Phase I Clinical and Pharmacological Study of O\textsuperscript{6}-Benzylguanine Followed by Carmustine in Patients with Advanced Cancer\textsuperscript{1}

Richard L. Schilsky,\textsuperscript{2} M. Eileen Dolan,\textsuperscript{3} Donna Bertucci, Reginald B. Ewesuedo, Nicholas J. Vogelzang, Sridhar Mani, Lynette R. Wilson, and Mark J. Ratain

Department of Medicine, Section of Hematology-Oncology, Cancer Research Center and Committee on Clinical Pharmacology, University of Chicago, Chicago, Illinois 60637

ABSTRACT

\textit{O\textsuperscript{6}}-benzylguanine (BG) is a potent inactivator of the DNA repair protein \textit{O\textsuperscript{6}}-alkylguanine-DNA alkyltransferase (AGT) that enhances sensitivity to nitrosoureas in tumor cell lines and tumor-bearing animals. The major objectives of this study were to define the optimal modulatory dose and associated toxicities of benzylguanine administered alone and in combination with carmustine to define the maximally tolerated dose and associated toxicities of carmustine administered with benzylguanine and to describe the pharmacokinetics of BG in humans and its effects on AGT depletion and recovery in peripheral blood mononuclear cells. Patients with histologically confirmed advanced solid tumors or lymphoma that had failed to respond to standard chemotherapy received BG as a 1-h i.v. infusion followed, 1 h later, by a 14-day washout (without therapy) period, patients received BG as a 1-h i.v. infusion followed, 1 h later, by a 15-min i.v. infusion of carmustine. Cycles of chemotherapy began 14 days after the last dose of BG and carmustine doses ranging from 10 to 120 mg/m\textsuperscript{2} and carmustine doses ranging from 13 to 50 mg/m\textsuperscript{2}. Plasma and urine samples were collected and analyzed for BG, and \textit{O\textsuperscript{6}}-benzyl-8-oxoguanine concentrations and AGT activity was determined in peripheral blood mononuclear cells.

There was no toxicity attributable to BG alone at any dose tested. Bone marrow suppression was the primary and dose-limiting toxicity of BG combined with carmustine and was cumulative in some patients. The neutrophil nadir occurred at a median of day 27, with complete recovery in most patients by day 43. Nonhematological toxicity included fatigue, anorexia, increased bilirubin, and transaminase elevation. Recommended doses for Phase II testing are 120 mg/m\textsuperscript{2} BG given with carmustine at 40 mg/m\textsuperscript{2}. BG rapidly disappeared from plasma and was converted to a major metabolite, \textit{O\textsuperscript{6}}-benzyl-8-oxoguanine, which has a 2.4-fold higher maximal concentration and 20-fold higher area under the concentration versus time curve than BG. AGT activity in peripheral blood mononuclear cells was rapidly and completely suppressed at all of the BG doses. The rate of AGT regeneration was more rapid for patients treated with the lowest dose of BG but was similar for BG doses ranging from 20–120 mg/m\textsuperscript{2}. In conclusion, coadministration of BG and carmustine is feasible in cancer patients, but the maximal dose of carmustine that can be safely administered with BG is approximately one-third of the standard clinical dose. Bone marrow suppression, which may be cumulative, is the dose-limiting toxicity of the combination. Prolonged AGT suppression is likely attributable primarily to the effect of \textit{O\textsuperscript{6}}-benzyl-8-oxoguanine.

INTRODUCTION

The DNA repair protein AGT\textsuperscript{4} (1) plays an important role in the protection of cells from the cytotoxic effects of alkylnitrosoureas and methylating agents. AGT removes adducts from the \textit{O\textsuperscript{6}} position of guanine in DNA through covalent binding of the alkyl group to a cysteine residue on the protein within the active site (1). During this process, irreversible inactivation of the protein occurs and the synthesis of new protein molecules is required to regenerate AGT activity.

