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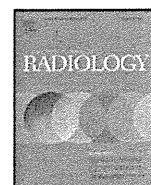
Role of perfusion-weighted imaging at 3 T in the histopathological differentiation between astrocytic and oligodendroglial tumors

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ABSTRACT

Objective: The differentiation of oligodendroglial tumors from astrocytic tumors is important clinically, because oligodendroglial tumors are more chemosensitive than astrocytic tumors. This study was designed to clarify the usefulness of 3 T MR perfusion imaging (PWI) in the histopathological differentiation between astrocytic and oligodendroglial tumors. This is because there is a growing interest in the diagnostic performance of 3 T MR imaging, which has the advantages of a higher signal-to-noise ratio (SNR) and greater spatial and temporal resolution.
Materials and methods: This study retrospectively included 24 consecutive patients with supratentorial, WHO grade II and III astrocytic and oligodendroglial tumors (7 astrocytic, 10 oligoastrocytic, and 7 oligodendroglial tumors) that were newly diagnosed and resected between November 2006 and December 2009 at Hiroshima University Hospital. These patients underwent dynamic susceptibility contrast-enhanced (DSC) PWI relative cerebral blood volume (rCBV) measurements before treatment. Astrocytic tumors were designated as the astrocytic group, and oligoastrocytic and oligodendroglial tumors as the oligodendroglial group. The regions of interest with the maximum rCBV values within the tumors were normalized relative to the contra-lateral white matter (rCBVmax).
Results: The average rCBVmax of astrocytic tumors (2.01 ± 0.68) was significantly lower than that of the oligoastrocytic (4.60 ± 1.05) and oligodendroglial tumors (6.17 ± 0.867) ($P < 0.0001$). A cut-off value of 3.0 allowed to differentiate the oligodendroglial group from the astrocytic group at 100% sensitivity and 87.5% specificity.
Conclusion: The rCBVmax values obtained from 3 T MR PWI may be useful as an adjunct to the postoperative histopathological diagnosis of glioma patients.

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1. Introduction

The differentiation of gliomas containing oligodendroglial components from astrocytic tumors is important clinically, because oligodendroglial and oligoastrocytic tumors are more chemosensitive than astrocytic tumors [1]. However, the differentiation of these tumors is sometimes difficult, because of the ambiguous criteria. Although a histopathological evaluation remains the reference standard for the histological subtyping of gliomas, this method is limited by issues such as subjective criteria, heterogeneity within tumors, and tissue sampling errors. Attempts have been made to enhance the prognostic information obtained by the histological classification of gliomas. Therefore, additional supportive information is required histopathological classification. Recently, dynamic susceptibility contrast-enhanced (DSC) MR perfusion imaging (PWI) has been used as part of a combined approach with histopathology in order to help to accurately subtype brain tumors [2,3].

There is evidence that MR PWI may be a sensitive marker of the microvascular density in gliomas. Relative cerebral blood volume (rCBV) measurements are closely correlated with angio-

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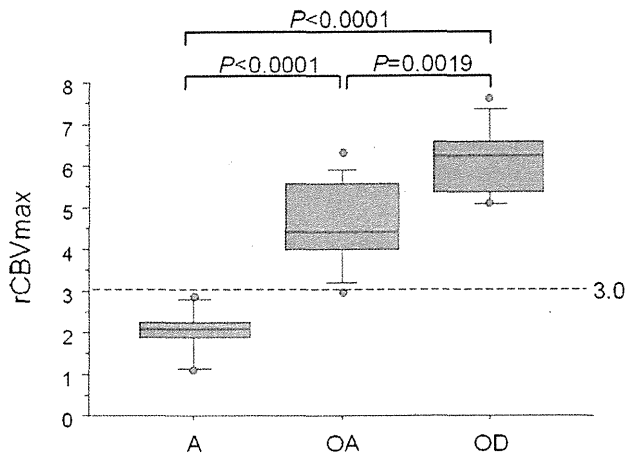[3] Tel: +81 3 3353 8111; fax: +81 3 5269 7438.

Fig. 1. Box plots of rCBVmax values for astrocytic tumors, oligoastrocytic tumors, and oligodendroglial tumors along with their respective standard deviation values. The cut-off line of 3.0 yields 100% sensitivity and 87.5% specificity for differentiating the oligodendroglial group (oligoastrocytic tumors and oligodendroglial tumors) from the astrocytic group. A, astrocytic tumors; OA, oligoastrocytic tumors; OD, oligodendroglial tumors.

graphic and histologic markers of tumor vascularity [4]. Perfusion image-based brain tumor characterization is currently performed by assessing the maximum ratio between an rCBV area within the glioma and the unaffected contralateral rCBV value (rCBV-max) [5]. Oligodendrogliomas often have an attenuated network

of branching capillaries resembling a chicken wire pattern. Most oligodendroglial tumors (oligodendrogliomas and oligoastrocytomas) exhibit higher rCBVmax values than astrocytic tumors [3].

Some reports have demonstrated the usefulness of PWI in the assessment of hemodynamics in gliomas and differentiation of oligodendroglial tumors from astrocytic tumors [3]. However, these reports used 1.5 T MR imaging, and significant overlaps of rCBV-max values were observed between astrocytic and oligodendroglial tumors. Recently, with the integration of 3 T imaging into clinical practice, there is a growing interest in the diagnostic performance of 3 T MR imaging techniques with respect to the established magnetic field strength of 1.5 T [6,7]. MR performed at higher magnetic field strengths has the advantages of higher signal-to-noise ratio (SNR) and magnetic susceptibility; this results in improved spatial and temporal resolution [6,7]. Therefore, these advantages may help improve the accuracy of differential diagnoses of gliomas. Therefore, this study was designed to characterize the hemodynamics within gliomas by using 3 T MR PWI and clarify whether this approach help the postoperative histopathological diagnosis in the differentiation between astrocytic and oligodendroglial tumors.

2. Materials and methods

2.1. Patients and histological diagnosis

This study retrospectively included 24 consecutive patients with supratentorial, WHO grade II and III astrocytic and oligodendroglial tumors that were newly diagnosed and resected between November 2006 and December 2009 at Hiroshima University
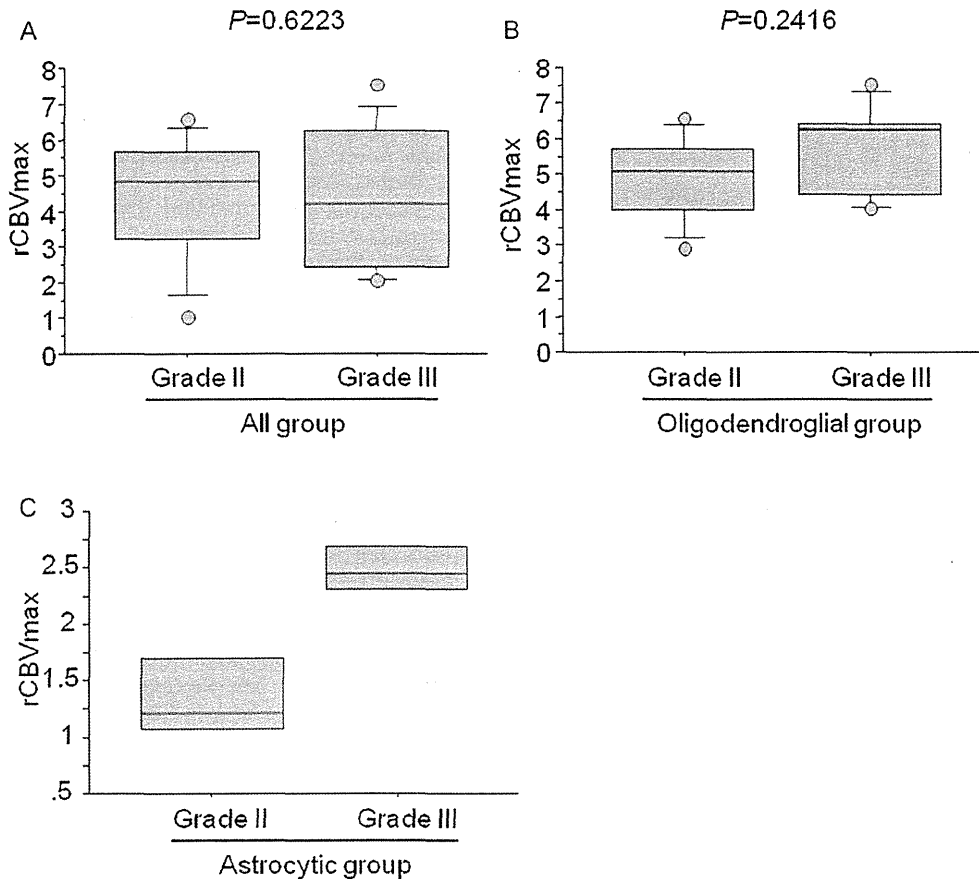

Fig. 2. (A) Box plots of rCBVmax values for grade II and III tumors in the astrocytic and oligodendroglial groups along with their respective standard deviation values. (B) Box plots of rCBVmax values for grade II and III tumors in the oligodendroglial group along with their respective standard deviation values.

Table 1
Patient demograhics, histologic diagnosis, and rCBVmax values.

