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Phase I Trial of Polifeprosan 20 With Carmustine Implant Plus Continuous Infusion of Intravenous O^6 -Benzylguanine in Adults With Recurrent Malignant Glioma: New Approaches to Brain Tumor Therapy CNS Consortium Trial

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Abstract

Purpose—This phase I trial was designed to (1) establish the dose of O^6 -benzylguanine (O^6 -BG) administered intravenously as a continuous infusion that suppresses O^6 -alkylguanine-DNA alkyltransferase (AGT) levels in brain tumors, (2) evaluate the safety of extending continuous-infusion O^6 -BG at the optimal dose with intracranially implanted carmustine wafers, and (3) measure the pharmacokinetics of O^6 -BG and its metabolite.

Patients and Methods—The first patient cohort (group A) received 120 mg/m² of O^6 -BG over 1 hour followed by a continuous infusion for 2 days at escalating doses presurgery. Tumor samples were evaluated for AGT levels. The continuous-infusion dose that resulted in undetectable AGT levels in 11 or more of 14 patients was used in the second patient cohort. Group B received the optimal dose of O^6 -BG for 2, 4, 7, or 14 days after surgical implantation of the carmustine wafers. The study end point was dose-limiting toxicity (DLT).

Results—Thirty-eight patients were accrued. In group A, 12 of 13 patients had AGT activity levels of less than 10 fmol/mg protein with a continuous-infusion O^6 -BG dose of 30 mg/m²/d. Group B

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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patients were enrolled onto 2-, 4-, 7-, and 14-day continuous-infusion cohorts. One DLT of grade 3 elevation in ALT was seen. Other non-DLTs included ataxia and headache. For up to 14 days, steady-state levels of O^6 -BG were 0.1 to 0.4 $\mu\text{mol/L}$, and levels for O^6 -benzyl-8-oxoguanine were 0.7 to 1.3 $\mu\text{mol/L}$.

Conclusion—Systemically administered O^6 -BG can be coadministered with intracranially implanted carmustine wafers, without added toxicity. Future trials are required to determine if the inhibition of tumor AGT levels results in increased efficacy.

INTRODUCTION

Despite advances in tumor biology, there has been little impact on the outcome of patients with malignant glioma. The median survival of patients with malignant gliomas in the United States is less than 2 years.¹ As a result, novel therapeutic approaches are imperative. Most malignant gliomas recur within 2 cm of the original resection site and within the radiation field. Polifeprosan 20 with carmustine implant (Gliadel; Guilford Pharmaceuticals, Baltimore, MD) delivers carmustine (BCNU) locally to the tumor site without significant systemic or local adverse effects.^{2,3} This biodegradable copolymer is implanted during surgery and releases carmustine locally for approximately 2 weeks.⁴ It has been evaluated in three phase III trials, and it prolongs survival when placed at the initial tumor resection or at recurrence.^{5–7}

O^6 -alkylguanine-DNA alkyltransferase (AGT) is a DNA repair protein known to remove and repair O^6 -alkylguanine lesions introduced by alkylating agents such as carmustine or temozolomide.⁸ AGT activity has been shown to be a major factor in the resistance of tumor cells to alkylating agents.^{8–10} O^6 -benzylguanine (O^6 -BG) is a low molecular weight substrate that inactivates AGT, requiring de novo synthesis to replenish the protein.¹¹ In vitro and in vivo studies using tumor cell lines and intracranial and subcutaneous brain tumor xenografts demonstrate that O^6 -BG treatment before carmustine increases its therapeutic effectiveness.^{10,12,13} In animals and humans, systemically administered O^6 -BG severely limits the amount of intravenous carmustine that can be given as the repair mechanisms of normal cells also are affected by exposure to O^6 -BG.^{10,12–16}

Prior studies demonstrated that 100 mg/m² of O^6 -BG given intravenously completely inhibits AGT activity in brain tumors.^{17,18} Combining systemic O^6 -BG with locally delivered carmustine could provide a synergistic cytotoxic effect on the tumor without increased systemic toxicity. Since carmustine is released over several weeks from wafers, tumor AGT activity would need to be suppressed by O^6 -BG during this period to maximize potential therapeutic benefit.