There is an inverse relationship between the level of AGT and the sensitivity of tumor cells grown in culture and as xenografts to the cytotoxic effects of alkylnitrosoureas (1). Increased AGT activity has been found in many human solid tumors including colon cancer (2), malignant melanoma (3), lung cancer, gliomas (4, 5), and others (6), and may account for the relative ineffectiveness of nitrosourea therapy in these diseases. Inactivation of AGT leads to an enhancement of the cytotoxic effects of chloroethylnitrosoureas (e.g., carmustine) and methylating agents (e.g., dacarbazine, temozolomide) in both cell culture and animal tumor xenograft models (7–9).

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\textsuperscript{2}To whom requests for reprints should be addressed: Division of the Biological Sciences, University of Chicago, 5841 South Maryland Avenue, MC1000, Chicago, IL 60637. Phone: (773) 834-3914; Fax: (773) 834-3915; E-mail: rschilsk@medicine.bsd.uchicago.edu.

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\textsuperscript{4}The abbreviations used are: AGT, \textit{O\textsuperscript{6}}-alkylguanine-DNA alkyltransferase; BCNU, bischloronitrosourea, carmustine; BG, \textit{O\textsuperscript{6}}-benzylguanine; MTD, maximally tolerated dose; PBMC, peripheral blood mononuclear cell; BG max, maximal dose of BG; DLT, dose-limiting toxicity; D\textsubscript{1}CO, carbon monoxide diffusion capacity; AUC, area under the concentration versus time curve; 8-oxo-BG, \textit{O\textsuperscript{6}}-benzyl-8-oxoguanine.
Therefore, various strategies have been attempted to deplete cells of AGT and thereby increase the sensitivity of tumor cells to carmustine and related agents.

Agents that methylate DNA, such as streptozotocin and dacarbazine, deplete AGT activity incompletely and produce significant clinical toxicity (10). BG, a low-molecular-weight AGT substrate, binds readily to the same cysteine residue on AGT that is used for alkyl group transfer and inactivates the protein stoichiometrically, requiring micromolar concentrations and only minutes of exposure to completely inactivate AGT (11, 12). Once AGT is inactivated, cells become vulnerable to killing by nitrosoureas because lesions that are present at the O\textsuperscript{6} position of guanine cannot be repaired until new AGT synthesis occurs (13–16). There is a strong correlation between the degree of sensitization that can be achieved and the level of AGT activity in cells, with little or no BG-induced enhancement of BCNU cytotoxicity occurring in cells that express low levels of AGT and the greatest enhancement observed in cells with high AGT activity (13). BCNU preceded by BG treatment results in significantly greater growth inhibition of human brain and colon tumor xenografts in nude mice compared with that observed in animals treated with BCNU alone (8, 17).

Preclinical toxicology studies in mice and dogs revealed BG alone to be nontoxic. When combined with BCNU, bone marrow toxicity was dose-limiting, and the MTD of BCNU was 2- to 3-fold lower in mice and 6-fold lower in dogs than in the absence of BG (18, 19).

On the basis of the strong preclinical evidence that BG administration could potentially reverse resistance to alkylntrosoureas, we conducted a Phase I study of the combination of BG and BCNU in patients with advanced cancer. The major objectives of the study were to define the optimal modulatory dose and associated acute and cumulative toxicities of BG administered alone and in combination with BCNU; to define the MTD of BCNU administered with BG; to determine the time course of AGT depletion and recovery in PBMCs after administration of BG; and to describe the pharmacokinetics of BG in humans.

PATIENTS AND METHODS

Patient Selection. Patients with histologically confirmed advanced solid tumors or lymphoma that had failed to respond to standard therapy or for which no standard therapy was available were eligible to participate in this study. Patients with malignant gliomas were eligible if they were on a stable steroid dose for at least 2 weeks before enrollment and met all of the other eligibility criteria. Patients with malignant melanoma were required to undergo a computed tomography head scan within 2 weeks of entry on-study to determine whether asymptomatic brain metastases were present. Other eligibility criteria included measurable or assessable disease by computed tomography, magnetic resonance imaging, radiograph or physical examination; age of at least 18 years; Karnofsky performance status of at least 60%; life expectancy of at least 8 weeks and adequate organ function defined as: a WBC count of at least 3500/\text{\mu l}, platelets of at least 100,000/\text{\mu l}, and hemoglobin at least 10 g/dl; total bilirubin level less than 1.7 mg/dl; aspartate aminotransferase less than twice the upper limit of normal; and serum creatinine level less than 1.7 mg/dl or measured creatinine clearance of at least 60 ml/min. Patients must have been off of all previous anticancer therapy for at least 4 weeks (6 weeks if the previous therapy included mitomycin C or a nitrosourea) and must have recovered from the toxic effects of any prior therapy. Patients were excluded from the study if they had a significant cardiac, pulmonary, neurological, endocrine, gastrointestinal, rheumatological, dermatological, or allergic disorder that would make administration of the therapy hazardous or would obscure the interpretation of adverse effects. Pregnant and lactating women were also excluded, and all of the patients with reproductive potential were required to use an effective contraceptive method during treatment and for 2 months after completion of treatment if they were sexually active. All of the patients gave written informed consent according to institutional and federal guidelines.

