Activity of Temozolomide in the Treatment of Central Nervous System Tumor Xenografts


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ABSTRACT

The activity of 8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (temozolomide) in the treatment of a panel of xenografts derived from ependymoma, medulloblastoma, and childhood and adult high-grade glioma was evaluated in athymic nude mice bearing s.c. and intracranial tumors. Temozolomide administered daily for a total of five doses demonstrated marked activity against a panel of Mer+ xenografts despite marginal to moderate activity of 1,3-bis(2-chloroethyl)-1-nitrosourea. The growth delays produced by temozolomide in these xenografts were 1.8-7.5-fold greater than those produced by procarbazine. Although temozolomide demonstrated marginal activity against the Mer+ cell line D341 Med when a 5-day schedule was used, a high-dose 1-day schedule resulted in moderate activity. Temozolomide produced increases in median survival of 1285% (adult glioma D-54 MG), 323% (childhood glioma D-456 MG), and 68% (ependymoma D612 EP). Pretreatment of mice with O6-benzylguanine increased temozolomide-induced mortality, requiring reduction of the dosage from 1200 to 750 mg/m2 on the single-day regimen. O6-Benzylguanine pretreatment of mice bearing Mer+ D341 Med increased the growth delay of temozolomide, in duplicate experiments, from 3.1 to 4.8 and 1.1 to 4.9 days. These studies suggest that temozolomide may be active in the treatment of a broad spectrum of central nervous system cancers, including Mer+ tumors resistant to 1,3-bis(2-chloroethyl)-1-nitrosourea.

INTRODUCTION

The failure to identify a cohort of chemotherapeutic agents active in the treatment of malignant CNS5 tumors is primarily responsible for the dismal prognosis associated with these neoplasms (1). Although alkylating agents have marked activity against neuronal tumors (2-5), tumors develop drug resistance, which subsequently leads to tumor progression and, frequently, death. Identifying agents active against malignant gliomas is even more problematic, presumably due to a high incidence of de novo drug resistance (6). Accordingly, the recent observation that 8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (temozolomide) produced responses in patients with high-grade glioma (7, 8) provided the rationale for evaluating the activity of this agent in a panel of human CNS tumor xenografts that are representative of the most common histiotypes seen with these cancers.

Temozolomide is an imidazole tetrazine, and its precise mechanism of action is similar to that of dacarbazine, notably conversion to the active methylating agent MTIC. Unlike dacarbazine, however, which requires metabolic dealkylation (a relatively inefficient process in humans compared with rodents) to form MTIC, temozolomide undergoes chemical conversion to MTIC under physiological conditions (9).

In this study we evaluated temozolomide against a panel of CNS tumor xenografts derived from ependymomas, medulloblastomas, and childhood and adult high-grade gliomas, and demonstrated marked antineoplastic activity against tumors in both s.c. and i.c. sites. Furthermore, we evaluated the modulation of temozolomide-induced toxicity and antitumor activity seen following O6-benzylguanine-mediated depletion of AGAT activity. The combination of O6-benzylguanine plus temozolomide was more active than treatment with temozolomide alone.

MATERIALS AND METHODS

Animals. Male or female athymic BALB/c mice (nu/nu genotype, 6 weeks or older) were used for all studies as described previously (10).

Xenografts. A panel of human CNS tumor-derived xenografts was used for all studies. D425 Med and Daoy were derived from medulloblastoma as described previously (11, 12). D-212 MG and D-456 MG were derived from childhood glioblastoma multiforme as described previously (13, 14). D528 EP and D612 EP were derived from posterior fossa ependymomas in children ages 2 and 3 years, respectively. D-245 MG was derived from an adult glioblastoma multiforme as described previously (15). D-54 MG is the Duke University subtype of A-172 established by Giard et al. (16).

Drugs. Temozolomide was provided in all studies by Schering-Plough Research Institute (Kenilworth, NJ). O6-Benzylguanine was synthesized as described previously (17). Procarbazine was provided by Hoffman LaRoche Inc. (Nutley, NJ). Temozolomide was given either once a day or daily for five doses via i.p. injection in a solution of 10% DMSO in 0.9% saline at a volume of 90 ml/m2. O6-Benzylguanine was administered 1 h prior to temozolomide via i.p. injection at a dose of 90 mg/m2 in 40% PEG-400 in 0.9% saline at a volume of 90 ml/m2 (14). BCNU was given as a single i.p. injection at a dose of 100 mg/m2 in 3% ethanol at a volume of 90 ml/m2. Procarbazine was given at a dose of 700 mg/m2 as a single i.p. injection in 0.9% saline at a volume of 90 ml/m2 daily for 5 consecutive days (15). BCNU was purchased from Sigma (St. Louis, MO).