This trial's objectives were to (1) establish the continuous-infusion dose of O^6 -BG that completely suppresses AGT levels (group A), (2) evaluate the safety of increasing the duration of continuous-infusion O^6 -BG for up to 2 weeks at a dose that suppresses tumor AGT activity when combined with intracranially implanted polifeprosan 20 wafers with carmustine (group B), and (3) measure plasma concentrations of O^6 -BG and its metabolite, O^6 -benzyl-8-oxoguanine (8-oxoBG; groups A and B).

PATIENTS AND METHODS

This study was approved by the Cancer Therapy Evaluation Program at the National Cancer Institute (NCI) and the institutional review boards of all participating institutions. Informed consent was obtained from each patient. Accrual took place between May 2000 and September 2002 at the following institutions of the New Approaches to Brain Tumor Therapy: Cleveland Clinic (Cleveland, OH), Emory University (Atlanta, GA), Henry Ford Hospital (Detroit, MI), Johns Hopkins University (Baltimore, MD), University of Pennsylvania (Philadelphia, PA),

and Wake Forest University (Winston-Salem, NC). Pharmacokinetic analysis was performed at the University of Chicago (laboratory of M.E.D.; Chicago, IL).

Patients

Patients were eligible if they were 18 years or older and could provide informed consent. In addition, they had to have had a prior diagnosis of malignant glioma, a supratentorial (≥ 1 cm) recurrence, surgical treatment indicated, a Karnofsky performance status of 60% or higher, previous radiation therapy completed more than 3 months prior, and an intraoperative diagnosis of viable glioma. At least 4 weeks must have elapsed since the patients' last chemotherapy dose, and they could not receive additional chemotherapy during the first 56 days on this study. Normal hematologic, renal, and liver functions were required. Patients were ineligible if they had a bilateral or multifocal tumor, projected survival of less than 60 days, known hypersensitivity to nitrosoureas, prior malignancy, or if they were pregnant or breast-feeding.

Study Design

The objective for group A was to determine the continuous-infusion dose of O^6 -BG that resulted in undetectable AGT tumor levels in at least 11 of 14 patients. Continuous-infusion O^6 -BG was initiated 2 days presurgery with a bolus of 120 mg/m² over 1 hour followed by a continuous infusion of 30 mg/m²/d, which continued for the next 2 days. On the day of surgery, the patients underwent surgical debulking and implantation of up to eight polifeprosan 20 with carmustine wafers. During the surgical procedure, a sample of tumor was obtained, frozen, and stored for AGT analysis. The infusion of O^6 -BG stopped once the surgery was completed. Although a previous study demonstrated that a bolus of 100 mg/m² adequately depleted AGT levels for 18 hours, we chose a 20% higher dose to extend the time of depletion.¹⁸

Up to 14 patients were to be enrolled until 11 patients had unmeasurable tumor AGT activity. Tumor AGT depletion was defined as AGT levels of less than 10 fmol/mg protein. In the event that four patients had measurable AGT activity, the dose of continuous-infusion O^6 -BG was to be increased by 10 mg/m²/d. This decision rule was chosen to have a greater than 95% chance of concluding that O^6 -BG inhibited AGT when it was effective and a small chance ($P < .00001$) of concluding that it inhibited AGT when in reality it was not effective.

Group B was designed to evaluate the safety of increasing the duration of continuous-infusion O^6 -BG at a dose that suppresses tumor AGT activity when combined with intracranially implanted carmustine wafers. The bolus of O^6 -BG was administered at least 1 hour presurgery, and the continuous-infusion duration was increased in a stepwise fashion (2, 4, 7, and 14 days) postoperatively. Six patients were studied at each infusion's duration. At the time of wafer implantation, steady-state pharmacokinetics data were obtained. Toxicity was assessed during the initial 28 days, and at days 42 and 56. All patients were followed for 12 months unless they progressed or started another form of treatment. All were followed for survival status until death.

