Therapy of glioblastoma multiforme improved by the antimutagenic chloroquine

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Object. Therapy of malignant tumors is frequently curtailed by the emergence of chemoresistant cell clones. Experimentally, the authors have demonstrated that chemotherapy for glioma in rats is markedly improved by the administration of the antimutagenic quinacrine. They studied the effects of chloroquine, an antimutagenic with an optimal pharmacological profile for human use, as adjuvant for the treatment of patients with glioblastoma multiforme (GBM).

Methods. In a prospective controlled randomized trial, 18 patients with GBM underwent standard treatment with surgery, chemotherapy, and radiotherapy; nine received an additional 150-mg dose of chloroquine daily starting 1 day after surgery and continued through the observation period. Nine matched patients were included as controls. Neuroimaging studies and clinical response were periodically compared. The follow-up period ranged from 24 to 50 months. Survival time was defined as the main outcome measure.

Survival was significantly longer in chloroquine-treated patients than in controls (33 ± 5 and 11 ± 2 months, respectively [$p < 0.0002$]). At the end of the observation period, four patients (46%) treated with chloroquine were alive, two had evidence of tumor remission after 2 years; in another two, tumor recurrence developed after 2 and 4 years of remission, respectively. No control patient survived more than 22 months after surgery.

Conclusions. Chronic administration of chloroquine greatly enhanced the response of GBM to antineoplastic treatment. Because the cytotoxicity of chloroquine on malignant cells is negligible, these favorable results appear mediated by its strong antimutagenic effect that precludes the appearance of resistant clones during radiotherapy and chemotherapy.

KEY WORDS • glioblastoma multiforme • cancer therapy • chloroquine • mutagenesis

Glioblastoma multiforme is the most frequent primary brain tumor in adults. Despite advances in diagnostic and surgical techniques, its prognosis remains somber; survival time in patients has not increased substantially during the last few decades.^{7,21} A common drawback of antineoplastic treatment is the appearance of acquired chemoresistance, even in tumors that were initially susceptible.^{4,6} The development of cell resistance to chemotherapy may be due to the survival and growth of tumor cells originally resistant or to the emergence of mutant cell clones that develop a newly acquired resistance.^{9,12} Cell mutations are common in malignant neoplasms, and this feature is enhanced during chemotherapy.^{14,19,23}

In a previous experimental study involving cultured C6 glioma cells and C6 rat malignant glioma, we demonstrated that quinacrine, an antimalarial drug with strong antimutagenic properties, administered in adherence to a chronic schedule maintained unchanged, the initial cytotoxic effect of the antineoplastic carmustine, which led to a high rate of tumor resolution in animals and optimal carmustine-induced toxicity in cultured C6 glioma cells.²⁴

Because quinacrine lacks antineoplastic properties,² it was apparent that the optimization of carmustine treatment for experimental malignant glioma, achieved by the addition of the antimalarial quinacrine, was due to its strong antimutagenic effect.

Here, we report a trial in patients with GBM who, in addition to standard therapies, were treated with chloroquine, an antimalarial antimutagenic compound analog to quinacrine, which was selected for the clinical study because of its great chemical and pharmacological similarities to quinacrine, its good toxicological profile, its commercial accessibility, and the vast clinical experience of several decades of use for the treatment of human malaria and some autoimmune disorders.^{8,10}

CLINICAL MATERIAL AND METHODS

Patient Population

In an open prospective randomized controlled study nine patients with GBM received a daily 150-mg dose of chloroquine starting the day after surgery and continued throughout the entire observation period. Nine contemporary patients with GBM were selected as paired controls on the basis of initial neuroimaging features and histological confirmation. Figures 1 and 2 show the neuroimaging

Abbreviations used in this paper: GBM = glioblastoma multiforme; KPS = Karnofsky Performance Scale; MR = magnetic resonance.

Fig. 1. Initial neuroimaging studies obtained in chloroquine-treated patients. (*Upper Row*: Cases 1–3; *Center Row*: Cases 4–6; *Lower Row*: Cases 7–9, respectively). Each panel shows the most representative imaging sequence of the GBM.

study most representative of each patient included in both groups. No patient was excluded after initial selection. All patients underwent resection of the lesion, radiotherapy (total dose 6000 Gy), and four cycles chemotherapy (intravenous carmustine 200 mg/m² [Bristol Myers, Mexico]) once every 6 weeks. Patients were entered into the study between May 1998 and May 2000 and followed through September 2002. The committees of research and ethics approved the study. Signed informed consent was obtained from those patients who received chloroquine. All patients were followed for a mean of 35 ± 10 months (35 ± 12 months for chloroquine-treated patients and 37 ± 9 months for controls). No patient initially included was lost to follow up. Tumor size and the extent of resection were comparable in both groups (Table 1). The mean age was 34 ± 10 years for chloroquine-treated patients (range 18–47 years) and 36 ± 3 years for controls (range 24–51 years); the male/female ratio was 6:3 for chloroquine-treated patients and 5:4 for controls. The mean KPS score at the time of diagnosis were 87 ± 3 for chloroquine-treated patients and 88 ± 6 for controls.