AGAT Assay. 3H-labeled methylated DNA was used to measure AGAT activity in s.c. xenografts as described previously (14, 17).

Dose Selection Studies. The doses of temozolomide LD10 on 5-day and 1-day schedules were defined by log-probit analysis of cohorts of 6-20 mice treated at dosages ranging between 150 and 1500 mg/m2 as described previously (18).

Xenograft Transplantation. Tumors were transplanted (s.c.) into the right flank as described previously with inoculation volumes of 50 µl (19). Intracranial tumor transplantation into the right cerebrum was performed with inoculation volumes of 10 µl using a 27-gauge needle equipped with a sleeve allowing 3.5-mm penetration as described previously (19).

Tumor Measurements. s.c. tumors were measured every 3-4 days with vernier calipers (Scientific Products, McGraw, IL). The tumor volume was calculated according to the following formula: (width)2 x (length)/2.

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2 To whom requests for reprints should be addressed, at Duke University Medical Center, Department of Pathology, Box 3156, Durham, NC 27710.

3 The abbreviations used are: CNS, central nervous system; temozolomide, 8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one; MTIC, 5-(3-methyl-1-triazen-1-y)imidazole-4-carboxamide; i.c., intracranial; AGAT, O6-alkylguanine-DNA alkyltransferase; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; LD10, dose lethal to 10% of treated animals.

4 H. S. Friedman and D. D. Bigner, unpublished data.

2853
Temozolomide and CNS Tumors

Treatment Regimen (Temozolomide Experiments). After s.c. injection of tumor homogenate and attainment of a median tumor volume exceeding 200 mm³, groups of 8–10 randomly selected mice were started on treatment with temozolomide or 0.9% saline on Day 1. After i.c. injection of tumor homogenate, groups of 8–10 randomly selected mice were started on treatment on the day that represented 50% of the time in days between the initial tumor inoculation and the median day of death, as previously defined in i.c. tumor-bearing mice receiving no therapy.

Treatment Regimen (Temozolomide Plus O6-Benzylguanine Experiments). The mortality of temozolomide (using a single-dose regimen) plus O6-benzylguanine treatment was defined in animals bearing s.c. xenografts. Evaluation of the antineoplastic activity of temozolomide (1-day regimen), with or without O6-benzylguanine, was conducted in a manner similar to the studies above using the 5-day regimen.

Assessment of Response. The response of s.c. xenografts was assessed by delay in tumor growth and by tumor regressions. Growth delay, expressed as T-C, is defined as the difference in days between the median time for the tumors of treated and control animals to reach a volume five times that of the initial treatment volume. Tumor regressions are defined as a decrease in tumor volume over two successive measurements. Statistical analysis was performed using the Wilcoxon rank order test for growth delay and Fisher’s exact test for tumor regressions as described previously (19). The response of i.c. xenografts was assessed by comparing the median survival time between treated and control groups. Statistical analysis was performed using the Wilcoxon rank order test.

AGAT Suppression. Suppression of baseline AGAT activity in Daoy xenografts was determined at 4, 12, and 24 h after a single i.p. dose of temozolomide at 1200 mg/m².

RESULTS

Dose Selection Studies

The LD10 for temozolomide, given as a single daily i.p. injection on Days 1–5 or Day 1, was 411 mg/m² and 1200 mg/m², respectively.

AGAT

The activity of AGAT ranged from undetectable in D-54 MG to 94.0 ± 30.3 (mean ± SD) fmol/mg protein in D341 Med (Table 1).

s.c. Xenograft Therapy

Toxicity. Of the 127 animals treated with the 5-day regimen of temozolomide (411 mg/m²/day), 12 (94%) died of drug toxicity. The median nadir weight loss was 13.0%. Of the 39 animals treated with procarbazine (700 mg/m²/day), none died of drug toxicity. The median nadir weight loss was 18.1%. Of 40 animals treated with BCNU (100 mg/m²/day), none died of drug toxicity. The median nadir weight loss was 4.5%.

