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Activity of High-Dose Cyclophosphamide in the Treatment of Childhood Malignant Gliomas

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Seventeen patients less than or equal to 20 years of age with newly diagnosed (n = 10) or recurrent (n = 7) malignant gliomas (anaplastic astrocytoma and glioblastoma multiforme) were treated with cyclophosphamide in association with hematopoietic cytokines (GM-CSF or G-CSF). Cyclophosphamide was given at a dose of 2 g/m^2 daily for 2 days at 4-week intervals. Toxicity consisted of grade IV neutropenia and thrombocytopenia in 95% and 48% of cycles, respectively. There were no cyclophosphamide-related cardiac, pulmonary, or urothelial toxicities observed. Four of 10 patients with newly diagnosed disease demonstrated responses (three complete and one partial responses; one CR was only of 2 months duration). None of the seven patients with recurrent tumors demonstrated a response. We conclude that high-dose cyclophosphamide warrants further evaluation in children with newly diagnosed malignant glioma. Med. Pediatr. Oncol. 30:75–80, 1998.

Key words: cyclophosphamide; childhood brain tumor; anaplastic astrocytoma; glioblastoma multiforme

INTRODUCTION

High-grade glial tumors are among the most malignant of childhood brain tumors. With conventional therapy, consisting of surgery and irradiation, survival at 5 years following the diagnosis of glioblastoma multiforme has been less than 10% [1–4]. These tumors are rarely amenable to complete surgical resection due to their infiltrative nature. While radiation therapy is commonly administered postoperatively and leads to prolongation of survival [3,5], long-term remissions remain rare.

The activity of alkylating agents in the treatment of these tumors has been demonstrated in laboratory and clinical studies [6–9]. However, drug resistance, manifest de novo or following initial tumor response, is frequently seen, with resultant tumor progression and death. A variety of mechanisms of drug resistance has been postulated, but such mechanisms are not well characterized [10,11].

An alternative approach to overcoming alkylator resistance may be dose escalation, due to the observation that alkylator-resistant cells are generally only five- to tenfold more resistant than sensitive cells, in striking distinction to the 1,000-fold increase in resistance which can be seen with antimetabolites or MDR-susceptible agents [11]. The availability of recombinant hematopoietic cytokines has made such dose escalation feasible. Our previous phase I study of cyclophosphamide with granulocyte-macrophage colony-stimulating factor (GM-CSF) demonstrated several responses in patients with recurrent glioma [12]. We therefore initiated a phase II study of high-dose cyclophosphamide in the treatment of both newly diagnosed and recurrent childhood malignant gliomas.

METHODS

Patient Selection

Children up to 20 years of age were eligible for treatment if they had histological confirmation of a malignant

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glioma (anaplastic astrocytoma or glioblastoma multiforme), with magnetic resonance imaging (MRI) documentation of measurable residual tumor (minimum of 1.5 cm^2 as product of largest perpendicular measurements). A postoperative MRI scan must have been obtained within 72 hours of surgery in any child with newly diagnosed tumor and for any child with recurrent tumor who underwent repeat resection. The estimated life expectancy must have been at least 8 weeks, and Lansky score (<18 years old) or Karnofsky score (18–20 years old) at least 60%. Minimal hematological values for entry into the study included a hemoglobin ⩾8.0 g/dl, absolute neutrophil count (ANC) ⩾1,500/μl, and platelet count ⩾100,000 cells/μl. Patients were required to have a serum creatinine ⩽2.0 mg/dl, SGOT and bilirubin ⩽1.5 × normal values, adequate pulmonary function as measured by diffusing capacity (DLCO, ⩾75% predicted), resting cardiac ejection fraction ⩾55% with normal wall motion on a gated nuclear angiography scan, and recovery from acute toxicity of any recent therapy. Prior treatment with radiation must have been completed at least 12 weeks prior to registration in the study. Prior treatment with chemotherapy must have been completed at least 6 weeks prior to registration in the study. No dose escalation of dexamethasone was permitted after study entry or the patient was deemed inevaluable for response. Informed consent was obtained from all patients' families; children ⩾12 years old were required to give signed consent.

