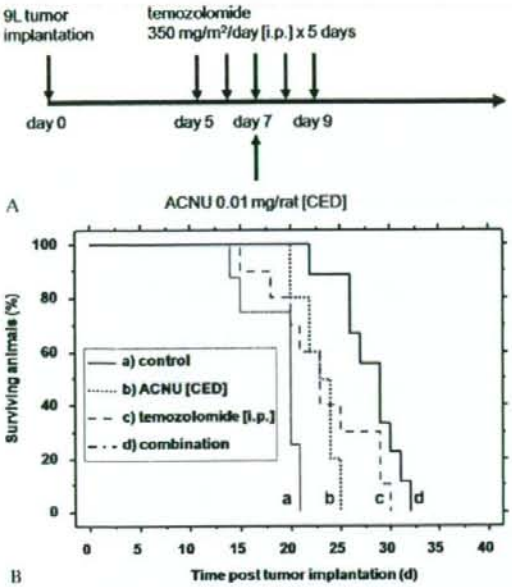

Figure 5: : Combined treatment using CED of ACNU plus systemic administration of temozolomide. (A) Experimental design of the survival study. Daily intraperitoneal injections of temozolomide (350 mg/m²/day) were performed 5–9 days after tumor implantation, and CED infusion of saline or ACNU at 0.01 mg/rat was performed 7 days after tumor implantation. (B) Survival of treated animals is indicated by Kaplan–Meier curves. Animals were treated with CED of saline (a), CED of ACNU alone (b), systemic temozolomide alone (c) or CED of ACNU plus systemic temozolomide (d)

No rats developed any discernable signs, including neurological deficits.

Enhanced antitumor effect of combined treatment using CED of ACNU with systemic temozolomide in a 9L rat brain tumor model

As shown in *Figure 5*, all animals in the control group died due to tumor progression by day 21, and mean survival was only 18.9 days (median: 20.0 days). Animals receiving CED of ACNU at half MTD (0.01 mg/rat) died by day 25, with a mean survival of 22.9 days (median: 23.5 days). This group exhibited significantly improved survival rate compared with the control group ($p<0.001$, log-rank test). Animals receiving systemic administration of temozolomide died by day 30, with a mean survival of 23.3 days (median: 23.0 days). This group also exhibited significantly improved survival rate compared with the control group ($p<0.05$). Animals treated using CED of ACNU plus systemic temozolomide died by day 32, with a mean survival of 28.0 days (median: 29.0 days). This group exhibited significantly improved survival rate compared with the control group ($p<0.001$). This group also demonstrated significantly enhanced survival rate compared with the CED group and the temozolomide group ($p<0.001$ and $p<0.05$, respectively).

DISCUSSION

With methods of local administration, including CED, highly cytotoxic agents with extensive distribution in the CNS have often resulted in brain damage such as leucoencephalopathy and brain atrophy[28]. Hence, good candidates for CED administration into brain tumors would ideally be agents with the highest possible therapeutic index against tumor cells compared with normal neuronal cells. Because of their selective toxicity against tumor cells, immunotoxins are excellent candidates for local delivery[7,11–14]. However, even with the administration of immunotoxin, adverse events mediated by non-specific binding in underlying pathophysiologic processes have been reported[12], indicating that increase in antitumor effect and reduction of neurotoxicity of the agents delivered via CED are required. In this study, we developed an effective method of combined treatment, including CED of ACNU plus irradiation or systemic administration of temozolomide, without increase in neuronal toxicity.

Combined-modality treatment improves therapeutic results if chemotherapeutic agents and radiation have synergistic interactions in tumor cells. For instance, local irradiation has been combined with systemic chemotherapy in treatment of glioma, since spatial cooperation exists when a drug kills cells outside the radiation target area. CED is a local infusion technique and several studies have pointed out that inhomogeneous drug distribution within tumor tissue after CED inevitably results in partial response and local recurrence of tumor[29,30]. By combining CED of ACNU with whole-brain irradiation or systemic temozolomide, we expected that radiotherapy or systemic chemotherapy would cover the cells not exposed to the agents delivered via CED, and that spatial cooperation would be achieved. In this study, we demonstrated that both methods of combined treatment significantly prolonged the survival of tumor-bearing rats, with a lower dose of ACNU than in our previous study.

Another promising characteristic of combined treatment using CED of ACNU and irradiation is that nitrosoureas can sensitize malignant glioma cells to radiation damage. A synergistic effect of BCNU and irradiation was observed in previous *in vivo* animal studies[31]. X-ray-mediated increase in BCNU-induced DNA cross-linking is considered as the mechanism by which cell kill is increased by combined treatment with such agents[32]. The same mechanism was involved in the present study at the DNA molecular level, since ACNU induces the same alkylating reactions and DNA interstrand cross-linking reactions as BCNU.

Temozolomide induces the same alkylating reaction as ACNU, which yields O_6-alkylguanine[33]. Clinically, temozolomide was given concomitantly with radiotherapy, and this treatment schedule yielded some advantage in antitumor efficacy[1]. First, it makes possible an increase in dose intensity. Second, continuous administration of alkylating agents results in depletion of O_6-methylguanine-DNA methyltransferase (MGMT), which causes the chemoresistance of tumor cells to alkylating agents. The abundant O_6-alkylguanine

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produced by continuous administration of temozolomide might deplete MGMT and enhance the efficacy of ACNU. In ongoing clinical protocols, a similar treatment concept is being applied using double-alkylating agents, such as BCNU, procarbazine and vincristine, in patients with malignant glioma[34,35].

The principal aim of this study was to develop a new therapeutic concept of use of CED with irradiation or systemic chemotherapy. Our multimodal approach yielded impressive effects and offers a potentially effective method for treating brain tumor xenografts. Current studies in our laboratory also suggest that other low-dose chemotherapeutic agents administered via CED combined with irradiation or systemic chemotherapy markedly enhance efficacy in treatment of brain tumor xenografts (data not shown) and support our approach. We believe that the findings presented in this study represent significant progress towards future clinical application of combinations of treatment with CED of ACNU for malignant glioma.

CONCLUSION

Combined treatment using CED of hydrophilic nitrosourea with irradiation or systemic administration of temozolomide improved therapeutic outcome in a brain tumor xenograft model. The results of our study suggest that multimodal approach with local chemotherapy via CED, radiotherapy and systemic chemotherapy is a promising strategy for treatment of brain tumors.

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Prognostic significance of surgery and radiation therapy in cases of anaplastic astrocytoma: retrospective analysis of 170 cases

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Object. The purpose of this retrospective study was to estimate the prognostic impact of treatment parameters for 170 patients with anaplastic astrocytoma (AA).

Methods. Survival outcome and prognostic factors were analyzed for 170 patients with AA. In the multivariate analysis, site of lesion (frontal or parietal lobe, $p = 0.002$), extent of surgery (total or subtotal resection, $p = 0.001$), Karnofsky Performance Scale status (0–2, $p = 0.021$), age (≤ 50 years, $p = 0.024$), and total dose of radiation therapy (> 60 Gy, $p = 0.029$) were significant favorable prognostic factors.

In the analysis of groups according to extent of surgery, patients who underwent total or subtotal resection had a significantly more favorable prognosis than did patients who underwent partial resection or biopsy (5-year survival rate 54.0% for total or subtotal resection compared with 17.5% for partial resection or biopsy; median survival time [MST] 62.6 months compared with 22.9 months [$p < 0.0001$, log-rank test]; hazard ratio [HR] 0.67; and 95% confidence interval [CI] 0.52–0.85 [$p = 0.001$]).

In the analysis of groups according to total radiation dose, the group of patients who received doses greater than 60 Gy had a significantly more favorable prognosis than did the group who received 60 Gy or less (5-year survival rate 45.0% for patients who received doses greater than 60 Gy compared with 21.1% for those receiving 60 Gy or less; MST 48.9 months compared with 21.6 months [$p = 0.0006$, log-rank test]; HR 0.96; 95% CI 0.93–0.99 [$p = 0.029$]).

Conclusions. The most important parameter in the treatment of AA was extent of surgery, and total radiation dose was the second most important factor. Resection of as much of the tumor as possible and delivery of a total radiation dose of greater than 60 Gy seem to be required for local control of AA.

