Table 4. Treatment-Related Neurotoxic Effects*

	No. in WBRT + SRS Group (n = 65)				No. in SRS-Alone Group (n = 67)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Acute toxic effects	2	1	1	0	3	3	2	0
Seizure	0	0	1	0	1	2	1	0
Other	2	1	0	0	2	1	1	0
Late toxic effects	3	0	2	2	1	0	0	2
Radiation necrosis	1	0	0	2	0	0	0	1
Leukoencephalopathy	1	0	2	0	0	0	0	0
Other†	1	0	0	0	1	0	0	1
Radiological leukoencephalopathy	2	3	2	0	1	1	0	0

Abbreviations: SRS, stereotactic radiosurgery; WBRT, whole-brain radiation therapy.
*From the National Cancer Institute's Common Toxicity Criteria version 2.0.[16]
†Other effects included 1 case of slight lethargy (grade 1) in the WBRT + SRS group and 1 case each of seizure (grade 4) and headache (grade 1) in the SRS-alone group.

Table 5. Univariate Analysis of Development of New Metastases at Distant Brain Sites

	Actuarial Rate, %		Log-Rank *P* Value
	6 mo	12 mo	
Treatment group			
WBRT + SRS	17.5	41.5	.003
SRS alone	49.9	63.7	
Age, y			
<65	34.5	55.9	.65
≥65	33.9	49.0	
Sex			
Male	32.7	51.5	.39
Female	36.3	55.9	
No. of brain metastases			
1	27.3	39.2	.03
2-4	42.4	69.9	
Primary tumor site			
Lung	29.5	52.0	.40
Other	43.1	55.9	
Primary tumor status			
Stable	32.8	44.8	.20
Active	37.1	69.6	
Extracranial metastases			
Stable	29.5	38.4	.02
Active	37.3	69.3	
KPS score			
70-80	43.2	57.4	.05
90-100	29.9	50.8	
Chemotherapy after brain treatment			
Yes	37.1	59.0	.33
No	32.9	50.0	

Abbreviations: KPS, Karnofsky Performance Status; SRS, stereotactic radiosurgery; WBRT, whole-brain radiation therapy.

ing SRS alone ($P = .36$), including 1 and 2 patients with grade 3 toxicity, respectively, in each group. The symptoms developed a median of 6 days after initiation of treatment (range, 1-64 days) in the WBRT + SRS group and 10 days (range, 1-86 days) in the SRS-alone group. Symptomatic late neurologic radiation toxic effects were observed in 7 patients in the WBRT + SRS group and in 3 patients in the SRS-alone group ($P = .20$). Toxic effects were experienced for a median of 15.6 months (range, 6.7-59.4 months) in the WBRT + SRS group and 6.2 months (range, 5.8-8.1 months) in the SRS-alone group. There were 3 cases of radiation necrosis (grade 1, n = 1; grade 4, n = 2), 3 cases of leukoencephalopathy (grade 1, n = 1; grade 3, n = 2), and 1 case of slight lethargy (grade 1) in the WBRT + SRS group. In patients receiving SRS alone, the following effects were observed: 1 case of radiation necrosis (grade 4), 1 of seizure (grade 4), and 1 of headache (grade 1). Radiation necrosis was diagnosed using positron emission tomography or surgical resection in all cases. Radiological findings consistent with leukoencephalopathy were observed in 7 patients in the WBRT + SRS group and in 2 patients in the SRS-alone group ($P = .09$). Three of these 9 patients also experienced symptomatic leukoencephalopathy; the other 6 patients were asymptomatic.

Brain Tumor Recurrence

Brain tumor recurrence at either distant or local sites in the brain was observed in 63 patients (23 in the WBRT + SRS group and 40 in the SRS-alone group). The 12-month actuarial brain tumor recurrence rate was 46.8% (95% CI, 29.7%-63.9%) in the WBRT + SRS group and 76.4% (95% CI, 63.3%-89.5%) in the SRS-alone group ($P < .001$).

Fifty-five patients had new brain metastases at distant sites (21 in the WBRT + SRS group and 34 in the SRS-alone group). The 12-month actuarial rate of developing new brain metastases was 41.5% (95% CI, 24.4%-58.6%) in the WBRT + SRS group and 63.7% (95% CI, 49.0%-78.4%) in the SRS-alone group ($P = .003$) (Figure 2B).

The multivariate analysis revealed that WBRT + SRS was associated with a reduced risk of recurrence (hazard ratio, 0.32; 95% CI, 0.18-0.58; $P<.001$) (TABLE 5 and TABLE 6).

During the follow-up period, 122 patients (92% of the total patients enrolled) had at least 1 follow-up MRI scan performed. In total, 581 follow-up MRI scans were performed; of these, 87 scans (15%) demonstrated new brain metastases; these 87 "event scans" were obtained in 55 patients. Sixteen percent of these "event scans" (14/87) were associated with neurologic symptoms at the time of the MRI examination.

A total of 247 metastases received initial treatment with SRS (117 in the WBRT + SRS group and 130 in the SRS-alone group). Follow-up MRI was available for 210 metastases (85%). The actuarial local tumor control rate at 12 months was 88.7% (95% CI, 80.1%-97.3%) in the WBRT + SRS group and 72.5% (95% CI, 60.3%-84.7%) in the SRS-alone group ($P=.002$) (FIGURE 3). The histopathological type (adenocarcinoma vs others) was not shown to be a significant factor ($P=.90$). The multivariate analysis also showed significantly better tumor control by WBRT + SRS treatment (hazard ratio, +4.83; 95% CI, 2.00-11.65; $P<.001$).

Salvage treatment for progression of brain tumor was required significantly more frequently in patients receiving SRS alone (29 patients) than in the WBRT + SRS group (10 patients) ($\chi^2=12.33$; $P<.001$). Salvage WBRT was applied in 11 patients in the SRS-alone group but was not used in any patients in the WBRT + SRS group. Salvage SRS was used in 19 patients in the SRS-alone group and in 9 patients in the WBRT + SRS group.

Systemic and Neurologic Functional Preservation

Systemic functional preservation rates (KPS score ≥70) at 12 months were 33.9% (95% CI, 22.2%-45.4%) in the WBRT + SRS group and 26.9% (95% CI, 16.3%-37.5%) in the SRS-alone group ($P=.53$). The decrease in the KPS score to below 70 was attributed to neurologic causes in 17 patients (29%) in the WBRT + SRS group and 14 (22%) in the SRS-alone group.