Patient	Sex	Age	Histology	rCBVmax
Astrocytic group				
1	F	45	Diffuse astrocytoma	1.03
2	M	50	Difluse astrocytoma	1.86
3	M	51	Diffuse astrocytoma	1.21
4	M	42	Anaplastic astrocytoma	2.12
5	F	64	Anaplastic astrocytoma	2.85
6	M	44	Anaplastic astrocytoma	2.03
7	M	11	Anaplastic astrocytoma	2.25
Oligodendroglial group				
8	F	49	Oligoastrocytoma	5.60
9	M	58	Oligoastrocytoma	5.72
10	F	49	Oligoastrocytoma	4.02
11	M	48	Oligoastrocytoma	3.52
12	M	51	Oligoastrocytoma	4.63
13	F	34	Oligoastrocytoma	2.93
14	M	81	Anaplastic oligoastrocytoma	5.00
15	F	68	Anaplastic oligoastrocytoma	4.04
16	M	19	Anaplastic oligoastrocytoma	4.22
17	F	24	Anaplastic oligoastrocytoma	6.23
18	M	31	Oligodendroglioma	5.12
19	M	35	Oligodendroglioma	6.60
20	M	47	Oligodendroglioma	6.19
21	M	40	Oligodendroglioma	5.06
22	M	81	Anaplastic oligodendroglioma	6.46
23	F	48	Anaplastic oligodendroglioma	7.55
24	M	34	Anaplastic oligodendroglioma	6.23

Hospital. The study population comprised 16 males and 8 females; the median age was 46.0 ± 16.9 (11–81 years). Surgical specimens were histologically examined by an experienced neuropathologist (YT, 24 years of experience) blinded to clinical and MRI data. The histopathological assessment, carried out according to the criteria of the revised World Health Organization (WHO) classification, revealed 7 astrocytic tumors (3 grade II diffuse astrocytomas and 4 grade III anaplastic astrocytomas), 10 oligoastrocytic tumors (6 grade II oligoastrocytomas and 4 grade III anaplastic oligoastrocytomas), and 7 oligodendroglial tumors (4 grade II oligodendrogliomas and 3 grade III anaplastic oligodendrogliomas). For statistical purposes, the astrocytic tumors (7 patients) were designated as the astrocytic group, and the oligoastrocytic tumors and oligodendroglial tumors (17 patients) as the oligodendroglial group.

The study protocol was approved by the institutional review board of our institution; written informed consent from the patients was waived. To protect patient privacy, we removed all identifiers from our records upon the completion of our analyses.

2.2. Conventional MR imaging

MR examinations were performed using a 3 T system (Signa EXCITE 3.0 T; GE Medical Systems, Milwaukee, WI, USA) with a circularly polarized head coil. All patients underwent MRI studies that included at least unenhanced and contrast-enhanced transverse T1-weighted-, unenhanced transverse T2-weighted-, and unenhanced transverse fluid-attenuated inversion-recovery (FLAIR) images. The transverse T1-weighted spin-echo MR sequence was performed using the following parameters: repetition time ms/echo time ms, 400/17; field of view (FOV), 22 cm × 22 cm; matrix size, 288 (frequency), 192 (phase); section thickness, 6 mm; section gap, 1.0 mm; signal acquired, 2. The contrast-enhanced T1-weighted sequences were obtained after administering 0.1 mmol gadolinium compound per kg body weight. The transverse fast spin-echo T2-weighted sequence was performed using the following parameters: repetition time ms/echo time ms, 4800/100; FOV, 22 cm × 22 cm; matrix size, 512 × 320; echo train length, 18; section thickness, 6 mm; section gap, 1.0 mm; 2 signals.

Transverse FLAIR images were acquired using fast and interleaved multi-section sequences with the following parameters: repetition time ms/echo time ms, 10,000/150; inversion time, 2400 ms; FOV, 22 cm × 22 cm; matrix size, 288 × 160; echo train length, 16; section thickness, 6 mm; section gap, 1.0 mm; 1 signal.

2.3. Perfusion imaging and region of interest analysis

PWI was obtained with a single-shot, gradient-echo-type echo-planar imaging (section thickness, 6 mm; gap, 1.0 mm; TR, 2000 ms; TE, 40 ms; FOV, 26 cm × 22 cm; matrix, 128 × 128; NEX, 1; slice, 15; phase, 40; acquisition time, 1 min 20 s). After the first 5 acquisitions, a bolus of gadolinium (0.1 mmol/kg) was injected with a mechanical injection pump (3 ml/s) through a 20-gauge intravenous catheter, followed by a 20-ml saline flush as previously reported [8]. The images were inspected for overall image quality and motion artifacts and were then transferred to a workstation for post-processing with a dedicated software package (Functool Performance, GE Medical Systems). On a voxel-by-voxel basis, the software calculated the curves of signal intensity changes over time, T_2^* relaxation rate (ΔR_2^*), the baseline to be subtracted from the ΔR_2^* curve, and area under the fitted ΔR_2^* curve. The ΔR_2^* was calculated using the equation: $\Delta R_2^* = -\ln(S_t/S_0)/\text{TE}$, where S_t is the signal intensity at time t, S_0 is the precontrast signal intensity (excluding the first one or two images acquired while reaching the steady-state MR signal), and TE is the echo time. The baseline was estimated by calculating the average background intensity prior to onset and after completion of the transient signal intensity change. Color-coded rCBV maps were generated by the numerical integration of ΔR_2^* for the first-pass bolus through each pixel on the basis of kinetic principles for non-diffusible tracers.

For each slice, one region of interest (ROI) of 50 mm^2 was placed within the tumor on the area showing the most elevated CBV on color perfusion maps. Six more ROIs were positioned in the contralateral normal white matter. Margins of gliomas were delineated on T2-weighted and FLAIR images. To minimize confounding factors in relative CBV analysis, the size of the ROIs were kept constant for all tumors. Special attention was given to avoid placing ROIs over blood vessels—especially larger ones. The maximum CBV

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values of all intra-tumoral ROIs, and the mean CBV of the contra-lateral ROIs were calculated. The rCBVmax values were then expressed as a ratio of the maximum rCBV value within the tumor to the mean CBV value in the contra-lateral normal appearing white matter. This approach has been demonstrated to provide the best inter- and intra-observer reproducibility [5]. Two authors (TS and FY, with 14 and 18 years of experience in brain MR imaging, respec-tively) who were blinded to the histological diagnosis, identified the ROIs after arriving at a consensus.

2.4. Statistical analysis

Data were analyzed using the SPSS (version 16.0) software package (SPSS Inc., Chicago, IL, USA). Multiple comparison tests, including the Tukey–Kramer test, were used for statistical com-parison of the rCBVmax values between astrocytic, oligoastrocytic, and oligodendroglial tumors. Correlations between the subgroups of histology and patients characteristics (age, sex, and rCBVmax value) were calculated using Fisher's exact probability test. The Mann–Whitney test was used for statistical comparison of rCBV-max values between grade II and III tumors in all groups, as well as grade II and III tumors in the oligodendroglial group. A P value < 0.05 indicated a statistically significant difference.

3. Results

The demographics of the patient population, histologic diagno-sis, and rCBVmax values are shown in Table 1. The average rCBVmax of astrocytic tumors (2.01 ± 0.68) was significantly lower than that of oligoastrocytic tumors (4.60 ± 1.05) and oligodendroglial tumors (6.17 ± 0.867; Fig. 1). Furthermore, the average rCBVmax of oligodendroglial tumors was significantly greater than that of the oligoastrocytic tumors. A threshold value of 3.0 allowed dif-ferentiation between the oligodendroglial and astrocytic groups at 100% sensitivity and 87.5% specificity with only one over-lapped patient (Fig. 1; Table 2).

No significant correlation was observed between the subgroups of histology, age (cut-off, mean age: 46 years), and sex (Table 2). No significant differences were observed in the average rCBVmax between grade II and III tumors in the two groups or grade II and III tumors in the oligodendroglial group (Fig. 2A and B). However, the rCBVmax values of anaplastic astrocytomas tended to be greater than those of diffuse astrocytomas (Fig. 2C). Representative images are shown in Figs. 3–5.

4. Discussion

Differentiating oligodendroglial tumors from astrocytic tumors is often difficult by anatomic MR imaging. This is because the MR images suffer from non-specificity and the inability to depict any-thing beyond morphologic aberration. However, with the addition of non-anatomic, physiology-based MR imaging methods such as perfusion MR imaging, more sophisticated tumor characterizations that can provide insight into underlying biological characteristics are now possible. Previous reports showed that most oligoden-droglial tumors exhibit higher rCBVmax values than astrocytic tumors, irrespective of tumor grade [3]. Histopathologically, Schif-fer et al. described the unique vascularity of oligodendrogliomas, in which an increased vascular density was observed in both low and high-grade oligodendrogliomas [9]. Subsequently, Lev et al. suggested that different perfusion patterns in astrocytic and oligo-dendroglial tumors are probably associated with the fine capillary network typically observed in oligodendrogliomas [3]. However, previous studies evaluated these tumors using 1.5 T MR imaging and found significant overlaps of the rCBV values between astro-

cytic and oligodendroglial tumors [2,3,10]. Our data demonstrated that the rCBVmax value obtained from 3 T MR PWI could differ-entiate the oligodendroglial group from the astrocytic group with less overlaps. This is the first reported study to clarify the role of 3 T MR PWI in the differentiation between astrocytic and oligoden-droglial tumors. The cut-off ratio of 3.0 yielded 100% sensitivity and 87.5% specificity for the differentiation of the oligodendroglial group from the astrocytic group. We hypothesize that the differ-ence between previous results and those of the current study are due to the advantages of using 3 T MR imaging.

DSC PWI benefits from the advantages of 3 T MR imaging [7,11]. The increased SNR and magnetic susceptibility of 3 T compared to those of 1.5 T results in images of brain tumors that are far more reliable [7]. Data can be obtained using smaller voxels (enhanced spatial resolution) at shorter time intervals (enhanced time reso-lution) by exploiting the increased SNR offered by 3 T MR systems. Furthermore, the frequent use of T_2^* sequences in perfusion studies also offers some potential advantages. In particular, the increased susceptibility effect in addition to shorter relaxation times at 3 T results in increased sensitivity to transitory T_2^* changes due to the bolus of contrast medium during capillary passage [6]. It has been suggested that these advantages allow the evaluation to be sensi-tive to the fine capillary network of oligodendroglial tumors and could lead to a clear differentiation between the oligodendroglial and astrocytic groups.

