was statistically significant ($p < 0.01$, chi-square test with continuity correction).

Speech function recovered in all patients within, on average, 6.1 ± 4.2 months after surgery (range 1–15 months, median 6 months). The time to speech recovery was significantly associated with the type of intraoperative change in CCEP recordings ($p < 0.01$, Kruskal-Wallis test) and was, on average, 1.8 ± 1.0, 5.5 ± 1.0, and 11.0 ± 3.6 months, respectively, if the response was unchanged, was decreased, or had disappeared. In this way, the absence of intraoperative CCEP changes had 100% sensitivity, specificity, and positive predictive value for the recovery of language function within 3 months after surgery. Additionally, the time to speech recovery was significantly associated with lesion location, taking, on average, 1.8 ± 1.0, 6.7 ± 1.2, and 8.8 ± 4.9 months, respectively, in cases of frontal, insular, and parietal neoplasms ($p < 0.02$, Kruskal-Wallis test).

Other evaluated factors, namely patient age, sex, tumor histopathological grade, lesion resection rate, maximum stimulus intensity, peak latency, and direction of stimulation during CCEP recording, did not demonstrate a statistically significant association with postoperative impairment of speech function and the time to its recovery.

Illustrative Cases

Case 8

History and Examination. A 33-year-old man experienced several episodes of generalized seizures. He had no other neurological symptoms or signs, and his speech function was normal. Magnetic resonance imaging demonstrated a nonenhancing intraaxial brain tumor centered in the left inferior parietal lobule, which had a hypointense signal on T1-weighted images and a slightly heterogeneous hyperintense signal on FLAIR images. Intracarotid amobarbital test revealed that the left cerebral hemisphere was language dominant.

Operation. Tumor removal was performed during awake craniotomy and continuous CCEP monitoring with stimulation of the TLA and recordings from the FLA. In total 95% resection of the lesion was attained under the guidance of cortical and subcortical mapping of language-related structures. During removal of the neoplasm, paraphasia, repetition failure, and dysarthria were noted, whereas an initially normal CCEP response demonstrated an abrupt decrease in amplitude and occasionally disappeared (Fig. 2). The histopathological diagnosis was oligodendroglioma.

Postoperative Course. After surgery the patient experienced the same speech disorders as intraoperatively, which recovered very slowly but became normal 15 months later. He did not have epileptic seizures during follow-up.

Case 13

History and Examination. A 58-year-old generally healthy man experienced generalized seizures. Magnetic resonance imaging revealed nonenhancing intraaxial brain tumor in the left parietal lobe. There were no other neurological symptoms, and the patient was initially followed up with anticonvulsants and regular neuroimaging at another hospital. Two years later, however, the appearance and gradual enlargement of a contrast-enhanced area at the peripheral part of the lesion was noted. Malignant transformation was suspected, and the patient was sent to our clinic for surgical treatment. On admission the patient was neurologically intact, and his speech function was normal. Magnetic resonance imaging revealed a tumor centered in the left inferior parietal lobule, which had hypointense signal on T1-weighted images and heterogeneous hyperintense signal on FLAIR images. The contrast-enhanced lesion was located within the left insula. Intracarotid amobarbital test revealed that the left cerebral hemisphere was language dominant.

First Operation. Two-stage surgical removal of the tumor was planned. Initially, the contrast-enhanced insular tumor was totally resected with the patient under general anesthesia. Histopathological examination revealed anaplastic oligodendroglioma. At the end of surgery chronic grid electrodes were implanted subdurally for detailed cortical mapping before resection of the residual neoplasm.

First Postoperative Course. The postoperative course was uneventful. The patient's speech function remained normal. Cortical mapping using grid electrodes identified the FLA in the posterior part of the inferior frontal gyrus and the inferior part of the precentral gyrus. The TLA was located in the middle and posterior parts of the superior temporal gyrus and in the inferior parietal lobule.