O^6 -BG was provided by NCI and prepared by the pharmacy staff according to the guidelines provided. After reconstitution of the drug in the 40% PEG-buffered diluent (20 mmol/L potassium phosphate, 5 mmol/L EDTA, and 20% volume-to-volume ratio glycerol), the appropriate dose was administered by continuous infusion for up to 2 weeks. O^6 -BG is stable for 24 hours after preparation, and thus the bag/cassette containing the drug was changed daily.

Polifeprosan 20 wafers with carmustine were provided by Guilford Pharmaceuticals (Baltimore, MD). The aim was to cover the surface of the resection cavity with eight wafers. The number of implanted wafers was recorded. Corticosteroid doses were based on an individual patient's needs and were tapered as indicated.

Dose Modification for Toxicity

For the purposes of this study, drug-related toxicity was arbitrarily defined as any of the following outcomes without other explanation in the 28 days after implantation: 40-point decline in the Karnofsky performance status (sustained for at least 2 weeks), two or more episodes of status epilepticus with therapeutic anticonvulsant levels, a brain abscess requiring surgical intervention, and WBC count of less than 2,000/mm³; ANC of less than 1,000/mm³; platelets of less than 50,000/mm³ or hemoglobin of less than 8 gm/dL; ALT, AST, alkaline phosphatase, or blood urea nitrogen of more than 5×the upper limit of normal (ULN); bilirubin of more than 1.5× the ULN; creatinine of more than 3× the ULN; and proteinuria (> 10 gm/L) or unexplained gross hematuria, dyspnea on exertion, or treatment-related death. Any death related to the use of *O*⁶-BG and/or carmustine wafers would stop enrollment until all data were formally reviewed.

AGT Activity

Extracts were prepared from tumors by homogenization in 50 mmol/L Tris (pH 7.5), 0.1 mmol/L EDTA, and 5 mmol/L dithiothreitol. AGT activity was determined as described previously.¹⁹ Briefly, cell extracts were incubated with [³H]-methylated DNA substrate (5.77 Ci/mmol). The DNA was precipitated by adding ice-cold prechloric acid at a final concentration of 0.25 N, which was then hydrolyzed in 0.1 N HCL at 70°C for 30 minutes. The modified bases were eluted on a C18 reverse-phase column using 10% methanol/0.05 M ammonium formate pH 4.5 at 37°C. Each assay was performed with a positive control cell line (DAOY or DuPro cell extract) and negative control (CHO cell extract). Protein concentration was determined by the Bradford method.²⁰ The results were expressed as femtomole of *O*⁶-methylguanine released from DNA per mg of protein.

Pharmacokinetic Measurements

Plasma concentrations of *O*⁶-BG and 8-oxoBG were measured before administration of *O*⁶-BG (120 mg/m² over 1 hour followed immediately by 30 mg/m²/d continuous infusion) and at 24, 48, 72, and 96 hours after administration of *O*⁶-BG bolus. Whole blood samples (7 mL) were collected in sodium-heparinized vacutainers and centrifuged for 10 minutes at 1,500 ×g. Samples were stored at −70°C until analysis. *O*⁶-BG and 8-oxoBG were measured by high-performance liquid chromatography as described previously with slight modifications.²¹ Aliquots of plasma were spiked with an internal standard, *O*⁶-[*p*-fluorobenzyl]guanine, extracted with ethyl acetate, and centrifuged at 2,500 ×g for 20 minutes, and the supernatant was evaporated to dryness under nitrogen. Samples were reconstituted in mobile phase (32% methanol/10 mmol/L potassium phosphate buffer; pH 7.5) and separated isocratically using a Waters Bondapak 125Å 4 μ phenyl column (3.9 × 300 mm) at 30°C and a flow rate of 1.0 mL/min. Retention times were 21.5, 24.6, and 28.9 minutes for 8-oxoBG, *O*⁶-BG, and *O*⁶-[*p*-fluorobenzyl]guanine, respectively. Samples were monitored at 280 nm using a UV detector and at 295 excitation and 360 emission on a fluorescence detector. The limit of detection for both *O*⁶-BG and 8-oxoBG was determined to be 10 ng/mL.

