High-dose BCNU with autologous bone marrow rescue for recurrent glioblastoma multiforme

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Eleven patients with recurrent malignant glioma were treated with single high doses of BCNU ranging from 600 to 1400 mg/sq m. To prevent the characteristic late myelosuppression observed after conventional doses of BCNU, autologous bone marrow harvested just before drug treatment was infused 24 to 36 hours after therapy. Higher doses of BCNU causes earlier and more profound myelosuppression; one patient died of pancytopenia, breakdown of the gut epithelium, and Clostridium septicemia 10 days after receiving 1400 mg/sq m of BCNU. All patients experienced transient emesis; four developed transient elevation of hepatic enzymes, two reversible interstitial pulmonary infiltrates, and two who received 1400 mg/sq m BCNU suffered irreversible cortical damage. Eight patients receiving 600 to 1200 mg/sq m demonstrated reconstitution of polymorphonuclear leukocytes and platelets within at least 30 days after treatment. With a follow-up time of up to 19 months, four patients improved, three stabilized, and three deteriorated and died. The median survival time was 7 months. Computerized tomography performed on patients receiving constant corticosteroids showed diminished contrast enhancement and mass effect in eight patients. High-dose BCNU at doses up to 1200 mg/sq m with marrow rescue is a feasible approach to the treatment of patients with glioblastoma.

KEY WORDS • malignant glioma • BCNU • bone marrow • glioblastoma • recurrent tumor

PATIENTS with malignant gliomas,18 which constitute one-third of primary brain tumors, have a median survival of less than 12 months following subtotal resection and whole-brain irradiation. Efforts to improve this survival time have led to the evaluation of new chemotherapeutic agents against brain tumors induced or implanted in animals. In these animal models, the most active agents are the low molecular weight, lipid-soluble nitrosoureas (including BCNU and CCNU), at maximum dosage. The use of BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) in the treatment of patients with glioblastoma multiforme has been associated with a marginal improvement in survival time. This limited effectiveness in man may be a result of dose limitations. Although pulmonary, renal, and hepatic toxicities occur, the principal dose-limiting effect of BCNU is a characteristic delayed, prolonged, and cumulative myelosuppression. Recent progress in the preservation and reinfusion of autologous bone marrow has allowed us to provide protection against this hematological toxicity in patients receiving high-dose BCNU as treatment for recurrent glioblastoma. We have infused freshly obtained and refrigerated autologous bone marrow 24 to 36 hours after high-dose drug administration.

Clinical Materials and Methods

Eleven patients (seven male and four female), aged 17 to 57 years, had clinical and computerized tomography (CT) evidence of recurrent glioblastoma after initial biopsy or partial resection and irradiation (whole-brain irradiation 4500 rads, with a boost to the tumor bed of 1000 to 1500 rads). The median interval between tumor diagnosis, primary treatment, and recurrence was 10.6 months (range 5 to 18 months). Ten days prior to baseline evaluation of tumor size and neurological state, each patient was placed on a dose of corticosteroids that provided maximum symptomatic benefit. This dose was kept constant over an 8-week study period and then reduced to the lowest level tolerated.

Prior to treatment, and at least weekly thereafter,
the following studies were performed for each patient: complete blood count, blood urea nitrogen (BUN), creatinine, serum glutamic oxaloacetic transaminase (SGOT), lactate dehydrogenase, and alkaline phosphatase. Contrast-enhanced CT scans, chest x-ray films, and pulmonary function and creatinine clearance studies were performed before treatment and 8 weeks later.

With the patients under general anesthesia, bone marrow was aspirated from the anterior and posterior iliac crest by previously described techniques. The aspirated marrow was anticoagulated with 15% (vol/vol) citrate dextrose solution-A (ACD) and preservative-free heparin (10 units/ml final concentration). The marrow was passed through stainless steel screens and collected in polyethylene bags; the volume obtained averaged 950 ml and contained 2 \times 10^{10} cells (mean). The bag containing the marrow was centrifuged at 2000 G for 10 minutes, following which a portion of the supernatant, containing suspended fat, was removed. The final volume averaged 600 ml, and had a packed red-cell volume of 45% to 55%. The marrow was refrigerated at 4°C until infusion. The viability of granulocyte-monocyte colony-forming cells (CFU-c), tested by a modification of the method of Pike and Robinson, exceeded 90%.