The actuarial rates of neurologic preservation at 12 months were 72.1% (95% CI, 58.8%-85.4%) with WBRT + SRS and 70.3% (95% CI, 55.6%-85.0%) with SRS alone ($P=.99$) when neurologic preservation was defined as a lack of any worsening of the neurologic grade on follow-up examination, compared with the pretreatment grade. In total, 85 patients (38 in the WBRT + SRS group and 47 in the SRS-alone group) did not have neurologic symptoms when brain metastases were diagnosed (grade 0). Among the 47 patients who had a pretreatment grade of 1 to 3, an improvement in neurologic status was observed at least once in 9 patients and 10 patients in the respective groups ($\chi^2=1.32$; $P=.24$). Deterioration of neurologic function was observed in 43 patients, including 7 who initially experienced improvement after treatment (22 in the WBRT + SRS group and 21 in the SRS-alone group; $\chi^2=0.09$; $P=.75$). This deterioration was attributed to either original or distant brain metastases in 13 patients (59%) in the WBRT + SRS group and 18 patients (86%) in the SRS-alone group ($\chi^2=3.78$; $P=.05$). Late neurologic radiation toxic effects were the cause of deterioration in 4 and 2 patients in each group, respectively. Either meningeal dissemination or spinal cord metastases induced neurologic deterioration in 5 and 1 patient in each group, respectively.

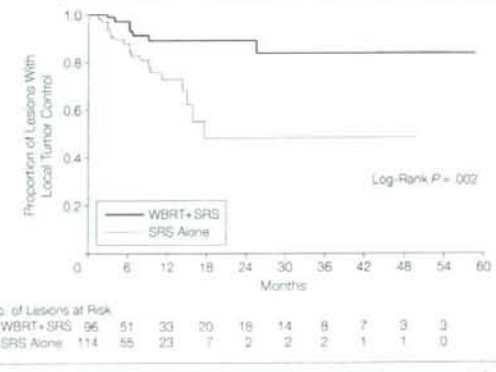
Neurocognitive function was optionally assessed using the Mini-Mental State Examination (MMSE). Among the 44 patients (25 in the WBRT + SRS group and 19 in the SRS-alone group) who lived 12 months or longer, MMSE data were available in 28 patients at least once (16 in the WBRT + SRS group and 12 in the SRS-alone group) at the median follow-up times of 30.5 months (range, 13.7-58.7 months) with WBRT + SRS and 20.7 months (range, 13.3-53.8 months) with SRS alone. The median MMSE pretreatment score was 28.0 (range, 23-30) in the WBRT + SRS

Table 6. Multivariate Analysis of Development of New Metastases at Distant Brain Sites

	Hazard Ratio (95% CI)	*P* Value
Treatment group (WBRT + SRS)	0.32 (0.18-0.58)	<.001
No. of brain metastases (2-4)	1.69 (0.97-2.93)	.06
Extracranial metastases (active)	2.00 (1.17-3.64)	.01
KPS score (70-80)	2.14 (1.17-3.93)	.01

Abbreviations: CI, confidence interval; KPS, Karnofsky Performance Status; SRS, stereotactic radiosurgery; WBRT, whole-brain radiation therapy.

Figure 3. Local Tumor Control



There was a statistically significant increase in local tumor control in patients receiving whole-brain radiation therapy (WBRT) plus stereotactic radiosurgery (SRS) ($P=.002$).

group and 27.0 (range, 23-30) in the SRS-alone group. The median score at the final follow-up was 27.0 (range, 21-30) in the WBRT + SRS group and 28.0 (range, 18-30) in the SRS-alone group.

COMMENT

Stereotactic radiosurgery is a method of delivering high doses of focal radiation to a tumor while minimizing irradiation of the adjacent normal tissue. This approach was originally developed by the Swedish neurosurgeon Lars Leksell as a substitute for direct surgical intervention.[21] Stereotactic radiosurgery is now available worldwide, and it is increasingly used to treat brain metastases because it is less invasive compared with direct surgical intervention, although a direct randomized comparison of the 2 modes has not been performed to date.

Whole-brain radiation therapy has been a standard treatment for brain metastases for several decades.[1-4,6,7,17] In more recent years, the importance of focal aggressive therapy combined with WBRT has been increasingly recognized.[1-22-24] Andrews et al[4] recently reported the results from RTOG 9508, a multi-institutional phase 3 trial of 333 patients with 1 to 3 brain metastases who received WBRT with or without SRS boost. A statistically significant improvement in median survival with the addition of SRS was seen in patients with a single brain metastasis.

To reduce the risk of late radiation effects,[1,2,3] WBRT is increasingly being omitted from the initial management strategy.[6-13] There is not yet a general consensus regarding the risks and benefits of omitting up-front WBRT. One study showed a trend toward improved survival among patients who received SRS alone,[12] whereas another study showed a trend toward worse survival among patients who received SRS alone.[10] A retrospective multi-institutional review of SRS alone vs SRS with WBRT in 569 patients failed to show any difference in survival between the 2 groups.[7] In a single-institution prospective randomized trial comparing WBRT with observation in patients who underwent conventional surgery,[6] a large increase in intracranial relapse and a concomitant increase in death due to neurologic causes were identified in the non-WBRT group; however, no survival difference was identified in that study. In the present study, no significant survival difference was observed between the groups receiving WBRT + SRS and SRS alone, although the number of patients was not large enough to allow detection of any differences that were smaller than we had assumed. In addition, no significant difference in the frequency of death due to neurologic causes was observed. Moreover, these results were obtained in spite of the rather large increase in intracranial failure when WBRT was omitted. A further observation of note from the present trial was the significant increase in local failure with SRS alone, even though the radiation dose in these patients was considerably higher than that administered to patients receiving WBRT + SRS. We have adapted the 30% reduced dose of SRS in the WBRT + SRS group, which could have lowered local control of the brain metastasis in the WBRT + SRS group. However, we have observed opposite results in this study; the local control rate was significantly higher in the WBRT + SRS group than in the SRS-alone group. This observation lends merit to the value of fractionation, which might help overcome some radiation resistance mechanisms, such as hypoxia.

Also of concern in this context is that higher brain recurrence rates are associated with neurologic deterioration.[6] In a previous randomized study of surgery with or without WBRT,[6] the time to neurologic deterioration was dramatically longer in the WBRT group, although no difference in functional independence was observed. In the current study, no significant difference in the preservation of neurologic function was observed. However, the present study might have less ability to detect small differences, and the present assessment of neurologic function was not conducted with sophisticated measures that might have detected differences between patient groups.

