Phase I study of Gliadel™ wafers plus temozolomide in adults with recurrent supratentorial high-grade gliomas

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Both Gliadel™ wafers [1,3-bis(2-chloroethyl)-1-nitrosourea] and temozolomide (TEMO) have been shown in independent studies to prolong survival of patients with recurrent malignant glioma following surgery and radiotherapy. On the basis of preclinical evidence of synergism between Gliadel wafers and TEMO, a phase I study was designed to evaluate the toxicity of combining these 2 agents in the treatment of patients with recurrent supratentorial malignant glioma. All patients had surgical resection of the tumor at relapse, and up to 8 Gliadel (3.85%) wafers were placed in the surgical cavity following resection. Two weeks after surgery, TEMO was given orally daily for 5 days. Cohorts of 3 patients received TEMO at daily doses of 100 mg/m^2, 150 mg/m^2, and 200 mg/m^2, respectively. Patients were assessed for toxicity 4 weeks after start of the first course of TEMO. Contrast-enhanced MRI of the brain was used to assess tumor response after the first cycle of TEMO. Patients with stable disease or response after the first cycle of TEMO were allowed to continue treatment at the same dose every 4 weeks for 12 cycles or until disease progression or unacceptable toxicity. Ten patients with a median age of 47 years (range, 22-66 years) were enrolled in this study. There were 7 patients with glioblastoma multiforme and 3 patients with anaplastic astrocytoma. Three patients were treated with TEMO at the first dose level of 100 mg/m^2, 4 at the second dose level of 150 mg/m^2, and 3 at the third dose level of 200 mg/m^2. The 10 patients received a median of 3 cycles (range, 1-12 cycles) of TEMO following placement of Gliadel wafers. The treatment was well tolerated, with only 1 patient suffering grade III thrombocytopenia at the highest dose level. Two patients at each dose level had no evidence of disease progression after treatment. Four patients suffered progressive disease on therapy. Our study demonstrates that TEMO can be given safely after placement of Gliadel (3.85%) wafers. The recommended dosage for TEMO for a phase II study of this combination is 200 mg/m^2 per day for 5 days. *Neuro-Oncology 3, 246–250, 2001 (Posted to Neuro-Oncology [serial online], Doc. 00-070, June 21, 2001. URL <neuro-oncology.mc.duke.edu>)*

The prognosis for adults with recurrent supratentorial malignant gliomas is extremely poor when using current standards of treatment (Fine, 1994). Nitrosoureas have been the mainstay of therapy for these patients, either along with or after radiotherapy (Chang et al., 1983; Walker et al., 1980). In a recent randomized placebo-controlled trial in adults with recurrent malignant gliomas who had failed surgery and radiation, with or without systemic chemotherapy, the use of Gliadel™

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[2]Abbreviations used are as follows: ANC, absolute neutrophil count; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; DLT, dose-limiting toxicity; MTD, maximum tolerated dose/dosage; TEMO, temozolomide.

wafers (BCNU)[2] prolonged survival by 8 weeks compared with placebo, with no significant toxicity (Brem et al., 1995). However, responses induced by BCNU are short-lived and have not produced durable remissions in patients with these tumors.

TEMO, an imidazole tertazinone, is a DNA methylating agent similar to dacarbazine. Unlike dacarbazine, which requires hepatic dealkylation to form the active methylating agent 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC), TEMO undergoes spontaneous chemical conversion to 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide under physiologic conditions (Horspool et al., 1990). TEMO has recently been shown to be active in adult patients with newly diagnosed and recurrent malignant gliomas (Friedman et al., 1998; Yung et al., 1999, 2000). Phase I studies have shown that the dose-limiting toxicity (DLT) of the drug is myelosuppression (Hammond et al., 1999; Newlands et al., 1992). The MTD of single-agent TEMO is 200 mg/m^2 per day daily for 5 days given every 4 weeks. Although significant responses have been obtained in patients with newly diagnosed and recurrent malignant glioma (Yung et al., 1999, 2000), sustained, disease-free survival has not been achieved in these patients with this agent.