Total doses of BCNU from 600 to 1400 mg/sq m surface area were administered 4 to 16 hours after bone marrow harvest. The drug was dissolved in ethanol and diluted with 0.85% NaCl to a final volume of 250 ml. Prophylaxis against emesis was provided by a separate intravenous infusion of perphenazine in 5% ethanol and diluted with 0.85% NaCl to a final volume of 10 ml/kg. Prophylaxis against emesis was also provided by a standard antiemetic protocol based on severity of symptoms. No patient developed increased intracranial pressure and became somnolent after harvesting, but responded to therapy within 24 hours. Administration of 1400 mg/sq m BCNU was associated with acute and permanent central nervous system toxicity. One hour after the second 700 mg/sq m dose of chemotherapy, one patient (Case 11) experienced his first grand mal seizure, following which he became akinetic and mute, with diffuse slowing of the electroencephalogram. Another patient (Case 10) suffered apparent worsening of memory within 48 hours of his chemotherapy. No patient experienced symptoms during bone marrow reinfusion. The bone marrow dose averaged 3.6 ± 0.5 (mean ± 1 SD) \times 10^8 cells/kg (range 1.5 to 5.1 \times 10^8/kg). There was no cell loss across a standard blood recipient filter.

Increasing doses of BCNU from 600 to 1400 mg/sq m caused progressively earlier and more profound myelosuppression (Table 1). Patients receiving total doses of 600 mg/sq m had mild neutropenia and thrombocytopenia with nadirs 24 to 34 days after therapy. In contrast, total doses of 1200 and 1400 mg/sq m resulted in progressively earlier leukopenia and thrombocytopenia (nadirs at 8 to 16 days). Thus, higher doses produced periods of risk from severe myelosuppression before the grafted marrow could repopulate the peripheral blood compartment. All patients treated at the two highest doses had leukocyte counts of less than 500/ml for at least 8 days and required platelet transfusion therapy. The patient in Case 11 (who received 1400 mg/sq m) had a platelet count of less than 30,000 at the time of his death from neurological causes on Day 45. Another patient (Case 9) became pancytopenic, developed diarrhea, and died of Clostridium septicemia 10 days after BCNU therapy. Postmortem examination revealed extensive erosions of the large and small intestine.

Serial measurement of SGOT and alkaline phosphatase revealed reversible elevations within 4 weeks of therapy in four patients. Two patients developed asymptomatic interstitial pulmonary infiltrates within 2 weeks of chemotherapy. These infiltrates subsided spontaneously within 10 days of their appearance, but one patient was left with mild restrictive pulmonary function abnormalities when tested 7 months later. It should be noted that patients received corticosteroids throughout their course of therapy which may have reduced the potential for improved pulmonary changes. No alteration in urinalysis, BUN, or creatinine clearance was noted.

Ten of 11 patients could be evaluated for functional

\[ \text{Results of Treatment} \]

Despite neurological symptoms and signs of intracranial hypertension, 10 of the 11 patients tolerated general anesthesia and bone marrow aspiration. One patient developed increased intracranial pressure and became somnolent after harvesting, but responded to hyperosmolar mannitol infusion. With increasing experience, the use of hyperosmolar (20%) mannitol became routine to stabilize patients in the post-harvest period.

Two patients were treated at each of the following dose levels: 600, 800, 1000, and 1200 mg/sq m BCNU. Three patients received 1400 mg/sq m. Since the first three patients developed superficial cutaneous erythema in association with peripheral intravenous infusion of BCNU and one patient had obvious phlebitis, routine placement of a Hickman line preceded all subsequent chemotherapy. Three patients had emesis lasting 24 hours. Administration of 1400 mg/sq m BCNU was associated with acute and permanent central nervous system toxicity. One hour after the second 700 mg/sq m dose of chemotherapy, one patient (Case 11) experienced his first grand mal seizure, following which he became akinetic and mute, with diffuse slowing of the electroencephalogram. Another patient (Case 10) suffered apparent worsening of memory within 48 hours of his chemotherapy. No patient experienced symptoms during bone marrow reinfusion. The bone marrow dose averaged 3.6 ± 0.5 (mean ± 1 SD) \times 10^8 cells/kg (range 1.5 to 5.1 \times 10^8/kg). There was no cell loss across a standard blood recipient filter.

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Ten of 11 patients could be evaluated for functional
**TABLE 1**

*Myelosuppression following high-dose BCNU and stored autologous bone marrow*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>BCNU Dose (mg/sq m)</th>
<th>WBC Nadir (cells/μl)</th>
<th>Platelets Nadir (cells/μl)</th>
<th>Day</th>
<th>Platelets Recovery to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>600</td>
<td>3400</td>
<td>43,000</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>1500</td>
<td>50,000</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>&lt;100</td>
<td>26,000</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>&lt;100</td>
<td>18,000</td>
<td>12</td>
<td>18</td>
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<td>5</td>
<td>1000</td>
<td>500</td>
<td>14,000</td>
<td>14</td>
<td>20</td>
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<tr>
<td>6</td>
<td>1000</td>
<td>400</td>
<td>20,000</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>1200</td>
<td>&lt;100</td>
<td>10,000</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>1200</td>
<td>&lt;100</td>
<td>10,000</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>9</td>
<td>1200</td>
<td>&lt;100</td>
<td>15,000</td>
<td>9</td>
<td>18</td>
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<td>8</td>
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<td>11</td>
<td>1400</td>
<td>&lt;100</td>
<td>14,000</td>
<td>14</td>
<td>&gt;35</td>
</tr>
</tbody>
</table>

