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Phase I study of BCNU and intravenous 6-mercaptopurine in patients with anaplastic gliomas*

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Summary. On the basis of response rates of up to 50%, BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] is the primary drug used in the chemotherapy of anaplastic gliomas. Preclinical data obtained in several experimental systems show that the cytotoxicity of chloroethylnitrosoureas can be increased by the concomitant use of thiopurines. In this phase I trial, patients with anaplastic gliomas received standard-dose BCNU (200 mg/m^2 × 1) in combination with escalating doses of intravenous 6-mercaptopurine (200, 350, 500, and 750 mg/m^2 daily × 3), with BCNU being given on day 3 to maximize the effect of the drugs on cellular DNA. No increase in hematologic toxicity was demonstrated as the dose of 6-mercaptopurine was increased. Responses and stabilization of disease were observed in several patients. Due to the safety of and the evidence of activity found for this regimen in the present trial, 750 mg/m^2 6-mercaptopurine has been incorporated into subsequent studies.

Introduction

BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea; carmustine] is the standard drug used to treat anaplastic gliomas. In phase II studies, response rates approaching 50% have been obtained [15, 19], and randomized studies by the Brain Tumor Study Group demonstrated a significant increase in the number of 18-month survivors when treatment with BCNU was added to surgery and radiotherapy [16, 17]. However, although these studies confirmed the activity of BCNU, they showed it to have only a minimal effect on long-term survival [3, 16, 17].

A synergistic cytotoxic effect has been demonstrated following the addition of thiopurines (6-thioguanine or 6-mercaptopurine) to the nitrosoureas nimustine (ACNU), BCNU, or 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride (PCNU) in several experimental systems [2, 5–8, 12, 13, 18]. The combination of 6-thioguanine (6-TG) and ACNU markedly increases survival in the L-1210 murine leukemia system when each is used at doses that fail to have any significant effect when given alone [6, 7]. Similar effects have been observed for this combination in the Lewis lung-carcinoma model, with a less striking but significant effect being noted in B16 melanoma [7]. This combination results in less than additive mortality and no more than additive marrow suppression [5]. The cytotoxic effect of BCNU on the 9L rat brain-tumor line is potentiated by 50% following the addition of 6-TG [2]. In this system, treatment with BCNU and 6-TG results in a 50% increase in the number of DNA cross-links and a 30% increase in sister chromatid exchanges as compared with exposure to BCNU alone.

A similar effect has been demonstrated in human glioma xenografts [13, 18], against which the combination of chloroethylnitrosoureas (CENU) and thiopurines is significantly more active than is either type of agent given alone in producing growth delays in subcutaneous tumors and in enhancing survival in mice with intracranial tumors. Furthermore, this effect is clearly schedule-dependent; potentiation is much greater when the CENU is given on the 4th day of thiopurine treatment as opposed to the 1st day. The mechanism of this interaction is thought to depend on the incorporation of the thiopurine into DNA in the place of guanine. Such a site appears to be more susceptible to attack by CENU, and an increase in the number of irreparable DNA adducts results [8, 18].

Thiopurines are variably absorbed when given orally [20]. Studies on the parenteral administeration of thiopurines are limited, but both 6-TG and 6-mercaptopurine (6-MP) have safely been used in phase I trials

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Table 1. Patients' characteristics

Number of patients enrolled	41
Median age (range)	44 (7–69) years
Gender (M/F)	28/13
Prior therapy:	
Radiation	40
Surgery	28
Chemotherapy	10
Histology:	
Glioblastoma multiforme	22
Anaplastic astrocytoma	13
Anaplastic oligodendroglioma	4
Malignant glioma	1
Not available	1