Study Design. The initial goal of the study was to define the dose of BG that produced maximal AGT suppression in human PBMCs without excessive toxicity and to define the time course of this inhibition. Once this dose of BG was defined, cohorts of patients were to be treated at a fixed dose of BG and at increasing doses of BCNU to determine the MTD of BCNU given with BG. Thus, patients who were enrolled in the study initially received BG as a 1-h i.v. infusion without BCNU. After a 14-day washout period, patients received the assigned dose of BG as a 1-h i.v. infusion followed, 1 h later, by a 15-min i.v. infusion of BCNU. Cycles of chemotherapy were repeated every 6 weeks as long as the patient’s tumor was stable or responding to treatment and the patient did not experience DLT.

The starting dose of BG was 10 mg/m\textsuperscript{2}. Escalation of the BG dose occurred in increments of 100% until maximal AGT suppression was observed in PBMCs 6 h after dosing or until the appearance of grade 1–2 toxicity attributable to BG. Dose escalation of BG was to continue until AGT suppression of at least 90% was observed in at least two of three patients in two successive dose cohorts (BG max). During the period of BG dose escalation, all of the patients received a fixed dose of BCNU of 13 mg/m\textsuperscript{2}. Once BG max was defined, all of the subsequent patients were to receive BG max, and the BCNU dose was to be escalated in cohorts of at least three patients until the MTD of BCNU was defined. Planned BCNU dose levels were 25 mg/m\textsuperscript{2}, 40 mg/m\textsuperscript{2}, 60 mg/m\textsuperscript{2}, 80 mg/m\textsuperscript{2}, and 100 mg/m\textsuperscript{2}. No intrapatient dose escalation was permitted. Midway through the study, we became aware of data from other trials that suggested that AGT suppression in PBMCs did not correlate with the extent of AGT suppression in solid tumor tissues; that BG doses greater than 80 mg/m\textsuperscript{2} were required to completely suppress AGT activity in tumor tissue; and that doses of at least 100 mg/m\textsuperscript{2} were required to completely suppress AGT activity in brain tumors (20, 21). We, therefore, studied additional cohorts of patients at BG doses higher than those determined to be BG max in PBMCs.

Definition of Study End Points. BG max was defined as stated above. DLT of BCNU was defined as the dose of BCNU that produced at least grade 3 nonhematological toxicity (except alopecia, nausea, or vomiting) or grade 4 hematological toxicity lasting more than 3 days, or fever in the presence of an absolute neutrophil count of less than 1000/\text{\mu l}. The MTD of BCNU was defined as the dose of BCNU that produced DLT in more than
two of six patients during the first cycle of chemotherapy. The recommended Phase II dose was one dose level below the MTD of BCNU.

**Drug Supply.** BG (NSC 637037) was supplied by the National Cancer Institute, Division of Cancer Treatment and Diagnosis, in 100-mg vials containing white lyophilized powder with 670 mg mannitol, USP, and sodium hydroxide to adjust pH to 7–8.5. Once diluted, each ml of solution contained 3.3 mg of BG; 22 mg of mannitol, USP; 0.4 ml of polyethylene glycol 400; and approximately 0.6 ml (pH 7) phosphate buffer. BCNU was commercially available from Bristol-Myers Squibb and was prepared for administration according to package labeling.