*WBC = white blood cells.

improvement (Karnofsky scale) over a follow-up period of 2 to 12 months following a single course of treatment (Table 2). Despite the low level of general function prior to treatment, four patients showed clinical improvement, and three had no evidence of progression following therapy. Two patients (Cases 1 and 3) were able to resume employment, which had been previously terminated. For the 10 evaluable patients, the duration of improvement or no progression lasted 8 and 4 months (median), respectively. Cases 1 and 3 were re-treated with BCNU and autologous marrow following relapse. Case 1 showed clinical and CT improvement after a second course of 600 mg/sq m, given 9 months after the first course. The other patient (Case 3) deteriorated, and cystic necrosis of the tumor was seen on CT scan following BCNU treatment at 800 mg/sq m. The median survival period for our 10 evaluable patients was 7 months (1 + to 19+ months), with three patients still alive 16+, 17+, and 19+ months from the date of tumor recurrence.

Contrast-enhanced CT scans performed 6 to 8 weeks after treatment, while the patient was receiving constant steroids, have shown unequivocal response in two of the 10 patients, seen as diminution in the area of contrast enhancement (Table 3). The remainder, including the two whose clinical condition had deteriorated, showed no change in tumor bulk. However, in eight of the 10 patients, serial scans demonstrated a marked reduction in contrast enhancement, and eight

**TABLE 2**

*Characteristics and clinical response in 11 patients with a single course of treatment*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex, Age (yrs)</th>
<th>Disease Duration (mos)</th>
<th>Clinical Response*</th>
<th>Survival Time† (mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 38</td>
<td>9.5</td>
<td>80%–90%↑</td>
<td>19+†</td>
</tr>
<tr>
<td>2</td>
<td>F, 41</td>
<td>9.0</td>
<td>60%–60%↑↑↑</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>M, 40</td>
<td>5.0</td>
<td>80%–85%↑↑↑</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>F, 29</td>
<td>18.0</td>
<td>85%–80%↑↑</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>M, 27</td>
<td>18.0</td>
<td>70%–80%↑↑↑</td>
<td>17+</td>
</tr>
<tr>
<td>6</td>
<td>M, 51</td>
<td>5.0</td>
<td>60%–60%↑↑↑</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>F, 17</td>
<td>12.0</td>
<td>80%–85%↑↑↑</td>
<td>16+</td>
</tr>
<tr>
<td>8</td>
<td>F, 34</td>
<td>12.0</td>
<td>70%–60%↑↑↑</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>M, 44</td>
<td>4.0</td>
<td>75%–75%↑↑↑</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>M, 57</td>
<td>5.0</td>
<td>75%–75%↑↑↑</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>M, 42</td>
<td>13.0</td>
<td>75%–60%↑↑↑</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Patient performance rated by a modification of the Karnofsky scale. Four of the 11 patients improved, and two remained the same.

†Median survival time: 7 months. Three patients are still alive.

‡Two courses of BCNU.

**TABLE 3**

*Computerized tomography changes following high-dose BCNU*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>BCNU Dose (mg/sq m)</th>
<th>Tumor Bulk</th>
<th>Contrast Enhancement</th>
<th>Low Density</th>
<th>Ventricles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>600</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>120%</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
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<td>NC</td>
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<td>↓</td>
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<tr>
<td>7</td>
<td>1200</td>
<td>130%</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>8</td>
<td>1200</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>9</td>
<td>1400</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
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<td>11</td>
<td>1400</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
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</tr>
</tbody>
</table>

Total: 11 patients (13 courses) 2/101 8/91 8/91 8/91

*Computerized tomography scans at 6- to 8-week intervals at isodose or diminished corticosteroids. NC = no change; ↓ = diminished; ↑ = increased.