Second Operation. Three weeks later the second-stage awake craniotomy was performed. Intraoperative brain mapping revealed the same location of the FLA as was defined with cortical stimulation using chronic grid electrodes; however, the TLA was identified only in the middle and posterior parts of the superior temporal gyrus. Continuous CCEP monitoring was performed with stimulation of the FLA and recordings from the TLA. During resection of the deep part of the neoplasm, paraphasia and repetition failure were noted, whereas subcortical electrical stimulation resulted in short-term speech arrest. It was accompanied by a decrease in the CCEP response in the posterior part of the superior temporal gyrus up to 20% and an increase in peak latency, although these parameters were stable in the middle part of the gyrus (Fig. 3). Approximately 30 minutes later partial recovery of the CCEP response was noted. Nevertheless, to avoid an irreversible deterioration in language function, further resection of the neoplasm was abandoned. In total 40% tumor removal was attained.

Second Postoperative Course. The postoperative course was uneventful. Despite the presence of speech production disorders in the early postoperative period, language function gradually recovered and became normal by 4 months after surgery. The patient received a course of fractionated radiotherapy (60 Gy) with concomitant and adjuvant chemotherapy (nimustine hydrochloride), which resulted in stabilization of the residual tumor.

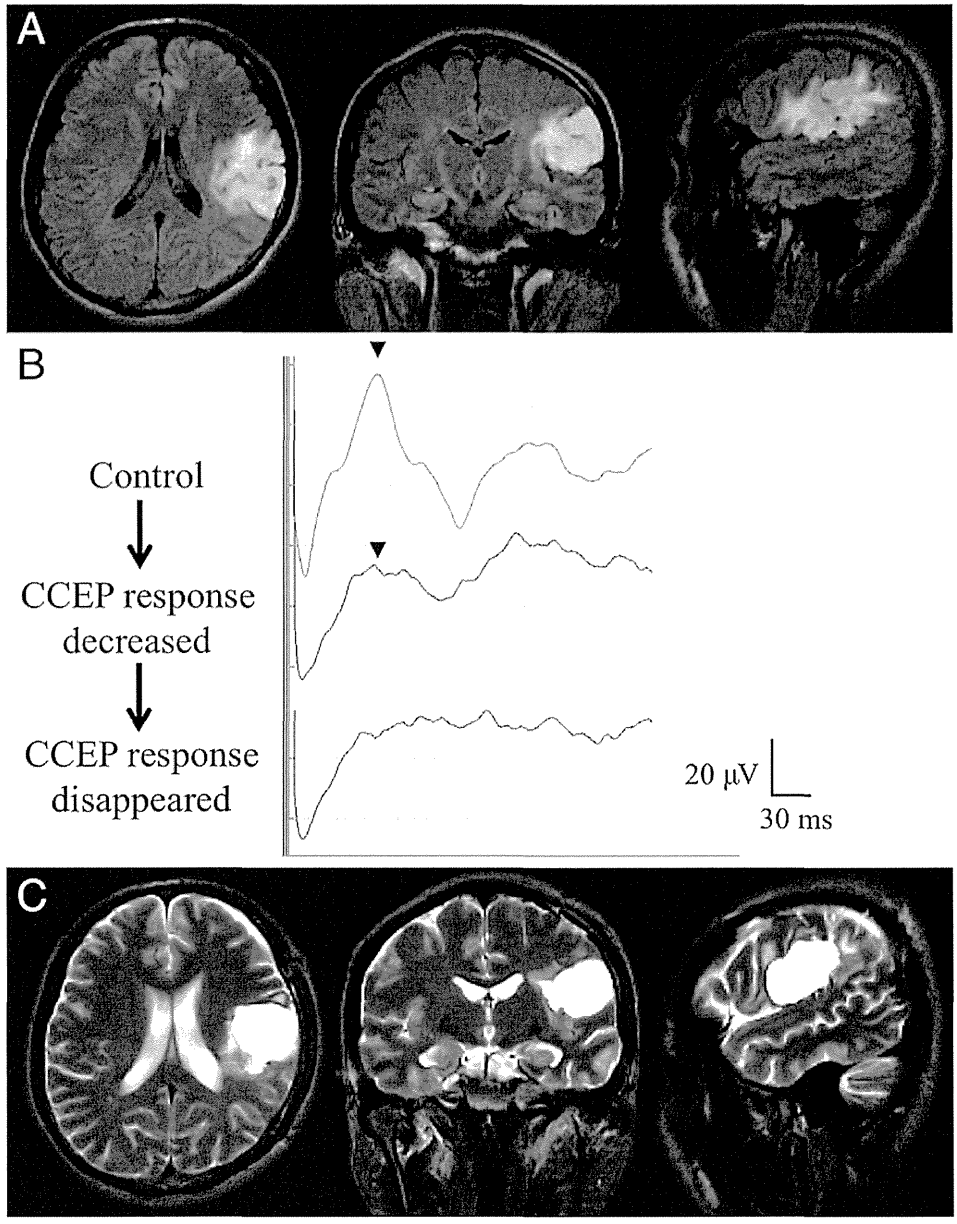

