Modulation of $O^6$-Alkylguanine-DNA Alkyltransferase-mediated Carmustine Resistance Using Streptozotocin: A Phase I Trial

Timothy J. Panella, David C. Smith, S. Clifford Schold, Miriam P. Rogers, Eric P. Winer, Robert L. Fine, Jeffrey Crawford, James E. Herndon II, and Donald L. Trump


ABSTRACT

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) resistance may be mediated by repair of chloroethylated guanine before stable cross-linking occurs. Guanine adducts may be repaired by the enzyme $O^6$-alkylguanine-DNA alkyltransferase ($O^6$-AGAT). Such repair irreversibly inactivates $O^6$-AGAT. Streptozotocin (STZ) forms adducts at the $O^6$ position of guanine; repair of these adducts consumes $O^6$-AGAT. In vivo STZ potentiates BCNU cytotoxicity. The purpose of this trial was to determine the maximum tolerated dose of BCNU that can be administered together with STZ. The STZ dose was 500 mg/m2/day for 4 days and was not escalated. BCNU was given 4 h after the third dose of STZ at a starting dose of 75 mg/m2. A total of 43 patients were entered in the study. There were 4 dose escalations, reaching a maximum tolerated BCNU dose of 175 mg/m2. At this dose, thrombocytopenia was the dose-limiting toxicity (one patient, 25-49 x 10^3/\mu l; 2 patients, <25x10^3/\mu l); neutropenia was less severe (2 patients, 2.0-3.9 x 10^3/\mu l; 1 patient, 1.0-1.9 x 10^3/\mu l). Two other commonly seen toxicities were elevations in the serum alkaline phosphatase and mild elevations in the serum creatinine. Peripheral blood lymphocyte $O^6$-AGAT levels decreased from a mean of 212 fmol/mg protein pretherapy to 8.2 fmol/mg protein on day 3 prior to BCNU $P = 0.03$. Three partial responses were seen. There were no therapy-related fatalities, and toxicity was easily managed. This study established that 150 mg of BCNU can be administered safely together with STZ, 500 mg/m2/day for 4 days. Additional studies are required to determine whether $O^6$-AGAT-mediated BCNU resistance is suppressed.

INTRODUCTION

BCNU1 (Bristol-Meyers-Squibb, Evansville, IN) exerts its primary cytotoxic effect through the formation of DNA interstrand cross-links. Cross-link formation is a stepwise process beginning with the transfer of a chloroethyl group from BCNU to the $O^6$ position of guanine (1). Following alkylation, a stable cross-link or covalent bond forms between guanine and its complementary cytosine (2). The cross-linking process requires up to 8 h and, if not repaired, will disrupt DNA function.

$O^6$-AGAT is an enzyme which removes alkyl groups from the $O^6$ position of guanine (3, 4). Irreversible inactivation of the protein occurs when it accepts alkyl groups, and it has been referred to as a suicide enzyme (5). $O^6$-AGAT has been found in most human tissues, and most human tumors contain amounts of $O^6$-AGAT similar to or greater than the tissue from which they originate (1, 3, 4, 6). Approximately 20% of cell lines derived from human tumors are deficient in this enzyme: such deficiencies are thought to be associated with in vitro culture (6-8). These $O^6$-AGAT-deficient cell lines are very sensitive to the cytotoxic effects of agents that alkylate the $O^6$ position of guanine (7-10). After BCNU treatment, Smith and Brent (10) demonstrated a large number of DNA cross-links in cell lines deficient in $O^6$-AGAT. Cross-links were not detectable in cell lines that contained $O^6$-AGAT (10). Transfection of the bacterial $O^6$-AGAT gene into $O^6$-deficient mammalian cells results in decreased sensitivity to BCNU (11). Cells high in $O^6$-AGAT can be sensitized to nitrosoureas by inhibition of enzyme activity. Recovery of the enzyme occurs by resynthesis within 24 h of depletion in cultured lymphocytes and HT-29 cells (12-15).