Although surgery and SRS are both focal treatments, SRS is less invasive and may be repeated more often than surgical intervention.[11] The optimal timing of these interventions is an issue that remains open for debate. Our results suggest that the early detection of a brain recurrence and early salvage brain treatment may prevent neurologic deterioration and neurologic death, even when WBRT is not included in the initial treatment. However, study participants more frequently undergo physical and radiological examinations than do patients in the community. Given that the majority of new brain metastases were initially detected in asymptomatic patients, studies assessing the benefits of scheduled imaging should be conducted in the future.

In conclusion, our findings demonstrated that SRS alone without up-front WBRT was associated with increased brain tumor recurrence; however, it did not result in either worsened neurologic function or increased risk of neurologic death. With respect to patient survival, the control of systemic cancer might outweigh the frequent recurrence of brain tumors. Therefore, SRS alone could be a treatment option, provided that frequent monitoring of brain tumor status is conducted.

Author Contributions: Dr Aoyama had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Drafting of the manuscript: Aoyama, Shirato, Tago, Nakagawa, Hayakawa.
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Statistical analysis: Aoyama, Tago, Kobashi.
Administrative, technical, or material support: Aoyama, Shirato, Tago, Nakagawa, Toyoda, Hatano, Kenjyo, Oya, Hirota, Shioura, Kunieda, Inomata, Hayakawa, Katoh.
Study supervision: Aoyama, Shirato, Tago, Nakagawa, Hatano, Kenjyo, Oya, Hirota, Kunieda, Kobashi.
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> The only true hope for civilization—the conviction of the individual that his inner life can affect outward events and that, whether or not he does so, he is responsible for them.
> —Stephen Spender (1909-1995)

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Clinical–patient studies

Hypofractionated radiotherapy boost for dose escalation as a treatment option for high-grade spinal cord astrocytic tumor

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Key words: astrocytoma, ependymoma, glioma, hypofractionated radiotherapy boost, radiotherapy, spinal cord

Summary

Purpose: To retrospectively analyze the outcome of post-operative radiotherapy for spinal cord glioma with the emphasis on the hypofractionated radiotherapy boost for dose escalation as a treatment option for high-grade spinal cord astrocytic tumors.

Materials and methods: Forty-one patients with spinal cord glioma received post-operative radiotherapy between 1979 and 2003. The median age was 34 years (range, 10–66 years). Median follow-up was 49 months (range, 5–291 months). There were 12 low-grade astrocytic tumors, 11 high-grade astrocytic tumors, 16 low-grade ependymal tumors and 2 high-grade ependymal tumors. Among 11 patients with high-grade astrocytic tumors, 5 with anaplastic astrocytoma and 1 with glioblastoma received hypofractionated radiotherapy boost for dose escalation. The median total dose of the conventional radiotherapy was 45.5 Gy in 19 fractions (range, 30.0–60.0 Gy). The median normalized total dose (using daily dose of 2.0 Gy and an α/β ratio of 2.0) of the hypofractionated radiotherapy boost was 131 Gy_2 (range, 85–249).

Results: The Kaplan–Meier survival rates at 10 years from the date of the first surgery were 64% for the entire group, 47% for the astrocytic tumors and 84% for the ependymal tumors, respectively ($P = 0.009$). Among 11 patients with high-grade astrocytic tumors, the actuarial survival rate at 10 years was 35%. The actuarial survival rates at 10 years were 67% for those who received hypofractionated radiotherapy boost for dose escalation, and 20% for those who did not ($P = 0.47$).

Discussion: The results for ependymal tumors and low-grade astrocytic tumors were comparable to those reported in the literature. Hypofractionated radiotherapy boost for dose escalation may help to prolong the survival of patients with high-grade astrocytic tumors.

Introduction

Spinal cord glioma is a rare disease with an expected 5-year overall survival rate of 54–82% [1,2]. Survival strongly depends on the pathological grade and subtypes of the disease. Long-term motor and sensory function is related to the extent of the surgical removal, and possibly to the adjuvant post-operative radiotherapy. Spinal ependymal tumors usually have a low-grade histology, and a 5-year survival rate of 83–100% [3,4]. Astrocytic tumors are often malignant and have a 5-year survival rate of 50–64% [1,4,5]. The benefits of post-operative radiotherapy in reducing relapse of the tumor after partial resection has been suggested by retrospective analyses both for ependymal tumors [4,6] and astrocytic ones [7,8], but its value has been questioned for children with astrocytic tumors [8]. It would be worthwhile to publish the long-term outcome of post-operative radiotherapy for spinal gliomas.

High-grade astrocytic tumors have a worse outcome, with a 5-year overall survival rate of 0–24% [1,9–12]. The benefits of chemotherapy in addition to radiotherapy are still under investigation [13]. Meanwhile hypofractionated radiotherapy boost for dose escalation for high-grade brain gliomas has been suggested to prolong the progression-free survival compared to the conventional dose radiotherapy albeit with increased toxicity [14–16]. The hypofractionated radiotherapy boost is far beyond the tolerance dose of the spinal cord, which means that sensorimotor function will deteriorate if a high dose is used for spinal cord glioma. However, the sensorimotor function of patients with spinal high-grade astrocytic tumors is usually very poor at the time of initial diagnosis, before treatment has begun [4,13]. Because of this pre-existing dysfunction, patients with high-grade astrocytic tumors would lose little sensorimotor function after hypofractionated radiotherapy boost. After conducting an ethics evaluation, we adopted hypofractionated radiotherapy boost as an option for patients with high-grade astrocytic tumors at the thoracic or lumbar spinal cord.

In this study, we have updated the long-term outcome of radiotherapy for spinal cord glioma. In addition, the efficacy and adverse effects of hypofractionated radiotherapy boost were investigated.

Materials and methods

Study group

Forty-one patients were treated with post-operative irradiation for spinal cord glioma at our institution between 1979 and 2003. Twenty were male, and 21 female. The median age was 34 years (range, 10–66).

Tumor characteristics

Pre-operative and pre-radiotherapeutic radiological investigation varied according to the time period during which the investigation was performed. A magnetic resonance imaging (MRI) was performed in 33 patients, a computed tomography (CT) in 2, a CT-myelogram in 2, and a myelogram in 4. The main location of the tumors was the cervical spinal cord in 15 patients, the thoracic in 12, the conus medullaris in 9, and the cauda equina in 5. All patients had histopathological confirmation of their diagnosis by neuropathologists. The patients were classified into four groups according to the World Health Organization classification: 12 low-grade astrocytic tumors (1 pilocytic astrocytoma, 10 diffuse astrocytomas, 1 oligoastrocytoma), 11 high-grade astrocytic tumors (10 anaplastic astrocytomas, 1 glioblastoma), 16 low-grade ependymal tumors (4 ependymomas, 11 myxopapillary ependymomas, 1 ependymoma with astrocytoma) and 2 high-grade ependymal tumors (2 anaplastic ependymomas). There were 2 patients who were initially diagnosed with a low-grade astrocytic tumor, and experienced recurrence within a year. Since the surgical specimens in the second operation for these 2 patients showed high-grade glioma, the 2 patients were included in the high-grade group.