KEY WORDS • **anaplastic astrocytoma** • **surgery** • **radiation therapy** • **survival analysis** • **multivariate analysis**

MALIGNANT gliomas and astrocytomas account for approximately 50% of primary CNS tumors in adults: glioblastomas multiforme account for approximately 30%, AAs for approximately 10%, and low-grade astrocytomas for approximately 10%. It has been reported that the median survival period of patients with AA is 10 to 40 months (~ 20–30 months on average).[6,16,20,22]

It has been reported as well that the major prognostic factors for patients with AA are age, KPS status, and extent of surgery. In some studies investigators have also shown that the presence of ringed contrast enhancement, score on the Ki 67 labeling index, total radiation dose, the presence of convulsion, microvascular density, and expression of vascular endothelial growth factor are prognostic factors.[1,2,5,6,8,16,20,22]

Despite the availability of combined multimodality treatment, AA has an unfavorable prognosis. Surgery and radiation therapy are essential for radical treatment of malignant astrocytoma, but an optimal treatment regimen has not yet been established. The purpose of this study was to evaluate the outcome of treatment in 170 patients with AA and to estimate the prognostic factors and contribution of each parameter of treatment by using multivariate analysis.

Clinical Material and Methods

Patient Population

Data were obtained in 170 consecutive patients with AA (109 men and 61 women; median age 44 years) who were treated between May 1981 and March 2002. The median follow-up period was 37.9 months (range 2.2–189 months). The characteristics of the patients in this study are shown in Table 1. All patients had intracranial primary tumor(s) for

Abbreviations used in this paper: AA = anaplastic astrocytoma; CI = confidence interval; CNS = central nervous system; CT = computed tomography; EBRT = external-beam radiation therapy; HR = hazard ratio; IORT = intraoperative radiation therapy; KPS = Karnofsky Performance Scale; MR = magnetic resonance; MST = median survival time; WBRT = whole-brain radiation therapy.

which the histopathological diagnosis was Grade III astrocytoma (Grade III oligoastrocytoma or other Grade III gliomas were excluded). The histopathological diagnosis was determined based on the findings of at least one pathologist and one neurosurgeon. Histopathological grading was determined on the basis of the latest World Health Organization classification. The final follow-up date was May 23, 2003. Astrocytomas that were initially treated as Grade III lesions and changed to Grade IV during the course of follow up were included in this study. Astrocytomas that were not Grade III at the time of initial treatment were excluded.

The KPS status was determined according to the criteria of the Eastern Cooperative Oncology Group before treatment.[12] This was accomplished using enhanced CT scans and/or T_2-weighted or enhanced T_1-weighted MR images. Preoperative and postoperative MR imaging was routinely performed after 1987. The size of the tumor was measured as accurately as possible by more than one radiologist and neurosurgeon. Because of the difficulty in measuring tumor volume in cases treated early in the study, the methods for assessing the size of the tumor were unified into measurement of its long axis.

Surgery and Chemotherapy

The extent of surgery was evaluated on postoperative MR images and/or CT scans obtained within 72 hours postsurgery. Comparing these with preoperative MR and/or CT images, gross-total resection of the tumor was defined as resection with no macroscopic residual tumor, subtotal resection was defined as more than 75% resection, and partial resection was defined as less than 75% resection. Biopsy (including open procedures and needle biopsy) that was performed only for the purpose of histological diagnosis was defined as less than 10% resection. Although there was uncertainty in evaluating the extent of surgery before the advent of MR imaging, this factor was assessed based on CT findings and the surgeon's judgment as far as possible. Surgery (or biopsy) was performed before radiation therapy and chemotherapy in almost all cases. Chemotherapy was routinely combined as much as possible. Patients who had renal dysfunction, hepatic dysfunction, or poor KPS status or who did not give consent for chemotherapy did not receive it. Nimustine hydrochloride (2–3 mg/kg weekly) was mainly used in adjuvant chemotherapy.

Radiation Therapy

All patients were treated with 4- to 10-MV photons (almost all of them with 10-MV photons) by using a linear accelerator, and all were immobilized in a resinous shell during the treatment. The standard dose of WBRT was 30 Gy in 15 fractions (plus local boost irradiation of 30 Gy in 15 fractions). The WBRT field included the cerebrum, cerebellum, and brainstem (above the posterior cranial fossa), and parallel-opposed lateral fields were used. The extended local irradiation field included the whole T_2-weighted high-intensity region visualized on MR images or a 2- to 3-cm margin around the tumor, and usually more than two fields were used. The fractionation regimen was as follows: 1) conventional fractionation (2 Gy/day with a total of 10 Gy delivered in five fractions within 1 week); 2) uneven fractionation (a combination of high-dose and low-dose fractions [4–5 Gy on Day 1 and 1–1.5 Gy/day between Days 2–5] with a total of 7–9 Gy delivered in three–five fractions/week); and 3) hyperfractionation (1.2 Gy/fraction, two fractions/day for a total of 12 Gy delivered in 10 fractions within 1 week). The conventional fractionation regimen was mainly used from 1981 through 1985, the uneven fractionation regimen from 1986 through 1995, and the hyperfractionation regimen from 1995 through 2003. A single dose of 15 Gy delivered using 8- to 10-MeV electrons was used for IORT.

TABLE 1

*Characteristics in 170 patients with AA who underwent surgery and radiation therapy**

Characteristic	No. of Patients
sex	
male	109
female	61
histological findings of tumor: AA (Grade III)†	170
site of lesion	
frontal lobe	74
parietal lobe	17
temporal lobe	36
occipital lobe	5
basal ganglia or thalamus	13
cerebellum	8
brainstem	3
other	14
extent of resection	
total	56
subtotal	20
partial	43
biopsy only	51
chemo	
nimustine hydrochloride	117
none	53
fractionation regimen	
conventional	49
uneven	76
HF	45
completion of tx	
completed	161
suspended	9

* Chemo = chemotherapy; HF = hyperfractionation; tx = treatment.
† According to the World Health Organization revised classification.

Treatment-Related Toxicity

Toxicity was clinically diagnosed on the basis of common toxicity criteria (version 2.0). Patients in whom radiographically confirmed changes without recurrence were demonstrated (such as ≥ Grade 3 leukoencephalopathy-associated radiological findings, or brain necrosis that was diagnosed on the basis of changes seen on follow-up CT or MR images, clinical examination, and/or histopathological findings) were classified as suffering treatment-related toxicity.

Statistical Analysis

Survival time was calculated from the date of first treatment to the date of death. Progression-free survival was calculated from the date of first treatment to the date of first progression (local recurrence or distant metastasis). Survival curves were analyzed using the Kaplan–Meier method and the log-rank test. The Cox proportional hazards model

and a stepwise method were used for multivariate analysis of prognostic factors. The patient's sex, age, KPS status, site of lesion, tumor size, treatment with or without chemotherapy, extent of surgery, treatment with or without IORT, total dose of radiation therapy, period of EBRT, irradiation field (combination of WBRT or extended local irradiation alone), fractionation regimen of radiation therapy, and period of treatment were used as continuous or discrete variables in multivariate analysis (Table 2). Analyses of prognostic factors were performed for all patients whose tumor diagnosis was Grade III glioma. Statistical relationships between significant prognostic factors were analyzed using the chi-square test.

Results

Data from patients with AA treated between May 1981 and March 2002 at our institution were analyzed. At least two cases of Grade III astrocytoma (1.1%) were histologically proven to be Grade IV astrocytoma at the time of recurrence. One hundred sixty-one patients completed the course of treatment, but nine did not because of deterioration of their general condition due to uncontrolled tumor growth. At the final follow-up date, 110 patients (64.7%) were dead and 60 (35.3%) were alive. Of the patients who had died, 102 (92.7%) died of primary disease and eight (7.3%) died of intercurrent disease.

Survival analysis of the 170 patients showed that the MST was 33.6 months and that the 2- and 5-year survival rates were 57.1 and 33.8%, respectively (Fig. 1). Two-year and 5-year progression-free survival rates were 49.3 and 30.2%, respectively.