Drug resistance mediated via multiple mechanisms plays an important role in treatment failure of patients with recurrent gliomas (Belanich et al., 1996; Friedman et al., 1997; Hotta et al., 1994; Jaeckle et al., 1998). Hence, chemotherapy with single agents such as BCNU or TEMO is destined to fail in a significant proportion of patients. A therapeutic strategy that incorporates 2 or more agents with different mechanistic pathways is more likely to offer a better chance for tumor control.

In a recent preclinical study, a synergistic effect on tumor control was demonstrated with sequential administration of BCNU and TEMO to animals bearing malignant glioma xenografts (Plowman et al., 1994). We therefore conducted a phase I study of Gliadel wafers followed by oral TEMO in patients with recurrent malignant gliomas to determine the MTD of TEMO that can safely be given following placement of Gliadel wafers.

Patients and Methods

Study Design

This phase I study was conducted at Duke University Medical Center between December, 1998, and January, 2000, and was designed to determine the MTD of TEMO when given 2 weeks after surgical implantation of Gliadel wafers in the tumor cavity. The study was approved by the Institutional Review Board at Duke University Medical Center.

Eligibility Criteria

Eligibility for this study included age >18 years; histologically confirmed recurrent, supratentorial glioblastoma multiforme, anaplastic astrocytoma, or gliosarcoma and pathology reviewed at Duke University Medical Center before study entry; at least 6 weeks between prior radiotherapy or chemotherapy; Karnofsky performance scale >70%; stable or decreasing dose of steroids; hematocrit >29%; ANC >1500/μl; platelet count >125,000/μl; blood urea nitrogen and serum creatinine <1.5 times the upper limit of laboratory normal; serum bilirubin <1.5 times the upper limit of normal; serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase <2.5 times the upper limit of normal; life expectancy >12 weeks; and written informed consent approved by the institutional review board. Patients were excluded if they required immediate radiation therapy; were neurologically unstable; had persistent vomiting; had previous malignancies; were positive for human immunodeficiency virus; were of child-bearing potential (unless they had a negative pregnancy test and were willing to use an effective method of contraception); were treated with temozolomide or BCNU previously; or had spinal metastases on MRI.

Treatment

Gliadel wafers. A Gliadel wafer (3.85%) (Aventis, Collegeville, Penn.) is a sterile, off-white to pale-yellow wafer approximately 14 mm in diameter and 1-mm thick. This wafer is a biodegradable polyanhydride copolymer containing the active ingredient BCNU (carmustine) incorporated into a polymer matrix. This hydrophobic polymer matrix consists of polycarboxyphenoxypropane and sebacic acid in a 20:80 molar ratio. Each 200-mg Gliadel wafer contains 7.7 mg of BCNU that is homogeneously distributed throughout the copolymer matrix. The absolute concentration of BCNU per wafer is expressed as a percentage of the total weight of the wafer. The number of Gliadel wafers placed was dependent on the size of the surgical cavity created at the time of resection, with a maximum of 8 wafers.

A baseline MRI of the brain was obtained within 72 h of surgery and implantation of the wafers in all patients.

Temozolomide. TEMO (Temodal; Schering Plough Corporation, Kenilworth, N.J.) is supplied as a machine-filled white, opaque, preservative-free, 2-piece hard gelatin capsule available in 250-, 100-, 20-, and 5-mg strengths. TEMO was administered on an empty stomach. The dose was rounded to the nearest 5 mg.

Temozolomide dose escalation. TEMO dose escalation was carried out in cohorts of 3 new patients using a starting dose of 100 mg/m^2 per day and escalating to 150 and 200 mg/m^2 per day daily for 5 days. Dose escalation beyond 200 mg/m^2 per day was not allowed, as that was the MTD of TEMO in previous phase I studies of this agent (Hammond et al., 1999).

Definition of the MTD of TEMO. The dose level was escalated in successive cohorts of 3 patients so long as DLT was not observed during the first course of treatment. DLT was defined as greater than grade III nonhematologic or grade IV hematologic toxicity. If 1 of 3 patients suffered DLT, a further 3 patients were to be treated at the same dose level, with no further DLT possible if dose escalation were to proceed. If 2 instances of DLT were encountered at any dose level, MTD was considered to have been reached; a total of 6 patients were to

be treated at the previous dose level to assure its tolerability. The MTD was the highest dose causing DLT in no more than 1 of 6 patients at that dose level.