FIG. 2. Case 8. Oligodendroglioma in a 33-year-old man. Preoperative MR images **(A)** demonstrated tumor in the left inferior parietal lobule. The neoplasm was resected during awake craniotomy, and intraoperative CCEP monitoring was performed with stimulation of the TLA and recordings from the FLA. During surgery, paraphasia, repetition failure, and dysarthria were noted. An initially normal CCEP response demonstrated a decrease in amplitude and occasionally disappeared **(B)**. The resection rate was 95% **(C)**. After surgery, the patient experienced prolonged speech dysfunction with slow recovery within 15 months of follow-up.

Discussion

The technique of CCEP monitoring is based on electrical stimulation of one cortical area and recording the averaged response from another, which permits evaluation of their functional interconnections.[4,7,8,17,20,21] Previous reports have demonstrated that this neurophysiological method can be useful for assessing the language network in patients with epilepsy and that the evolved response is well correlated with the location of the FLA and TLA.[4,7,21] In the present study we showed for the first time that CCEP recordings can be effectively performed during awake craniotomy for brain tumors and are feasible for the evaluation of language, since the evolved response was closely related to intraoperative and postoperative speech function, as well as to its prognosis after surgery.

Direct comparison of the results of CCEP monitoring presented herein with those previously reported by other groups is difficult. To the best of our knowledge, all previous studies have been based on recordings performed after subdural implantation of chronic grid electrodes in patients with seizures, whereas in the present study CCEP monitoring was applied during the resection of brain neoplasms. Moreover, the quantitative and qualitative differ-