Survival time was the main outcome measure. Clinical follow-up examination was performed approximately every 3 weeks. Drug toxicity was periodically monitored in blood by hematological, renal, and liver function testing. The KPS score was recorded. Periodically MR im-

aging and computerized tomography studies were conducted to monitor tumor relapse. Clinical response was graded at 1 and 2 years after surgery, as proposed by MacDonald, et al.:¹⁸ 1) complete response, total disappearance of tumor or edema on MR imaging; 2) partial response, greater than 50% reduction of tumor size on MR imaging accompanied by improvement or stability of neurological status; and 3) progressive disease, increase in tumor size on MR imaging or clinical deterioration. Overall survival, measured from the time of surgery until death or current survival, was estimated using the Kaplan–Meier method.

RESULTS

Overall survival in chloroquine-treated and control patients is shown in Fig. 3. Four patients (46%) from the chloroquine-treated group survived until the end of the observation period (June 2002), a mean of 31 ± 6 months (95% confidence interval 11–55) after surgery. In two of these four there was no imaging-documented evidence of tumor and optimal clinical status, and in the other two evidence of tumor regrowth was revealed on their final follow-up studies, 24 and 50 months postoperatively, respectively; a new course of chemotherapy and radiotherapy supplemented with chloroquine-treatment was initiated at the end of the study (July 2002); the final imaging studies

Fig. 2. Initial neuroimaging studies obtained in control patients. (Upper Row: Cases 10–12; Center Row: Cases 13–15; Lower Row: Cases 16–18, respectively). Each panel shows the most representative imaging sequence of the GBM.

obtained in these four patients are shown in Fig. 4. In control patients mean survival was 10.6 ± 2 months (95% confidence interval 6.6–14.6) ($p < 0.0002$); all died before the 24-month follow up. Mean survival in chloroquine-treated patients who finally died of complications related to the tumor was 20 months and 11 months for controls ($p < 0.008$).

One year after surgery clinical response in chloroquine-treated patients was complete in five (56%) and partial in four (44%); at that time all chloroquine-treated patients were alive and without progressive disease. In control patients clinical response was complete in one (11%), partial in two (22%), and progressive disease in two (22%); at that time four control patients (45%) had died (Table 1). Two years after surgery clinical response in chloroquine-treated patients was complete in three (33%) and progressive in one (11%); five patients (56%) had died. At this time, all control patients had died.

In the chloroquine-treated group the first patient (Case 1) died at 18 months after surgery; however, an MR image obtained 1 month before had demonstrated no evidence of tumor activity (Fig. 5). The patient died at home of acute bronchopneumonia due to aspiration (according to the medical report). The family did not authorize an autopsy study. In the chloroquine-treated group one patient (Case 8) underwent a second surgery for cytoreduction 6 months

after the beginning of therapy. Histopathological comparison, which was performed without knowledge on the source of the specimen, showed that the most recent tissue specimen had a significant reduction of mitotic index (45–50 compared with 4–5 per microscopic field) and less anaplasia than the specimen obtained at initial surgery. Sequential images obtained in the chloroquine-treated patient (Case 6) who survived more than 50 months are shown in Fig. 6. Independent histopathological reexamination of the tumor in those patients from the chloroquine-treated group with long survival confirmed the initial diagnosis of GBM.

In this study the only secondary reaction that could be attributable to chloroquine was the increased frequency of seizures in chloroquine-treated patients compared with controls (five and zero, respectively). Seizures have been reported as an occasional reaction to chloroquine;²⁵ however, their incidence is also high in the natural history of brain neoplasms. Nonetheless, seizures were well controlled by standard antiepileptic treatment. There were no retinal abnormalities at ophthalmological evaluation in any patient from the chloroquine-treated group.