Differentiating oligodendroglial tumors from astrocytic tumors is important clinically because these two tumor types are well-defined, clinicopathological entities with distinct biological and prognostic characteristics [1]. Patients with oligodendroglial tumors are more likely to respond to chemotherapy and enjoy a longer progression-free survival than patients with astro-cytic tumors of comparable malignancy grade [12]. However, neither clear diagnostic criteria nor reliable markers exist for oligodendroglioma—in part, this diagnosis rests on subjective crite-ria. The clinical need to distinguish oligodendroglial from astrocytic tumors appears to have resulted in a widening of the histolog-ical criteria for oligodendroglial tumors and an increase in the frequency of oligodendroglial tumors as well as a decrease in astrocytic tumors [13]. Consequently, the number of patients with astrocytic tumors in this study was low. Furthermore, the distinc-tion between mixed oligoastrocytic tumors and oligodendroglial or astrocytic tumors is difficult and varies between different pathologists [13]. Some of the difficulties in the classification of oligoastrocytic tumors include the definition of oligodendroglial vs. astrocytic differentiation at the histological level and the mini-mal amount of oligodendroglial or astrocytic tumor cells required for the diagnosis of oligoastrocytic tumors [14]. The current data showed that the average rCBVmax of oligodendroglial tumors was significantly higher than that of oligoastrocytic tumors. Recently, Chang et al. reported the rCBV data of 134 patients with supra-tentorial gliomas. Their data showed the normalized median CBV values of 21 non-enhanced oligodendrogliomas (1.24, 0.83–2.27) to be higher than those of 13 non-enhanced oligoastrocytomas (0.95, 0.59–1.37) [15]. These results suggest that the more oligoden-droglial component a tumor has, the higher is the rCBVmax value observed in the tumor. Therefore, the rCBVmax values obtained from 3 T MR PWI may also help to the postoperative histopatho-logical diagnosis in the differentiation between oligoastrocytomas and oligodendrogliomas.

The current study found no significant difference in the average rCBVmax value between grade II and III tumors in the astrocytic and oligodendroglial groups. Several previous studies have sug-gested that rCBV measurement may improve the grading of gliomas [3,16–18]. However, Lev et al. and Arvinda et al. indicated that low-grade oligodendrogliomas show high rCBV foci and thus confound the reliability of rCBV values in distinguishing high-grade gliomas
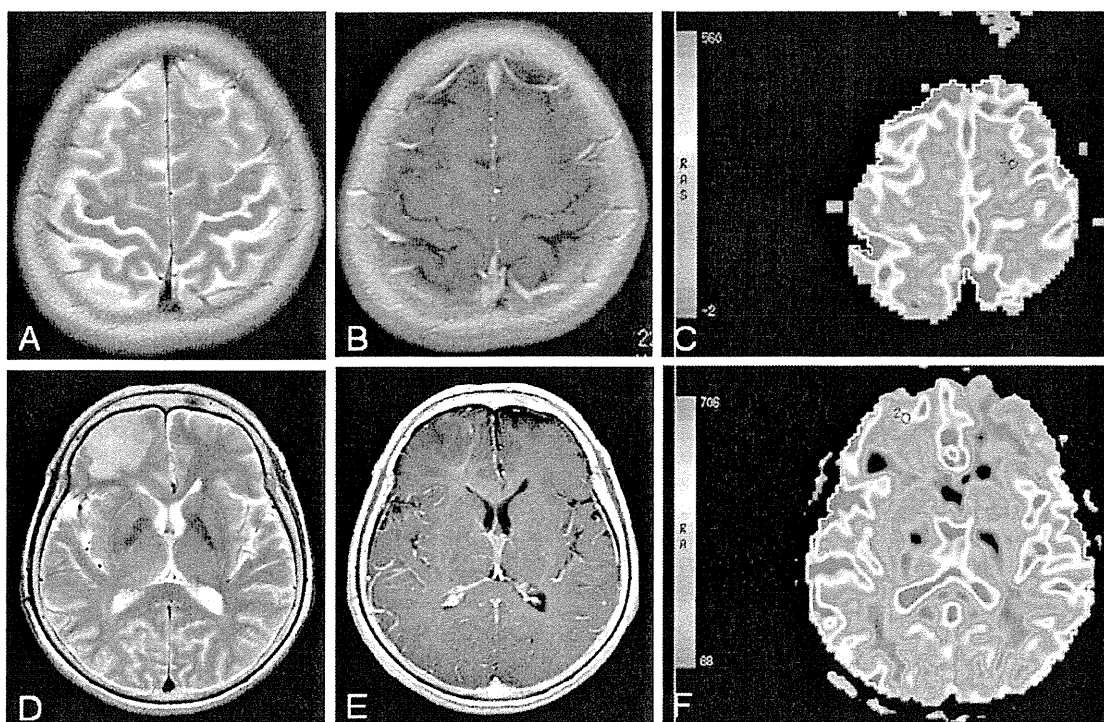
Fig. 3. (A–C) A representative case of a diffuse astrocytoma. (A) Axial transverse T2-weighted image shows a left frontal tumor. (B) Axial contrast-enhanced transverse T1-weighted image shows the tumor without enhancement. (C) The rCBV map shows that the rCBVmax value of the tumor is 1.03. (D–F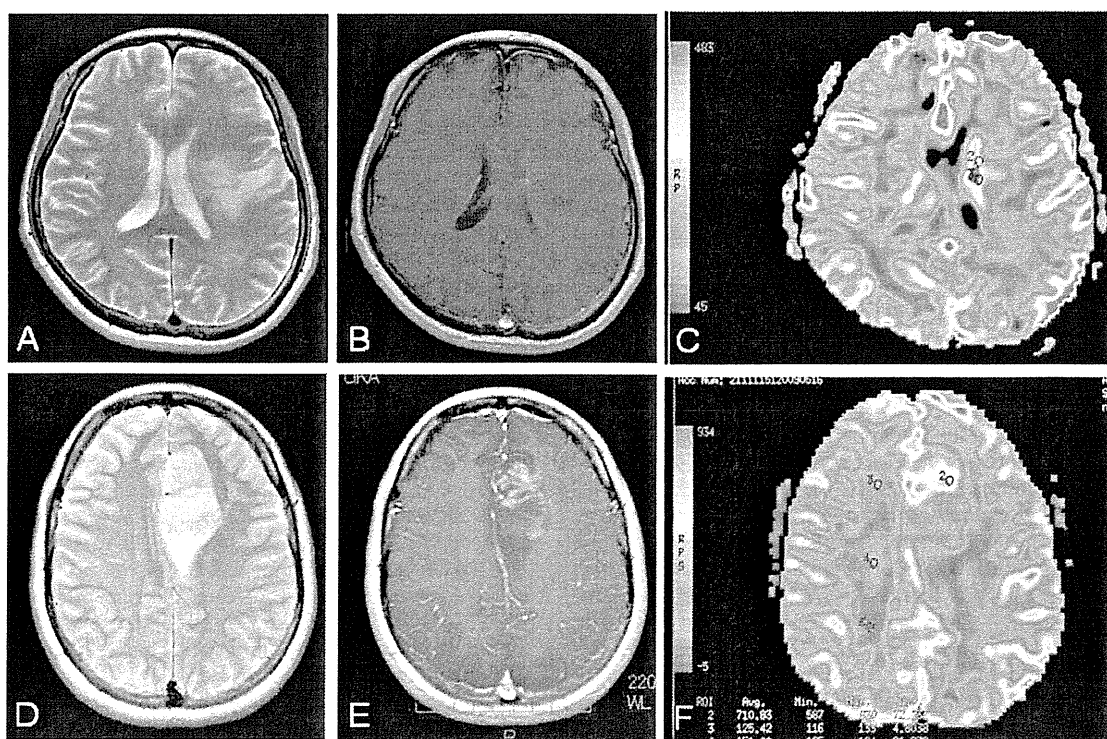) Representative case of an anaplastic astrocytoma. (D) Axial transverse T2-weighted image shows a right frontal tumor. (E) Axial contrast-enhanced transverse T1-weighted image shows the tumor with partial enhancement. (F) The rCBV map shows that the rCBVmax value of the tumor is 2.85.

Fig. 4. (A–C) A representative case of an oligoastrocytoma. (A) Axial transverse T2-weighted image shows a left frontal tumor. (B) Axial contrast-enhanced transverse T1-weighted image shows the tumor with partial enhancement. (C) The rCBV map shows that the rCBVmax value of the tumor is 5.72. (D–F) A representative case of an anaplastic oligoastrocytoma. (D) Axial transverse T2-weighted image shows a left frontal tumor. (E) Axial contrast-enhanced transverse T1-weighted image shows the tumor with partial enhancement. (F) The rCBV map shows that the rCBVmax value of the tumor is 6.23.

Table 2
Subgroups of histology and patients characteristics.