Treatment Schedule

Cyclophosphamide was administered at a dose of 2 g/m^2/day for 2 successive days with courses repeated every 4 weeks for a total of four courses. The drug was administered over a 1-hour period with twice maintenance (3,000 ml/m^2/day) intravenous hydration and mesna uroprotection. Patients received either GM-CSF or G-CSF depending on availability and third party coverage, to ameliorate neutropenia. GM-CSF was administered subcutaneously at a dosage of 250 $\mu g/m^2$ twice daily beginning 24 hours after the second dose of cyclophosphamide for each course. G-CSF was administered subcutaneously at a dosage of 10 μg/kg daily beginning 24 hours after the second dose of cyclophosphamide for each course. Treatment with GM-CSF or G-CSF continued until the ANC exceeded 1,000 cells/μl or 10,000 cells/μl, respectively, on two consecutive days after the chemotherapy-associated nadir. Courses of chemotherapy were repeated every 4 weeks provided that hematologic recovery had occurred (ANC ⩾1,000 cells/μl, platelets ⩾100,000 cells/μl). Therapy for children with newly diagnosed tumors was designed to consist of four courses of chemotherapy followed by external beam radiotherapy. Chemotherapy was to be discontinued in any child who demonstrated progressive tumor or unacceptable toxicity, with immediate referral for radiotherapy with or without prior repeat resection. Therapy for children with recurrent tumors was designed to continue at monthly intervals for a total of four courses or until progressive tumor or unacceptable toxicity was experienced.

Evaluation of Tumor Response

Patients were evaluated by neurological examination prior to each course of therapy. MRI scan of the brain was performed pre- and postgadolinium contrast injection following the second and fourth courses. Responses were defined by standard radiographic criteria. Complete response (CR) was defined as disappearance of all radiographic evidence of measurable tumor. Partial response (PR) was defined as >50% reduction in the product of the largest perpendicular diameters of all lesions. Stable disease (SD) was defined as ⩽50% reduction and ⩽25% increase in size. Progressive disease (PD) was defined as a >25% increase in such dimensions.

Evaluation of Toxicity

Complete blood counts, including differential leucocyte counts and platelet counts, were performed twice weekly or more frequently if indicated. Renal function, hepatic enzymes, and serum electrolytes were checked prior to each course of treatment to assess nonhematologic toxicity. Pulmonary function tests and gated nuclear cardiac angiography were performed at study entry and following the second and fourth courses.

Statistics

Cyclophosphamide was to be studied as long as evidence existed that 30% or more of patients could be expected to respond. The following stopping criteria were employed where P denotes the true probability of tumor response:

No. of patients tested	Maximum number of responders	Confidence that $P < 0.3$
9	0	96.0
14	1	97.4

Accrual was to be stopped by the above criteria, recognizing that if the true response rate is 30% the protocol will be closed 4% of the time with nine patients and 2.6% of the time with 14 patients.

RESULTS

Patient Demographics

Seventeen children with malignant glioma were treated with cyclophosphamide (Table I). Ten children had newly diagnosed tumors (7 glioblastoma multiforme, 3 anaplastic astrocytoma) and seven had recurrent tumors (4 glioblastoma multiforme, 3 anaplastic astrocytoma). The seven patients with recurrent tumors had previously been treated with surgery (n = 7), radiation therapy (n