DISCUSSION

Clinical response and survival in patients treated with

TABLE 1
Clinical characteristics of patients with GBM*

Group & Case No.	Age (yrs), Sex	Resec-tion (%)	KPS Score	Clinical Response		Survival (mos)
				1 Yr	2 Yrs	
CQT						
1	46, F	100	90	complete	—	18†
2	28, M	60	90	partial	—	18†
3	18, M	60	70	complete	—	23†
4	34, F	80	90	partial	—	18†
5	47 M	50	70	partial	—	22†
6‡	30, M	90	100	complete	complete	50
7	35, M	100	70	complete	complete	27
8	42, F	70	100	partial	progression	24
9	22, M	80	100	complete	complete	24
controls						
10	51, M	60	90	—	—	6†
11	42, M	80	90	partial	—	14†
12	24, F	80	90	—	—	6†
13	49, M	60	80	—	—	5†
14	25, F	100	100	progression	—	13†
15	34, M	60	90	progression	—	14†
16	33, F	100	90	partial	—	13†
17	26, M	100	90	complete	—	22†
18	40, F	60	80	—	—	3†

* CQT = chloroquine-treated; — = not applicable.

† The patient died.

‡ This patient suffered tumor recurrence after 50 months of follow up.

chloroquine were improved over those in controls ($p < 0.002$), as well as those reported in large series of collaborative trials involving carmustine.^{7,29} The long-term survival rate of 46% in the chloroquine-treated patients seems to be due to the effects of this drug rather than any

other internal variable. This is supported by the finding that, in a large series of patients with GBM who underwent the standard protocol of surgery, radiotherapy, and chemotherapy, only 8% of our institution's patients survived a similarly long period.¹⁷

Fig. 3. Kaplan-Meier estimates of survival after surgery in GBM patients. Significantly longer survival was demonstrated in patients treated with chloroquine in addition to standard therapy than in controls.

Fig. 4. Cases 6 through 9. Representative images obtained in the four GBM patients with long survival. *Upper Left:* Case 6. Fifty months after surgery the tumor recurred. *Upper Right:* Case 7. Twenty-seven months after surgery no evidence of tumor was found. *Lower Left:* Case 8. Twenty-four months after surgery the tumor recurred. *Lower Right:* Case 9. Twenty-four months after surgery no evidence of tumor was found.

There are several decades of clinical experience with the use of chloroquine for the treatment of various parasitic and immune-mediated disorders; however, to our knowledge, this is the first clinical experience with chloroquine as adjuvant in the treatment of GBM. The schedule of chloroquine treatment—continuous administration in conjunction with radiotherapy and the pulses of chemotherapy—was designed to test it on the hypothetical basis

Fig. 5. Case 1. Magnetic resonance image obtained 17 months after surgery in a chloroquine-treated patient. There was no evidence of tumor activity; nevertheless, the patient died at home 1 month later of complications related to acute bronchopneumonia.

Fig. 6. Case 6. Long-term sequential images from a chloroquine-treated patient obtained prior to therapy (*upper left*); 1 and 2 years after surgery, with no evidence of recurrence (*upper right*) and (*lower left*); after 4 years of follow up at which time the tumor relapsed (*lower right*).

of its antimutagenic activity that would prevent the appearance of mutant clones among cells surviving the initial cytotoxic action of carmustine and radiotherapy.^{14,26}

Chloroquine and quinacrine bind strongly to nucleic acids, particularly to CG sequences of DNA reinforcing its structural configuration and preventing mutagenesis. This action has been demonstrated in viruses, bacteria, and many eukaryotic cells, including various cancer cell lines.^{1,3,11,16,27} In addition to this conspicuous effect, chloroquine acts as immunomodulator by inhibiting phospholipase A2 and tumor necrosis factor.^{20,28} It has been shown that quinacrine inhibits the mechanisms of outward cell transport of vincristine in epithelial cancer cells, apparently potentiating its cytostatic effects.^{13,15} Additionally, chloroquine improves the cell mechanisms of DNA repair from the damage induced by alkylating therapy.^{5,22} It is possible that, in addition to antimutagenesis, these effects also participate in the observed beneficial action of chloroquine in GBM therapy.

The dose and duration of chloroquine treatment in this study were determined based on clinical experiences derived in the long-term treatment of autoimmune disorders. The daily dose of 150 mg is considered low in pharmacological studies.¹⁰ Thus, in further trials it could be increased to equal the dose used to treat acute malaria (300 mg daily). This measure would seem reasonable during the periods of radiotherapy and carmustine-based chemotherapy.

CONCLUSIONS

We found that chronic administration of chloroquine greatly enhanced the response of GBM to antineoplastic

agents. Because of the low toxicity and good pharmacological profile of chloroquine, it can be tested in other forms of cancer. A long-term double-blind controlled study in a large number of patients with GBM is underway at our institution.

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