**Pretreatment and Follow-Up Studies.** Before the initiation of therapy, all of the patients had a history and physical examination, assessment of Karnofsky performance status, chest radiograph, 12-lead electrocardiogram, determination of tumor measurements, dipstick urinalysis, and routine laboratory studies that included a complete blood count with differential WBC count, electrolytes, urea, creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, and alanine aminotransferase and aspartate aminotransferase levels. Baseline pulmonary function studies including D_l CO and arterial blood gases were performed on all of the patients. Complete blood counts were assessed weekly during therapy and daily if grade 4 neutropenia or thrombocytopenia was documented. History and physical examination, determination of performance status, toxicity evaluation, and serum chemistries were performed every 2 weeks and before each cycle of therapy. Disease assessment and pulmonary function tests were performed after every two cycles of therapy. A complete response was defined as the disappearance of all clinical, biochemical, and radiographic evidence of the tumor for a minimum of 4 weeks and the absence of no disease-related symptoms. A partial response was defined as at least a 50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions lasting at least 4 weeks during which time no new lesions had appeared. Minor response was defined as objective decrease of disease but less than that required for a partial response. Disease progression was defined as ≥25% increase in the sum of the products of the perpendicular diameters of all of the measured lesions over the smallest sum observed or as the appearance of any new lesions or the clear worsening of assessable disease.

**Plasma Sampling and Pharmacokinetic Studies.** Patients were admitted to the General Clinical Research Center at the University of Chicago for administration of the initial dose of BG. Whole-blood samples were collected in sodium-heparinized vacutainers before treatment, at 30 and 60 min during the infusion of BG and at 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 24, and 48 h after the completion of the infusion. Plasma was obtained by centrifugation at 2500 rpm for 10 min. Urine was collected at various time periods up to 48 h after completion of the infusion. Samples were stored at −70°C until analysis. Total plasma and urine concentrations of BG and 8-oxo-BG were measured by high-pressure liquid chromatography using methods that have been described previously (22). For determination of AGT activity in PBMCs, blood (40 ml) was collected twice before the BG infusion was begun and at 1, 6, 10, 24, 48, 72, 168, and 336 h postinfusion. Within an hour of collection, an equal volume of RPMI medium was added, and the diluted blood was layered on Ficoll-Paque (Histopaque 1077). After centrifugation at 400 × g for 30 min, the lymphocyte layer was removed and resuspended in 15 ml PBS and centrifuged at 250 × g for 10 min at room temperature. RBCs were lysed by the addition of 6 ml of deionized water for 15 s after which 2 ml of 3.6% NaCl and 15 ml of PBS were added. The samples were centrifuged at 250 × g for 10 min. Final pellets were resuspended in 50 mM Tris (pH 7.5), 0.1 mM EDTA, and 5 mM DTT.

The pharmacokinetics of BG were analyzed using noncompartmental methods with WinNonlin (PharSight Corp., Apex, NC). The terminal elimination t1/2 of BG was estimated from the data using compartmental methods with WinNonlin (PharSight Corp., Apex, NC). The terminal elimination t1/2 of BG was estimated from the data using compartmental methods with WinNonlin (PharSight Corp., Apex, NC). The terminal elimination t1/2 of BG was estimated from the data using compartmental methods with WinNonlin (PharSight Corp., Apex, NC). The terminal elimination t1/2 of BG was estimated from the data using compartmental methods with WinNonlin (PharSight Corp., Apex, NC). The terminal elimination t1/2 of BG was estimated from the data using compartmental methods with WinNonlin (PharSight Corp., Apex, NC).
λ is the terminal elimination rate constant. The latter was based on visual inspection of the data. When $C_{\text{t,0}}$ was below the limit of quantitation, a concentration of BG (15 ng/ml) or 8-oxo-BG (10 ng/ml) equivalent to one-half of the limit of quantitation was used for the calculation of the AUC.