Surgical treatment

All patients underwent a surgical procedure prior to the initial radiation therapy; 5 patients underwent a gross total resection, 32 a subtotal or partial resection, and 4 a biopsy.

Radiation therapy

Conventional radiation therapy excluding the hypofractionated radiotherapy boost for dose escalation

Treatment was given to the primary tumor site with or without additional irradiation to the entire spine or entire central nervous system. In general, the gross tumor volume (GTV) was a gadolinium-enhanced area on MRI as well as high-intensity areas on T2-weighted MRI. The clinical target volume (CTV) was determined by adding 2–4 cm margins to the cranial and caudal sides to the GTV. The planning target volume (PTV) margin was 1 cm for each side. Treatment was delivered with mega-voltage X-ray (4, 6, or 10 MV), except in 2 patients who had been treated by cobalt gamma ray 13 and 19 years ago. Local irradiation was given using single posterio-anterior and/or oblique wedge-pair fields. For the low-grade astrocytic tumors and low-grade ependymal tumors with post-operative residual disease, we treated the patients with 40–46 Gy in 20–23 fractions. For the patients who received conventional radiotherapy, the median total dose was 46 Gy in 23 fractions (range, 35 Gy in 14 fractions to 50 Gy in 20 fractions) in the low-grade astrocytic tumors and 40 Gy in 20 fractions (range, 30 Gy in 12 fractions to 50.4 Gy in 28 fractions) in the low-grade ependymal tumors. In routine practice, the patients with high-grade glioma were treated with 50–54 Gy in 25–27 fractions. The median total dose was 50 Gy in 25 fractions (range, 46 Gy in 23 fractions to 60 Gy in 24 fractions) in the patients with high-grade gliomas who were treated with the conventional radiation therapy.

Hypofractionated radiotherapy boost for dose escalation

After conducting an ethics evaluation, the following research was conducted for patients with high-grade astrocytic tumors. Patients were candidates for hypofractionated radiotherapy boost for dose escalation if the high-grade astrocytic tumors existed in the thoracic or lumbar spinal cord and the upper edge of the PTV did not involve the cervical spinal cord or the spine at a higher level. Patients with a disseminated tumor were not candidates for this treatment. The definitive loss of function that would occur after this treatment was explained to all patients and their families in detail. In all cases, the treatment was applied only with the written informed consent of the patients and their families.

The hypofractionated radiotherapy boost was delivered using the supra-tolerable dose for the spinal cord. We used various dose fractionation schedules depending on the length of the field, surrounding critical organs, and the general condition of the patient. We defined an irradiation dose of more than 70 Gy_2 or more in normalized total dose (NTD) assuming a 2 Gy daily dose with α/β of 2.0 as the hypofractionated radiotherapy boost for dose escalation. We used 6 or 10 MV X-rays with two to three portals to reduce the spread of the dose to surrounding normal tissue, except in one patient who received posterior single portal irradiation. All treatments were given to the involved areas with the same margin as conventional radiotherapy.

Adverse effect assessment

Neurologic motor function was scored and classified into four groups (excellent, good, fair, or poor/dead) at the time of initial diagnosis based on our previous study [4] (Table 1), and these scores were compared to those obtained at the patient's last visit. Other adverse events were scored according to the National Cancer Institute

Table 1. Neurological motor function classification

Excellent	Intact or minimal neurological deficit, no functional impairment
Good	Mild neurological deficit, ambulating without braces or aid, no functional impairment
Fair	Moderate neurological deficit, ambulating with braces and/or aid, significant functional impairment
Poor	Quadriplegic or paraplegic, wheel chair dependent, significant functional impairment

Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE v3.0).

Statistics

Follow-up time was calculated from the first date of the surgery. The median follow-up time for the entire group was 53 months (range, 5–291), and that of the 6 patients who received hypofractionated radiotherapy boost, 19 months (range, 8–124). Survival rates were determined with the Kaplan-Meier method. Univariate analyses were performed using the log-rank test.

Results

Entire group

By the time of the last follow-up, 13 patients had died. The actuarial survival rates of the entire group at 5 and 10 years from the date of the first surgery were 73% and 64%. The actuarial survival rates at 5 and 10 years were 54% and 47% for astrocytic tumors, and 94% and 84% for ependymal tumors, respectively ($P=0.009$) (Figure 1). Among the 23 patients with astrocytic tumors, the 5- and 10-year actuarial survival rates were 68% and 57% for low-grade tumors, and 40% and 40% for high-grade ones ($P=0.056$) (Figure 2). The 5- and 10-year actuarial survival rates were 100% and 88% for low-grade ependymal tumors. There was a significant difference in survival between low-grade ependymal tumors and low-grade astrocytic tumors ($P=0.05$). The 2-year actuarial survival rates and progression-free rates were 53% and 18% for high-grade astrocytic tumors. The median survival for the high-grade astrocytic tumors was 21 months (range, 5–221).

For 11 patients with myxopapillary ependymoma, the actuarial 5- and 10-year survival rates were 100% and 80%, respectively. For 5 patients with ependymoma,

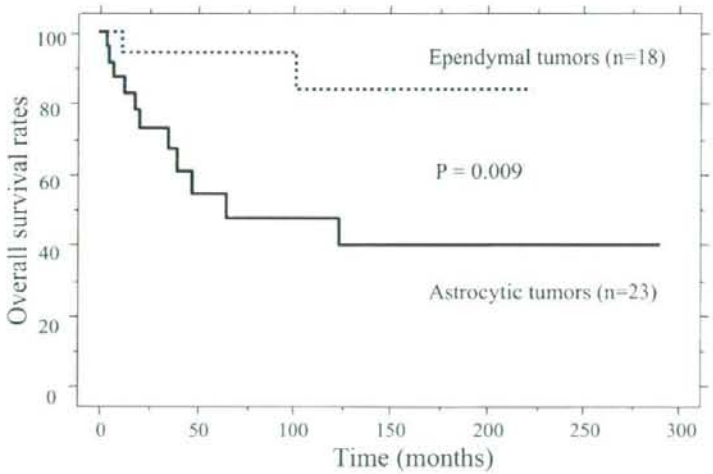

Figure 1. The actuarial survival curves for astrocytic tumors and for ependymal tumors.