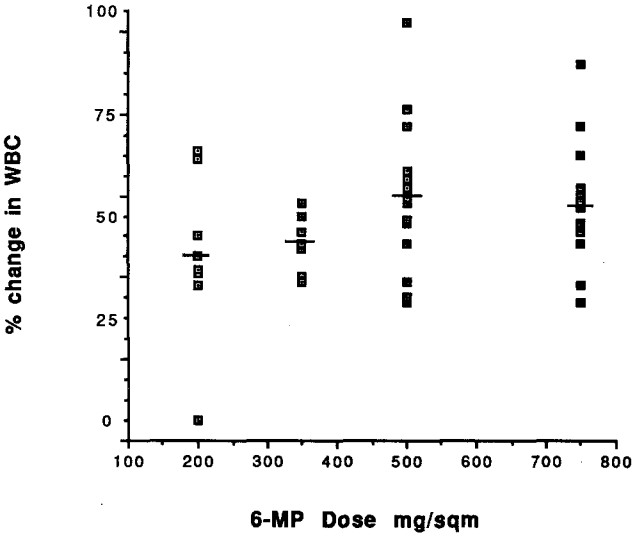

Fig. 1. Percentage change in WBC for cycle 1. *Bars* indicate mean values

[9, 21]. The available pharmacokinetic data indicate that high plasma levels can be obtained with little interpatient variability [9, 21]. On the basis of these observations, we undertook a phase I study of BCNU and escalating doses of intravenous 6-MP in patients with anaplastic gliomas. The treatment schedule was designed to take advantage of the potentiating effect of the thiopurine.

Patients and methods

Patients. Patients with a histologic diagnosis of glioblastoma multiforme, anaplastic astrocytoma, gliosarcoma, anaplastic oligodendroglioma, and mixed anaplastic glioma were eligible for this trial. In addition, subjects with primary brain-stem tumors were eligible if they showed unequivocal radiographic and clinical evidence of a progressive neoplasm. Patients were required to have a measurable, contrast-enhancing lesion as determined by computerized tomography (CT) or magnetic resonance imaging (MRI) of the head within 7 days of the first dose of chemotherapy and to have stable dosing with corticosteroids for at least 5 days prior to the baseline imaging study.

Other eligibility criteria included evidence of adequate bone marrow function (hemoglobin, ≥ 10 g/dl; absolute neutrophil count, $\geq 1500/\mu l$; platelet count, $\geq 100{,}000/\mu l$), renal function (serum creatinine, ≤ 1.5 mg/dl), hepatic function (conjugated bilirubin, <1.5 mg/dl; alkaline phosphatase, <2 times the normal value; SGOT, <2 times the normal level), and pulmonary function (carbon monoxide-diffusing capacity, $\geq 60\%$ of the expected value), as well as a Karnofsky performance status of at least 50%. Subjects who had undergone biopsy or surgical resection of their tumor were eligible for immediate therapy if a postoperative contrast-enhanced CT scan had been obtained within 72 h of surgery. Patients were excluded if they had received prior therapy with nitrosoureas or any chemotherapy or radiation within 8 weeks of study entry unless clear evidence of progression had arisen during that interval.

Study design. 6-MP was given intravenously at four dose levels (200, 350, 500, and 750 mg/m^2) on days 1–3 of each 6-week treatment cycle. BCNU was injected intravenously at a dose of 200 mg/m^2 on day 3 of each cycle. At least three patients were treated in each cohort at each participating institution (Duke University Medical Center and Evanston Hospital). If <2 patients displayed a granulocyte nadir of $\leq 500/\mu l$ or a platelet nadir of $\leq 50{,}000/\mu l$ (severe hematologic toxicity), the 6-MP dose was escalated to the next level. If ≥ 2 patients developed severe hematologic toxicity, an additional 3 patients were enrolled at that dose level for further characterization of the toxicity. The maximally tolerated dose was defined as the dose at which $\leq 33\%$ of the patients developed severe hematologic toxicity. Complete blood counts were obtained weekly throughout the course of therapy, with serum chemistries and liver-function tests being carried out on at least a biweekly basis. All patients who completed one cycle of therapy were considered to be evaluable for toxicity, which was scored according to the Common Toxicity Criteria of the National Cancer Institute.

Prior to each treatment cycle, patients were evaluated by neurologic examinations, head CT (or MRI), and pulmonary function testing. Criteria for retreatment included a hemoglobin value of ≥ 9.0 g/dl, an absolute granulocyte count of $\geq 1500/\mu l$, a platelet count of $\geq 100{,}000/\mu l$, a serum creatinine level of <1.5 mg/dl, a conjugated bilirubin value of <1.5 mg/dl, an alkaline phosphatase level of <2 times the normal value, an SGOT level of <2 times the normal value, and a carbon monoxide diffusion capacity amounting to $\geq 60\%$ of the expected value. The doses of BCNU and 6-MP were reduced by 25% in the presence of severe hematologic toxicity (as defined above). Treatment was given every 6 weeks until either evidence of progression or severe toxicity was noted or the patient refused further therapy. Response to therapy was assessed by neurologic examination and serial CT or MRI. Therapy was discontinued after the completion of seven (Evanston) or nine (Duke) cycles in patients with stable or responding disease.