	Subgroups of histology			P value
	Astrocytic group	Oligodendroglial group	Total	
Number of patients	7	17	24	
Age (mean)				0.6591
<46 years	4	7	11	
>46 years	3	10	13	>0.9999
Sex				
Male	5	11	16	
Female	2	6	8	
rCBVmax				0.0001*
<3.0	7	1	8	
>3.0	0	16	16	

Statistical analysis performed by Fisher's exact probability test.
* $P < 0.05$.

from low-grade ones [3,19]. Furthermore, the current study found no significant difference in the average rCBVmax between grade II and III tumors in the oligodendroglial group. Spampinato et al. analyzed the rCBV values of 22 patients with oligodendrogliomas or oligoastrocytomas and suggested that rCBV measurements are helpful in differentiating between low-grade and anaplastic oligodendroglial tumors [20]. However, their study included 5 patients with recurrent tumors after treatment (23%, 5/22 patients). Therefore, the inclusion of recurrent tumors in their study may be the cause of the differences between that study and the current one. The present series suggested that the grading of oligodendroglial tumors is difficult to make using rCBV assessments alone, due to their unique histological components. On the other hand, in the current study, the rCBVmax values of anaplastic astrocytomas tended to be higher than those of diffuse astrocytomas. Lev et al.

also found a significant positive correlation between the astrocytic tumor grade and rCBV values [3]. Therefore, our and their results suggest that glioma grading based on rCBV measurement should be examined while excluding oligodendroglial tumors.

Our study has some limitations. First, the study population was rather small at 24 patients; larger prospective studies should be planned in order to achieve more definitive conclusions regarding the use of rCBVmax values for patient management. Second, there was no direct correlation between the focus of the rCBVmax value and the histopathological correlation. Further investigation aiming to directly correlate imaging with the histopathological findings, such as the tumor removal with stereotactic technique, will strengthen the validity of rCBVmax values using 3 T MR PWI as a noninvasive imaging marker of tumor subtype. Third, oligodendroglial tumors have exhibited relevant genetic profiling with
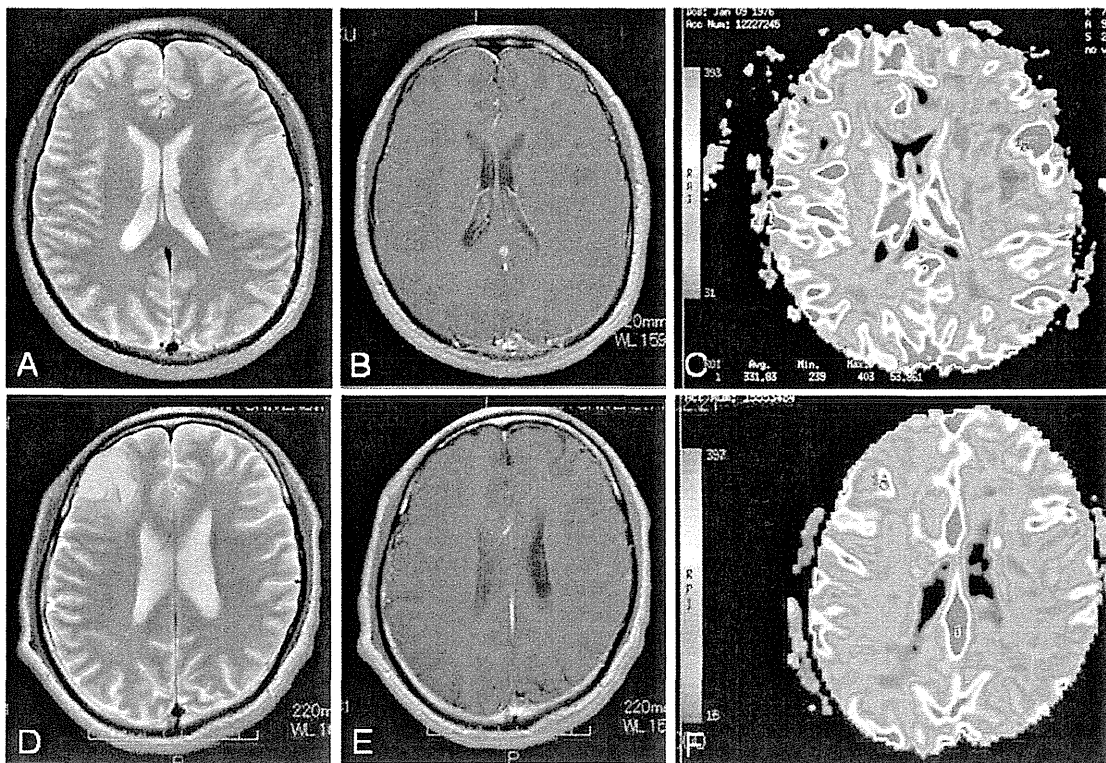

Fig. 5. (A–C) A representative case of an oligodendroglioma. (A) Axial transverse T2-weighted image shows a left frontal tumor. (B) Axial contrast-enhanced transverse T1-weighted image shows the tumor with faint partial enhancement. (C) The rCBV map shows that the rCBVmax value of the tumor is 5.12. (D–F) A representative case of an anaplastic oligodendroglioma. (D) Axial transverse T2-weighted image shows a right frontal tumor. (E) Axial contrast-enhanced transverse T1-weighted image shows the tumor without enhancement. (F) The rCBV map shows that the rCBVmax value of the tumor is 6.23.

1p/19q loss of heterozygosity. However, we did not analyze the relationship between our findings and the genetics of such tumors. In the future, further investigations aiming to correlate our findings with genetics are needed.

5. Conclusions

The present study is the first report to evaluate the role of 3 T MR PWI in differentiating between astrocytic and oligodendroglial tumors. The results showed that the rCBVmax values obtained from 3 T MR PWI may differentiate the oligodendroglial group from the astrocytic group with high sensitivity and specificity. Although only 3 T MR PWI could not clearly differentiate between these groups preoperatively, this approach may be useful as an adjunct to a post-operative histopathological diagnosis in patients presenting with gliomas.

Conflict of interest

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

Acknowledgement   -

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ORIGINAL ARTICLE

A Multicenter Phase I/II Study of the BCNU Implant (Gliadel® Wafer) for Japanese Patients with Malignant Gliomas

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Abstract

Carmustine (BCNU) implants (Gliadel® Wafer, Eisai Inc., New Jersey, USA) for the treatment of malignant gliomas (MGs) were shown to enhance overall survival in comparison to placebo in controlled clinical trials in the United States and Europe. A prospective, multicenter phase I/II study involving Japanese patients with MGs was performed to evaluate the efficacy, safety, and pharmacokinetics of BCNU implants. The study enrolled 16 patients with newly diagnosed MGs and 8 patients with recurrent MGs. After the insertion of BCNU implants (8 sheets maximum, 61.6 mg BCNU) into the removal cavity, various chemotherapies (including temozolomide) and radiotherapies were applied. After placement, overall and progression-free survival rates and whole blood BCNU levels were evaluated. In patients with newly diagnosed MGs, the overall survival rates at 12 months and 24 months were 100.0% and 68.8%, and the progression-free survival rate at 12 months was 62.5%. In patients with recurrent MGs, the progression-free survival rate at 6 months was 37.5%. There were no grade 4 or higher adverse events noted due to BCNU implants, and grade 3 events were observed in 5 of 24 patients (20.8%). Whole blood BCNU levels reached a peak of 19.4 ng/mL approximately 3 hours after insertion, which was lower than 1/600 of the peak BCNU level recorded after intravenous injections. These levels decreased to less than the detection limit (2.00 ng/mL) after 24 hours. The results of this study involving Japanese patients are comparable to those of previous studies in the United States and Europe.

Key words: BCNU implant, Gliadel® Wafer, malignant gliomas, phase I/II study, pharmacokinetic

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Introduction

Malignant gliomas (MGs) are highly malignant cancers with 5-year survival rates of 25% or less.[1] The outcomes of MG treatments have been unsatisfactory, and drugs available in Japan for the treatment of MGs are limited to certain chemotherapeutic agents such as temozolomide (TMZ, Temodar®; Merck, Whitehouse Station, New Jersey, USA). There is no standard method for the treatment of recurrent MGs.

A BCNU implant is a controlled-release preparation of carmustine (BCNU; an alkylation agent of the nitrosourea family) that is inserted into the brain. BCNU was first approved in 1979 in the United States (USA) for the treatment of multiple myeloma and other conditions. Because this drug is highly lipid-soluble and can cross the blood-brain barrier effectively, it has been used primarily by injection for the treatment of brain tumors in USA and Europe.