**AGT Activity.** The assay for alkyltransferase activity was performed as described previously (22). Briefly, alkyltransferase activity was measured as the removal of $O^2$-[3H]methylguanine from a 3H-methylated DNA substrate (5.8 Ci/mmol) after incubation with extract at 37°C for 30 min. The DNA was precipitated by adding ice-cold perchloric acid (0.25N) and was hydrolyzed by the addition of 0.1N HCl at 70°C for 30 min. The modified bases were separated by reverse-phase high-pressure liquid chromatography with 0.05M ammonium formate (pH 4.5) was performed as described previously (22). Briefly, alkyltransferase activity was measured as the removal of $O^2$-[3H]methylguanine from a 3H-methylated DNA substrate (5.8 Ci/mmol) after incubation with extract at 37°C for 30 min. The DNA was precipitated by adding ice-cold perchloric acid (0.25N) and was hydrolyzed by the addition of 0.1N HCl at 70°C for 30 min. The modified bases were separated by reverse-phase high-pressure liquid chromatography with 0.05M ammonium formate (pH 4.5) containing 10% methanol. Each assay was performed with a positive control cell line (Duoy cell extract) and lymphocytes from three normal volunteers. Protein was determined by the method of Bradford (23), and the results were expressed as fmol of $O^2$-methylguanine released from the DNA substrate per mg of protein.

**Statistical Considerations.** Comparisons of $D_{LT}$ post-therapy treatment. Because of the basis of this information, we recommend doses of BG at a BCNU dose of 40 mg/m$^2$ had previously had high-dose chemotherapy consisting of 1-$\beta$-d-arabinofuranosylcytosine, etoposide, cyclophosphamide, and carbamustine and an autologous stem-cell transplant as therapy for refractory lymphoma. On the basis of this information, we recommend doses of BG at 120 mg/m$^2$ with BCNU at 40 mg/m$^2$ for subsequent Phase II studies. The higher BG dose is recommended based on data reported by other investigators, which suggested that 120 mg/m$^2$ is the optimal dose to completely suppress AGT in tumor tissue.

In patients with DLT, the neutrophil nadir occurred between days 11 and 36 of the treatment cycle (median, day 27) with complete recovery in most patients by day 43. The platelet nadir occurred between days 17 and 27 of the treatment cycle (median, day 21) with complete recovery in most patients by day 37. Sepsis or febrile neutropenia were uncommon with only four episodes documented. Similarly, only three patients required transfusion of platelets at any time during their chemotherapy treatment. Because nitrosoureas are known to cause cumulative bone marrow toxicity, we tried to determine whether severe hematological toxicity was common during the second cycle of chemotherapy. Table 7 summarizes episodes of grade 3 or higher bone marrow suppression occurring during cycle 2 in patients treated with BCNU doses of 32 mg/m$^2$, 40 mg/m$^2$, and 50 mg/m$^2$ given with BG at doses of 40 mg/m$^2$ or higher. Dose-limiting hematological toxicity occurred in 4 of 6 patients treated with BCNU at 50 mg/m$^2$; in 3 of 18 patients treated with BCNU at 40 mg/m$^2$; and in 0 of 12 patients treated with BCNU at 32 mg/m$^2$. Prior therapy received by the patients with DLT is summarized in Table 6. It should be noted that one of the patients with DLT at a BCNU dose of 40 mg/m$^2$ had previously had high-dose chemotherapy consisting of 1-$\beta$-d-arabinofuranosylcytosine, etoposide, cyclophosphamide, and carbamustine and an autologous stem-cell transplant as therapy for refractory lymphoma.

The characteristics of the 78 patients enrolled in the study are listed in Table 1. Sixteen patients did not complete the first cycle of therapy and are, therefore, not assessable for toxicity or response; toxicity data on the remaining 62 patients form the basis of this report. In most cases, failure to complete the first cycle of therapy was attributable to rapid disease progression. Table 2 lists the dose levels evaluated in this study. A total of 121 cycles of chemotherapy were administered at BG doses ranging from 10 to 120 mg/m$^2$ and BCNU doses ranging from 13 to 50 mg/m$^2$.