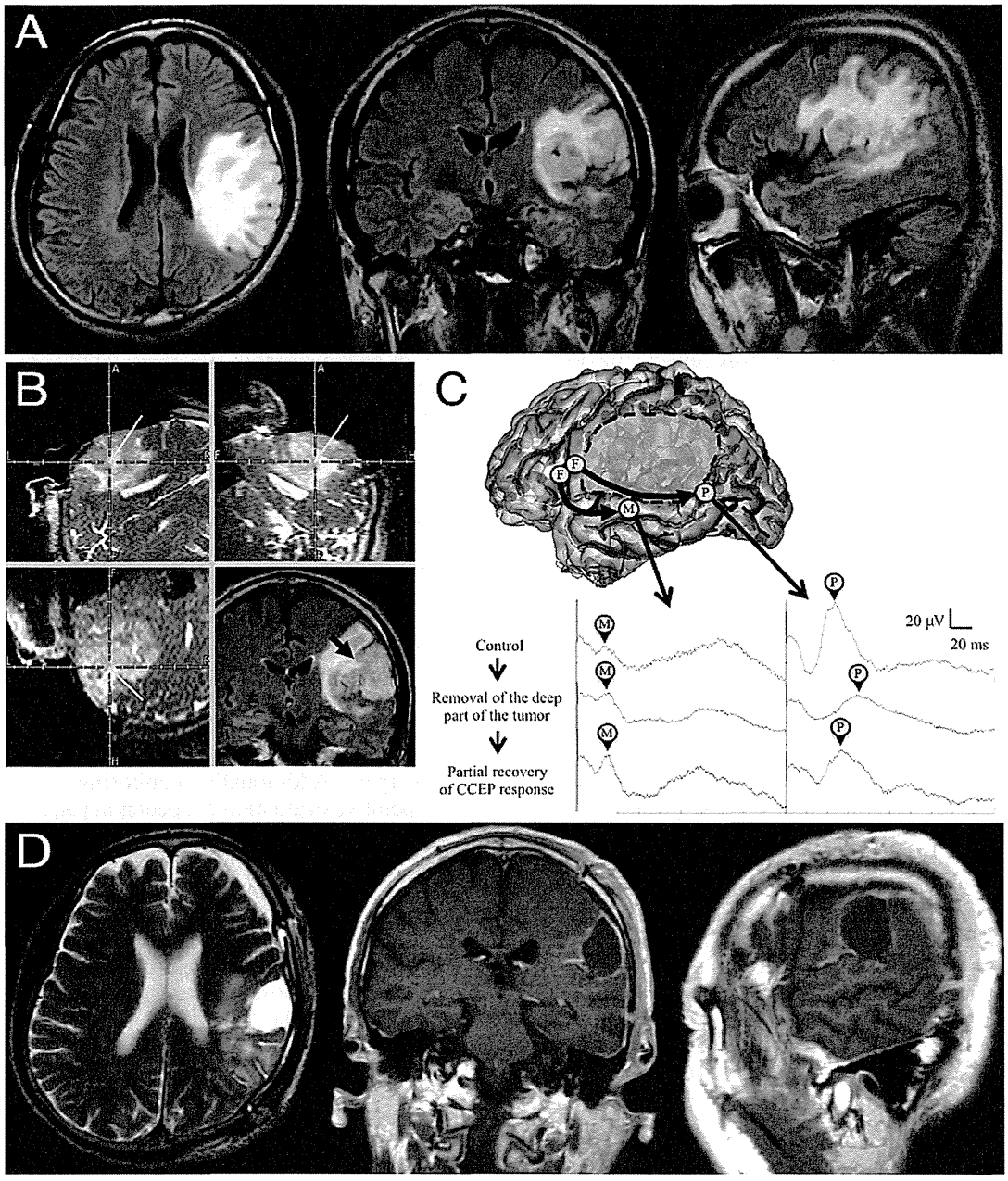

FIG. 3. Case 13. Anaplastic oligodendroglioma in a 58-year-old man. A neoplasm in the inferior parietal lobule **(A)** was resected during a second-stage awake craniotomy and intraoperative CCEP monitoring with stimulation of the FLA and recordings from the TLA. During removal of the deep part of the tumor, language function was impaired, whereas subcortical electrical stimulation resulted in short-term speech arrest (**B**, *black arrow*). It was accompanied by a decrease in CCEP response **(C)** in the posterior part of the superior temporal gyrus (P) up to 20% and an increase in peak latency, whereas these parameters recorded from the middle part of the gyrus (M) were stable. Approximately 30 minutes later some recovery of the CCEP response was noted. However, only partial resection of the tumor was attained **(D)** to avoid permanent postoperative speech dysfunction. After surgery the patient experienced paraphasias and repetition failures, with gradual recovery within 4 months of follow-up. *Curved arrows* (C) show the directions of stimulation, presumably along dorsal and ventral language pathways, and *arrowheads* (C) indicate peak corresponding to CCEP response. F = FLA.

ences in subcortical fibers interconnecting evaluated cortical areas, as well as the distance between stimulating and recording electrodes, are reflected in the intersubject variability of latency and amplitude of CCEP responses[3,4] and may differ in cases of nonstructural brain pathology and tumors adjacent to the area of interest, particularly because of the invasive and compressive effects of the latter. In their original study, Matsumoto et al.[21] investigated language-related CCEP in 8 patients with epilepsy. The response typically consisted of an early (N1) and late (N2) negative peak with latencies of 22–36 msec and 113–164 msec, respectively, during stimulation of the FLA and