TABLE I. Patient Characteristics at Study Entry

No.	Age/Sex	Diagnosis	Disease state	Tumor location	Prior therapy[b]
1	11/M	GBM[a]	Progressive	Occipital, temporal, spine	RT[a]
2	10/F	AA[a]	Recurrent	Midbrain	RT
3	4/F	GBM	Progressive	Thalamus	RT, '8-in-1'
4	7/F	GBM	Progressive	Temporal	RT
5	1.8/F	GBM	Recurrent	Frontal	Cisplatin, VP16, vincristine
6	18/M	AA	Persistent	Cerebellum, brainstem, spine	RT, carboplatin
7	15/M	AA	Recurrent	Pineal	RT, BCNU[a], AZQ[a]
8	20/M	GBM	Newly diagnosed	Frontal	—
9	5/M	GBM	Newly diagnosed	Parietal	—
10	8/F	GBM	Newly diagnosed	Thalamus	—
11	14/M	AA	Newly diagnosed	Frontal	—
12	4/M	GBM	Newly diagnosed	Temporal	—
13	12/M	AA	Newly diagnosed	Brainstem	—
14	20/M	GBM	Newly diagnosed	Temporal	—
15	13/M	GBM	Newly diagnosed	Temporal	—
16	5/M	GBM	Newly diagnosed	Parietal	—
17	12/F	AA	Newly diagnosed	Parietal	—

[a]GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; RT, radiation therapy; BCNU, 1,3 Bis(2-chloroethyl)-1-nitrosourea; AZQ, diaziquone.
[b]Prior therapy included surgery in all cases.

TABLE II. Number of Courses Administered, Tumor Response, and Patient Outcome

No.	Newly diagnosed (ND) or recurrent/ progressive (R)	No. of courses	Response following 1–2 courses	Response following 3–4 courses	Duration of response	Subsequent therapy	Outcome[b]
1	R[a]	1	PD[a]	—	—	—	Died, 6 months
2	R	2	PD	—	—	—	Died, 4 months
3	R	1	PD	—	—	—	Died, 7 months
4	R	2	PD	—	—	Chemotherapy	AWD[a], 5 months
5	R	1	PD	—	—	—	Died, 6 months
6	R	2	PD	—	—	Chemotherapy	AWD, 6 months
7	R	4	SD[a]	PD	—	Chemotherapy	AWD, 9 months
8	ND[a]	4	SD	PD	—	Chemotherapy, RT[a], ABMT[a], Surgery	Died, 18 months
9	ND	3	SD	CR[a]	14+ months	Chemotherapy, RT, ABMT	A,NED[a] 16+ month
10	ND	4	SD	PD	—	Chemotherapy, RT	AWD, 8 months
11	ND	2	PD	—	—	Chemotherapy, RT	AWD, 6 months
12	ND	3	SD	PD	—	Chemotherapy, RT, ABMT, Surgery	AWD, 26 months
13	ND	2	SD	—	—	—	Died, 2 months
14	ND	3	PR[a]	CR	2 months	Chemotherapy, RT	Died, 9 months
15	ND	4	PR	PR	4 months	Chemotherapy, RT	AWD, 16 months
16	ND	4	PR	CR	18+ months	Chemotherapy, RT, ABMT	A,NED, 19+ month
17	ND	4	SD	SD		RT	AWD, 9 months

[a]ABMT, high-dose chemotherapy with autologous bone marrow transplant; A,NED, alive with no evidence of disease; AWD, alive with disease; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; RT, radiation therapy; R, recurrent tumor; ND, newly diagnosed.
[b]Time measured from date of study entry.