**Toxicity.** No clinically significant toxicity was observed after administration of BG alone at any dose level. Bone marrow suppression was the primary toxicity and DLT of BG combined with BCNU. Hematological toxicity observed during cycle 1 is listed in Table 3. Grade 3 or higher toxicity was noted at BCNU doses as low as 25 mg/m$^2$ given with BG. As originally defined in the protocol, a BG dose of 40 mg/m$^2$ had dose-limiting hematological toxicity and three of four patients treated at a BCNU dose of 50 mg/m$^2$ had dose-limiting hematological toxicity. Further escalation of the BG dose was not associated with increased BCNU toxicity (Table 4), which suggests that BG doses of ≥40 mg/m$^2$ produced maximal AGT suppression in bone marrow progenitor cells. Table 5 summarizes cycle-one hematological toxicity data for BCNU doses of 32 mg/m$^2$, 40 mg/m$^2$, and 50 mg/m$^2$ given with BG at doses of 40 mg/m$^2$ or higher. Dose-limiting hematological toxicity occurred in 4 of 6 patients treated with BCNU at 50 mg/m$^2$; in 3 of 18 patients treated with BCNU at 40 mg/m$^2$; and in 0 of 12 patients treated with BCNU at 32 mg/m$^2$. Prior therapy received by the patients with DLT is summarized in Table 6. It should be noted that one of the patients with DLT at a BCNU dose of 40 mg/m$^2$ had previously had high-dose chemotherapy consisting of 1-$\beta$-d-arabinofuranosylcytosine, etoposide, cyclophosphamide, and carbamustine and an autologous stem-cell transplant as therapy for refractory lymphoma.
experienced grade 3 or 4 neutropenia or thrombocytopenia, which clearly indicated the potential for cumulative hematological toxicity to occur. One patient, initially thought to have treatment-related grade 4 bone marrow suppression after his second cycle of chemotherapy at BG and BCNU doses of 120 and 40 mg/m², respectively, was diagnosed with acute promyelocytic leukemia. This patient had a hepatocellular carcinoma and had previously been treated with pyrazaloacridine. Cytogenetic examination of the leukemic blasts revealed a t(15;17) translocation. No other cytogenetic abnormalities were noted. Given the low cumulative dose of BCNU received (80 mg/m²), this leukemia was unlikely to be related to the BG/BCNU therapy.

Nonhematological toxicity was uncommon and not clearly dose-related in this study. The most common toxicities of grade 2 or higher were fatigue, anorexia, increased bilirubin, and transaminase elevation. None of these events occurred in more than 15% of the patients. Because high cumulative doses of carmustine are known to be associated with pulmonary toxicity, we monitored D_L CO in these patients after every two cycles of therapy. Pulmonary function tests were performed prestudy in every patient, but only 30 patients returned for testing after two cycles of therapy. Among patients with both baseline and postcycle 2 measurements, mean D_L CO uncorrected fell approximately 9% (P = 0.002) and mean D_L CO corrected fell approximately 10% (P = 0.006). Twenty of the 30 patients had a decrease in the percentage of predicted D_L CO. Of these, the median percent decrease in the percentage of predicted D_L CO was 10% (range, 2–46%). Only four patients had a percent decrease in percentage of predicted D_L CO of ≥20%. These patients had been treated with BG/BCNU doses of 40/32, 80/25, 120/25, and 120/32. No patient experienced clinical signs or symptoms of pulmonary toxicity.

Antitumor Response. No complete or partial responses were observed.

Pharmacological Studies. We have previously reported results of pharmacological studies performed in 25 patients enrolled in this study and treated at BG doses ranging from 10 to 80 mg/m² (22). BG is rapidly eliminated from plasma (t_1/2, at 120 mg/m² averaged 1.9 h) and is replaced by a major metabolite, 8-oxo-BG. This biologically active metabolite of BG has a longer t_1/2 (averaging 4.0 h at 120 mg/m²) and a 12- to 29-fold higher AUC than BG.

Fig. 1 illustrates the relationship between BG dose and AUC for patients treated at doses ranging from 40 to 120 mg/m² of BG. As suggested by Fig. 1, the BG clearance decreases as the dose increases from 40 to 120 mg/m², with values of 40.9 ± 12.1, 34.7 ± 12.9, and 31.6 ± 10.6 liters/h/m², at BG doses of 40, 80, and 120 mg/m², respectively.