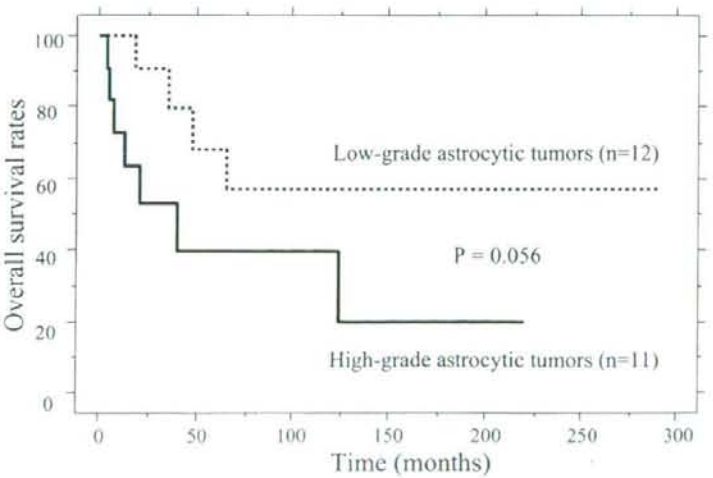

Figure 2. The actuarial survival curves for low-grade astrocytic tumors and for high-grade ones.

those rates were 100% and 100%. Tumor relapse at the cauda equina after radiotherapy was observed in a single patient with myxopapillary ependymoma, for whom the irradiation field had not included the cauda equina. In two with anaplastic ependymoma, one patient died at 12 months, and the other lived for 224 months.

The results of neurological assessment are shown in Table 2. Motor function at the time of initial diagnosis was excellent in 5, good in 23, fair in 7, and poor in 6 patients. At the last follow-up, motor function was scored as excellent in 10, good in 3, fair in 2, poor in 5, dead in 13, and unknown in 8. Improvement of motor function was observed in 9 patients, of whom 8 had good function and 1 fair at the initial diagnosis. No patient with poor function at the initial diagnosis showed improvement of motor function.

Hypofractionated radiotherapy boost for dose escalation

Of the 6 patients who received hypofractionated radiotherapy boost for dose escalation, there was 1 glioblastoma, and 5 were anaplastic astrocytomas as shown in Table 3A. The characteristics of 5 patients with anaplastic astrocytoma who did not receive hypofractionated radiotherapy boost are also shown in Table 3B. The median total NTD of the hypofractionated radiotherapy boost was 131 Gy_2 (range, 85–249 Gy_2). The actuarial 3-, 5- and 10-year survival rates were 67%, 67% and 67% for the patients who received a hypofractionated radiotherapy boost, and 40%, 20% and 20% for the others with high-grade astrocytic tumors ($P=0.47$) (Figure 3).

Table 2. Result of neurological assessment

	Initial diagnosis	Last follow-up
Excellent	5	10
Good	23	3
Fair	7	2
Poor	6	5
Dead	–	13
Unknown	–	8
Total	41	41

Effectiveness of the treatment was evaluated radiologically and histopathologically in one patient who suffered from marginal relapse. The patient had thoracic anaplastic astrocytoma (Table 3A, No. 6) and treated with 40 Gy in 10 fractions after 46 Gy in 23 fractions with the margin of field at the edge of high intensity in T2-weighted image, that is, the junction of C7 and T1 (C7/T1). The patient experienced marginal relapse at C7/T1. Surgical resection of the relapsed tumor showed that tumor relapse was observed also at the level of T3/4 which had received 80% of the prescribed dose but no tumor cell was detected at the level below T5 where more than 90% of the prescribed dose had been irradiated. Hypofractionated radiotherapy boost was interrupted for 6 days in one patient who experienced a severe decline in bowel movements during the treatment. NCI-CTCAE v3.0 Grade 3 leukopenia was seen in 2 patients during hypofractionated radiotherapy boost. No patients suffered from radiation-induced skin necrosis throughout the follow-up period. One patient treated by a single posterior field experienced severe subcutaneous induration and pruritus starting 3 years after the treatment. The patient was treated with a single posterior field, and was the initial patient for this treatment in our series. Two patients experienced severe lower limb pain for 3–6 months, which began at 6 months after the treatment, and decreased in intensity afterwards. Regarding neurologic motor function, one patient had good function and the rest poor before treatment. After the hypofractionated radiotherapy boost for dose escalation, the one with good function pre-treatment had been ambulant for 10 months, and was alive, using a wheelchair, and fully employed at 45 months after the treatment at the time of analysis. The poor function of the other 5 remained after treatment.

In the same patient who experienced marginal relapse, pathological examination of the normal structure was also performed which shoed that the spinal cord and meningus below the level of T5 was severely atrophied.

Table 3. Patients characteristics of high-grade astrocytic tumors treated with (A) hypofractionated radiotherapy boost for dose escalation; (B) conventional radiotherapy

No.	Sex	Age	Pathological diagnosis	Site	Treatment	NTD[a] (Gy_2)	Follow-up time (mo)	Final status	Cause of death
(A)									
1	M	54	Anaplastic astrocytoma	T7-10	40 Gy/16f + 40 Gy/4fr	165.0	123.9	Dead	Unknown
2	F	12	Anaplastic astrocytoma	T7-10	46 Gy/23f + 18.75 Gy/3fr	84.7	13	Dead	Dissemination
3	M	37	Glioblastoma	T1-4	9 Gy/6f + 40 Gy/4fr	127.9	8	Dead	Dissemination
4	F	31	Anaplastic astrocytoma	T12-L1	14 Gy/7f + 60 Gy/10fr	134.0	45	Alive	–
5	M	54	Anaplastic astrocytoma	T10-L2	40 Gy/16f + 42 Gy/7f + 40 Gy/4fr	249.0	29	Alive	–
6	F	23	Anaplastic astrocytoma	T1-T12	46 Gy/23fr + 40 Gy/10fr	106.0	18	Alive	–
(B)									
1	M	37	Anaplastic astrocytoma	T11-L1	52.5 Gy/23fr	57.2	21	Dead	Dissemination
2	F	34	Anaplastic astrocytoma	C1-C7	45.5 Gy/19fr	50.4	221	Alive	–
3	M	11	Anaplastic astrocytoma	T11-L1	50 Gy/20fr	56.3	5	Dead	Dissemination
4	F	34	Anaplastic astrocytoma	C4-C6	46 Gy/23fr	46.0	40	Dead	Dissemination
5	M	19	Anaplastic astrocytoma	C1-C4	50 Gy/25fr	50.0	5	Dead	Dissemination

[a]NTD (Gy_2): Normalized total dose using a daily dose of 2.0 Gy and α/β ratio of 2.0.