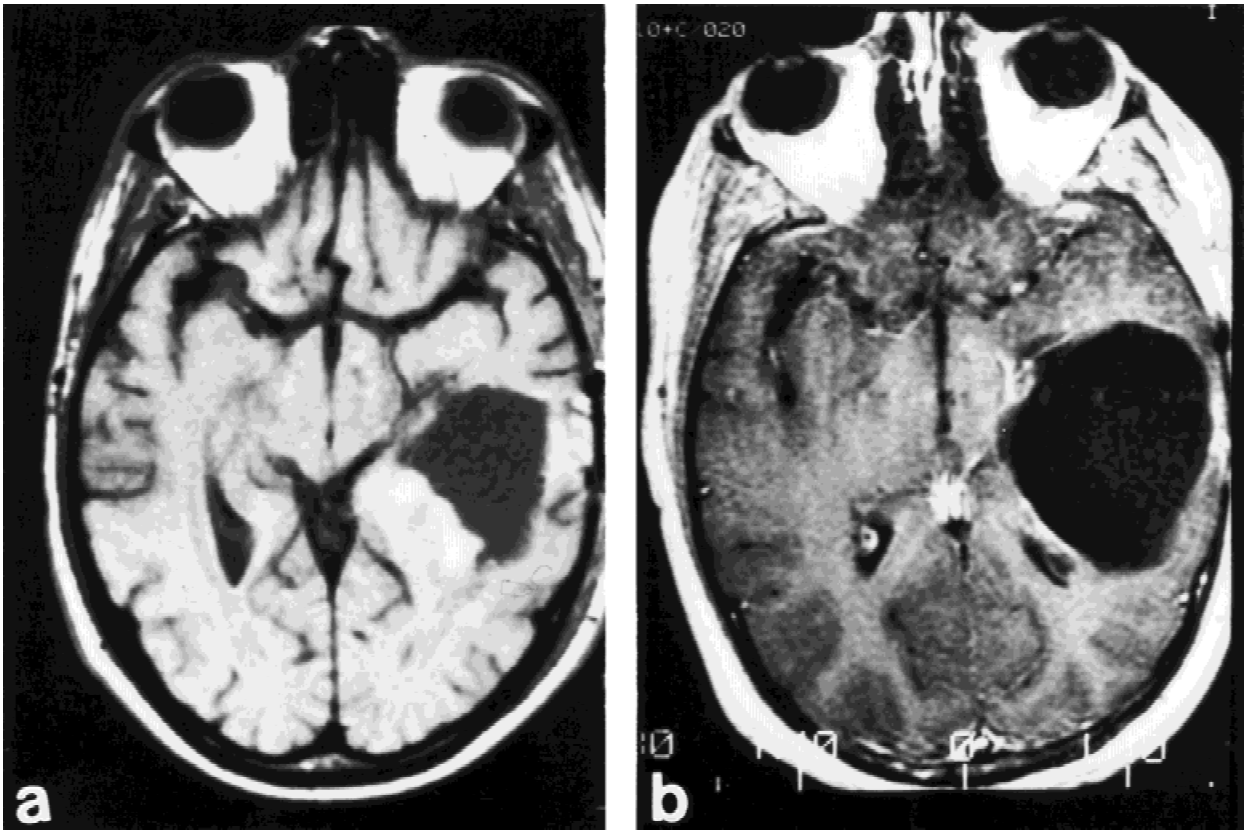

Fig. 1. **a:** Axial T1-weighted MRI with Gd-DTPA contrast of patient 14 in the immediate postoperative period, demonstrating a surgical cavity in the left posterior temporal region with adjacent residual enhancing tumor. Mass effect on the left occipital horn is evident. **b:** Following three courses of chemotherapy, there has been complete resolution of residual tumor enhancement and decreased mass effect on the left occipital horn.

= 6), and chemotherapy (n = 4). The patients with newly diagnosed tumors had undergone surgery alone.

Treatment

Table II details the number of courses of chemotherapy each child received and the reason for termination of therapy. In newly diagnosed patients, two patients received two courses, three received three courses, and five completed all four courses. Reasons for discontinuing therapy prior to the planned four courses in these patients were progressive disease (n = 3) and achievement of CR (n = 2) with alternate therapy subsequently administered per parental request. Among the seven patients with recurrent tumor, only one completed four cycles of chemotherapy. The remaining six patients developed progressive disease and were taken off study following one cycle (3 patients) or two cycles (3 patients).

Tumor Response

Objective tumor responses (CR or PR) to high-dose cyclophosphamide were observed in four of 10 patients (40%) with newly diagnosed malignant gliomas. Two 5-year-old children with parietal lobe GBM achieved a CR following three and four courses of chemotherapy, respectively. Both subsequently received involved-field radiotherapy followed by high-dose chemotherapy (cyclophosphamide and melphalan) with autologous bone marrow rescue. These two children remain disease-free for 14+ and 18+ months, respectively, since diagnosis. A 20-year-old patient with temporal lobe GBM achieved a CR following three courses of therapy (Fig. 1A and B), but developed tumor progression 2 months later during radiotherapy. A 13-year-old child with temporal lobe GBM completed four courses of therapy with a PR (Fig. 2A and B). He subsequently went on to receive radiotherapy, but developed tumor progression 4 months later. In this study, the overall tumor response rate (CR + PR) in children with newly diagnosed GBM and AA was 57% (4/7) and 0% (0/3), respectively.