STZ (Upjohn, Kalamazoo, MI) preferentially methylates DNA at the $O^6$ position of guanine. $O^6$-AGAT rapidly repairs this damage and is depleted. This repair is similar to that which follows BCNU adduct formation; however, the reaction is more efficient and consumes more $O^6$-AGAT (14, 16). In a BCNU-resistant colon tumor cell line exposed to STZ for 1 h, $O^6$-AGAT levels were reduced by 75%. Subsequent exposure to BCNU induced 300-400% enhancement of cell kill. As the duration of time between STZ and BCNU increases from 1-6 to 24 h, the degree of enhanced cytotoxicity is diminished (8). Gerson (16) found that, when streptozotocin was given to patients at 500 mg/m2/day for 4 days, a progressive decrease in lymphocyte $O^6$-AGAT activity was observed, reaching a maximum 73% reduction after 3 days of treatment (16).

Our desire was to further explore the ability of STZ to reduce $O^6$-AGAT activity and potentiate BCNU cytotoxicity. Since the side effects of BCNU and STZ are different, combining these two drugs appears feasible. BCNU induces bone marrow, hepatic, and pulmonary toxicity (17), while STZ induces gastrointestinal and renal abnormalities (18). Based on these background data, we undertook the following phase I trial to determine the MTD of BCNU which could be administered with full-dose STZ.

PATIENTS AND METHODS

Forty-three patients were entered in this trial between April 1990 and April 1991. Eligibility requirements included a histological diagnosis of malignancy that was refractory to standard treatment, 4 weeks from prior chemotherapy (6 weeks for mitomycin C or nitrosoureas), absolute granulocyte count $>1.5 \times 10^9/\mu l$, platelets $>100 \times 10^9/\mu l$, bilirubin $<2.0 \text{ mg/dl}$, and creatinine $<2.0 \text{ mg/dl}$. Patients gave informed consent, had an Eastern Cooperative Oncology Group performance status of 0-2, a life expectancy of &gt;12 weeks, and were older than 18 years of age.

The design of this phase I trial called for a standard dose and schedule of STZ (500 mg/m2/day for 4 days). BCNU was administered 4 h after the third dose of STZ (Fig. 1) and was escalated in cohorts of 6 patients. The BCNU dose started at 75 mg/m2 and was escalated in 25-mg/m2 increments. The fourth STZ dose was designed to maintain low $O^6$-AGAT while cross-linking occurred. Metoclopramide (100 mg), di-
The four most commonly seen toxicities were thrombocytopenia (Fig. 2), neutropenia (Fig. 3), elevations in serum alkaline phosphatase levels (Fig. 4), and mild elevations in serum creatinine concentrations. Thrombocytopenia was frequent and was dose limiting. While thrombocytopenia occurring during the first cycle of therapy was significant in 6 patients, only 3 required transfusions, and none had bleeding complications. Platelet nadirs usually occurred at day 28 with recovery of counts by day 42. This is the typical pattern following exposure to BCNU. Cumulative bone marrow toxicity was also observed. All 8 patients who received 3 or 4 cycles of therapy had a considerable decrease in platelet count by cycle 3 or 4 (pretreatment mean, 231 x 10^9/liter; nadir mean, 74 x 10^9/liter) and grade 3 thrombocytopenia was seen in 4 of these patients. The majority (n = 7) of these patients were receiving ≥125 mg/m^2 BCNU. Because of the cytotoxic potential of STZ, it is difficult to determine whether modulation of O^6-AGAT led to increased BCNU toxicity. In order to assess this issue, 3 patients who were given treatment as described above for the first cycle were given BCNU on day 1, prior to STZ, on the second cycle. Thrombocytopenia was less on the second cycle as compared to the first in these patients (first cycle mean platelet nadir, 115 x 10^9/liter; second cycle mean platelet nadir, 228 x 10^9/liter), suggesting that depletion of O^6-AGAT increased megakaryocyte sensitivity to BCNU.

The reductions in WBC were typically mild. There was one episode of fever with neutropenia which was treated with antibiotics and resolved. One patient had reactivation of herpes zoster. Two patients with locally advanced head and neck cancer had aspiration pneumonia which occurred during the hospitalization for chemotherapy and resolved without sequelae.