Conventional BCNU preparations were effective against brain tumors; however, increasing the dose level to achieve a higher efficacy caused severe adverse systemic reactions (bone marrow suppression, lung toxicity, etc.). A BCNU implant is a sterile disc-like formulation (approximately 14.0 mm in diameter and approximately 1.3 mm in thickness) containing BCNU. Under moisture-rich conditions, the biodegradable component of the preparation is gradually hydrolyzed leading to release of the active ingredient BCNU, which exerts an anti-tumor effect (Fig. 1). If this preparation is inserted in the vicinity of residual tumor tissue during surgical resection of MGs, the tumor cells can be directly and efficiently exposed to high levels of BCNU for a certain period of time starting immediately after surgery while avoiding bone marrow suppression, lung toxicity, and other negative effects. This preparation is thus expected to be beneficial for diminishing residual tumors and
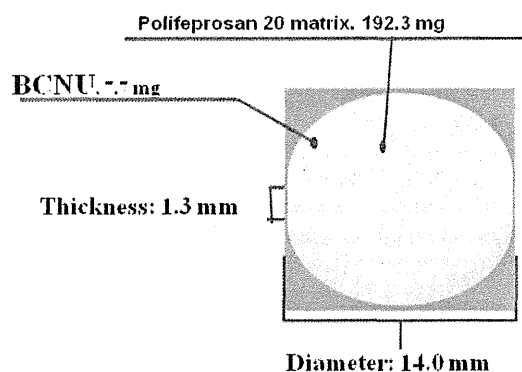
suppressing tumor growth. In a placebo-controlled, double-blind comparative study of patients with recurrent MGs, Brem et al.[2] reported that the cumulative death rate of glioblastoma (GBM) patients during the 6-month post-BCNU implant period was significantly lower than that in the placebo group (P = 0.013). In a placebo-controlled, double-blind comparative study of patients with newly diagnosed MGs, Valtonen et al.[3] reported that the survival rates of patients receiving BCNU implants were significantly higher than those of patients in the placebo group during the 12-month implant insertion period (P = 0.029). Westphal et al.[4] reported that the survival period was extended significantly by this preparation (P = 0.027). In these studies, the safety profile of BCNU implants was comparable to that of placebo, and no severe adverse events (bone marrow suppression, pulmonary fibrosis, etc.) due to BCNU implants were noted. On the basis of these clinical results, the BCNU implant is now recommended as an additional postoperative therapy for MGs in the treatment guidelines prepared by the National Comprehensive Cancer Network[5] and The National Cancer Institute (USA)[6] as well as the treatment guidelines prepared by the National Institute for Health and Clinical Excellence (UK).[7] However, in these clinical studies, radiotherapy was primarily utilized as concomitant therapy after BCNU implantation. These clinical studies were conducted between 1990 and 2002, and during that period, TMZ was approved only for the treatment of recurrent anaplastic astrocytoma. Therefore, combined therapy involving TMZ plus radiotherapy for newly diagnosed cases was not approved. In recent years, TMZ is often used as the standard therapy for MGs in combination with radiotherapy, and bevacizumab (BEV, Avastin®; Genentech, San Francisco, California, USA), an antivascular endothelial growth factor antibody. Therefore, it has recently been attracting attention as a new potential treatment for recurrent MGs. Retrospective reports on the safety and efficacy of BCNU implants in combination with these new treatments is available, but no prospective study has been carried out in compliance with Good Clinical Practice. Furthermore, the BCNU exposure level *in vivo* and the timing of its disappearance following insertion into the brain remain unknown. To evaluate the efficacy, safety, and pharmacokinetics of the BCNU implant combined with chemotherapy and radiation therapy after its insertion into the removal cavity in Japanese patients with MGs (newly diagnosed MGs and recurrent GBM), a prospective, uncontrolled, open-label, multicenter phase I/II study (NPC-08 study) was carried out from 2009

Polifeprosan 20 matrix. 192.3 mg

BCNU -.- mg

Thickness: 1.3 mm

Diameter: 14.0 mm

Fig. 1   BCNU implant configuration.

to 2012 after acquisition of the approval from the institutional review board of each participating facility. This paper will present the results of the survival survey conducted over 24 months after insertion of the BCNU implant, evaluations during the first 12 months after insertion, and the results of simultaneous BCNU blood level measurements.

Materials and Methods

This study (NPC-08 study) was carried out in compliance with ethical principles based on the Declaration of Helsinki, the study protocol, and Good Clinical Practice. Informed consent for treatment and postoperative follow-up was obtained from all patients. NPC-08 study was registered with ClinicalTrials.gov (number NCT00919737).

I. Patients

The study enrolled patients satisfying all of the following requirements: (1) presence of tumorous lesions in the cerebral parenchyma confirmed by magnetic resonance imaging (MRI), (2) age over 18 and less than 65, (3) Karnofsky performance status (KPS) 60 or over, and (4) histological suspicion of newly diagnosed MGs or recurrent GBM by intraoperative pathological diagnosis. Patients with recurrent GBM were enrolled in the study only when they had received prior conventional radiotherapy. The histopathological diagnosis was reviewed by a central pathological assessment committee separate from the participating facilities to ensure diagnosis by a third party. The required number of cases (24 cases) was defined under the consideration for previously reported adverse events in overseas (CSF leakage etc.). Concerning the required number (24 cases), each recurrent and newly diagnosed

MGs should include at least 8 cases to detect the expected side effect.

II. Procedures

A maximum of 8 sheets of BCNU implants were inserted into the removal cavity during surgery (maximum of 61.6 mg BCNU). Re-insertion during the study period was prohibited. On the 14th day following BCNU implant insertion, patients with newly diagnosed MGs received concomitant therapy, i.e., the standard therapy proposed by Stupp et al.[8] involving TMZ (75 mg·m-2day-1) plus radiation (60 Gy) for a maximum period of 6–7 weeks and adjuvant TMZ therapy with 1 cycle consisting of 5-day consecutive TMZ administration (150–200 mg·m^{-2}day^{-1}) and a subsequent 23-day cessation. For patients with recurrent GBM, appropriate adjuvant chemotherapy [e.g., chemotherapy with TMZ alone or TMZ plus Interferon- (INF-β)] was permitted (Fig. 2).

In this study, first we evaluated the status of the occurrence of adverse events carefully in a small number of patients (6 patients) at the efficacy and safety evaluation committee, and then, based on the judgment of the committee, we moved to a multicenter study with larger sample size.

Methods and Statistical Analyses

For efficacy evaluation, the overall survival (OS) rate at 24 months after insertion, median overall survival (mOS) period, and progression-free survival (PFS) rate at 12 months after insertion of BCNU implants were calculated by the Kaplan-Meier method. The following two population groups were an effective analysis set and are defined as a full analysis set (FAS) unless otherwise specifically noted:
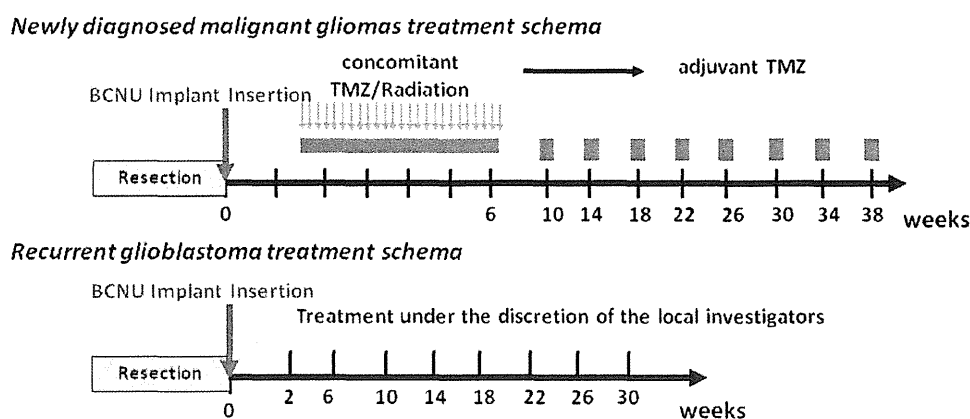

Fig. 2 Treatment schema. TMZ: temozolomide.

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(a) An FAS group consisting of all subjects enrolled in the clinical study excluding those who never underwent implantation with the present formulation and (b) A group verified to have GBM/other GBM (non-GBM) based on the central pathological diagnosis.

To evaluate PFS, tumor progression was rated based on the following criteria: the tumor was classified as progressive if its major diameter multiplied by its vertical dimension (short minor diameter) showed a > 25% increase in comparison with the preceding image showing the minimum value for each parameter or if any new lesion(s) appeared (McDonald criteria). Evaluation of MRIs was carried out by the efficacy and safety evaluation committee in accordance with the McDonald criteria, and the evaluators were blinded to the background variables of the subjects. For efficacy analysis, the OS rate, mOS period, PFS rate, and median PFS period were determined by the Kaplan-Meier method, and 95% confidence intervals (95% CIs) were calculated for each parameter. The OS time and PFS time were not analyzed in this study.

However, when OS and PFS were lower than 50% up to the cutoff time in a given patient population, a median survival period was calculated. One month was defined as 30 days, and 1 year was defined as 360 days. To determine safety profiles, adverse events and abnormal changes in laboratory parameters were evaluated until the 12th month in all patients who received BCNU implants in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Adverse events were classified in accordance with the Medical Dictionary for Regulatory Activities Japanese translation (MedDRA/J) version 14.0. The number of patients who experienced each event and the incidence of each event were analyzed in relation to severity. All evaluations performed by attending physicians were reviewed by the efficacy and safety evaluation committee.

For pharmacokinetic analysis, BCNU levels in the blood were measured periodically (before insertion and 3–6 hours, 24 hours, 72 hours, or 168 hours after insertion). Validation and BCNU measurement in blood samples were carried out by liquid chromatography-tandem mass spectrometry (LC/MS/MS) at Celerion Inc (Lincoln, Nebraska, USA).

Validation of the quantification method employed in this study confirmed good linearity of BCNU and the internal standard (d8-BCNU) within the range of quantification (2.00–100 ng/mL) (≥ 0.9952). The lower limit of quantitation was set at 2.00 ng/mL.

Table 1 Patient characteristics

		Newly diagnosed malignant gliomas (n = 16)	Recurrrent malignant gliomas (n = 8)
Age (years)	Mean	46.6	42.9
	SD	14.09	14.57
	Min	21	25
	Median	49.5	41
	Max	63	63
Male/Female		8/8	4/4
Preoperative tumor sizes	Mean	23.0	16.9
	SD	15.0	10.5
	Min	2.0	3.5
(cm²)	Median	22.5	22.6
	Max	62.4	26.3
Rate of tumor resection	Mean	91.9	87.3
	SD	8.5	17.0
	Min	80.0	55.0
(%)	Median	92.5	95.0
	Max	100	100
Number of BCNU implants	Mean	7.7	7.9
	SD	0.87	0.35
	Min	5.0	7.0
(sheets)	Median	8.0	8.0
	Max	8	8
Pre-insertion KPS score	60	1 (6.3)	0 (0.0)
	70	1 (6.3)	2 (25.0)
	80	4 (25.0)	1 (12.5)
(%)	90	7 (43.8)	3 (37.5)
	100	3 (18.8)	2 (25.0)
	≤70	2 (12.5)	2 (25.0)
	80≤	14 (87.5)	6 (75.0)
1st/2nd recurrence	1st	–	6 (75.0)
	2nd	–	2 (25.0)
History of medical treatment for tumor	Yes	–	7 (87.5)
	No	–	1 (12.5)

KPS: Karnofsky performance status.