Results

Between November 1987 and May 1989, a total of 41 patients were enrolled in this trial at the participating institutions (24 patients at Duke University Medical Center and 17 patients at Evanston Hospital). Their characteristics are summarized in Table 1. All but one of the patients had received prior radiation therapy, and most had undergone at least partial resection of their primary tumor. Glioblastoma multiforme was the most common histologic diagnosis. Two patients had brain-stem tumors that were inaccessible and were therefore not biopsied. At autopsy, one of these brain-stem tumors proved to be a glioblastoma multiforme; an autopsy was not performed in the second case.

In all, 39 of the 41 patients were evaluable for hematologic toxicity. Of the 2 patients who were not evaluable, 1 refused blood counts and 1 was admitted to the hospital during his first cycle due to progressive disease and no further count was obtained. Figures 1 and 2 show the percentage change observed in the WBC and platelet counts of each evaluable patient during cycle 1 of therapy. There was no significant change in the hematologic toxici-

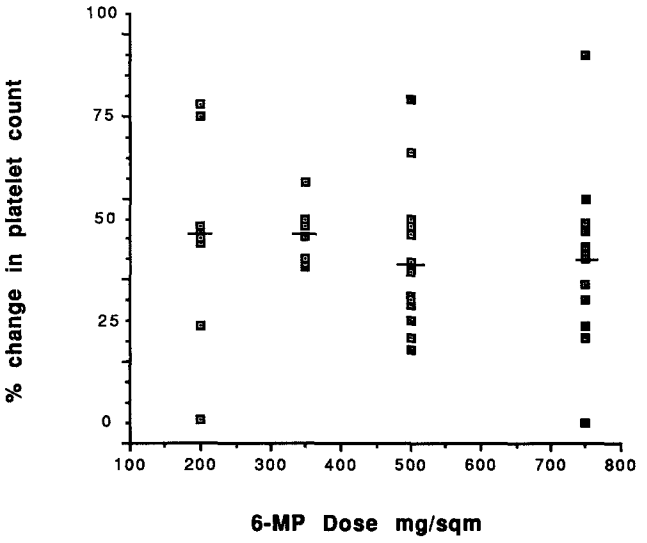

Fig. 2. Percentage change in platelet count for cycle 1. *Bars* indicate mean values

ty, and the mean change in both hematologic parameters remained stable as the dose of 6-MP was escalated.

Table 2 details the significant hematologic toxicities seen and includes the number of patients in each cohort and the number of cycles delivered. The median number of therapy cycles delivered per patient was 2 (range, 1–9), and a total of 135 cycles were given. Five patients each completed a total of seven cycles, and two received nine cycles. It is noteworthy that four of these patients, including the two who completed nine cycles, were in the cohort receiving the highest dose of 6-MP (750 mg/m^2). Cumulative hematologic toxicity was demonstrated in patients treated with multiple cycles, although it was mild in most cases. Of 26 patients who received >1 cycle, 6 developed grade 3 WBC toxicity (11 episodes, 4 in 1 patient receiving 350 mg/m^2), 5 experienced grade 3 platelet toxicity (5 episodes), and 1 developed grade 3 anemia requiring transfusion. No correlation was found between the dose and the incidence of cumulative toxicity.

In only two cases was severe hematologic toxicity documented, and both patients died during the study period. One subject treated with 500 mg/m^2 6-MP presented at the clinic on day 10 with weakness and hypovolemia. Her counts included a WBC of 400/μl [absolute neutrophil counts (ANC), 224/μl], and a platelet count of 33,000/μl. She was admitted to the hospital and died 3 days later after her family had requested only supportive care. No infection or progression of her disease was documented. The second patient, a 17-year-old man who was treated with 750 mg/m^2 6-MP, developed status epilepticus on day 28 of his first cycle of therapy. He was admitted to the intensive care unit and showed progressive deterioration of his neurologic status. CT and MRI scans revealed encephalomalacia consistent with radionecrosis and no evidence of intracranial bleeding. Cerebrospinal fluid assays showed no evidence of infection. This patient was thrombocytopenic on admission (platelet count, 86,000/μl) and became pancytopenic during the hospitalization period, displaying a nadir WBC of 800/μl (ANC, 400/μl), a platelet nadir of 23,000/μl, and a hemoglobin value of 8.8 g/dl in the setting of persistent upper gastrointestinal bleeding. He died 11 days after his admission (day 39). These were the only severe hematologic toxicities (as defined in the protocol) seen during the first cycle of therapy. One other patient treated with 500 mg/m^2 6-MP developed severe anemia (hematocrit, 20.3%; hemoglobin, 6.9 g/dl) on day 22 of his first cycle of therapy and required transfusion.