No patient with a recurrent malignant glioma responded to high-dose cyclophosphamide (n = 7). One patient with a recurrent anaplastic astrocytoma achieved disease stabilization following two courses of therapy, but developed progressive disease following the fourth course. The remaining six patients developed progressive disease after one course (n = 3) or two courses (n = 3) of therapy.

Toxicity

Treatment-related toxicity consisted of myelosuppression, with grade IV neutropenia and thrombocytopenia occurring after 95% and 48% of chemotherapy courses,

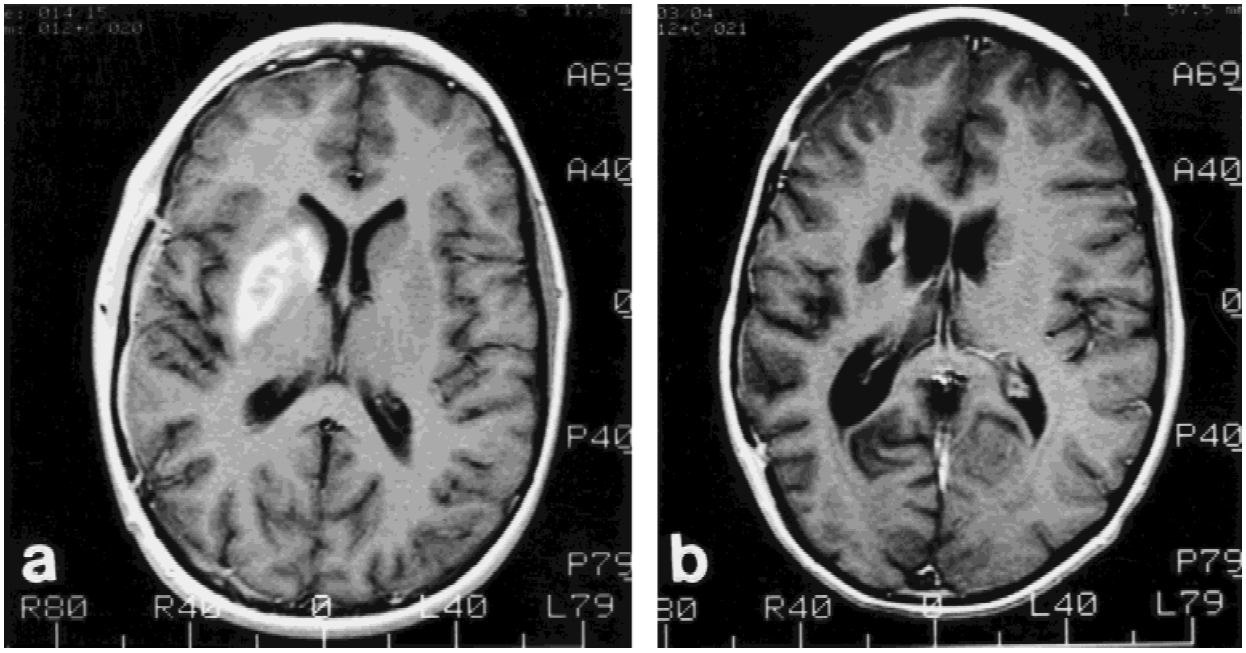

Fig. 2. **a:** Postoperative axial T1-weighted MRI with Gd-DTPA contrast of patient 13 reveals a contrast-enhancing mass in the region of the right basal ganglia with minimal mass effect. **b:** Following four courses of chemotherapy, a near-total resolution of contrast-enhancing mass has been achieved, leaving a residual cavity.

respectively. The mean duration of grade 4 thrombocytopenia and neutropenia was 3 and 6 days, respectively. No delay in retreatment with cyclophosphamide was noted in any patient. Fifty-eight percent of the 46 courses administered were complicated by subsequent admission to the hospital for the treatment of fever with neutropenia with a mean hospitalization time of 4 days. Nausea was well controlled with ondansetron in most cases. There was no cyclophosphamide-related cardiac, pulmonary or urinary bladder toxicity, and there were no treatment-related deaths. No patient demonstrated any adverse CNS effects secondary to the hydration regimen. Three documented infections (sepsis) were noted which all promptly responded to antibiotics. Two patients experienced hypersensitivity to GM-CSF.