Statistical Considerations

Demographic and clinical characteristics were summarized using appropriate descriptive statistics. Categorical data were summarized with frequencies and percents and continuous data with medians and ranges. Toxicities were tallied by treatment cohort. Survival time was calculated from the start of therapy until death from any cause, and survival was estimated using the Kaplan-Meier method.²² CIs were calculated using standard methods. Analyses of demographic and clinical characteristics and toxicities were performed using SAS Version 9.1 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

Forty-two patients were accrued to this study. Demographic and clinical characteristics by treatment group are presented in Table 1. Thirty-nine patients (93%) received eight polymer wafer implants. One patient (2%) each received four, five, and six wafers.

Treatment Administration for Group A

Fourteen patients received 120 mg/m² of *O*⁶-BG over 1 hour, followed by a continuous infusion of 30 mg/m²/d of *O*⁶-BG for at least 48 hours presurgery. Twelve had undetectable AGT in the tumor samples at the time of surgery (ie, 48 hours after the *O*⁶-BG bolus).²³ One tumor sample was too small for measurement of AGT activity. The *O*⁶-BG bolus of 120 mg/m² followed by a continuous-infusion dose of 30 mg/m²/d was used in group B.

Treatment Administration for Group B

For treatment group B, continuous infusion was successfully increased to the 14-day time point. Six patients were enrolled at the 2-, 4-, and 7-day continuous-infusion cohorts, and no dose-limiting toxicities (DLTs) were observed. Six patients were initially enrolled at the 14-day time point. However, in four out of the first six patients treated, *O*⁶-BG began precipitating in the intravenous catheter after 10 days, so these infusions were temporarily stopped. With a new supply of *O*⁶-BG, four additional patients were accrued to this cohort without precipitation. All 10 patients in the cohort were evaluated for toxicity. One patient developed grade 3 elevation in ALT. Although *O*⁶-BG was not the likely cause, it prompted the investigator (K.J.) to stop the therapy. As a result, this was considered a DLT.

All significant toxicities related to carmustine polymer or *O*⁶-BG are presented in Table 2. The only grade 4 toxicity was one cerebrospinal fluid leak. Although an infection and CNS hemorrhage were noted in one patient in the 14-day infusion cohort, these occurred after the 28-day evaluation period for DLTs.

Pharmacokinetics

Plasma concentration of *O*⁶-BG and 8-oxoBG were measured in patients before and after *O*⁶-BG infusion for up to 48 hours using high-performance liquid chromatography with UV and fluorescence detection (Fig 1). In group A, the maximum serum concentration (*C*_{max}) of 8-oxoBG varied from 0.4 to 5.8 μmol/L at 24 hours after *O*⁶-BG infusion with the mean of 2.4 μmol/L. The number of patient samples analyzed for 8-oxoBG was *n* = 13 (presurgery), *n* = 14 (24 hours postsurgery), *n* = 14 (48 hours), *n* = 10 (72 hours), and *n* = 8 (96 hours). The plasma concentration of *O*⁶-BG was 6× lower with a mean of 0.4 μmol/L. Only six patients had interpretable results for *O*⁶-BG because of coeluting peaks detectable by UV and fluorescence. Specifically, *n* = 3 (presurgery), *n* = 4 (24 hours post-surgery), *n* = 3 (48 hours), *n* = 3 (72 hours), and *n* = 3 (96 hours) were analyzed. *O*⁶-BG and 8-oxoBG are equally effective as AGT inactivators in cells.²⁴

In group B, a total of 28 patients were treated with 120 mg/m² of *O*⁶-BG over 1 hour (given at least 1 hour presurgery) followed by 30 mg/m²/d of continuous-infusion *O*⁶-BG immediately postsurgery (Fig 2). The length of the infusion increased from 2 to 14 days. Plasma concentrations of 8-oxoBG (ng/mL) and *O*⁶-BG (ng/mL) were evaluated. The *C*_{max} of 8-oxoBG occurred at 1 hour after the *O*⁶-BG infusion, with values ranging from 5.7 to 38.6 μmol/L (*n* = 26) and a mean value of 17.7 μmol/L. For 8-oxoBG, the results were *n* = 18 (presurgery), *n* = 24 (day of surgery), *n* = 24 (1 day postsurgery), *n* = 16 (2 days), *n* = 9 (3 days), *n* = 12 (4 days), *n* = 3 (5 days), *n* = 3 (6 days), *n* = 10 (7 days), *n* = 5 (12 days), and *n* = 5 (14 days). The mean *C*_{max} for *O*⁶-BG was 15× lower at 1.1 μmol/L. The steady-state levels