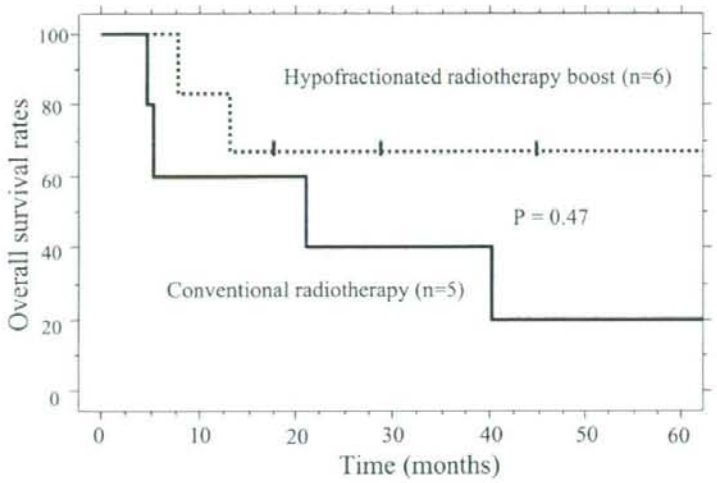

Figure 3. The actuarial survival curves for patients who received conventional radiotherapy and for patients who received hypofractionated radiotherapy boost for dose escalation.

The patient tolerated the surgery for the relapsed tumor without serious complications.

Discussion

In many cases, spinal ependymoma can be totally resected, and often no irradiation is required after the surgery [4,17,18]. However, for patients who receive a partial resection, or have a tumor with a high proliferation index [17], post-operative radiotherapy has been recommended to reduce relapse of the tumor [4]. Gilhuis et al. [6] reported that three out of three patients who did not receive radiotherapy relapsed, whereas three out of eleven patients who received radiotherapy relapsed after a partial resection of the ependymoma. Total resection is usually impossible for myxopapillary ependymoma, which often has residual disease or dissemination at the cauda equina. Gilhuis et al. [6] showed that three out of three patients who did not receive radiotherapy relapsed, whereas one out of nine who received radiotherapy relapsed. We have not seen any relapse of the tumor when we used a radiation field that covered the cauda equina. Since we have seen a relapse at the cauda equina in one girl who did not receive irradiation for the cauda equina, and had only been treated on the tumor bed after a partial resection of a myxopapillary ependymoma, we strongly recommend that the cauda equina be included as the clinical target volume for this disease. The results in this study showed that conventional radiotherapy after the partial resection of ependymal tumors did not increase morbidity. The survival rate at 5 years was 93% in 18 patients in Linstadt et al.'s series [12], 83% in 59 patients in Waldron et al.'s series [3], 100% in 10 patients in McLaughlin et al.'s series [2], 97% in 35 patients in Schild et al.'s series [19], and 94% in 25 patients in Wahab et al.'s series [5] (Table 4A). The actuarial survival rates at 5 and 10 years were 94% and 84%, respectively, for the ependymal tumors in our series, which is consistent with the other reports.

The actuarial overall survival rates of 5 and 10 years for astrocytic tumors were 54% and 47%, respectively. This is consistent with the previous reports: the 5-year survival rates were 53% in 15 patients in Linstadt et al.'s series [12], 55% in 23 patients in Jyothirmayi et al.'s series [10], 58% in 12 patients in McLaughlin et al.'s series [2], 64% in 24 patients in Wahab et al.'s series [5], and 54% in 52 patients in Rodrigues et al.'s series [1] (Table 4B). The 5- and 10-year actuarial survival rates were 68% and 57% for low-grade astrocytic tumors, which was lower than for ependymal tumors. Long-term morbidity due to adjuvant conventional radiotherapy was shown to be minimal. Post-operative radiotherapy for a low-grade astrocytoma is a reasonable option, with a possibly

Table 4. Result of post-operative radiation therapy for spinal cord (A) ependymal tumors; (B) astrocytic tumors; (C) high-grade astrocytic tumors

Author (year)	Patients	5-year survival rates (%)
(A)		
Linstadt et al. (1989)	18	93
Waldron et al. (1993)	59	83
McLaughlin et al. (1998)	10	100
Schild et al. (1998)	35	97
Wahab et al. (1999)	25	94
(B)		
Linstadt et al. (1989)	15	53
Jyothirmayi et al. (1997)	23	55
McLaughlin et al. (1998)	12	58
Wahab et al. (1999)	24	64
Rodrigues et al. (2000)	52	54
(C)		
Linstadt et al. (1989)	3	0
Cohen et al. (1989)	19	0
Jyothirmayi et al. (1997)	6	0
McLaughlin et al. (1998)	4	24
Rodrigues et al. (2000)	15	20

increased progression-free rate [20]. It may not be necessary to use ionizing irradiation on pediatric patients if a total gross resection has been achieved [8]. The scale for the assessment of neurological outcome in this study can be criticized to be to too simple and we hope to analyze the sensorimotor function using more precise scale in a prospective study in near future.

The results of the high-grade gliomas in the literature are all dismal (Table 4C). Of the 6 patients in Jyothirmayi et al.'s series [10], none survived more than 2 years. Of the 3 patients in Linstadt et al.'s series [12], none survived more than 8 months. The median survival was 6 months following surgery in 19 patients in Cohen et al.'s series [9]. The 5-year progression-free survival was 20% in the 15 patients in Rodrigues et al.'s series [1]. In our series, the projected actuarial 5-year survival rates of high-grade glioma were 35%, and, particularly for patients treated with hypofractionated radiotherapy boost for dose escalation, the projected actuarial 5-year survival rates were 67%. Because of careful treatment planning, no serious non-spinal cord injuries such as renal failure or skin ulcers were observed. The severe subcutaneous skin induration and pruritus in the initial patient after posterior single field irradiation could have been avoided if a more sophisticated treatment had been used. Sensorimotor dysfunction was acceptable for the rest of the patients. For the patient with good motor function before treatment, the decision to get a hypofractionated radiotherapy boost had been a difficult one. However, that patient was fully employed at 45 months after surgery, and had accepted the decision as the right one. The appropriate margin for this treatment may be larger than the T2-high region considering the marginal relapse in our series. Kyoshima et al. [21] have reported about the surgical cordotomy for a patient with spinal high-grade astrocytic tumor. They showed that the tumor infiltrated more rostrally within the parenchyma of the spinal cord than the level indicated by a T2-weighted MR image [21], which is consistent with our pathological findings at the relapse.