Table 3 shows the results of univariate and multivariate analyses of prognostic factors for the 170 patients. In the multivariate analysis, site of the lesion ($p = 0.002$), extent of surgery ($p = 0.001$), KPS status ($p = 0.021$), age ($p = 0.024$), and total dose of radiation therapy ($p = 0.029$) were significant prognostic factors. Patient sex, tumor size, treatment with or without chemotherapy, treatment with or without IORT, period of EBRT, irradiation field, fractionation regimen of radiation therapy, and period of treatment were not statistically significant in multivariate analysis.

The patients in whom primary lesions were located in the frontal or parietal lobe had a significantly more favorable prognosis than did the patients who had primary lesions in other sites (MST 48.9 months compared with 22.6 months [$p < 0.0001$, log-rank test]). The patients who had a good KPS score before treatment had a significantly more favorable prognosis than did those in whom a poor KPS score was noted (MST 41 months compared with 10 months [$p < 0.0001$, log-rank test]). The patients who were 50 years of age or younger had a significantly more favorable prognosis than did the patients who were older than 50 years of age (MST 44.9 months compared with 19.0 months [$p < 0.0001$, log-rank test]).

Figure 2 shows survival curves according to extent of surgery. The patients who underwent total or subtotal resection of the tumor showed a significantly more favorable prognosis than did those who underwent partial resection or biopsy only (5-year survival rate 54.0% compared with 17.5%; MST 62.6 months compared with 22.9 months [$p < 0.0001$, log-rank test]; HR 0.67; 95% CI 0.52–0.85 [$p = 0.001$]). In the survival analysis based on fine distinctions, the MSTs of patients in the total resection, subtotal resection, partial resection, and biopsy-only groups were 86.4, 61.6, 22.9, and 23.4 months, respectively ($p < 0.0001$, log-rank test [data not shown]). The prognosis of

TABLE 2

*Parameters of variables in univariate and multivariate analyses in 170 patients with AA**

Variable	Value
sex (D)	
male	109
female	61
mean age in yrs (C)	43.1 ± 17.5
KPS status (D)	
0–2	136
3–4	34
site of lesion (D)	
frontal or parietal lobe	91
other	79
mean tumor size in cm (C)	5.1 ± 2.0
tx w/ chemo (D)	
yes	117
no	53
extent of resection (D)	
total or subtotal	76
partial or biopsy only	94
tx w/ IORT (D)	
yes	28
no	142
irradiation field (D)	
combination of WBRT	39
extended local	131
fractionation regimen (D)	
conventional	49
uneven	76
HF	45
mean total dose in Gy (C)	63.0 ± 11.0
mean period of EBRT in days (C)	49.9 ± 14.1
period of tx (D)	
1981–1992	85
1992–2002	85

* Means are expressed ± standard deviations. Abbreviations: (C) = continuous variable; (D) = discrete variable.

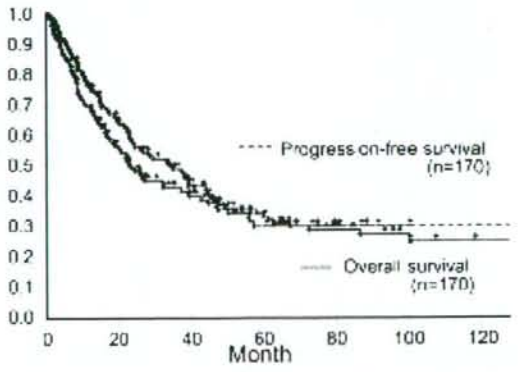

FIG. 1. Graph showing overall and progression-free survival in 170 patients with AA. In this and subsequent graphs, values on the y axis denote the percentage of survival.

TABLE 3

*Results of univariate and multivariate analyses of data in 170 patients with AA**

Variable	Univariate Analysis			Multivariate Analysis		
	HR [Expβ]	χ^2	p Value	HR [Expβ]	χ^2	p Value
sex (D)	0.921	0.671	0.413	NS	NS	NS
age (C)	0.980	11.43	<0.001	0.986	5.078	0.024
KPS status (D)	0.596	19.73	<0.001	0.748	5.314	0.021
site (D)	0.672	16.74	<0.001	0.717	9.873	0.002
tumor size (C)	0.930	2.397	0.122	NS	NS	NS
chemo (D)	0.849	2.553	0.110	NS	NS	NS
extent of op (D)	0.591	27.73	<0.001	0.665	10.71	0.001
IORT (D)	0.839	2.066	0.151	NS	NS	NS
irradiation field (D)	0.723	8.922	0.003	NS	NS	NS
fractionation regimen (D)	0.600	9.922	0.007	NS	NS	NS
total dose (C)	0.962	14.56	<0.001	0.960	4.763	0.029
period of EBRT (C)	0.994	0.531	0.466	NS	NS	NS
period of tx (D)	0.576	30.80	<0.001	NS	NS	NS

* Exp = exponential; NS = not significant.

patients in the total resection group was the most favorable, and significantly so, whereas the prognosis of those in the subtotal resection group was slightly worse, although the difference was not significant (p = 0.55, log-rank test). Nevertheless, the prognosis of the patients in the subtotal resection group was significantly more favorable than for those in the partial resection and biopsy-only groups (p = 0.007, log-rank test).

Figure 3 shows survival curves according to the total dose of radiation therapy. The group treated with a high dose (> 60 Gy, 91 patients) showed a significantly more favorable prognosis than did the one treated with a low dose (≤ 60 Gy, 79 patients) (5-year survival rate in the high-dose group was 45.0% compared with 21.1% in the low-dose group; MST was 48.9 months compared with 21.6 months ([p = 0.0006, log-rank test]; HR 0.96; 95% CI 0.93–0.99 [p = 0.029]).

Figure 4 shows survival curves according to extent of surgery (≥ subtotal resection or ≤ partial resection) and total radiation dose (> 60 Gy or ≤ 60 Gy). Patients were divided into three categories (Group A, those who underwent ≥ subtotal resection and received a > 60-Gy radiation dose; Group B, those who underwent ≤ partial resection and received a > 60-Gy radiation dose, or ≥ subtotal resection and ≤ 60 Gy; and Group C, those who underwent ≤ partial resection and received a ≤ 60-Gy radiation dose). The prognosis of the patients in Group A was significantly more favorable than that of patients in Group B or C (5-year survival rates: Group A, 66.2%; Group B, 25.7%; and Group C, 14.8% [p < 0.0001, log-rank test]; HR 0.40, 95% CI 0.28–0.56 for Group A, and HR 1.27, 95% CI 0.98–1.67 for group B [p < 0.0001]). The groups in which either surgery or radiation dose was insufficient showed significantly poor prognosis.

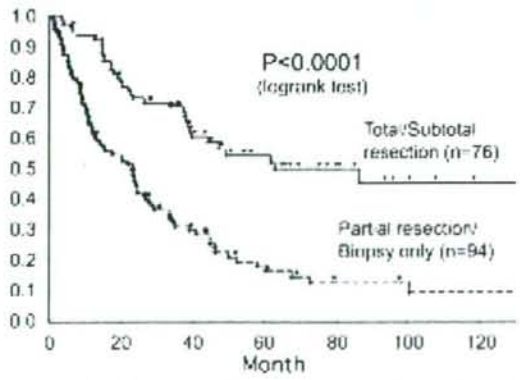

FIG. 2. Survival curves plotted according to extent of surgery (total or subtotal resection compared with partial resection or biopsy only) in 170 patients with AA.

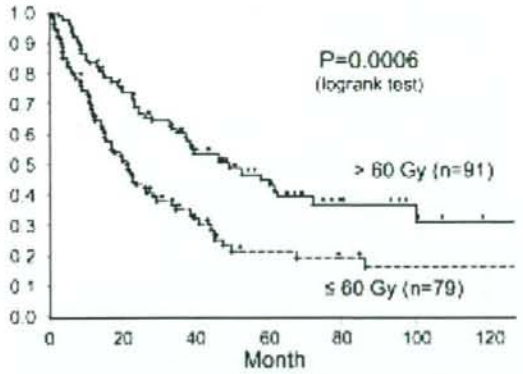

FIG. 3. Survival curves plotted according to total radiation dose (> 60 Gy or ≤ 60 Gy) in 170 patients with AA.