Results

Table 1 outlines the patient characteristics. During this study, BCNU implants were inserted in a total

of 24 patients. At intraoperative pathological consultations, these 16 newly and 8 recurrent patients were diagnosed as MGs or GBM. However, after the central pathological diagnoses of the 16 newly diagnosed MGs during the central review were GBM in 9 cases and other tumors in 7 cases (3 cases of anaplastic oligodendroglioma, 2 cases of oligodendroglioma, and 1 case each of anaplastic ganglioglioma and oligoastrocytoma). Of the 8 recurrent GBMs, the diagnoses were GBM in 4cases and other tumors in 4 cases (1 case each of anaplastic oligodendroglioma, anaplastic oligoastrocytoma, anaplastic astrocytoma, and high-grade glioma).

In 6 of 24 patients, whole blood BCNU levels were measured. The ages (mean ± SD) were 45.4 ± 14.05 years, 46.6 ± 14.09 years, and 42.9 ± 14.57 years in the entire population, patients with newly diagnosed MGs, and patients with recurrent MGs, respectively. There were 12 male (50.0%) and 12 female (50.0%) patients, indicating no gender bias. The duration of illness were 16.7 ± 28.88 months, 5.7 ± 15.07 months, and 38.8 ± 37.71 months in the entire population, patients with newly diagnosed MGs, and patients with recurrent MGs, respectively. The preoperative tumor sizes were 21.0 ± 13.8 cm², 23.0 ± 15.0 cm², and 16.9 ± 10.5 cm² in the entire population, patients with newly diagnosed MGs, and patients with recurrent MGs, respectively. The median tumor sizes were 22.6 cm², 22.5 cm² and 22.6 cm² in the entire population, patients with newly diagnosed MGs, and patients with recurrent MGs, respectively. The rates of tumor resection were 90.3 ± 11.8%, 91.9 ± 8.5%, and 87.3 ± 17.0% in the entire population, patients with newly diagnosed MGs, and recurrent MGs, respectively. The rates of median tumor resection were 92.5%, 92.5%, and 95.0% in the entire population, patients with newly diagnosed MGs, and patients with recurrent MGs, respectively. The number of patients with a pre-insertion KPS score over 80 was 20 (83.3%) in the entire population, 14 (87.5%) in patients with newly diagnosed MGs, and 6 (75.0%) in patients with recurrent MGs. Of the recurrent MGs patients, recurrence occurred once in 6 cases (75.0%) and twice in 2 cases (25.0%). All recurrent MG patients received conventional radiotherapy (local), and 7 of these patients (87.5%) had a history of medical treatment for the tumor. Tumor resection in the newly diagnosed MG patients was partial removal: 9 cases (56.3%) and total removal: 7 cases (43.7%). Tumor resection in the previous treatment of recurrent MG patients was biopsy: 2 cases (25.0%), partial removal: 4 cases (50.0%), and total removal: 2 cases (25.0%). In this study, the period from the first operation to

the second was less than 1 year in 4 recurrent MG patients (50.0%). Eight sheets of BCNU implants were inserted in 21 of 24 patients. One patient each received 7, 6, and 5 sheets.

After surgery, standard TMZ plus conventional radiotherapy was utilized for all newly diagnosed MG patients (n = 16). For recurrent MG patients, TMZ alone (n = 7) or TMZ plus INF-β therapy (n = 1), BEV therapy (n = 2), or IMRT therapy (n = 1) was utilized.

I. Efficacy

Using the Kaplan-Meier method, OS rates at 12 and 24 months for patients with newly diagnosed MGs were 100% and 68.8%, respectively (95% CI: 40.5–85.6%). The mOS in this group could not be calculated (Fig. 3). For patients with recurrent MGs, the OS rate at 6 months was 87.5% (95% CI: 38.7–98.1%), the OS rate at 12 months was 62.5% (95% CI: 22.9–86.1%), the OS rate at 24 months was 25.0% (95% CI: 3.7–55.8%), and the mOS was 12.0 months (361 days) (Fig. 3). In subgroup analysis of patients according to histological type, the 16 patients with newly diagnosed MGs were divided into the GBM group and the non-GBM group. In the GBM group (n = 9), the OS rate at 24 months and the mOS were 44.4% (95% CI: 13.6–71.9%) and 20.2 months, respectively. In the non-GBM group (n = 7), the OS rate was 100%. For patients with recurrent MGs, the OS rate at 12 months and the mOS were 50.0% (95% CI: 5.8–84.5%) and 8.6 months, respectively, in the GBM group (n = 4). In the non-GBM group (n = 4), the OS rate was 75.0% (95% CI: 12.8–96.1%) and the mOS was 12 months. According to the Kaplan–Meier method, the PFS rate at 6 months was 75.0% (95% CI: 46.3–89.8%) and
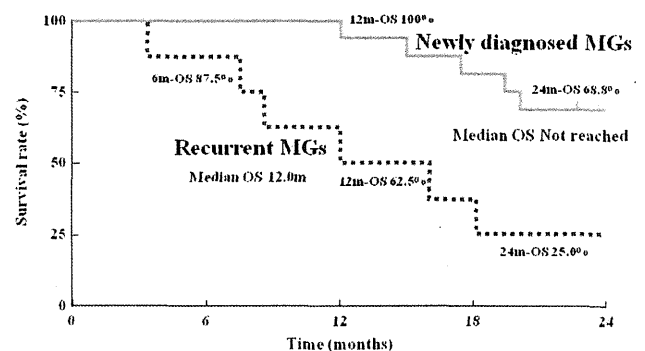
Fig. 3 Kaplan-Meier curve of survival period/rate. MGs: malignant gliomas, OS: overall survival rate, m: months, 6m-OS: the overall survival rates at 6 months, 12m-OS: the overall survival rates at 12 months, 24m-OS: the overall survival rates at 24 months.

that at 12 months was 62.5% (95%CI: 34.9–81.1%) in patients with newly diagnosed MGs (Fig. 4). When this group was subdivided, the PFS rate at 12 months was 55.6% (95% CI: 20.4–80.5%) in the GBM group and 71.4% (95% CI: 25.8–92.0%) in the non-GBM group. For patients with recurrent MGs, the PFS rate at 6 months was 37.5% (95% CI: 8.7–67.4%), the PFS rate at 12 months was 25.0% (95% CI: 3.7–55.8%), and the median PFS was 170
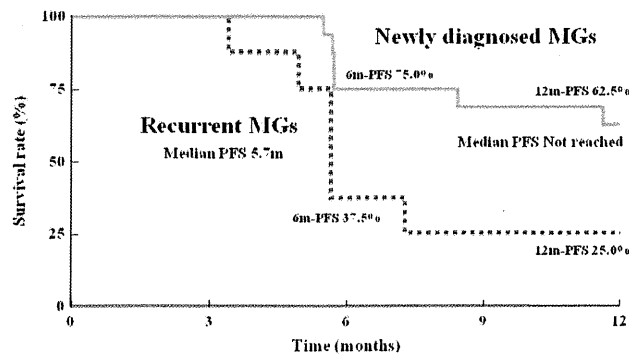

Fig. 4 Kaplan-Meier curve of progression-free survival period/rate (judged by the efficacy and safety evaluation committee). MGs: malignant gliomas, PFS: progression-free survival rate, m: months, 6m-PFS: progression-free survival rate at 6 months, 12m-PFS: progression-free survival rate at 12 months.

days (Fig. 4). When this group was subdivided, the PFS rates at 6 monthsand 12 months were both 25.0% (95% CI: 0.9–66.5%) in the GBM group and 50.0% (95% CI: 5.8–84.5%) in the non-GBM group. Figure 5 shows gadolinium contrast-enhanced T_1 MRIs before insertion, within 3 days of insertion, and 6 months and 12 months after insertion of BCNU implants in a patient with recurrent GBM (first relapse). A tumor, 5 cm in size, was noted in the left frontal lobe, and 8 sheets of the BCNU implant were inserted. Subsequently, TMZ alone (220–260 mg/day) was applied for 9 cycles. Even at 12 months after insertion, there was no tumor growth or any other changes observable on MRI images.