J. Neurosurg. | Volume 54 | April, 1981

457
FIG. 1. Computerized tomography in Case 1. A: Eight days prior to high-dose BCNU therapy (8 months after irradiation) showing recurrent left frontal mass and surrounding edema. B: One month after therapy with isodose steroids. C: Five months after therapy with diminished steroids. D: Seven months after therapy showing recurrent periventricular tumor. E: Nine months after the original therapy (1½ months after repeat high-dose BCNU).

of the 10 showed a reduction in the size of the area of low density surrounding the tumor (that is, evidence of diminished cerebral edema). Examples of these changes are shown in Figs. 1 and 2. Unexplained CT findings included enlargement of the lateral ventricles in the majority of patients following high-dose chemotherapy.

Discussion

These studies demonstrate the safe administration of single doses of BCNU (600 to 1200 mg/sq m) followed by autologous marrow infusion in the treatment of recurrent glioblastoma multiforme. However, we remain uncertain about the value of this approach in comparison to the standard intravenous method of BCNU administration. Our results may be no better than those reported at customary dose levels. Clinical improvement or stabilization was noted in six of our eight patients given up to 1200 mg/sq m, and followed 4+ to 19+ months. Clear response (by CT evaluation) was noted as decreased bulk in 20%, and diminished enhancement on CT was noted in six of the eight. These results are similar to those of Fay, et al. who treated glioblastoma with high-dose BCNU, and found that five of seven patients were at least partial responders.

These studies come at a time when there is considerable pessimism regarding nitrosourea chemotherapy of glioblastoma. Although clinical studies with BCNU date back to 1962, and now number in excess of 200 reports, the use of this drug has been associated with only marginal improvements in survival times. Delayed myelosuppression represents the major restriction on single and cumulative BCNU doses. Thus, single-dose BCNU in excess of 400 mg/sq m produces significant myelosuppression and thrombocytopenia. In most clinical studies, cumulative myelosuppression limits the majority of patients to less than 1200 mg/sq m of BCNU during their entire postoperative course. The hope of providing this dose as a single injection prompted the use of autologous marrow.

Following marrow infusion, we did not observe the characteristic late leukopenia and thrombocytopenia that occur 4 to 6 weeks after BCNU administration. Instead, patients who received BCNU, 800 to 1400 mg/sq m, reached leukocyte and platelet nadirs 10 to 15 days after therapy, remained suppressed for approximately 10 days, and then returned to adequate white cell and platelet levels as a presumed result of grafted marrow. With one exception (Case 9, treated at 1400 mg/sq m), intercurrent infection and/or
bleeding was not seen. The use of viable, refrigerated bone marrow to avoid chemotherapy-induced myelotoxicity is not new, and takes advantage of the short biological half-life of BCNU. Initial experience suggests that this technique cannot be used in individuals with preexisting marrow suppression.

In patients with glioblastoma, recurrence of tumor not treated by chemotherapy, surgical debulking, or techniques for control of cerebral edema is usually followed by clinical deterioration and death within 3 to 5 months. Four of our eight evaluable patients receiving less than 1200 mg/sq m showed improvement in their modified Karnofsky scale of functional ability while on constant or diminished steroid doses, and three were not worsened when evaluated 8 and 4 months, respectively, after high-dose BCNU. The median survival time for evaluable patients is 7 months. The most significant benefit appears to have been associated with doses of BCNU of less than 1200 mg/sq m. Above this dose, unacceptable central nervous system toxicity was encountered which may be related to the BCNU-necrotizing arteriolitis recently described by others. However, preliminary evaluation of necropsy material from our patients treated at 1400 mg/sq m fails to show evidence of vascular changes or damage to normal cortex that would not be explained by prior irradiation.

In our study, CT evidence of change complemented clinical improvement. Two patients showed clear diminution in tumor bulk. Six of the eight patients treated with BCNU at 1200 mg/sq m or below showed diminished enhancement of tumor mass when scanned at isodose of diminished steroids 8 weeks after high-dose BCNU. Five of these six had functional improvement or had stabilized at this time. Diminished contrast enhancement is thought to reflect tumor response to therapy. These changes are unlikely to represent an artifact of primary surgery or irradiation, as 4 to 18 months had passed since these therapies. Decreased peritumoral edema was noted on CT scan in five of our eight patients treated at 1200 mg/sq m or less of BCNU. Unexpectedly, seven of the eight developed ventricular enlargement without clinical features of hydrocephalus. Serial daily lumbar punctures performed on Case 1, 9 months after the first treatment, produced no change in the clinical state. The significance of these CT findings is uncertain, but they are noteworthy in the light of another therapeutic approach which was not associated with CT evidence of improvement. Ongoing evaluations of active patients include neuropsychological tests, positron emission tomography, and brain electrical activity mapping to further evaluate posttreatment changes.

As a result of this initial experience, we have begun to treat newly diagnosed glioblastoma patients with 600 mg/sq m BCNU before and 1 1/2 months after irradiation.

Acknowledgment

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