Figure 5 shows survival curves according to total radiation dose in the 56 patients who underwent gross-total resection of tumor. The patients treated with a high dose (> 60 Gy, 33 patients) showed a significantly more favorable prognosis than the ones treated with a low dose (≤ 60 Gy, 23 patients). The 5-year survival rate in the high-dose group was 65.6%, compared with 38.3% in the low-dose group ([p = 0.04, log-rank test]; HR 0.67; 95% CI 0.44–1.00 [p = 0.05]).

Treatment-associated brain necrosis was seen in 10 (5.9%) of the 170 patients (Table 4). The median period before brain necrosis occurred was 28.3 months (range 8.2–76.2 months). The mean total radiation dose for patients with brain necrosis was 70.6 ± 12.9 Gy, and that for patients without brain necrosis was 62.5 ± 10.7 Gy (mean ± standard deviation, p = 0.02). The total radiation dose was significantly higher in patients in whom brain necrosis was noted. In the group of patients who underwent chemotherapy, five (4%) of 117 had brain necrosis, and in the group of patients who underwent IORT, three (11%) of 28 had brain necrosis. No significant relationships were found between brain necrosis, use of IORT, and use of chemotherapy. Four of the 10 patients with brain necrosis died; however, as shown in the table, no patient died of radiation-induced brain necrosis. Most of the patients with brain necrosis showed few neurological symptoms.

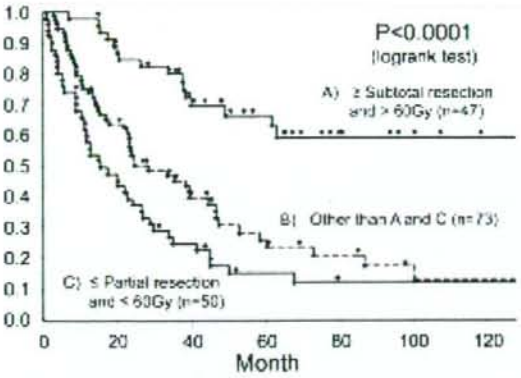

FIG. 4. Survival curves plotted according to extent of surgery and total radiation dose (Group A, ≥ subtotal resection and > 60 Gy [47 patients]; Group B, ≥ subtotal resection and ≤ 60 Gy, or ≤ partial resection and > 60 Gy [73 patients]; and Group C, ≤ partial resection and ≤ 60 Gy [50 patients]).

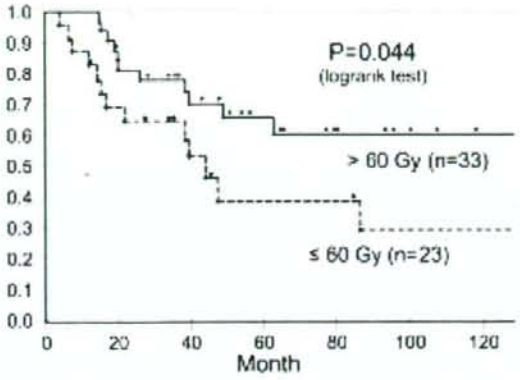

FIG. 5. Survival curves plotted according to total radiation dose (> 60 Gy or ≤ 60 Gy), limited to the patients who underwent gross-total tumor removal (56 patients).

Discussion

Significant prognostic factors for patients with AA in multivariate analysis were as follows: site of the lesion, extent of surgery, KPS status, patient's age, and total dose of radiation therapy. According to these results, factors on the patient's side, such as site, KPS, and age, account for the majority of prognostic factors. As shown in previous studies, these factors have a great impact on the prognosis of patients with high-grade glioma.[2,6,8,11,16,20,22]

Extent of surgery was the most powerful prognostic factor in the treatment parameters, and total dose of radiation therapy was the second most important prognostic factor. Several authors have reported that extent of surgery has a strong correlation with the prognosis of patients, and the results of our study are compatible with those of the other studies.[11,16] Curran et al.[3] suggested that resection did not have a great impact on prognosis in patients with glioblastoma multiforme located in the supratentorial area compared with the impact of radiation alone. However, in this study of AAs located in the supratentorial area (132 tumors), the prognosis of patients who underwent total or subtotal resection was significantly more favorable than that of patients who underwent partial resection or biopsy only (MST 86.4 compared with 24.7 months, $p < 0.0001$). Although there are various opinions about the significance of surgery,[1,6,20] the results of the aforementioned study imply that surgery is an essential modality and that the extent of resection has a great impact on prognosis in patients with AA.

It is known that malignant glioma is a radioresistant tumor both in vitro and in vivo, and it is difficult to control the lesion by using irradiation alone.[14,16,19] The results of our study also support the suggestion that resection of as much of the tumor as possible is a precondition for a favorable prognosis. According to our results, total radiation dose was the second most significant prognostic factor in the parameters of treatment. This finding has no relation to the presence of postoperative residual tumor: patients in the higher total dose group, even those who underwent total or subtotal resection, showed a significantly more favorable prognosis (80 patients, 5-year survival rates 63.1% compared with 31.7% [p = 0.003, log-rank test; data not shown]). Also, as shown in Fig. 5, a radiation dose of more

TABLE 4

*Characteristics in 10 patients with treatment-associated brain necrosis**

Case No.	Fractionation Regimen	Dose of EBRT (Gy)	IORT	Chemo	Extent of Resection	Site of Lesion	Time to Brain Necrosis (mos)	Status at Final FU
1	uneven	58	no	no	biopsy	multiple	8.2	disease-specific death
2	uneven	54	no	yes	biopsy	frontal	9.6	disease-specific death
3	uneven	61.5	no	no	total	frontal	23.4	alive
4	uneven	63	no	yes	total	frontal	55.5	alive
5	uneven	63	no	no	partial	frontal	33.2	alive
6	HF	72	no	no	total	frontal	12.8	alive
7	HF	72	yes	no	total	frontal	76.2	alive
8	HF	72	no	yes	subtotal	frontal	35.4	alive
9	HF	72	yes	yes	subtotal	frontal	11.6	dead of intercurrent disease
10	HF	72	yes	yes	partial	temporal	34.7	disease-specific death

* biopsy = biopsy alone; FU = follow up; total = gross-total resection.

than 60 Gy significantly contributes to improvement in the patients' prognosis, even in cases in which gross-total resection of the tumor was successfully performed. This result supports the suggestion that postoperative radiation therapy at an insufficient dose leads to local recurrence, even if the primary lesion has been totally resected, and that combined radiation therapy is essential for treatment of AA.

Malignant glioma often spreads microscopically beyond the macroscopic margin visualized on MR or CT images.[7,9] It is therefore thought to be difficult to resect microscopic malignant cells invading peripheral tissue around the tumor. On the other hand, malignant glioma is radioresistant,[14,16] and it is therefore difficult to eliminate macroscopic tumor by using radiotherapy alone. It seems that the role of surgery is removal of the bulk of the tumor, and that the role of radiation therapy is elimination of residual microscopic malignant cells. Surgery and radiation therapy have distinct roles, and both modalities seem to be essential for the treatment of Grade III glioma. Results of survival analysis taking into account the combination of these modalities seem to support this opinion (Fig. 4).

Miller et al.[8] reported that total radiation dose was one of the significant prognostic factors. The results of our study are consistent with their results in that total radiation dose had an impact on the prognosis of patients with malignant glioma. However, these authors reported that no additional benefit could be obtained for doses greater than 60 Gy, whereas the results of our study are quite different in this point. This difference may be due to the number of patients in the Miller study (16 with AA and 66 with glioblastoma multiforme) who received radiation therapy at that time. In the present study, which includes a much larger cohort (170 patients with only AAs), we suggest that postoperative radiation therapy at a dose of more than 60 Gy is required for local control, and this speculation seems to be valid from the viewpoint of biological and clinical radiosensitivity of malignant glioma cells.[14,19]

Use of chemotherapy was not a significant prognostic factor in this study, either in univariate or multivariate analysis, and its use was not significantly related to brain necrosis. The role of chemotherapy in high-grade glioma is controversial. Nevertheless, several investigators have reported a small but significant benefit for survival in patients with high-grade glioma.[4,21] Although the impact of chemotherapy is not superior to that of surgery and radiotherapy, further investigation of optimal timing, dose intensity, and the optimal drug combination is required. Use of IORT was also not a significant prognostic factor in multivariate analysis, and the significance of IORT for treatment of high-grade glioma is controversial. Several investigators have suggested that there is no significant benefit of IORT,[10] whereas others have suggested that IORT is effective for patients with high-grade glioma, but the survival benefit has not been clarified.[13,15] The prognostic significance of IORT in patients with Grade III astrocytoma was not shown in this study.