During this study, non-responders to TMZ received either BEV therapy (1 newly diagnosed GBM patient and 1 recurrent GBM patient) or IMRT therapy (1 recurrent anaplastic astrocytoma patient). BEV therapy for the newly diagnosed GBM patient involved 5 cycles of treatment (330 mg/day) after recurrence at 8.4 months after insertion of BCNU implants. The patient died 12 months (362 days) after insertion of the BCNU implants. BEV therapy for the second recurrence GBM patient involved 10 cycles of treatment (500 mg/day) at 5.7 months after insertion of the BCNU implants. The patient died at 18.2 months (546 days) after insertion of the BCNU implants. IMRT therapy was applied at a dose of 60 Gy to the enhanced area and 50 Gy to the area around the lesion after the
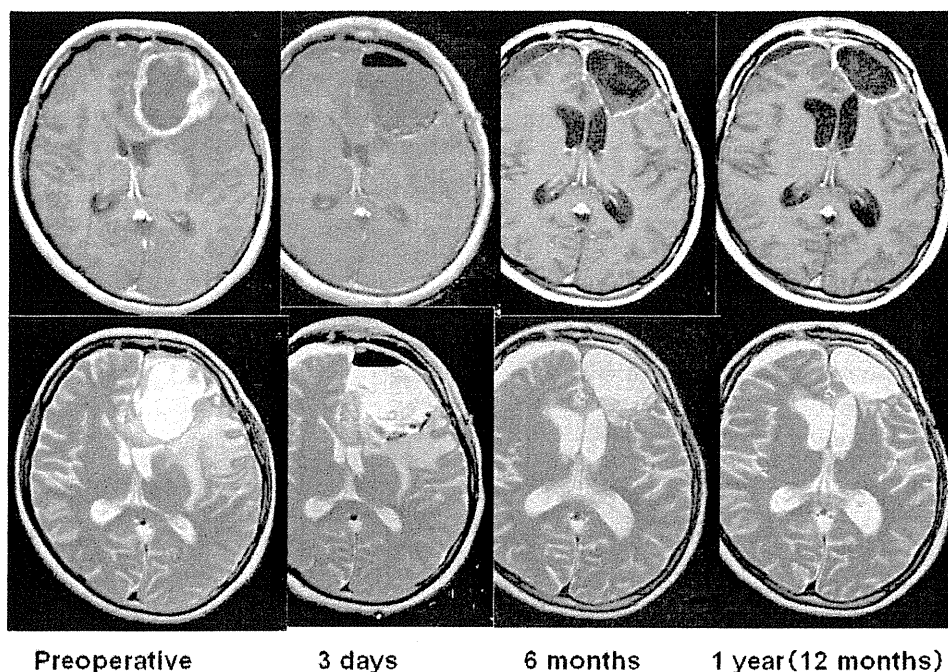

Fig. 5 Time course of magnetic resonance (MR) imaging findings (1st relapse of recurrent glioblastoma), axial gadolinium contrast-enhanced T_1-weighted MR images (*upper row*), and T_2-weighted MR images (*lower row*).

second recurrence at 7.3 months after insertion of the BCNU implants. The patient died at 16.1 months (483 days) after insertion of the BCNU implants.

II. Safety

Adverse events were noted in 24 of 24 (100%) patients who received BCNU implants and adverse events attributable to BCNU implants were 13 of 24 patients (54.2%). Major adverse events (over 20%, Table 2) listed in descending order of incidence were fever (18 cases, 75.0%); alopecia (16 cases, 66.7%); constipation (14 cases, 58.3%); headache (13 cases, 54.2%); nausea (12 cases, 50.0%), wound complication and leukocytopenia (11 cases each, 45.8%); brain neoplasm (10 cases, 41.7%); vomiting, malaise, brain edema, and lymphopenia (9 cases each, 37.5%); anorexia and hemiparesis (8 cases each, 33.3%); insomnia (7 cases, 29.2%); aphasia, seizure, and increase in blood creatine phosphokinase (CPK; 6 cases each, 25.0%); and pruritus, facial swelling, radiation-induced skin injury, and weight loss (5 cases each, 20.8%). Of these adverse events, severe events (grade 3) included brain neoplasm (7 cases, 29.2%), hemiplegia (6 cases, 25.0%), brain edema (4 cases, 16.7%), and aphasia (3 cases, 12.5%). Adverse, life-threatening events or those causing disabilities (grade 4) included tumor progression (3 cases, 4.2%).

Within 12 months (360 days) of BCNU implant insertion, 3 patients died from tumor progression. None of the deaths had causal relationships with the investigational drug. Within 24 months of BCNU implant insertion, 6 patients died in addition to the above-mentioned 3 patients (9 deaths in total). The cause of death was progressive disease (PD) in 5 cases (2 newly diagnosed MGs and 3 recurrent MGs) and respiratory failure in 1 case (newly diagnosed MG). None of these deaths had causal relationships with the investigational drug.

Frequently noted adverse events attributable to BCNU implants (adverse reactions, Table 3) were brain edema (6 cases, 25.0%); fever and lymphocytopenia (3 cases each, 12.5%); and nausea, vomiting, headache, hemiparesis, anorexia, and increase in alanine aminotransferase (ALT; 2 cases each, 8.3%). None of these adverse reactions were rated as grade 4 or worse. There were 6 cases of grade 3 events in 5 of 24 patients (20.8%) including brain edema (2 cases), hemiparesis (2 cases), increase in ALT (1 case), and increase in CPK (1 case). None of the patients experienced convulsion, poor wound healing, infection, meningitis, or hydrocephalus as an adverse reaction. The adverse reactions listed above appeared within 3 months of BCNU implant insertion.

Among the patients who did not respond to TMZ

Table 2 Number of patients (%) who experienced adverse events according to Common Terminology Criteria for Adverse Events (CTCAE) grade (events with an incidence over 20%)

System organ class/event name	Cases (%) (n = 24)			
	All grades		Grade 3 or higher	
All adverse events	24	(100.0)	19	(79.2)
Gastrointestinal disorders				
Nausea	12	(50.0)	–	
Constipation	14	(58.3)	–	
Vomiting	9	(37.5)	–	
General disorders and administration site conditions				
Malaise	9	(37.5)	–	
Fever	18	(75.0)	1	(4.2)
Injury, poisoning, and procedural complications				
Wound complication	11	(45.8)	–	
Nervous system disorders				
Aphasia	6	(25.0)	3	(12.5)
Headache	13	(54.2)	–	
Brain edema	9	(37.5)	4	(16.7)
Hemiparesis	8	(33.3)	6	(25.0)
Seizure	6	(25.0)	1	(4.2)
Psychiatric disorders				
Insomnia	7	(29.2)		
Metabolism and nutrition disorders				
Anorexia	8	(33.3)	–	
Skin and subcutaneous disorders				
Pruritus				
Facial swelling	5	(20.8)	–	
Alopecia	5	(20.8)	–	
Radiation-induced skin	16	(66.7)	–	
injury	5	(20.8)	–	
Neoplasms (benign, malignant, and unspecified)				
Brain neoplasm	10	(41.7)	7	(29.2)
Investigations				
Lymphopenia	9	(37.5)	2	(8.3)
Blood creatine phosphokinase increased	6	(25.0)	2	(8.3)
Weight loss	5	(20.8)	–	
Leukocytopenia	11	(45.8)	2	(8.3)

MedDRA/J Version 14.0. Event name: The same event name seen in the same patient was counted as one case. If severity differed between multiple episodes of the same event, then the most severe episode was selected. System organ class: If there were multiple event names within the same system organ class in the same patient in one line, the patient was counted as one. Incidence (%) = No. of patients developing the event/All patients studied × 100.

Table 3 Number of patients (%) who attributable to BCNU implants according to Common Terminology Criteria for Adverse Events (CTCAE) grade

System organ class/event name	Cases (%) (n = 24)			
	All grades		Grade 3 or higher	
All adverse reactions	13	(54.2)	5	(20.8)
Gastrointestinal disorders				
Nausea	2	(8.3)	–	
Abdominal discomfort	1	(4.2)	–	
Vomiting	2	(8.3)	–	
General disorders and administration site conditions				
Hypothermia	1	(4.2)	–	
Fever	3	(12.5)	–	
Edema	1	(4.2)	–	
Nervous system disorders				
Hyperesthesia	1	(4.2)	–	
Memory disorder	1	(4.2)	–	
Aphasia	1	(4.2)	–	
Heterotropia	1	(4.2)	–	
Headache	2	(8.3)	–	
Homonymous hemianopsia	1	(4.2)	–	
Urinary incontinence	1	(4.2)	–	
Brain edema	6	(25.0)	2	(8.3)
Monoparesis	1	(4.2)	–	
Hemiparesis	2	(8.3)	2	(8.3)
Hemiplegia	1	(4.2)	–	
Reproductive system and breast disorders				
Irregular menstruation	1	(4.2)	–	
Metabolism and nutrition disorders				
Anorexia	2	(8.3)	–	
Investigations				
C-reactive protein increased	1	(4.2)	–	
Alanine aminotransferase increasd	2	(8.3)	1	(4.2)
Lymphocyte decreased	3	(12.5)	–	
Platelet decreased	1	(4.2)	–	
Blood creatine phosphokinase increased	1	(4.2)	1	(4.2)
Leukocyte increased	1	(4.2)	–	

※ MedDRA/J Version 14.0. Event name: the same event name seen in the same patient was counted as one case.

and who received BEV therapy (1 newly diagnosed GBM patient and 1 recurrent GBM patient) or IMRT therapy (1 recurrent anaplastic astrocytoma patient), leukocytopenia (grade 2) was noted in a patient who underwent BEV therapy and alopecia (grade 2) and malaise (grade 2) were noted in a patient who underwent IMRT therapy.

III. Pharmacokinetics

BCNU levels in whole blood were measured in 6 of the patients who received BCNU implants. The age of these 6 patients (mean ± SD) was 45.5 ± 15.7 years (21–61 years), body weight was 59.2 ± 14.2 kg (42.9–77.1 kg), median number of BCNU implant sheets inserted was 8 sheets (5–8 sheets: 8 in 4 cases, 7 and 5 in 1 case each), and the administration of BCNU at a median dose level were 61.6 mg (38.5–61.6 mg). As shown in Fig. 6, BCNU levels reached a peak approximately 3 hours after insertion and ranged from 6.49 ng/mL to 19.4 ng/mL (10.19 ± 4.77 ng/mL). After 24 hours, levels were in the vicinity of or below the lower limit of quantification (2.00 ng/mL).