Gastrointestinal toxicity consisted of nausea, vomiting, and elevations in serum alkaline phosphatase levels. Emesis was easily controlled with metoclopramide, lorazepam, and diphenhydramine. One patient had persistent, severe emesis requiring hospitalization and increased doses of antiemetics. Elevations of alkaline phosphatase due to therapy (Fig. 4) were sometimes difficult to distinguish from those related to tumor growth.

### RESULTS

**Patient Characteristics.** The patient characteristics are summarized in Table 1. The majority of patients had either colorectal cancer or head and neck cancer. Most patients had received prior chemotherapy and/or radiotherapy; 6 patients had received nitrosoureas previously. The median number of prior regimens was one.

**Toxicity.** Four dose escalations occurred (Table 1), reaching a maximum BCNU dose of 175 mg/m^2 with a total of 66 treatment cycles administered. MTD, as determined by cycle 1 therapy, was reached at 175 mg/m^2. At this dose, there were 3 of 6 patients with reversible grade 3 or 4 bone marrow and/or hepatic toxicity. Two more patients were added to this dose level to better define toxicity and to obtain lymphocyte O^6-AGAT levels.

<table>
<thead>
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<th>Total no. of patients</th>
<th>43</th>
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<td>Male</td>
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| Mean age (range) (yr) | 56 (30-73) |

<table>
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<td>Head and neck</td>
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<td>Renal</td>
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<tr>
<td>Melanoma</td>
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</tr>
<tr>
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<tr>
<td>Prior nitrosourea</td>
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<tr>
<td>X-ray therapy only</td>
<td>4</td>
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<tr>
<td>Surgery only</td>
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<table>
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<th>BCNU dose (mg/m^2)</th>
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<tr>
<td>75</td>
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<td>150</td>
<td>10</td>
</tr>
<tr>
<td>175</td>
<td>8</td>
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* There was one patient each with pancreatic, esophageal, gastric, bladder, salivary, carcinoid, leiomyosarcoma, adenoid cystic, skin squamous cell, non-small cell lung, mycosis fungoides, and adenocarcinoma of unknown primary.
Seven of the 18 patients with elevated alkaline phosphatase levels had documented progressive disease in the liver or bone; however, the increase in 11 patients was not associated with progressive disease and reversed after treatment. At dose level 5, only one of 5 patients with an elevated alkaline phosphatase level had progressive disease in liver or bone. There were no consistent elevations of transaminases or bilirubin levels. Elevations in serum creatinine were rarely greater than 1.5-fold normal and always resolved prior to the next treatment cycle. These elevations occurred equally at each dose level and were believed to be secondary to STZ. One patient complained of shortness of breath after her first cycle of treatment, and pulmonary function testing revealed a forced expiratory volume in 1 s ≤1 liter. Her chest radiograph appeared normal, and no further treatment was given. Her symptoms gradually resolved, and she died 1 year later of progressive disease. There was no clear evidence of pulmonary toxicity. There was one death from tumor progression occurring 30 days after treatment. Except for this patient, all patients were evaluable for toxicity at 42 days. Four patients had a combined toxicity consisting of thrombocytopenia, mild neutropenia, mild elevation in creatinine, and elevation in alkaline phosphatase. These toxicities resolved after the first treatment cycle, reoccurred during the second treatment cycle, and again resolved.

The major reason patients were removed from study was disease progression. Four patients did not continue due to toxicity. This includes the one patient who died, one patient with forced expiratory volume in 1 s < 1 liter, one patient with serious thrombocytopenia, and one patient with an elevated bilirubin level most likely secondary to disease progression. Two patients had a decrease in performance status, and 4 patients requested that chemotherapy be discontinued for reasons other than toxicity (2 who desired no further therapy, 2 who were unable to travel).

Partial responses occurred in one patient with adenocarcinoma of unknown primary presenting as a cervical lymph node (level 1), one patient with squamous cell carcinoma of the head and neck (level 3), and one patient with rectal carcinoma (level 5). None of the responders had previously been treated with a nitrosourea. Most patients had progressive disease, 5 had stable disease (two patients for > 7 months), and no complete responses were seen.