Activity. In the 5-day regimen, temozolomide was active in the therapy of all but 1 xenograft with statistically significant (P < 0.01) growth delays ranging between 40.8 days in D-54 MG to 120+ days in D-456 MG (Table 2). In this regimen, temozolomide produced statistically significant (P < 0.01) tumor regressions in all xenografts except D341 Med, which has the highest levels of AGAT of all of the xenografts tested. The 1-day regimen of temozolomide, however, produced a growth delay of 10.9 days with 1 of 8 regressions in D341 Med. An additional experiment was conducted that reduced the dose to 1050 mg/m² for 1 day. This regimen produced a growth delay of 8.6 days with 5 of 9 tumor regressions. BCNU produced growth delays ranging from 4.1 days against D-212 MG to 18.3 days against D612 EP (Table 3). BCNU produced statistically significant (P < 0.01) tumor regressions against D612 EP and D528 EP. Procarbazine produced growth delays ranging from 7.5 days against D-212 MG to 48.9 days against D612 EP (Table 3). Procarbazine produced statistically significant (P < 0.01) tumor regressions against D-456 MG, D612 EP, and D528 EP. No tumor regression was seen in any animal receiving vehicle.

Intracranial Xenograft Therapy with Temozolomide (5-Day Regimen)

Toxicity. Of the 30 animals treated with temozolomide (411 mg/m²/day), 2 died of drug toxicity.

Activity. Temozolomide produced statistically significant (P < 0.01) increases in median survival in all xenografts studied, yielding increases of 68% in D612 EP, 323% in D-456 MG, and 1285% in D-54 MG (Table 4). Tumors with lower AGAT levels exhibited a greater response to temozolomide.

Temozolomide (1-Day Regimen) with or without O6-Benzylguanine

Toxicity. Pretreatment of mice with O6-benzylguanine (90 mg/m²) resulted in greater sensitivity to temozolomide. The LD10 for temozolomide was decreased to 750 mg/m².

Activity (s.c. Xenografts). The activity of temozolomide (750 mg/m², single dose) against s.c. D341 Med xenografts was increased by pretreatment with O6-benzylguanine, with growth delays of −3.1 and 1.1 days produced by temozolomide alone and 4.8 and 4.9 days produced by temozolomide plus O6-benzylguanine (Table 5). Temozolomide alone produced no tumor regressions, but temozolomide plus O6-benzylguanine produced 3 of 10 and 1 of 9 regressions in this xenograft model.

AGAT Suppression

AGAT activity in Daoy xenografts was reduced to undetectable levels 4 h after temozolomide administration. Partial recovery of activity occurred at 12 h (15% of baseline) and 24 h (27% of baseline).

DISCUSSION

Temozolomide, the 3-methyl derivative of mitozolomide, is a second generation imidazotetrazine with activity against a spectrum of murine tumors (9) and superb delivery to all body tissues including the CNS (20, 21). Despite severe and unpredictable thrombocytopenia observed with mitozolomide in clinical trials (22), temozolomide was advanced to clinical trial, in part due to the observation of its spontaneous chemical conversion to MITC without the need for metabolic activation. Initial Phase I trials of single-dose i.v. and, subsequently, p.o 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 (7, 8).

Previous clinical trials have supported the activity of other methylating agents against brain tumors. Procarbazine is a N-methylhydrazine derivative that has been used extensively, both alone and with other agents [notably vincristine and 1-(2-chloroethyl)-3-cyclohexyl-
Table 2 Activity of temozolomide against s.c. CNS tumor xenografts