Discussion

This study (NPC-08 study) was designed to evaluate the efficacy, safety, and pharmacokinetics of BCNU implants with chemotherapy (including TMZ) and radiotherapy for Japanese patients with newly diagnosed MGs or recurrent GBM (under conditions indicated for BCNU in USA and Europe). Of the 24 patients who received BCNU implants (newly diagnosed MG, 16 cases; recurrent MG, 8 cases), the survival rate for patients with newly diagnosed
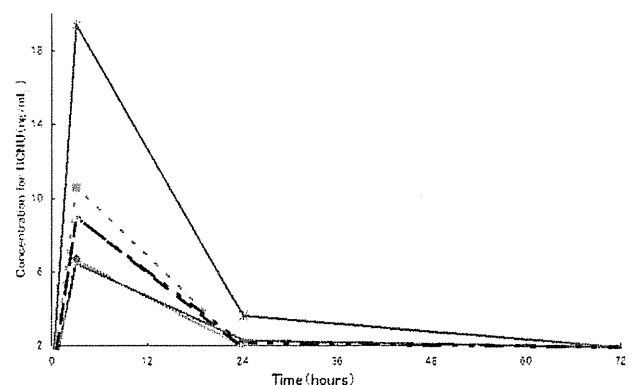
Fig. 6 Time course of BCNU levels in whole blood. Six Japanese patients with malignant gliomas received the maximum blood concentration of BCNU at about 3 hours after implant placement was 19.4 ng/mL. The lower limit of quantitation (2.00 ng/mL).

MGs was 100.0% at 12 months and 68.8% at 24 months, making it impossible to calculate the median survival time in this group. These results were superior those of placebo-controlled double-blind comparative studies[3,4] conducted in USA and Europe in which the OS rate at 12 months was 59.2% (95% CI: 50.4–68.0%) and the mOS was 13.8 months (95% CI: 12.1–15.1 months). However, these studies from USA and Europe were not combined therapy involving TMZ plus radiotherapy for newly diagnosed MGs cases. In NPC-08 study, combined TMZ plus radiotherapy was applied to all patients with newly diagnosed MGs after insertion of BCNU implants. According to previous reports[9–13] examining combined TMZ plus radiotherapy after insertion of BCNU implants, the OS rate at 12 months and 24 months was 56.8–81.0% and 13.0–47.0%. Furthermore, the OS rate at 6 months with recurrent MG was 87.5% in NPC-08 study. According to a clinical study by Brem et al.,[2] the OS rate at 6 months in patients with recurrent MG was 60%. Thus, although the number of patients studied was small, the results of the NPC-08 study were comparable to the results of USA and European studies.

Potential adverse effects of BCNU implants were noted in 13 of 24 patients (54.2%). Severe adverse reactions were noted in 5 of 24 patients (20.8%), although none were live threatening. Important adverse events requiring close attention include brain edema, seizure, poor wound healing, infection, headache, hemiparesis, meningitis, and hydrocephalus according to clinical reports from USA and Europe. We compared the adverse event data in NPC-08 study with the data from three placebo-controlled double-blind comparative studies conducted in USA and Europe (Table 4).

The incidence of Brain edema was higher in the NPC-08 study (25.0%, 6/24 patients) than that in the double-blind studies (4.9%, 12/246 patients). However, there was no significant difference between the BCNU implant group and the placebo group regarding brain edema on grade 3 or worse in the double-blind studies. Brain edema can also be caused by tumor resection, MGs themselves, dose of steroid and so on, therefore, the expression of the brain edema is necessary to be careful, it is necessary to consider the administration of steroid drug. In this study, brain edema of any CTCAE grade occurred in 6 of 24 patients. There were no patients with brain edema of CTCAE grade 4. Two patients developed brain edema of CTCAE grade 3. These two patients were not from a specific facility. We examined the occurrence rate of brain edema by each patient's background in the Japanese studies, but were unable to find any tendency due to the

small sample size. Nearly 700 patients were enrolled in foreign studies. Among those patients, 3.8% (26/676) developed brain edema of any CTCAE grade, and 1.9% (12/676) developed brain edema of CTCAE grade 3 or 4. On the other hand, the total number of patients enrolled in the Japanese study was small (24 patients). We consider that this small number of patients (denominator) may have contributed to a large difference; i.e., in the present case, higher rate of brain edema occurrence than in foreign studies. In addition, we infer that one of the reasons that brain edema were more often observed in Japan than in foreign countries is as follows: the protocol for the Japanese study has described "brain edema, convulsion, cerebrospinal fluid (CSF) leakage, and limited hypofunction" as notable adverse events that were reported in foreign studies, and requested investigators and clinical research coordinators to carefully watch for these adverse events. This may have encouraged physicians to conduct CT/MRI testing more frequently than is required in the protocol, leading to observation of higher occurrence rate of brain edema. In summary, although the occurrence rate of brain edema was higher in the Japanese study than in the foreign studies, it is difficult to determine whether the Japanese patients are more likely to develop brain edema than the foreign patients because of the small number of the Japanese patients enrolled in this study. This question should be addressed in future studies. Seizure is one of the complications of brain tumors and neurosurgical interventions, and its incidence differed little between NPC-08 study and the combined double-blind studies. There was no difference between the BCNU implant group and the placebo group in terms of overall incidence or incidence of seizure grade 3 or worse. One of the 3 double-blind studies, Brem et al.[2] reported that the median day of onset of seizures was faster in the BCNU implant group (3.5 days) than in the placebo group (55.5 days) (Wilcoxon test: P = 0.01). In the NPC-08 study, the median day of onset of seizures was 91.5 days.

Since the number of patients studied was small, these results were not clear that the day of onset of seizures was tended to be faster by BCNU implant. However, it is necessary to consider the administration of anticonvulsant drugs. None of the patients was experienced poor wound healing after craniotomy as adverse reactions in the NPC-08 study. In the combined double-blind studies, the incidence of poor healing was slightly higher in the BCNU implant group (7.3%, 18/246 patients) than in the placebo group (3.2%, 8/248 patients). Therefore, the expression of poor wound healing is necessary to

Table 4 Comparison of the number of patients (incidence) who experienced major adverse reactions in the NPC-08 study and in the combined double-blind comparative studies

System organ class/Event name	The NPC-08 study			Double-blind studies[2,10,13]					
	All grades			All grades			Placebo		
	All grades	Grade 3	Grade 4	All grades	Grade 3	Grade 4	All grades	Grade 3	Grade 4
Total patients	24			246			248		
Brain edema	6 (25.0)	2 (8.3)	–	12 (4.9)	2 (0.8)	3 (1.2)	12 (4.8)	4 (1.6)	2 (0.8)
Seizure	–	–	–	31 (12.6)	7 (2.8)	2 (0.8)	39 (15.7)	11 (4.4)	2 (0.8)
Major seizure	–	–	–	1 (0.4)	–	–	2 (0.8)	–	1 (0.4)
Poor healing	–	–	–	18 (7.3)	4 (1.6)	–	8 (3.2)	1 (0.4)	–
Infection	–	–	–	13 (5.3)	2 (0.8)	3 (1.2)	16 (6.5)	2 (0.8)	–
Headache	2 (8.3)	–	–	28 (11.4)	8 (3.3)	–	22 (8.9)	8 (3.2)	1 (0.4)
Hemiplegia	1 (4.2)	–	–	24 (9.8)	5 (2.0)	–	34 (13.7)	15 (6.0)	–
Monoparesis	1 (4.2)	–	–	–	–	–	–	–	–
Hemiparesis	2 (8.3)	2 (8.3)	–	–	–	–	–	–	–
Meningitis	–	–	–	5 (2.0)	2 (0.8)	–	1 (0.4)	–	–
Hydrocephalus	–	–	–	2 (0.8)	1 (0.4)	1 (0.4)	1 (0.4)	–	–

MedDRA/J Version 14.0. Event name: The same event name seen in the same patient was counted as one case. If severity differed between multiple episodes of the same event, then the most severe episode was selected. Incidence (%) = No. of patients who experienced adverse reaction / all patients studied × 100.

be careful. Infection and meningitis were not observed in the NPC-08 study. In the combined double-blind studies, the overall incidence of this event and the incidence of grade 3 or worse differed little between the BCNU implant group and the placebo group, and the incidence was also high in the placebo group.

The incidence of headache was not high in the NPC-08 study (8.3%) and differed little between the BCNU implant group and the placebo group. The incidence of hemiparesis was slightly higher in the combined double-blind studies. Hydrocephalus did not develop in any patient in the NPC-08 study. In the combined double-blind studies, the incidence of hydrocephalus was approximately 0.8% in the BCNU implant group, which was comparable to its incidence in the placebo group. All of the important adverse events discussed above were symptoms accompanying a brain tumor or surgical resection of the tumor.

For pharmacokinetic analysis, BCNU levels in the blood were measured at multiple time points after surgery. The administration of BCNU at a median dose level of 61.6 mg (38.5–61.6 mg) to 6 patients caused a mean peak BCNU level of 10.19 ng/mL. BCNU has been administered intravenously and inserted into the removal cavity for the treatment of brain tumors in USA and Europe. According to a report[14] describing the pharmacokinetics of intravenous BCNU injection, the peak BCNU level in the blood averaged 6.2 μg/mL, whereas the peak level following insertion in the brain averaged 0.01 μg/mL.

Thus, the BCNU level in the blood after insertion into the brain was much lower (1/600) than that after intravenous injection, and BCNU disappears from the blood almost completely within 24 hours of insertion into the brain.

Systemic administration of BCNU often induces severe adverse events such as leucopenia and thrombocytopenia. Insertion of BCNU implants into the brain is expected to markedly reduce systemic adverse events as compared with intravenous BCNU.

Taken together, these results indicate that when insertion of BCNU implants into the brain (maximum of 8 sheets containing a maximum of 61.6 mg BCNU) was followed by chemotherapy or radiotherapy in patients with newly diagnosed or recurrent MGs, there are no major safety concerns associated with the use of BCNU implants. The BCNU implant is now recommended as a treatment option along with the surgical resection of MGs on the basis of established treatment guidelines. The data from this clinical study was comparable to previous data from USA and Europe with respect to efficacy and safety. Therefore, from a risk/benefit viewpoint, the use of BCNU implants is recommended.

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Conflicts of Interest Disclosure

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