The relative AGT activity in PBMCs was determined after administration of BG. The rate of AGT regeneration was more rapid for patients receiving the lowest dose of BG (10 mg/m²); however, the rate of regeneration was similar for doses ranging from 20 to 120 mg/m². AGT inactivation curves have been published for the initial 25 patients (22). Fig. 2 provides data for a total of 50 patients.

DISCUSSION

Inactivation of AGT is a potentially important strategy to improve the clinical utility of the alkylating nitrosoureas. This study and others (21) clearly demonstrate that BG completely suppresses AGT activity in PBMCs and tumor tissues at nontoxic doses. Coadministration of BG and BCNU is feasible in cancer patients but, as expected based on pre-clinical data, the maximal dose of BCNU that can be safely administered in the presence of BG is approximately one-third of the standard clinical dose. Clinicians must monitor the BCNU dose particularly carefully in the presence of BG because even small increments in BCNU dosage can produce severe toxicity when AGT is inactivated. Patients and clinicians must also be alert to the possibility of cumulative bone marrow suppression and the potential for severe hematological toxicity to occur in the second or later cycles of therapy, even in patients who do not experience significant toxicity during the initial treatment cycle. Although pulmonary toxicity is not clinically significant after two cycles of therapy, we cannot rule out the possibility of pulmonary dysfunction after higher cumulative doses of BCNU.

The study design that we used clearly illustrates the advantages and disadvantages of using PBMCs as surrogates for tumor tissue in assessing biochemical events. PBMCs are easily
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BG and 8-oxo-BG as a function of BG dose. AUC was determined as described in the text. AUC values for BG are 1.1 ± 0.6, 2.7 ± 1.2, and 4.2 ± 1.4 µg/h/ml and for 8-oxo-BG are 19.4 ± 9.7, 52.6 ± 15.2, and 80.9 ± 19.0 µg/h/ml at BG doses of 40, 80, and 120 mg/m², respectively.

Fig. 1. Plot of AUC for BG and 8-oxo-BG as a function of BG dose. AUC was determined as described in the text. AUC values for BG are 1.1 ± 0.6, 2.7 ± 1.2, and 4.2 ± 1.4 µg/h/ml and for 8-oxo-BG are 19.4 ± 9.7, 52.6 ± 15.2, and 80.9 ± 19.0 µg/h/ml at BG doses of 40, 80, and 120 mg/m², respectively.

Fig. 2. AGT activity relative to pretreatment values in PBMCs of patients after a 1-h infusion of BG. Lymphocytes were isolated from blood at various times and AGT activity determined. This plot represents data from 50 patients including data from the initial 25 patients that have been published previously (22).

that tumor responses would occur. A number of possible explanations exist for the apparent lack of tumor efficacy. Only 24 patients received a BCNU dose of 40 mg/m² or higher and, of these, only 10 received BG at a dose of 120 mg/m². Treatment of a heterogeneous group of only 10 patients at the recommended Phase II doses or above is not an adequate test of antitumor efficacy. It is also reasonable to consider whether this schedule of BG administration is sufficient to produce sustained AGT depletion throughout the period of time when BCNU-induced DNA cross-links are most likely to occur, i.e., 12–18 h after BCNU administration (24). Synthesis of new AGT molecules after BG depletion occurs at different rates in different tissues. Persistence of BG or 8-oxo-BG at sufficient concentration is necessary to insure that newly synthesized AGT is inactivated or the removal of alkylnitrosourea monoadducts from DNA is likely to occur, thereby limiting the effectiveness of BCNU. Pharmacological data from this study demonstrates that plasma concentrations of 8-oxo-BG of at least 100ng/ml are detectable in most patients at 10 h post-BG dosing, and Spiro et al. (21) have demonstrated undetectable AGT activity in tumor biopsies obtained 18 h after a BG dose of 120 mg/m². Thus, it seems likely that the present schedule of administering BG over a 1-h period prior to BCNU is adequate to suppress AGT throughout the period of BCNU-induced DNA cross-link formation.

Phase II studies of the combination of BG and BCNU are ongoing or planned in patients with malignant gliomas, myeloma, colorectal cancer, melanoma, soft tissue sarcoma, and pediatric brain tumors. Recommended dosage for these studies is BG 120 mg/m² given over a period of 1 h followed 1 h later by BCNU 40 mg/m².

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