All 26 patients who completed >1 cycle of therapy were capable of receiving the 2nd cycle at the prescribed dose and on schedule. Of 18 patients who received >2 cycles, 3 required reductions in the dose of both 6-MP and BCNU for the 3rd cycle or subsequent courses due to hematologic toxicity; a total of 5 cycles were given at reduced doses to these 3 patients.

Pulmonary toxicity as demonstrated by a decline in carbon monoxide-diffusing capacity (DLCO) to <60% of the predicted value was seen in 11 patients (28%). All 26 of the subjects who underwent serial DLCO assessments showed a decline. Although one patient displayed a marked decline (from 75% to 54%) following her first cycle of therapy, most of our subjects showed a slow, progressive decline over several cycles. No renal or hepatic toxicity was observed during this study.

Therapy was discontinued due to progression of disease in the majority of patients (n = 23). Other reasons for cessation of therapy included pulmonary toxicity (n = 7), completion of the study (n = 7), death (n = 2), and refusal of further therapy (n = 1). One patient was lost to follow-up after his fourth cycle of therapy. Among the 15 subjects who received only 1 cycle of therapy, the major reason for discontinuation was disease progression (n = 11), the other causes being pulmonary toxicity (n = 1), death (n = 2), and refusal of further therapy (n = 1).

Although response was not the primary end point of this study, several patients showed a clear response to the therapy as manifested by a reduction in the size of their tumors

Table 2. Hematologic toxicity profile: grade 3 or greater

6-MP dose (mg/m^2)	Patients (n)	Total cycles	Number of cycles resulting in toxicity of grade >2		
			Platelets	WBC	Hb
200	8	20	1 (1)	0	0
350	6	17	4 (1)	0	
500	15	49	3 (3)	6 (5)	2 (2)
750	12	48	3 (3)	5 (4)	0

Figures in parentheses indicate the number of patients developing the respective toxicity. Hb, Hemoglobin

on imaging studies. Ten patients were judged to have achieved a reduction in the size of their tumor masses or stabilization of their disease for variable periods, although quantitative measurements of tumor volume were not performed. One complete response was achieved by a 29-year-old man with an obligodendroglioma who received nine cycles of therapy at 750 mg/m^2. He subsequently relapsed and died of progressive disease at 38 months after his entry into the study.

Discussion

BCNU has been shown to exert activity against human brain tumors without producing an advantage in survival. Efforts to improve the efficacy of therapy in this setting have focused on the administration of active agents in combination. In the present trial, BCNU was used with 6-MP in a combination that has been shown to produce a synergistic effect in preclinical studies [2, 5–8, 12, 13, 18]. Two prior trials have used 6-MP together with a CENU for the therapy of brain neoplasms [10, 11]; in both studies, 6-MP was given orally at either 21 or 33 days following the administration of the CENU. Neither of these studies demonstrated a significant benefit for the addition of 6-MP on this schedule to a combination regimen that included a CENU.

The present study demonstrates that BCNU and 6-MP can safely be used on this schedule in patients with brain tumors at intravenous doses of up to 750 mg/m^2 6-MP $\times 3$. These doses are considerably higher than both the standard oral dose of 6-MP (50–100 mg/day) and the oral dose previously given in brain-tumor studies (100 mg/m^2 6 h $\times$ 12). Although 6-MP was given in this study as a daily bolus, the total dose delivered at the highest dose level (2250 mg/m^2 over 3 days) was comparable with the maximally tolerated dose delivered by continuous infusion in a previous phase I study (2400 mg/m^2 over 48 h) [21]. At doses greater than 750 mg/m^2 out this study crystallization of 6-MP has been demonstrated in the kidneys of patients receiving bolus therapy [4]. No potentiation of hematologic toxicity was demonstrated in this study and a number of patients were capable of completing multiple cycles of therapy.

The incidence of pulmonary toxicity observed in the present study (28%) is within the range previously reported for BCNU given as a single agent (20%–30%) [1, 14], and this value was probably increased by our use of DLCO as a criteria since few of our patients were symptomatic. It is noteworthy that at the higher doses given a number of patients completed the study, receiving either seven or nine cycles of therapy. Although this may have been attributable to the increased number of patients enrolled at the two highest dose levels, it suggests the possibility that the efficacy of this combination may be enhanced at these levels.

On the basis of these findings, we have initiated a phase III trial in which this combination, with 6-MP being given at the highest dose (750 mg/m^2), has been incorporated into one of the treatment arms. Accrual of patients into this trial is ongoing.

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