were 0.7 to 1.3 $\mu\text{mol/L}$ for 8-oxoBG and 0.1 to 0.4 $\mu\text{mol/L}$ for O^6 -BG. For O^6 -BG, only 16 patients had interpretable results because of other peaks detectable both by UV and fluorescence: $n = 10$ (presurgery), $n = 13$ (day of surgery), $n = 10$ (1 day postsurgery), $n = 7$ (2 days), $n = 3$ (3 days), $n = 7$ (4 days), $n = 7$ (5 days), $n = 4$ (6 days), $n = 8$ (7 days), and $n = 4$ (12 days).

Thirteen of the 14 patients in group A and 25 of the 28 patients in group B have died. Median survival for group A was 7.2 months (95% CI, 3.7 to 15.6 months), and median survival for group B was 8.5 months (95% CI, 5 to 11.3 months).

DISCUSSION

Alkylating drugs, such as carmustine and temozolomide, are the most effective agents in patients with primary brain tumors. Although systemic carmustine results in tumor responses in some patients, its benefit in adjuvant trials is modest. Placement of locally administered carmustine using sustained-release polymers prolonged survival for 8 to 10 weeks in randomized placebo-controlled trials in this patient population.^{5–7} In addition, this is devoid of systemic toxicities. Thus, improving the efficacy of locally administered carmustine is a reasonable therapeutic strategy.

AGT, a DNA repair protein present in a significant percentage of high-grade gliomas, confers relative resistance to treatment with temozolomide and carmustine.⁸ In 167 patients treated with carmustine, patients with low tumor AGT levels lived longer than patients with high AGT levels.²⁵ Similarly, survival in patients treated with radiation and temozolomide was correlated with the absence of the AGT repair protein.²⁶ These studies suggest that the efficacy of carmustine could be enhanced by depletion of tumor AGT.

O^6 -BG is a low molecular weight substrate that inactivates AGT. Preclinical studies combining O^6 -BG with carmustine suggest that this results in improved tumor control and survival.^{10, 12, 13} However, systemically administering the O^6 -BG and carmustine markedly enhances myelosuppression and reduces the maximum tolerated dose of systemic carmustine from 200 mg/m^2 to 40 mg/m^2 .¹⁵ In contrast, local delivery of high doses of carmustine directly to the tumor results in minimal systemic exposure to carmustine.²⁷ Therefore, the combination of local carmustine delivered via polymer wafer and O^6 -BG should minimize systemic carmustine toxicity while maximizing the efficacy of the carmustine within the tumor.

This hypothesis was tested in a preclinical F98 rat brain tumor model. F98 expresses high levels of AGT and is resistant to treatment with alkylating agents. Rats treated with carmustine polymer alone showed no increased survival compared to controls, whereas combination treatment of O^6 -BG with carmustine polymer resulted in significant prolonged survival. This suggests that O^6 -BG potentiates the effects of locally delivered carmustine, and for tumors expressing AGT, it may be necessary for carmustine to provide a meaningful benefit.²⁸

This study aimed to identify a continuous-infusion dose of O^6 -BG that would suppress tumor AGT activity for 2 weeks and to assess the local and systemic toxicity of O^6 -BG combined with implanted carmustine wafers. The results demonstrate that 120 mg/m^2 of O^6 -BG followed by 30 $\text{mg/m}^2/\text{d}$ by continuous infusion reduces tumor AGT levels to an undetectable level for 48 hours. In group A patients, the plasma levels of O^6 -BG and 8-oxoBG, the active metabolite of O^6 -BG, at 48 hours was 0.4 μM for O^6 -BG and 1.2 μM for 8-oxoBG (Fig 1).