Although the number of patients was too small to come to definitive conclusions, the preliminary outcome of the hypofractionated radiotherapy boost for dose escalation was encouraging. Further investigation is required to confirm the benefit and indication of this treatment. Surgical cordotomy may also provide the similar results in survival but the latency between the treatment and the loss of the neurological function in our method would be preferable for the quality of life. Patients with dissemination or a tumor in the cervical spine would not be candidates for this treatment. Recent advances in radiotherapy, such as intensity-modulated radiotherapy or particle therapy with careful monitoring of the patient position, would make it easier to concentrate the dose to the spinal cord, without causing damage to the surrounding normal tissues [22,23]. The most important step to take when considering this treatment is a pathological diagnosis by neuro-pathologists, and sufficient agreement concerning ethical considerations from the community as well as from the patient.

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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).

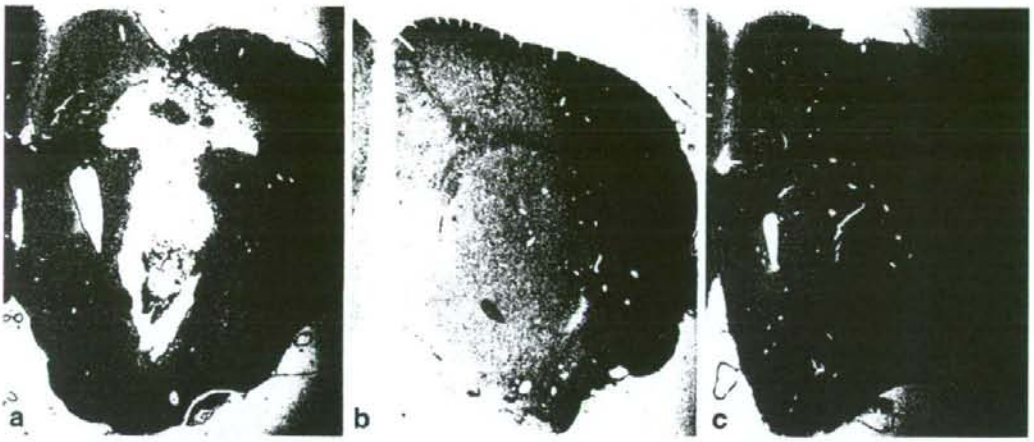

Fig. 1 Local tissue toxicity of ACNU administered via CED in the normal adult rat brain. Rat brains were treated with a single CED infusion of ACNU at different seven doses (0.2, 0.1, 0.04, 0.02, 0.01, 0.004, or 0.002 mg/rat). Representative H&E sections from three groups on the 30th day after CED. Extensive 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).

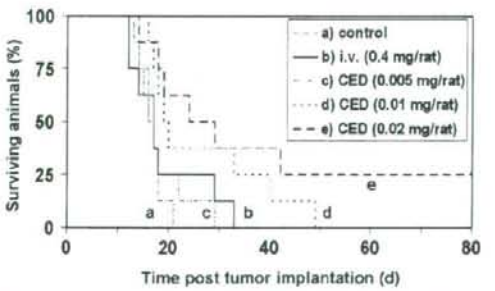

Fig. 2 Treatment of rats bearing 9 L tumors with CED or i.v. administration of ACNU. Seven days after tumor implantation within the brain, rats were treated with CED of saline (**a**), i.v. administration of ACNU at 0.4 mg/rat (**b**), and CED of ACNU at 0.005 mg/rat (**c**), 0.01 mg/rat (**d**), and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

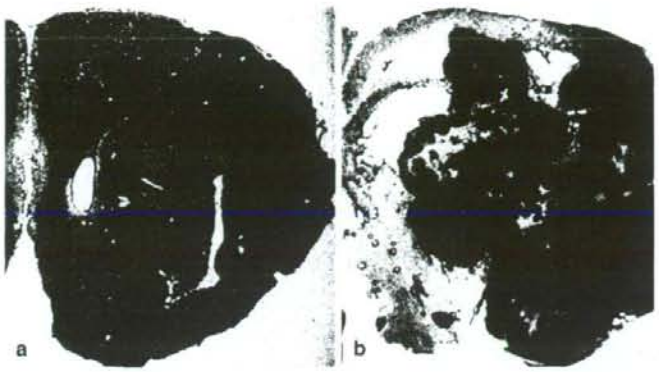

Fig. 3 Representative brain sections from surviving and non-surviving animals. (**a**) Brain section obtained from one of the survivors treated by CED of ACNU at 0.02 mg/rat. Neither survivor had residual tumor. (**b**) Brain section from a rat of the control group showing a typical tumor found in all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

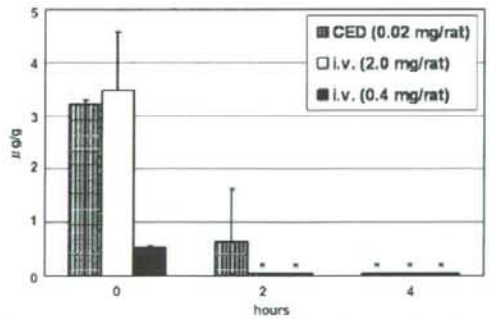

Fig. 4 Tissue concentrations of ACNU in the normal rat brain following single CED infusion and bolus i.v. injection. Drug concentrations were measured by high-performance liquid chromatography assay for ACNU. *: below the detection limit of 0.05 μ g/g

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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EXPRESSIVE AND RECEPTIVE LANGUAGE AREAS DETERMINED BY A NON-INVASIVE RELIABLE METHOD USING FUNCTIONAL MAGNETIC RESONANCE IMAGING AND MAGNETOENCEPHALOGRAPHY

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OBJECTIVE: It is known that functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG) are sensitive to the frontal and temporal language function, respectively. Therefore, we established combined use of fMRI and MEG to make reliable identification of the global language dominance in pathological brain conditions.

METHODS: We investigated 117 patients with brain lesions whose language dominance was successfully confirmed by the Wada test. All patients were asked to generate verbs related to acoustically presented nouns (verb generation) for fMRI and to read three-letter words for fMRI and MEG.