DISCUSSION

The outlook for children with malignant gliomas remains dismal. The addition of chemotherapy to the traditional therapies of surgery and radiation led, in a randomized trial, to a modest but statistically significant improvement in survival [8]. Further improvements have not been achieved, despite newer multiple-agent chemotherapy regimens [13]. There is a compelling need to identify alternate agents with activity against these malignant tumors.

We elected to study cyclophosphamide because of the ability to administer the drug in a dose-intensive fashion, and because of in vitro and preliminary clinical data suggesting anti-glioma activity [6]. In this study, we found that in seven patients who had received prior radiotherapy (n = 6) and chemotherapy (n = 4), no tumor responses were achieved. However, in 10 newly diagnosed patients treated following partial tumor resection only, four responses (3 CR, 1 PR) were seen with one of the CR of only 2 months duration. Although a Fisher exact test gives a two-sided *P* value of 0.10, suggesting that this difference may be chance, it may also reflect true differences in tumor response. Radiation-induced resistance to alkylating agents may have contributed to the lack of therapeutic efficacy in the cohort of patients with recurrent tumors; a variety of biochemical mechanisms underlying resistance in this setting have been postulated.

Such disparity in phase II responses between untreated and treated patients has been observed previously [15,16]. Horowitz et al. [15] reported apparent failure of the alkylating agent, melphalan, to produce tumor responses in a group of heavily pretreated patients with recurrent rhabdomyosarcoma. Subsequent study of this agent in newly diagnosed patients led to the demonstration of significant anti-tumor activity, confirming the activity which had been seen previously in the murine xenograft model [15]. Their experience illustrated the inherent limitations of phase II drug trials in previously treated patients, and the authors concluded that testing of new agents in untreated cancer patients was justified if standard therapy was unsatisfactory, and if there was adequate preclinical evidence of tumor activity at clinically achievable drug concentrations. This approach has led to the increasingly frequent use of ''up-front phase II windows'' in testing new agents for activity against

poor-prognosis tumors. With similar reasoning, Finlay et al. [9] have favored the use of experimental, intensive chemotherapy with autologous marrow support in the setting of certain newly diagnosed malignant brain tumors, including GBM and AA.

Despite early concerns that cyclophosphamide would not cross the blood-brain barrier, this agent has proved active in the treatment of medulloblastoma [17], pineoblastoma [18], germinoma [19], and pilocytic astrocytoma [20]. Duffner et al. [21] have reported responses to cyclophosphamide and vincristine in children less than 3 years of age with malignant gliomas. We now report tumor responses in four of 10 children and young adults with newly diagnosed malignant gliomas following treatment with high-dose cyclophosphamide. No responses were seen in seven patients with recurrent malignant gliomas. In a similar study of dose-intensified cyclophosphamide (1.18–2.25 g/m^2/day × 2 days) with GM-CSF used in the treatment of childhood malignant brain tumors, Abrahamsen et al. [22] reported no objective responses in five patients with recurrent malignant gliomas. However, in contrast to the results of our study, Abrahamsen et al. [22] observed no tumor responses to dose-intensified cyclophosphamide in six patients with newly diagnosed malignant gliomas. They therefore concluded that high-dose intensity cyclophosphamide is unlikely to benefit children greater than 3 years of age with malignant gliomas. However, on the basis of our preliminary data in an admittedly small cohort of patients, we are continuing to investigate the activity of high-dose cyclophosphamide with cytokine support in a larger group of children and young adults with newly diagnosed malignant gliomas. If the results reported in this study are confirmed in a larger cohort of patients, high-dose cyclophosphamide would warrant inclusion in a phase III trial for children with newly diagnosed malignant gliomas.

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