<table>
<thead>
<tr>
<th>Xenograft</th>
<th>Derivation</th>
<th>Experiment</th>
<th>Regimena</th>
<th>Median time to 5 times initial tumor volume of control tumors (days)</th>
<th>T-Cb</th>
<th>Regressionsc</th>
</tr>
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<tbody>
<tr>
<td>D-341 Med</td>
<td>Medulloblastoma</td>
<td>1</td>
<td>411 mg/m² × 5 days</td>
<td>30.3</td>
<td>3.5</td>
<td>2/9d</td>
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<tr>
<td>D-341 Med</td>
<td>Medulloblastoma</td>
<td>2</td>
<td>411 mg/m² × 5 days</td>
<td>31.4</td>
<td>0.5d</td>
<td>0/10d</td>
</tr>
<tr>
<td>D-341 Med</td>
<td>Medulloblastoma</td>
<td>3</td>
<td>1200 mg/m² × 1 day</td>
<td>28.9</td>
<td>10.9</td>
<td>1/8d</td>
</tr>
<tr>
<td>D-341 Med</td>
<td>Medulloblastoma</td>
<td>4</td>
<td>1025 mg/m² × 1 day</td>
<td>29.3</td>
<td>8.6</td>
<td>5/9</td>
</tr>
<tr>
<td>D528 EP</td>
<td>Ependymoma</td>
<td>1</td>
<td>411 mg/m² × 5 days</td>
<td>75.2</td>
<td>51</td>
<td>8/2 (2)</td>
</tr>
<tr>
<td>D528 EP</td>
<td>Ependymoma</td>
<td>2</td>
<td>411 mg/m² × 5 days</td>
<td>43.3</td>
<td>90+</td>
<td>9/9 (7)</td>
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<tr>
<td>D612 EP</td>
<td>Ependymoma</td>
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<td>411 mg/m² × 5 days</td>
<td>40.8</td>
<td>72.8</td>
<td>8/8</td>
</tr>
<tr>
<td>D612 EP</td>
<td>Ependymoma</td>
<td>2</td>
<td>411 mg/m² × 5 days</td>
<td>40.2</td>
<td>86.1</td>
<td>10/10</td>
</tr>
<tr>
<td>D-456 MG</td>
<td>Childhood GBM</td>
<td>1</td>
<td>411 mg/m² × 5 days</td>
<td>44.5</td>
<td>120+</td>
<td>7/7 (7)</td>
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<tr>
<td>D-456 MG</td>
<td>Childhood GBM</td>
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<td>411 mg/m² × 5 days</td>
<td>28.1</td>
<td>120+</td>
<td>8/8 (8)</td>
</tr>
<tr>
<td>D-212 MG</td>
<td>Childhood GBM</td>
<td>1</td>
<td>411 mg/m² × 5 days</td>
<td>39.5</td>
<td>56.2</td>
<td>9/9</td>
</tr>
<tr>
<td>D-212 MG</td>
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<td>2</td>
<td>411 mg/m² × 5 days</td>
<td>37.4</td>
<td>47.4</td>
<td>10/10</td>
</tr>
<tr>
<td>D-54 MG</td>
<td>Adult AA</td>
<td>1</td>
<td>411 mg/m² × 5 days</td>
<td>7.5</td>
<td>40.8</td>
<td>10/10 (1)</td>
</tr>
<tr>
<td>D-245 MG</td>
<td>Adult GBM</td>
<td>1</td>
<td>411 mg/m² × 5 days</td>
<td>20.5</td>
<td>108.3</td>
<td>8/8 (1)</td>
</tr>
<tr>
<td>D-245 MG</td>
<td>Adult GBM</td>
<td>2</td>
<td>411 mg/m² × 5 days</td>
<td>25.6</td>
<td>111.9</td>
<td>9/9</td>
</tr>
</tbody>
</table>

a Values not statistically significant (P < 0.01).

b Growth delay in days, defined as the difference between the median time for tumors in treated (T) and control (C) animals to reach five times the volume recorded at initiation of treatment.

c Defined as a decrease in tumor volume over two successive measurements, number of regressions/number treated. Numbers in parentheses, number of complete regressions.

d Values not statistically significant (P < 0.01).

e Abbreviations as used in Table 1.

Table 3 Treatment of CNS tumor xenografts growing s.c. in athymic nude mice with BCNU, procarbazine, or temozolomide

<table>
<thead>
<tr>
<th>Xenograft</th>
<th>Derivation</th>
<th>BCNUa</th>
<th>Procarbazinex</th>
<th>Temozolomidec</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-212 MG</td>
<td>Childhood GBM</td>
<td>4.1d</td>
<td>0/10d</td>
<td>2/10d</td>
</tr>
<tr>
<td>D-456 MG</td>
<td>Childhood GBM</td>
<td>6.1</td>
<td>1/10d</td>
<td>10/10 (2)</td>
</tr>
<tr>
<td>D612 EP</td>
<td>Ependymoma</td>
<td>18.3</td>
<td>9/10</td>
<td>10/10</td>
</tr>
<tr>
<td>D528 EP</td>
<td>Ependymoma</td>
<td>10.9</td>
<td>1/10</td>
<td>9/9</td>
</tr>
</tbody>
</table>

Abbreviations: GMB, glioblastoma multiforme.

a BCNU was given by single i.p. injection at a dose of 100 mg/m² in 30% ethanol at a volume of 90 ml/m².

b Procarbazine was given by i.p. injection at a dose of 700 mg/m² in 0.9% saline at a volume of 90 ml/m² daily for 5 consecutive days.

c Temozolomide was given by i.p. injection at a dose of 111 mg/m² in 10% DMSO in 0.9% saline at a volume of 90 ml/m² daily for 5 consecutive days.

d Growth delay in days, defined as the difference between the median time for tumors in treated (T) and control (C) animals to reach five times the volume recorded at initiation of treatment.

e Defined as a decrease in tumor volume over two successive measurements, number of regressions/number treated. Numbers in parentheses, number of complete regressions.