RESULTS: fMRI typically showed prominent activations in the inferior and middle frontal gyri, whereas calculated dipoles on MEG typically clustered in the superior temporal region and the fusiform gyrus of the dominant hemisphere. A total of 87 patients were further analyzed using useful data from both the combined method and the Wada test. Remarkably, we observed a 100% match of the combined method results with the results of the Wada test, including two patients who showed expressive and receptive language areas dissociated into bilateral hemispheres.

CONCLUSION: The results demonstrate that this non-invasive and repeatable method is not only highly reliable in determining language dominance, but can also locate the expressive and receptive language areas separately. The method may be a potent alternative to invasive procedures of the Wada test and useful in treating patients with brain lesions.

KEY WORDS: Expressive language function, Functional magnetic resonance imaging, Language dominance, Magnetoencephalography, Receptive language function

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Brain asymmetries have been of considerable interest in neurology for more than a century. Based on clinicopathological studies, the "classical mode" of language organization consists of a frontal "expressive" area for planning and executing speech and writing, and a temporal "receptive" area for analysis and identification of linguistic sensory stimuli. This basic scheme of language functions has generally been accepted, with the assumption that both expressive and receptive functions dominantly exist in the same hemispheric side.

The Wada test has been considered the most reliable method to determine language dominance. According to one of the largest studies performed to date, 4 and 96% of right-handed subjects with chronic epilepsy have speech dominance in the right and left hemispheres, respectively (3). Furthermore, several studies suggested the possibility of atypical language representation in patients with chronic epilepsy (20–30%) (9, 28). However, the procedure of successive anesthetization of each hemisphere by intracarotid injections of sodium amobarbital requires catheterization and irradiation. Furthermore, the Wada test results can only demonstrate a relative distribution of language functions across the two hemispheres. More detailed information on localization of specified language functions within a hemisphere is important for understanding the language networks, as well as the treatment of brain lesions.

The use of functional magnetic resonance imaging (fMRI) has recently been developed to identify the hemisphere with language dominance. Most language fMRI studies have observed activations in the inferior frontal gyrus (IFG) and middle frontal gyrus (MFG) using tasks such as word generation and categorization (16, 24, 29). Detection of the receptive language area by fMRI has been reported to be more difficult than that of the expressive language function, and the use of listening or sentence comprehension tasks has resulted in visualization of only a few pixels in the temporoparietal region (8, 16, 25, 26). In addition, a fundamental limitation of an fMRI-based brain mapping is the varying degrees of regional hemodynamic responses under pathological brain conditions (7, 10, 15). Therefore, a clinical interpretation of localized activations on fMRI remains complicated and controversial.

Magnetoencephalography (MEG) reflects intracellular electric current flow in the brain and allows accurate localization of the current dipole sources. Dipoles of MEG deflections that peaked at approximately 400 milliseconds after word presentation (late responses) have been observed to localize in the temporoparietal regions. These late responses have been considered to be related to the receptive language function (19, 20). We have also observed dense dipole clusters of the semantic late responses in the superior temporal gyrus (STG), supramarginal gyrus (SmG), and fusiform gyrus (FuG) of the suspected dominant hemisphere (11, 12). Therefore, we sought to use MEG not only as an additional diagnostic tool for identifying the language dominance, but also to localize the receptive language center.

In the present study, we describe a non-invasive method to locate the expressive and receptive language areas by co-utilizing fMRI and MEG. The language dominance determined by our method matched the results from the Wada test with 100% accuracy. The usefulness of the method was well demonstrated, especially in those patients who showed dissociated expressive and receptive language functions. The data show that this method is highly reliable and may be useful in the management of patients with brain lesions as well as in studying normal brain functions.

METHODS

Patients

The functional brain mapping using fMRI (with the verb generation task) and MEG was performed in 117 patients with brain lesions since August 1999 (>7 yr) after this project was approved by the Institutional Committee for Ethics (*Table 1*). fMRI studies with the abstract/concrete (A/C) categorization task were also performed in 106 patients. Ninety-seven patients also underwent the Wada test to confirm the dominant cerebral hemisphere for language functions. Six patients showed negative Wada test results owing to the steal effect of a large arteriovenous malformation (AVM) or an overdose. The final analyses were performed in 87 patients (48 men, 39 women), who underwent Wada test, fMRI, and MEG investigations. The mean age (± standard deviation) was 43.6 ± 14.1 years. The Edinburgh Handedness Inventory was used to estimate the patients' handedness (18). A written informed consent was obtained from the patient or his/her family before participation in the study.

Magnetic Resonance Protocols

Anatomic magnetic resonance imaging (MRI) and fMRI were performed during the same session with a 1.5-T whole-body magnetic resonance scanner with echo-planar capabilities and a standard whole-head transmit-receiver coil (Siemens Vision, Erlangen, Germany). During the procedures, foam cushions were used to immobilize the head.

Language fMRI

The patients were instructed to respond to all language tasks silently. fMRI data was acquired with a T2-weighted echo-planar imaging sequence (echo time, 62 ms; repetition time, 114 ms; flip angle, 90 degrees; slice thickness, 4 mm; slice gap, 2 mm; field of view, 260 mm; matrix, 64 × 128; 14 slices). Each fMRI session consisted of three dummy scan volumes followed by three activation and four baseline (rest) periods. During each period, five echo-planar imaging volumes were collected, yielding a total of 38 imaging volumes and 2 minutes 32 seconds in measurement time for each session. fMRI data of language-related semantic responses were acquired as follows. All subjects were examined with two different lexical semantic language paradigms; verb generation by listening to nouns and A/C categorization by reading words. All words for semantic tasks were selected from common Japanese words listed in the electronic dictionary of the National Institute for Japanese Language.

Verb Generation Task

For the auditory stimuli (duration ranges were between 400 and 600 ms), common concrete nouns spoken by a native Japanese speaker with a flat intonation were recorded and digitized with a sampling rate of 44,000 Hz. A backward playback of the sound files (reference sounds) was used to eliminate the primary auditory activation during the rest periods with the same inter-stimuli intervals (1600–2400 ms) as the active periods. The auditory stimuli were delivered binaurally via two 5-m-long plastic tubes terminating at a headphone. The sound intensity was approximately 95 dB sound pressure level at the subject's ear. Subjects were instructed to silently generate a verb related to each presented noun during the active periods and passively listen to the reference sounds during the rest periods.

A/C Categorization Task

Visual stimuli were presented on a liquid crystal display monitor with a mirror above the head coil allowing the patients to see the stimuli. Words consisting of three *Kana* letters (Japanese phonetic symbols) were presented in a 300-millisecond exposure time with interstimuli intervals ranging from 2800 to 3200 milliseconds. Patients were instructed to categorize the presented word silently into "abstract" or "concrete" based on the