Lymphocyte O6-AGAT levels uniformly decreased in the 6 patients from whom paired samples were obtained (Fig. 5). The level decreased from a pretreatment mean of 212 fmol/mg protein to a mean of 8.2 fmol/mg protein after 3 doses of STZ (P = 0.03). Variable pretreatment values seemed to decrease to similar posttreatment values. The O6-AGAT activity decreased by ≥89% in all patients in whom levels were measured.

Tumor tissue levels of O6-AGAT were assessed in two patients (one with melanoma and one with adenocarcinoma of unknown primary) before and after the administration of STZ. Pretreatment tissue specimens showed low levels of O6-AGAT (<100 fmol/mg protein), and there was no significant change...
following exposure to STZ. The patient with adenocarcinoma of unknown primary had a partial response despite the low measured O6-AGAT levels and prior exposure to multiple chemotherapeutic regimens.

**DISCUSSION**

The current study is based on preclinical and in vivo data which indicate a sustained and significant depletion of O6-AGAT by STZ when given on this schedule (16). The first three doses of STZ were given to deplete tissue O6-AGAT prior to BCNU. The fourth dose of STZ was designed to maintain low levels of enzyme while BCNU uptake, alklylation, and cross-linking occurred. This should enhance the cytotoxic effect of BCNU, based on the in vitro data showing an increased number of DNA cross-links in O6-AGAT-deficient cells.

Four dose escalations of BCNU were accomplished. The MTD of BCNU was 175 mg/m² when given in conjunction with STZ. This is lower than the single-agent dose (250 mg/ m²) MTD of BCNU as determined in phase I testing (17), suggesting a potentiation of toxicity, but is comparable to the dose administered in combination regimens. Based on the alternative regimen administered to 3 patients, it appears that the decreased MTD may be due to modulation of O6-AGAT by STZ. Detection of enhanced tumor cytotoxicity will require phase II testing.

No unusual toxicity occurred in this trial. Thrombocytopenia was dose limiting. Neutropenia, emesis, azotemia, and elevations in alkaline phosphatase concentrations were well tolerated.

Initial lymphocyte O6-AGAT levels varied from 433–49 fmol/mg protein. This 9-fold interpatient variation in O6-AGAT levels is slightly higher than that reported by others (4, 16, 19) and may be explained by our one very high value. The percentage decrease ranged from 46–100% with enzyme activity decreasing ≥90% in 7 of the 6 patients. This decrease is similar to in vitro decreases in O6-AGAT with STZ and other modulators (12, 14, 16, 19). Based on in vitro information, this level of enzyme inhibition should be adequate to sensitize tumor cells to BCNU. The tumor level of O6-AGAT was extremely low in the two patients whose tumors were assayed in this study, and the effects of STZ on tumor O6-AGAT remain unclear.

In the only other reported clinical trial of this combination (20), the nonoverlapping side effects led Lokich et al. (20) to combine STZ and BCNU in 1974. In their protocol, STZ and BCNU were given daily for 5 days. In this fashion, most of the BCNU would have been given prior to depletion of O6-AGAT. However, the toxicity profile was similar to the current study with an increase in leukopenia and thrombocytopenia when compared to the reported experience with BCNU given on the same schedule.

In summary, STZ was found to inhibit O6-AGAT in lymphocytes to the same extent as found in in vitro studies in which potentiation of BCNU cytotoxicity was reported. We reached an MTD of 175 mg/m² with thrombocytopenia as the dose-limiting toxicity. Systemic toxicity to this regimen of BCNU and STZ was not enhanced, and future work will involve phase II trials in patients with BCNU-sensitive tumors to determine whether augmented tumor toxicity will occur. Phase I trials directed at further inhibition of BCNU resistance are being developed. Several possibilities include the inhibition of O6-AGAT with O6-benzylguanine, inhibition of excision repair (21), inhibition of glutathione (22), or polyamine depletion (23).

**REFERENCES**

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