One of the problems is occurrence of toxicity, such as brain necrosis. Five of the 10 patients with brain necrosis in our study underwent uneven fractionation of radiotherapy. Brain necrosis occurred in those five despite relatively low doses of total radiation without IORT. A large single-fraction size (4–5 Gy/fraction) was used in this regimen. It is known that irradiation with a large single-fraction size is one of the risk factors of late toxicity, especially in the CNS.[23] Care should be taken when using a large single-fraction size for treatment of CNS tumors. Hyperfractionated radiation therapy uses a small single-fraction size (1.2 Gy/fraction); nevertheless, the standard dose is relatively high. Therefore, the use of IORT should be determined with caution. In our study, the significance of the fractionation regimen of radiotherapy combined with the use of IORT has not been shown in the multivariate analysis of patient survival. However, a large fraction size for EBRT or IORT seemed to be one of the causes of late toxicity.

The difference in influence of fraction size between malignant glioma and healthy CNS tissue may come from the difference between alpha/beta values in malignant tissue and those in healthy tissue from the viewpoint of radiation biology. It is reported that the alpha/beta value of malignant glioma is high (usually $\geq$ 10 Gy), whereas the alpha/beta value of healthy CNS tissue is low (usually $<$ 5 Gy).[17,18,24] This difference between alpha/beta values in tumor and healthy tissue means that healthy CNS tissue is more sensitive to an increase in the fraction size of irradiation. Based on these results, it seems that to avoid late toxicity a large fraction size of EBRT or IORT should not be routinely used for treatment of CNS tumors. However, as shown in Table 4, there was no treatment-related death in patients in our study. It is therefore thought that irradiation of more than 60 Gy will not lead to dangerous and intolerable toxicity. As shown by survival curves in Fig. 3, the survival benefit of irradiation with more than 60 Gy seems to exceed the risk of radiation-induced toxicity.

Conclusions

The results of this retrospective study showed significantly better survival in patients who underwent surgery and radiation therapy for treatment of AA. As complete a tumor resection as possible and combining surgery with high-dose irradiation seem to improve the prognosis of patients with these lesions. The MST of patients with AA has been extended by approximately one and a half times in recent decades, but the optimum dose, field of radiation therapy, fractionation regimen, and regimen of chemotherapy have not yet been established. Further investigation of treatment parameters is required for improvement of treatment protocols for AA.

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LABORATORY INVESTIGATION

Safety and efficacy of convection-enhanced delivery of ACNU, a hydrophilic nitrosourea, in intracranial brain tumor models

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Abstract Convection-enhanced delivery (CED) is a local infusion technique, which delivers chemotherapeutic agents directly to the central nervous system, circumventing the blood–brain barrier and reducing systemic side effects. CED distribution is significantly increased if the infusate is hydrophilic. This study evaluated the safety and efficacy of CED of nimustine hydrochloride: 3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride (ACNU), a hydrophilic nitrosourea, in rat 9 L brain tumor models. The local neurotoxicity of ACNU delivered via CED was examined in normal rat brains, and the maximum tolerated dose (MTD) was estimated at 0.02 mg/rat. CED of ACNU at the MTD produced significantly longer survival time than systemic administration ($P < 0.05$, log-rank test). Long-term survival (80 days) and eradication of the tumor occurred only in the CED-treated rats. The tissue concentration of ACNU was measured by high-performance liquid chromatography, which revealed that CED of ACNU at the dose of 100-fold less total drug than intravenous injection carried almost equivalent concentrations of ACNU into rat brain tissue. CED of hydrophilic ACNU is a promising strategy for treating brain tumors.

Keywords Brain tumor · Convection-enhanced delivery · High-performance liquid chromatography · Nimustine hydrochloride · Nitrosourea

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Abbreviations

ACNU	3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride
BBB	Blood-brain barrier
BCNU	1,3-bis-chlorethyl-1-nitrosourea
CED	Convection-enhanced delivery
CNS	Central nervous system
HBSS	Hanks balanced salt solution
H&E	Hematoxylin and eosin
i.v.	Intravenous
MTD	Maximum tolerated dose

Introduction

Prognosis for the patients with high-grade gliomas remains dismal. Recently, Stupp et al. [1] demonstrated that radiotherapy plus concomitant and adjuvant temozolomide, a novel oral alkylating agent, is well tolerated and improves survival in patients with newly diagnosed glioblastoma. However, the activity of temozolomide is still not satisfactory in malignant gliomas. Poor penetration of most anti-cancer drugs across the blood–brain barrier (BBB) into the central nervous system (CNS) remains a major obstacle in the application of systemic chemotherapy for intracranial malignancies [2, 3]. Even using agents that penetrate the BBB, tumoricidal drug concentrations are difficult to reach brain tumor tissue without incurring unacceptable systemic side effects.

Convection-enhanced delivery (CED) was introduced in 1994 as a strategy to overcome such difficulties

[4]. Utilizing bulk flow, CED allows the direct delivery of small or large molecules to a targeted site, offering an improved volume of distribution compared to simple diffusion. CED bypasses the BBB, delivers a high concentration of therapeutic agents to the injection site, provides wider distribution of therapeutic agents within the target site, and minimizes systemic exposure, resulting in fewer systemic side effects. In addition, CED provides homogeneous distribution of infusate, which drop off sharply at the edge in normal brain tissue, resulting in delivery of the therapeutic agent to the entire targeted region while limiting the potential for widespread neurotoxicity [5].

Nitrosoureas have been important in systemic chemotherapy for high-grade gliomas for decades. 1,3-bischlorethyl-1-nitrosourea (BCNU) had the most proven efficacy, but the effects on clinical outcome have been limited [6]. Dose escalation of BCNU to increase the efficacy against gliomas has been hampered by severe systemic toxicity to the bone marrow, lungs, and kidneys [7]. To avoid such systemic toxicities, local delivery methods, including direct injection and biodegradable polymers or wafers, have been used, but only offered modest improvements to the overall survival rates for patients with malignant gliomas [8–13]. Those delivery methods yielded limited diffusion and distribution of drug into the surrounding tissues, which is typically not more than a few millimeters [13].

Convection-enhanced delivery has the potential to deliver an efficient volume of BCNU to targeted sites without systemic exposure. BCNU could be safely and effectively administered via CED in the rat glioma model to shrink gliomas with little or no toxicity [14]. However, BCNU is not the ideal drug for CED because the log p of BCNU is 1.53, which means that BCNU is lipophilic [15] (log p is the log of the octanol/water partition coefficient [16]). For CED injection, it needs to dissolve in organic solvent like ethanol that has nonspecific cytotoxity in itself. Furthermore, the water solubility of drugs limits the volume of distribution within the brain tissue and CED distributed lipophilic drugs less widely than hydrophilic agents [11, 12].

3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(2-chloroethyl)-1-nitorosourea hydrochloride (ACNU), is the first water-soluble nitrosourea compound discovered in 1974 [17]. ACNU dissolves in water easily as a cationic ion. The log p of ACNU is 0.92 [17], which means that ACNU is lipophilic as well as hydrophilic, because ACNU changes from cationic ion to neutral compound under physiological conditions. In clinical protocols against high-grade gliomas, systemic administration of ACNU has proven efficacy but also dose-limiting myelotoxicity like BCNU [18, 19].

We hypothesized that CED of ACNU would be therapeutically advantageous over systemic administration for treating intracranial malignancies, because CED could distribute hydrophilic ACNU over the entire targeted region and deliver a high concentration of ACNU without systemic exposure. This study examined the safety and efficacy of CED with ACNU in rat 9 L brain tumor models.

Materials and methods

ACNU

ACNU was provided by Sankyo Co. Ltd. (Tokyo, Japan). Infusion solutions of ACNU were prepared by diluting ACNU in saline to a concentration of 10, 5, 2, 1, 0.5, 0.2, and 0.1 mg/mL.

Tumor cell line

The 9 L gliosarcoma cells (American Type Culture Collection, Rockville, MD, USA) were maintained as monolayers in a complete medium consisting of Eagle's minimal essential medium supplemented with 10% fetal calf serum, non-essential amino acids, and 100 U/mL penicillin G. Cells were cultured at 37°C in a humidified atmosphere consisting of 95% air and 5% CO_2.