Dose modifications. If patients demonstrated stable disease or improvement on neuroimaging after the first course of treatment and did not demonstrate DLT, they were allowed to continue on the same dose of TEMO for a total duration of 12 months unless tumor progression or unacceptable toxicity occurred. No intrapatient dose escalation was allowed.

If TEMO was not administered at the scheduled interval due to inadequate hematologic parameters, blood counts were obtained weekly until the ANC was >1500/μl and platelets were >100,000/μl. Patients were then treated with TEMO with the following dose adjustments: no dose modification for an ANC nadir >1000/μl and platelets >50,000/μl. Patients with ANC and platelet nadirs below these levels received a 50-mg/m^2 dose reduction with their next dose of the drug. Patients who failed to reach an ANC >1500/μl and platelets >100,000/μl beyond 3 weeks of scheduled therapy were taken off study as were those who required dose reductions below 100 mg/m^2 per day.

Evaluation During Treatment

In addition to blood counts, serum chemistries and MRI of the brain were obtained at 4-week intervals during treatment. Because all patients in this study had no evidence of disease at the time of study entry, response assessment was based on presence or absence of disease progression only.

Results

A total of 10 patients were enrolled on this study; 3 at the first dosage level, 4 at the second, and 3 at the third (Table 1). The median age of the patients at entry into the study was 47 years (range, 22-66 years). Histologic diagnosis included 7 patients with glioblastoma multiforme and 3 patients with anaplastic astrocytoma. The only toxicity noted was grade III thrombocytopenia in 1 patient with anaplastic astrocytoma treated at the third dose level of 200 mg/m^2 per day. The study was completed as planned, with no patient suffering DLT. The recommended dosage of TEMO for a phase II study of Gliadel wafers plus TEMO in patients with recurrent malignant glioma is therefore 200 mg/m^2 per day for 5 days.

The 10 patients received a median of 3 cycles (range, 1-12 cycles) of TEMO after placement of Gliadel wafers. Though assessment of tumor response was not a primary goal of this phase I study, 2 patients in each dosage level continued to have no evidence of disease during treatment. Four of these patients refused treatment with TEMO between 2 and 5 months after enrollment and were taken off study (Table 1). Two patients completed 12 months of TEMO with no evidence of disease before stopping therapy with this agent. Four patients suffered progression of disease on TEMO.

Discussion

BCNU, a nitrosourea compound, is a bifunctional alkylator that provides chloroethyl groups to the O^6 position of the guanine nucleotide in DNA. This chloroethyl adduct then forms a cross-link with another alkylated guanine nucleotide on the opposite strand leading to strand breakage and cell death (Plowman et al., 1994). The main mechanism of resistance to BCNU is through an increased production of alkylguanine alkyltransferase in the tumor cells, an enzyme that removes the alkyl group from the O^6 position of guanine prior to DNA cross-linking (Dolan and Pegg, 1997; Ludlum, 1990). Depleting the levels of this enzyme in tumor cells through use of an alkylguanine alkyltransferase substrate such as O^6-benzylguanine prior to administration of nitrosoureas has been shown to increase the sensitivity of these cells to subsequent nitrosourea exposure (Dolan et al.; 1991 Pegg, 1990). The mechanism of action of TEMO is through methylation of the O^6 position of guanine. Unlike the nitrosoureas that further induce cross-linking of DNA, methylation of DNA causes cellular apoptosis by initiation of repetitive cycles of mismatch repair

Table 1. Clinical features, treatment, response, and outcome of patients treated with Gliadel plus temozolomide

Pt. no.	Age (yrs)/ sex	Tumor type	Dose level of TEMO (mg/m^2 per day)	No. of cycles of TEMO	Response and duration	Reason off study	Outcome
1	66/M	GBM	100	2	NED, 3 months	Patient refusal	Dead
2	58/M	GBM	100	2	NED, 3 months	Patient refusal	Dead
3	52/M	GBM	100	1	PD	PD	Dead
4	62/M	GBM	150	3	PD	PD	Dead
5	62/M	GBM	150	1	PD	PD	Dead
6	33/F	AA	150	12	NED, 12 months	Completed study	Alive, NED
7	30/M	GBM	150	5	NED, 5 months	Patient refusal	Alive with disease
8	36/M	AA	200	12	NED, 12 months	Completed study	Alive, NED
9	22/F	AA	200[a]	4	PD	PD	Dead
10	43/F	GBM	200	1	NED, 1 month	Patient refusal	Alive with disease

None of the patients had measurable evidence of disease at entrance into the study. With the exception of patient No. 9, none of the patients experienced toxicity.

