study, we suggest the initiation of a Phase I trial of IT microcrystalline temozolomide for patients with NM.

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REFERENCES


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4 Unpublished data.
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