Animals and intracranial xenograft technique

All protocols used in the animal studies were approved by the Institute for Animal Experimentation of Tohoku University Graduate School of Medicine.

Male Fisher 344 rats weighing approximately 200 g were purchased from Charles-River Laboratories (Charles-River Japan Inc., Tsukuba, Japan). For the intracranial xenograft tumor model, 9 L gliosarcoma cells were harvested by trypsinization, washed once with Hanks balanced salt solution without Ca^{++} and Mg^{++} (HBSS), and resuspended in HBSS for implantation. Cells (5×10^5) in 10 μL HBSS were implanted into the striatal region of Fisher 344 rat brains as follows: under deep isoflurane anesthesia, rats were placed in a small-animal stereotactic frame (David Kopf Instrument, Tujunga, CA, USA). A sagittal incision was made to expose the cranium followed by a burr hole in the skull at 0.5 mm anterior and 3 mm lateral from the bregma using a small dental drill. Cell suspension (5 μL) was injected over 2 min at a depth of 4.5 mm from the brain surface; after a 2-minute wait, another 5 μL were injected over 2 min at a depth of

4.0 mm, and after a final 2-minute wait, the needle was removed and the wound was sutured.

CED

Convection-enhanced delivery of ACNU or saline was done using a volume of 20 μL as described previously [20]. Briefly, the infusion system consisted of a reflux-free step-design infusion cannula (as described [21]) connected to a loading line (containing ACNU or saline) and an olive oil infusion line. A 1-mL syringe (filled with oil) mounted onto a micro-infusion pump (BeeHive; Bioanalytical Systems, West Lafayette, IN, USA) regulated the flow of fluid through the system. Based on chosen coordinates, the infusion cannula was mounted onto stereotactic holders and guided to the target region of the brain through burr holes made in the skull. The following ascending infusion rates were applied to achieve the 20-μL total infusion volume: 0.2 μL/min (15 min) + 0.5 μL/min (10 min) + 0.8 μL/min (15 min).

Evaluation of toxicity

Healthy male Sprague–Dawley rats weighing approximately 200 g (Charles-River Japan Inc.) received a single 20-μL CED infusion of ACNU at doses of 0.2, 0.1, 0.04, 0.02, 0.01, 0.004, or 0.002 mg/rat (six per group). Rats were monitored daily for survival, weekly weights, and general health (alertness, grooming, feeding, excreta, skin, fur, mucous membrane conditions, ambulation, breathing, and posture). Three rats in each group were euthanized on the 30th or the 60th day after the CED treatment, and their brains were removed, fixed, subjected to paraffin sectioning (5 μm), and stained with hematoxylin and eosin (H&E).

Survival studies

Forty rats with 9 L tumor cells were randomly assigned to five groups: (a) the control group, receiving CED of saline ($n = 8$); (b) the systemic treatment group, receiving intravenous (i.v.) injection of ACNU at a dose of 0.4 mg/rat (2 mg/kg: clinically tolerable dose for i.v. administration [17]) ($n = 8$); and (c)–(e) CED groups, receiving CED of ACNU at a dose of 0.005 mg/rat ($n = 8$), 0.01 mg/rat ($n = 8$), and 0.02 mg/rat ($n = 8$). Seven days after tumor cell implantation, a single CED infusion (20 μL; 1 mg/mL or 0.5 mg/mL ACNU) or a bolus i.v. injection via a tail vein (0.4 mL; 0.1 mg/mL ACNU) was performed for each group. Rats were monitored daily for survival and general health. Animal weights were reported weekly. The study was terminated 80 days after tumor implantation, when the surviving animals were euthanized and their brains stained with H&E.

Results for the survival studies are expressed as a Kaplan–Meier curve. Survival between the treatment groups was compared with a log-rank test.

High-performance liquid chromatography for ACNU in rat brain tissue

Normal Sprague–Dawley rats weighing approximately 200 g (Charles-River Japan Inc.) were given a single 20-μL infusion by CED of ACNU at 0.02 mg/rat or a bolus i.v. injection of ACNU at 2.0 mg/rat or 0.4 mg/rat (nine rats per group). Three rats were sacrificed at 0, 2, or 4 h after the treatments. The appropriate brain hemisphere was perfused with phosphate buffered saline, surgically removed, and frozen. All samples were stored at -80°C to avoid deterioration until biochemical measurements were carried out (within a month of brain dissection). Phosphoric acid buffer (0.1 mol/L) was added to the tissues at an 80% ratio (v/w), and the tissue was homogenized using a mechanical homogenizer. Fluoranthene (0.8 μg, internal standard) and *n*-hexane (5 mL) was added to the homogenates (0.5 mL). The mixture was shaken for 5 min and centrifuged at 3,000 rpm for 5 min, then the *n*-hexane layer was extracted and evaporated. The remnant was dissolved in 6% acetonitrile (200 μL) and injected into the chromatographic column (4.6 × 150 mm^2; Nova-Pack C18; Waters, Milford, MA, USA). Analysis was conducted on LC-10A system (Shimadzu Co., Kyoto, Japan). The mobile phase consisted of 6% acetonitrile, refined water, and 1 g/L sodium heptanesulphonate (PIC B7) (77 : 23 : 0.4). All separations were performed isocratically at a flow rate of 1.0 mL/min at room temperature. ACNU was typically eluted in 3 min, and detected by ultraviolet at 254 nm.

Results

Toxicity of ACNU in normal rodent CNS

Dose-limiting local toxicity occurred at 0.04 mg/rat or over, establishing the maximum tolerated dose (MTD) at 0.02 mg/rat (Fig. 1). All animals that received CED of ACNU at 0.04 mg/rat or over had extensive tissue necrosis within the CNS (Fig. 1a). Animals receiving CED of ACNU at 0.02 mg/rat or under showed evidence of minor trauma at the site of the infusion cannula in the striatum but otherwise no apparent tissue toxicity (Fig. 1b, c).

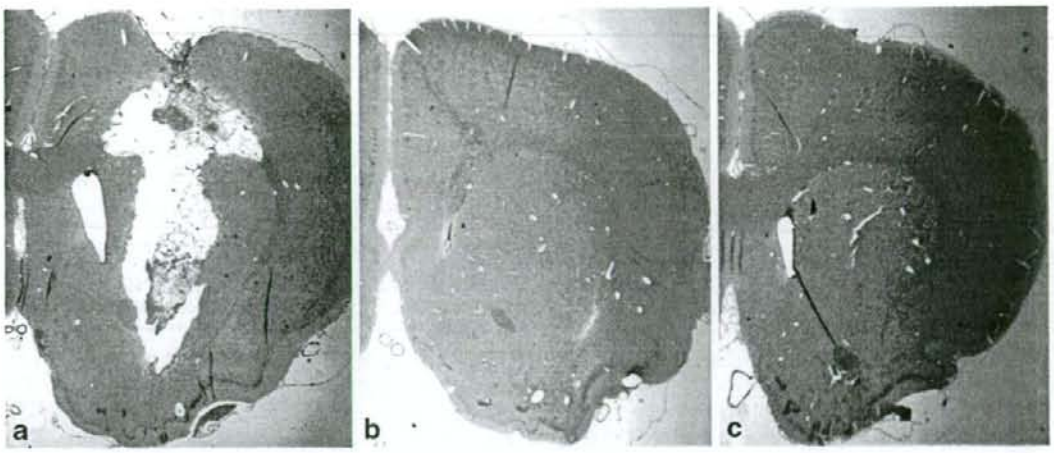

Fig. 1 Local tissue toxicity of ACNU administered via CED in the normal adult rat brain. Rat brains were treated with a single CED infusion of ACNU at different seven doses (0.2, 0.1, 0.04, 0.02, 0.01, 0.004, or 0.002 mg/rat). Representative H&E sections from three groups on the 30th day after CED. Extensive tissue injury was observed in animals treated with more than 0.04 mg/rat (**a**: 0.1 mg/rat). Rats treated with less than 0.02 mg showed no drug-induced damages (**b**: 0.02 mg/rat, **c**: 0.01 mg/rat)

No systemic toxicities were observed following CED of ACNU even at or over MTD. Furthermore, even the extensive CNS damage caused by ACNU resulted in no neurological symptoms.