1-nitrosourea, in the treatment of CNS tumors (23). Kumar et al. (24) first reported treatment of progressive primary or metastatic brain tumors with procarbazine, observing a response rate of 48%. Green et al. (25) used adjuvant procarbazine following surgery and radiotherapy in adults with high-grade glioma, with results comparable to activity of temozolomide against a broad spectrum of CNS xenografts growing s.c. and i.c. Temozolomide was active against the Merprocarbazine xenografts despite marginal to moderate activity of ependymomas, medulloblastomas, and childhood and adult high-grade gliomas (5, 15, 19) provided the opportunity for preclinical definition of the activity of novel antineoplastics against CNS tumors. The xenografts also provided the capability to perturb the mechanisms of resistance to these drugs. Initial studies demonstrated the marked activity of temozolomide against a broad spectrum of CNS xenografts growing s.c. and i.c. Temozolomide was active against the Mer xenografts D-54 MG and D-245 MG, which is not surprising since these tumor cells do not express the DNA repair protein AGAT, which is known to protect cells from alkylation damage at the O6 position of guanine. Indeed, temozolomide mediates AGAT depletion (28) and displays activity in cells that is inversely proportional to AGAT evaluated with procarbazine, observing a response rate of 48%. Green et al. (25) used adjuvant procarbazine following surgery and radiotherapy in adults with high-grade glioma, with results comparable to activity of temozolomide against a broad spectrum of CNS xenografts growing s.c. and i.c. Temozolomide was active against the Merprocarbazine xenografts despite marginal to moderate activity of ependymomas, medulloblastomas, and childhood and adult high-grade gliomas (5, 15, 19) provided the opportunity for preclinical definition of the activity of novel antineoplastics against CNS tumors. The xenografts also provided the capability to perturb the mechanisms of resistance to these drugs. Initial studies demonstrated the marked activity of temozolomide against a broad spectrum of CNS xenografts growing s.c. and i.c. Temozolomide was active against the Mer xenografts D-54 MG and D-245 MG, which is not surprising since these tumor cells do not express the DNA repair protein AGAT, which is known to protect cells from alkylation damage at the O6 position of guanine. Indeed, temozolomide mediates AGAT depletion (28) and displays activity in cells that is inversely proportional to AGAT activity (29). Temozolomide demonstrated marked activity against a panel of Mer" xenografts despite marginal to moderate activity of BCNU. The growth delays produced by temozolomide were 1.8–7.5-
fold greater than those produced by procarbazine. Furthermore, although temozolomide demonstrated marginal activity against the Mer line D341 Med when a 5-day schedule was used, a 1-day schedule resulted in moderate activity.

These results have several implications. First, although the activity of temozolomide against i.e. xenografts correlated inversely with tumor AGAT levels, this correlation was not observed with s.c. xenografts, suggesting that alternative mechanisms of resistance to temozolomide may exist, including non-AGAT-mediated repair of temozolomide-induced methylation. Second, temozolomide was highly active against Mer xenografts that were far less sensitive to BCNU or procarbazine, suggesting great promise for this agent in the treatment of tumors sensitive or resistant to these agents. Third, further evaluation of alternative treatment schedules (such as 1-day versus 5-day) is warranted to define the optimal regimen.

Studies were subsequently conducted to evaluate modulation of temozolomide-induced toxicity and activity following O6-benzylguanine-mediated depletion of AGAT. A single-dose temozolomide schedule was chosen to avoid the need for maintaining AGAT depletion for 5 days. Pretreatment with O6-benzylguanine increased the toxicity of temozolomide, necessitating a reduction in dose to 750 mg/m² from the LD₁₀₀ of 1200 mg/m², but it also increased the activity. These results are in contrast to those of Plowman et al. (30), which failed to demonstrate O6-benzylguanine-mediated enhancement of temozolomide activity. However, these investigators attempted to modulate activity against a Mer xenograft, which, of course, would not be expected to be sensitized by an AGAT inhibitor.

The current studies suggest that temozolomide may be active in the treatment of a broad spectrum of CNS cancers, including glioma, ependymoma, and medulloblastoma. The surprising and unanticipated activity of temozolomide against a spectrum of Mer xenografts derived from malignant glioma, medulloblastoma, and ependymoma suggests that it may also be active despite the presence of tumor AGAT activity. A Phase II trial of temozolomide in recurrent high-grade glioma patients, with quantitation of tumor AGAT levels prior to treatment, will soon start, allowing extension of the current studies to the clinic.

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