Patients in group B exhibited no local or systemic toxicity with the combination treatment. Plasma concentrations of O^6 -BG and 8-oxoBG in these patients were measured at various time points during the continuous infusion. Figure 2 demonstrates that the initial effect on plasma drug levels of the bolus followed by the continuous infusion continues for 48 to 72 hours. The

steady-state plasma drug levels stabilize at 72 hours and were found to be 0.7 to 1.3 μm for 8-oxoBG and 0.1 to 0.4 μm for O^6 -BG. Previous in vitro results indicate that the effective doses for 50% depletion of AGT activity in 30 minutes in HT29 colon tumor cell extract are 0.2 $\mu\text{mol/L}$ for O^6 -BG and 0.3 $\mu\text{mol/L}$ for 8-oxoBG.²⁴ In group A, we found absolute suppression of AGT activity at 48 hours, and we expected this suppression to last for the entire 2-week infusion period. However, when the group B data were analyzed using later time points, the steady-state drug levels were lower. The effect of 8-oxoBG plasma levels of 0.7 to 1.3 μm on brain tumor AGT levels cannot be determined from this study.

This study demonstrates that AGT levels in brain tumors can be suppressed using systemically administered O^6 -BG. No added toxicity was noted when this was combined with locally administered carmustine. This is in contrast to combining O^6 -BG with systemic carmustine. These data support proceeding with clinical trials designed to test the hypothesis that O^6 -BG will increase the efficacy of local carmustine and improve patient survival. These trials should include newly diagnosed patients and a higher concentration of carmustine polymers.²⁷ The pharmacokinetics raise the possibility that the continuous-infusion dose required to completely suppress AGT levels for 2 weeks may need to be higher.

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Fig 1.

Mean ($\pm$ standard deviation) of *O*⁶-benzylguanine (*O*⁶-BG; ■) and *O*⁶-benzyl-8-oxoguanine (8-oxoBG; ●) plasma concentration in group A patients. A total of 14 patients were treated with 120 mg/m² *O*⁶-BG over 1 hour followed immediately by a continuous infusion of 30 mg/m²/d *O*⁶-BG for 48 hours presurgery.

Fig 2.

A total of 26 patients were treated with 120 mg/m² of O^6 -benzylguanine (O^6 -BG) over 1 hour, administered at least 1 hour presurgery, and followed immediately by a 30 mg/m²/d of continuous-infusion O^6 -BG beginning on the day of surgery. The length of the infusion increased up to 14 days. Concentrations of O^6 -benzyl-8-oxoguanine (8-oxoBG; ●) and O^6 -BG (■) were evaluated before the O^6 -BG bolus, day of surgery, and various days postsurgery.

Table 1
Demographic and Clinical Characteristics of Patients by Study Group

Characteristic	Group A (n = 14)	Group B (n = 28)
Age, years		
Median	48	53
Range	28–74	30–70
Sex, % male	43	79
Race, % white	93	82
Karnofsky performance status, %		
Median	90	80
Range	60–100	60–100
Histology		
Glioblastoma multiforme, %	93	89
Anaplastic astrocytoma, %	7	11
No. of prior chemotherapies		
None, %	50	21
1, %	29	57
> 1, %	21	22
No. of prior surgeries		
1, %	71	57
> 1, %	29	43

Table 2
Grade 3 or 4 Toxicities at Least Possibly Related to Study Treatment by Group and Infusion Time Cohort

Toxicity	Group A(n = 14)	Group B (infusion cohort)				Total(%)
		2-Day(n =6)	4-Day(n = 6)	7-Day(n = 6)	14-Day(n = 10)	
Ataxia	0	0	0	0	2	5
CNS hemorrhage/infection without neutropenia	0	0	0	0	1*	2
Confusion	0	0	1	0	0	2
CSF leak	0	1	0	0	0	2
Headache	1	1	0	0	0	5
Intracranial pressure	0	0	0	0	1	2
Seizure	1	0	0	0	0	2

Abbreviation: CSF, cerebrospinal fluid.

* Toxicity occurred after the 28-day evaluation period for dose-limiting toxicity.