Abbreviations: TEMO, temozolomide; GBM, glioblastoma multiforme; NED, no evidence of disease; PD, progressive disease; AA, anaplastic astrocytoma.

[a]Patient experienced grade 3 thrombocytopenia.

(Friedman et al., 1992; Lage and Dietel, 1999; Liu et al., 1996; Wedge et al., 1996). The 2 main mechanisms of resistance to TEMO are through mismatch repair protein deficiency and increased production of alkylguanine alkyltransferase (Liu et al., 1996; Wedge et al., 1996; Yung et al., 2000).

In this context, it is possible that prior exposure to a nitrosourea can result in depletion of alkylguanine alkyltransferase levels and sensitize tumor cells to subsequent exposure to TEMO. In a preclinical study, Plowman et al. (1994) gave 400 mg/kg of TEMO 2 hours after BCNU at doses of 27 and 18 mg/kg, respectively, to athymic mice bearing subcutaneous SF-295 glioblastoma xenografts. The combination produced a 500% growth delay that exceeded the sum of the growth delays produced by each drug given alone. This formed the basis for our phase I study.

We used Gliadel wafers instead of intravenous BCNU to avoid the systemic toxicity of the drug. Studies of Gliadel wafers in animals have shown that intracerebral implants of BCNU-containing polymers produce high local concentrations of BCNU for approximately 2-3 weeks providing greater local brain exposure and diminished systemic effects compared with systemic dosing of BCNU (Grossman et al., 1992). Based on the animal model, one can surmise that significant concentrations of BCNU would be present in the tumor during the time TEMO is administered, allowing a pharmacologically relevant interaction between the 2 drugs. Toxicity was minimal when we used this combination in our study, with only 1 patient suffering grade III thrombocytopenia at the highest dose level of TEMO. We did no further dose escalation because the reported MTDs for this agent have not been over 200 mg/m^2 per day for 5 days given every 4 weeks, irrespective of the intensity of prior therapy (Hammond et al., 1999).

A recent phase I study evaluated the toxicity of escalating single doses of TEMO given orally, either before or after i.v. BCNU, given every 6 weeks in 45 patients with a variety of cancers refractory to standard therapy (Schold et al., 2000). TEMO was given 2 hours after or 4 hours before BCNU. The dose level of BCNU and TEMO ranged from 50 to 200 mg/m^2 and from 175 to 750 mg/m^2, respectively. Toxicity in this study, evaluated after the first course of therapy, was mainly hematologic, although 3 cases of pulmonary toxicity were observed during subsequent cycles of treatment. The MTDs of TEMO and BCNU were 400 and 100 mg/m^2, respectively, when TEMO was given before BCNU. The corresponding MTDs when TEMO was given after BCNU were 550 and 150 mg/m^2, respectively. Nine of 43 evaluable patients (including 7 patients with malignant glioma) demonstrated a partial response to treatment. The recommended doses and schedule for a phase II study of this combination from this study were BCNU 150 mg/m^2 i.v. followed 2 hours later by oral TEMO 550 mg/m^2 every 6 weeks. Although the drug combination can be given every 6 weeks repeatedly, unlike Gliadel wafers plus TEMO, toxicity from repeated systemic exposure to BCNU must be closely monitored.

In summary, we report on the results of a phase I study of Gliadel wafers plus oral TEMO in patients with recurrent malignant glioma. The combination did not produce any significant toxicity, even at the maximum dosage level of TEMO of 200 mg/m^2 per day for 5 days. A phase II study of the combination in patients with recurrent malignant glioma is currently underway at the Brain Tumor Center at Duke University. This study will define the therapeutic advantage of Gliadel Wafers plus TEMO in patients with recurrent malignant glioma and, ultimately, may lead to incorporation of this strategy for patients at initial diagnosis.

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