Anti-tumor efficacy of ACNU through CED or intravenous administration

The anti-tumor efficacy of ACNU delivered via CED at the tested MTD (0.02 mg/rat) and half MTD (0.01 mg/rat) was compared with that of ACNU administered systemically at 0.4 mg/rat in the intracranial 9 L tumor model. The control group received CED infusion of saline.

As shown in Fig. 2, all animals in the control group expired due to tumor progression by day 21 and mean survival was only 16.5 days (median, 16.5 days). Systemic treatment with ACNU showed no improvement in survival. All animals expired by day 33 and mean survival was 19 days (median, 17 days). Animals treated with CED of ACNU at the dose of 0.005 mg/rat also expired by day 29 and mean survival was 18.2 days (median, 16.5 days). There was no significant advantage compared with the control group. Animals treated with CED of ACNU at the dose of 0.01 mg/rat expired by day 49 and mean survival was 26.5 days (median, 19.5 days). Although this CED treatment group showed a slight improvement in survival, there was no significant advantage compared with the group receiving i.v. administration of ACNU. Animals treated with CED of ACNU at the MTD of 0.02 mg/rat showed significantly improved survival rate compared with i.v. administration of ACNU ($p < 0.05$, log-rank test); treatment at the 0.02 mg/rat resulted in two of eight animals (25%) surviving beyond day 80 (median, 26.5 days).

Histopathologic evaluation of brain tissue was done in all animals at death or after sacrifice. Animals showing clinical signs of tumor progression were euthanized. Two animals survived to the study end at day 80, in the group receiving CED infusion of ACNU at the MTD (0.02 mg/rat), and showed complete pathologic responses (Fig. 3a). Tumor progression was observed in the brains of all rats, which died (Fig. 3b).

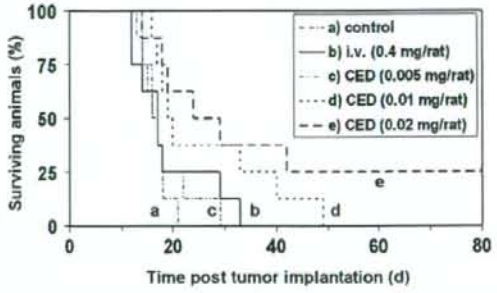

Fig. 2 Treatment of rats bearing 9 L tumors with CED or i.v. administration of ACNU. Seven days after tumor implantation within the brain, rats were treated with CED of saline (**a**), i.v. administration of ACNU at 0.4 mg/rat (**b**), and CED of ACNU at 0.005 mg/rat (**c**), 0.01 mg/rat (**d**), and 0.02 mg/rat (**e**). Eight animals per group

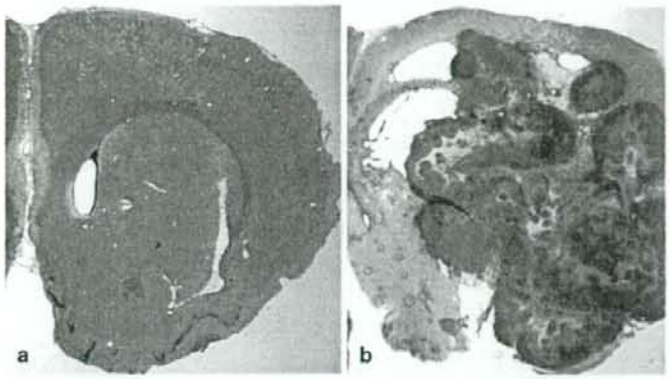

Fig. 3 Representative brain sections from surviving and non-surviving animals. (**a**) Brain section obtained from one of the survivors treated by CED of ACNU at 0.02 mg/rat. Neither survivor had residual tumor. (**b**) Brain section from a rat of the control group showing a typical tumor found in all non-surviving animals in which tumor progression led to death

Tissue concentration of ACNU following CED or intravenous administration

The mean tissue concentrations just after the treatment with CED of ACNU at the dose of 0.02 mg/rat, and i.v. injection of ACNU at 2.0 and 0.4 mg/rat were 3.21, 3.47, and 0.52 μg/g, respectively. CED of ACNU at the dose of 100-fold less total drug than i.v. injection carried an almost equivalent concentration of ACNU into rat brain tissue. The tissue concentration after treatment with CED at the dose of 0.02 mg/rat was almost as high as that of i.v. administration at the dose of 2.0 mg/rat, and was about six times as high as that of i.v. administration at the dose of 0.4 mg/rat. ACNU was completely cleared from the brain tissues within 4 h in all groups (Fig. 4).

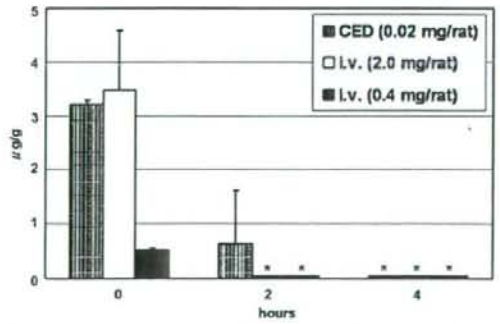

Fig. 4 Tissue concentrations of ACNU in the normal rat brain following single CED infusion and bolus i.v. injection. Drug concentrations were measured by high-performance liquid chromatography assay for ACNU. *: below the detection limit of 0.05 μ g/g

Discussion

Convection-enhanced delivery has shown considerable potential for the treatment of brain tumors, with some of the protocols now in clinical trials [5, 22]. ACNU is a hydrophilic nitrosourea with a proven efficacy against high-grade gliomas through systemic administration [18, 19]. Our studies demonstrated that combining ACNU with the CED technique provided safe and significant anti-tumor effects in animal brain tumor models.

To evaluate the safe dose of ACNU via CED, we performed the toxicity test in the normal brain parenchyma of intact rats. The established MTD was 0.02 mg/rat (1.0 mg/mL ACNU, 20 μL CED). This dose was far smaller than the clinically tolerable dose of 0.4 mg/rat for systemic administration, and CED at the dose of 0.02 mg/rat resulted in no systemic complication.

3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride is lipophilic as well as hydrophilic under physiological conditions ($\log p = 0.92$). Hydrophilic ACNU delivered via CED is expected to distribute over the extracellular space of the brain, gradually becoming lipophilic, then taken up into the surrounding cells, and manifesting the anti-cancer effect.

As confirmed by high-performance liquid chromatography, ACNU administered via CED yielded much higher drug levels in brain tissue than i.v. administration. The survival study using 9 L rat brain tumor models revealed that CED infusion at the MTD of ACNU produced significantly improved survival rate compared with i.v. administration, and the anti-tumor effect of ACNU delivered via CED was dose-dependent. These results demonstrated that CED enhanced

the anti-tumor effect of hydrophilic ACNU compared with i.v. administration.

Infusion of a high concentration of ACNU resulted in increased local CNS toxicity, which was ascribed to the non-specific cytotoxicity of ACNU. The local neurotoxicity strictly limited the therapeutic window of ACNU delivered via CED, so we could not attempt dose escalation to increase the anti-tumor effect of ACNU. Several studies have utilized drug encapsulation in nano-particles to overcome such non-specific cytotoxicity of anticancer drugs [23–25]. Encapsulation of drugs increases tissue tolerance by reducing the acute tissue exposure and slowing the rate of drug release. Encapsulated ACNU in nano-particles may allow a higher dose of ACNU to be delivered via CED.

The short-tissue retention time of ACNU was another limiting factor of the anti-tumor efficacy in our study. ACNU infused via CED was completely cleared from the brain tissue within 4 h. Unencapsulated and water-soluble agents are typically cleared from the brain in less than one day [23, 24]. Furthermore, if the molecular weight of the agent is < 200–400, free exchange takes place between plasma and brain extracellular water across the BBB [15]. The rapid clearance of ACNU may be partially due to its small molecular weight (309.15). To extend the drug residence, encapsulation of drugs in nano-particles as described above is also possible. Encapsulated agents have prolonged tissue residence time in CED compared with free agents [23, 24]. Combining drug encapsulation techniques with CED may reduce CNS toxicity as well as increase tissue retention and anti-tumor efficacy.

The survival rate of animals treated with CED of ACNU at the dose of 0.02 mg/rat (0.1 mg/kg) was 25%. Presumably the difference between survivors and non-survivors within the same CED group could be attributed to the inhomogeneous drug distribution within the tumors. Heterogeneous distribution of anti-cancer drugs results in partial response and local recurrence of brain neoplasms [5, 26]. Current ongoing clinical CED lacks monitoring or confirmation of the drug distribution [5, 22], although several infusion sites can be selected to optimize catheter placement and achieve homogeneous drug distribution over the entire targeted lesion [5]. Further animal studies with CED are needed to improve the drug distribution in human brain tumors.

Several studies support the applicability of ACNU administered via CED to clinical treatment of high-grade gliomas in humans. Locally injected ACNU into recurrent gliomas was effective in inducing tumor necrosis and inhibiting tumor growth [27]. Intraventricular administration of ACNU is safe and efficacious in the treatment of malignant gliomas [28–30]. The present study also suggests that CED of ACNU is capable of increasing efficacy in the field of glioma treatment.

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Neoplasm

Deficits in Japanese word spelling as an initial language symptom of malignant glioma in the left hemisphere

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Abstract

Background: A good performance status at diagnosis is a prognostic factor in patients with malignant glioma whose median survival is 24 months. As early diagnosis may improve their poor prognosis, we looked for currently unknown initial symptoms among patients in good performance status.

Methods: We chose 17 consecutive patients with malignant glioma in the left frontal and/or temporal lobe whose Karnofsky Performance Status was more than 80. At preoperative evaluation, we administered the Japanese version of the Western Aphasia Battery.

Results: The chief complaint was difficulty in speech (n = 6), headache/nausea (n = 4), seizures (n = 5), and uncinate fits (n = 1); one patient was symptom-free. Of the 17 patients, 14 exhibited no motor deficits. In 15 patients, the aphasia quotient exceeded 80, indicating that the overall language deficits were mild. However, in the reading section, their scores on the "spelled *kanji* (Japanese ideogram) recognition" test (full score = 10) were selectively low (5.3 ± 1.6 for right-handed individuals with frontal lesions, 6.1 ± 1.0 for right-handed patients with temporal lesions, 7.2 ± 2.0 for left-handed/bimanual individuals with frontal/temporal lesions). Their scores on the "spelling kanji" test were 3.0 ± 1.6, 4.8 ± 1.2, and 9.4 ± 0.6, respectively.

Conclusions: Our findings point to the importance of recognizing spelling deficits as an initial symptom of left hemisphere glioma in efforts to identify patients in good performance status whose prognosis may be improved. It would be important to determine if the spelling of alphabetic words is also impaired early in the clinical course of left hemisphere glioma.

Keywords: Aphasia; Diagnosis; Dyslexia; Glioma; Language; Spelling

1. Introduction

As the clinical course of malignant glioma often involves rapid progression, the tumor has usually invaded a large part of the brain before the patients undergo surgical treatment. Despite efforts to develop new strategies for the diagnosis and treatment of patients with GB, the most malignant type, the median survival continues to be less than 1 year [17,18,26,32]. A better KPS in the preoperative stage is reportedly an independent factor for a good prognosis [1,12,25]. In addition, patients with smaller tumors [7] and those who underwent extensive surgical resection [5,7,14,34] experienced significantly longer survival. Thus, for early diagnosis and treatment, it is essential to know early signs or symptoms in patients with malignant glioma and whose tumors are small and who are in normal performance status.

Among patients with left-sided brain tumors, 37% to 58% manifest language dysfunction [13,20] that tends to be associated with high-grade rather than low-grade glioma

Abbreviations: AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; AQ, aphasia quotient; fMRI, functional magnetic resonance imaging; GB, glioblastoma; KPS, Karnofsky Performance Status; MRI, magnetic resonance imaging; PET, position emission tomography; WAB-J, Western Aphasia Battery.

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[3,35]. Japanese patients exhibit unique deficits because the Japanese language system comprises 2 different orthographies, *kanji* and *kana* characters. Whereas kanji (morphograms or ideograms) contain semantic and phonetic representations, kana (phonograms or syllabograms) have only phonological meaning (Fig. 1). These 2 orthographies involve different subprocesses in the left hemisphere [27,31]. For example, patients with lesions in the left angular gyrus exhibit kana alexia and agraphia for both kana and kanji,

Fig. 1. Phonogram (kana), morphogram (kanji), and brief explanation of kanji spelling tests in the WAB-J. Kana have only phonetic value, whereas kanji have both phonetic and semantic values. For example, "sunny" is "はれ" in kana and "晴" in kanji; both are pronounced "ha-re." In kana characters, the sound-to-script correspondence is strictly one-to-one, so that, with very few exceptions, "は" and "れ" are pronounced "ha" and "re," respectively. Most kanji characters are constructed with a few components reflecting semantic values. In this example, "晴" consists of a left part (hen) "日" and a right part (tsukuri) "青"; they mean "sun" and "blue," respectively. Thus, "sunny day" comprises "sun" and "blue (sky)." In the "spelled kanji recognition" test, the correct answer is "晴" when the patient is given "日" and "青" (green arrows). In the "spelling kanji" test, the correct answer is "日" and "青" when the patient is given "晴" (yellow arrows). These tests correspond to the spelling-related test in the English version of the WAB [11].

Table 1
Clinical characteristics of patients with glioma in the left hemisphere

Case no.	Initial	Age/ sex	CC	Paresis	KPS (%)	Additional	Pathologic finding
Right-handers with left frontal glioma							
1	YK	38/F	Speech	Mild	90	Ins, TT	AA
2	MT	53/F	Speech	Mild	90	TT	GB
3	HS	63/M	Seizure	Mild	90	No	AO
4	SI	50/M	Headache	No	80	Ins, TT	AOA
5	SK	44/M	Seizure	No	100	Ins	AA
Right-handers with left temporal glioma							
6	YS	67/F	Speech	No	90	No	GB
7	TN	43/F	Seizure	No	100	Ins	AA
8	TN	54/M	Speech	No	90	No	GB
9	CN	68/F	Speech	No	90	No	GB
10	MS	53/M	Uncinate fit	No	100	No	AOA
11	JH	52/F	Headache	No	100	No	AOA
12	AN	76/M	Speech	No	90	No	GB
13	SO	50/F	Seizure	No	100	No	AA
14	TS	50/F	Headache	No	100	No	AA
Non–right-handers with left frontal or temporal glioma							
15	MI	50/M	Nausea	No	100	Ins	A-II
16	YI	63/F	Seizure	No	100	TT	GB
17	KH	54/M	Incidental	No	100	No	AOA

CC indicates chief complaint; F, female; M, male; Ins, insula; TT, temporal tip; A-II, astrocytoma grade II.

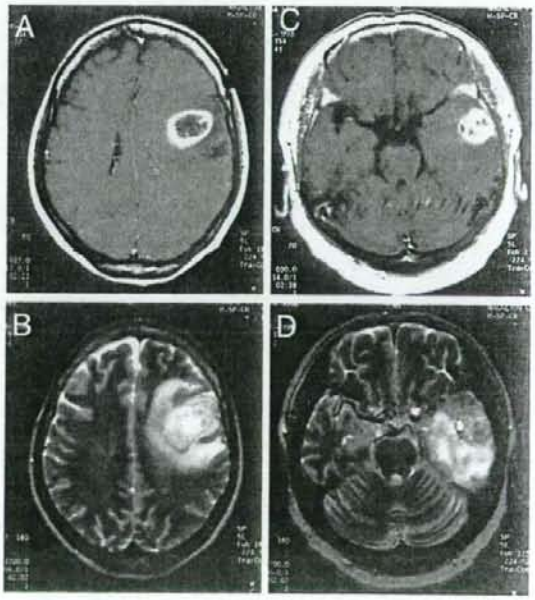

Fig. 2. Illustrative cases of patients with glioma in the left hemisphere. A: Gadolinium-enhanced T1-weighted MRI of a patient (case 2) with frontal glioma. B: T2-weighted MRI (case 2). C: Gadolinium-enhanced T1-weighted MRI of a patient (case 6) with temporal glioma. D: T2-weighted MRI (case 6).