recordings from the TLA, and 23–39 msec and 90–161 msec in the opposite direction. Recordings were successfully accomplished in the posterior language area after stimulation of the anterior language area in 7 of 8 patients, and in 3 of 4 patients when it was done in the opposite direction. Therefore, interconnections between anterior and posterior cortical language areas were considered bidirectional[21] in contrast to the classic model of language.[11] In our cases CCEP response frequently consisted of two negative peaks, but only the highest one evoked in any direction was evaluated because it was considered to be more suitable for the purpose of intraoperative monitoring. Since the median peak latency was 91 msec, it probably corresponded to N2, which mainly reflects the direct arrival of slow multisynaptic volleys via the cortico-subcortico-cortical pathway.[21] Moreover, in the present study the intraoperative CCEP response strongly depended on the direction of stimulation and was clearly bidirectional in only 4 patients. In Cases 9, 10, 12, and 13, responses could not be reliably observed in the FLA during stimulation of the TLA, while they were clearly identified in the opposite direction. It has been previously remarked that the CCEP response in the anterior language area during stimulation of the posterior language area is less well defined, because of the more scattered distribution of the projecting neurons within the latter and the subsequent activation of the smaller number of projecting neurons at one time with the stimulation technique applied.[21] Additionally, the degree of evoked response may be related to the size of pyramidal neurons, which are smaller in the posterior language area and may require greater stimulation intensity.[21] On the other hand, the strip electrodes for intraoperative CCEP recordings in our patients were positioned in the cortical areas, whose direct electrical stimulation resulted in speech abnormalities, while the best response in the TLA during stimulation of the FLA can be presented in spatially different areas.[21] In some way it can explain the unidentified CCEP response in the TLA after stimulation of the FLA in our Cases 1–3 and 5. Finally, the absence of a noted response from white matter within the resection cavity may be particularly caused by a predetermined delay in recordings of 10 msec after trigger, since subcortical CCEP responses usually have short latencies.[8]

It is evident that the intraoperative evaluation of CCEP may provide new insights into the neurophysiological organization of speech function. For example, according to the traditional model, the FLA and TLA are interconnected via a single neuronal pathway involving the arcuate fasciculus and superior longitudinal fasciculus.[11] However, this model cannot explain the different CCEP responses observed during speech impairment accompanying the removal of the deep part of the left inferior parietal glioma in Case 13: deterioration of peak latency and amplitude was noted in the posterior part of the superior temporal gyrus but not in its middle part. On the other hand, such a finding may favor the recently described dorsal and ventral pathways of the language network.[1,10,14,35] The former involves the arcuate fasciculus and superior longitudinal fasciculus and interconnects the FLA with the TLA and parietal cortex,[9] whereas the latter includes the uncinate fasciculus and inferior fronto-occipital fasciculus and interconnects the FLA with the TLA and occipital cortex.[1,10,35] In such a way, in our patient isolated deterioration of the CCEP response in the posterior part of the superior temporal gyrus may have been caused by alterations of the dorsal pathway but preservation of the ventral one.

Moreover, the application of intraoperative CCEP monitoring may have definite clinical importance. During awake craniotomy the majority of patients in the present study demonstrated speech dysfunction, which usually appeared during removal of the deep part of the tumor that affected the subcortical language pathways. Trinh et al.[40] noted that the presence of language and/or motor abnormalities not resolved by the end of surgery is associated with an 88% probability of a neurological deficit in the immediate postoperative period and that patients in such cases are more than 6 times more likely to have dysfunction at the 3-month follow-up.[40] In our series the changes in CCEP were constantly observed in the presence of intraoperative speech abnormalities, but the appearance of the latter was not always accompanied by alterations in the CCEP response. Changes in CCEP were variable and ranged from complete disappearance to a more or less prominent decrease followed by some recovery, which was associated with the time interval to restore language function. Of note, the absence of any intraoperative CCEP change had 100% positive predictive value for the recovery of speech function within 3 months after surgery. Additionally, monitoring CCEP facilitated intraoperative evaluation of speech in patients with a preexisting deficit (Cases 3 and 6) and was helpful in differentiating dysarthria caused by pyramidal tract injury from dysphasia (Case 1). Therefore, CCEP recording can be considered as a useful adjunct for assessing language during surgery and predicting its postoperative prognosis.

Decision making in regard to the optimal resection rate for glioma, whose removal under awake conditions is accompanied by the appearance of speech disturbances, is always difficult. It is evident that complete elimination of the neoplasm can be beneficial to patient survival, but its attainment at the cost of permanent postoperative speech dysfunction is hardly acceptable.[22] The majority of persistent neurological deficits after awake craniotomy for glioma removal is related to the dissection of eloquent subcortical structures,[40] which can be identified with diffusion tensor imaging or intraoperative electrical stimulation. The former imaging technique, however, is susceptible to known inaccuracies, even if performed intraoperatively,[28,32,37] whereas positive subcortical mapping through the resection cavity is associated with the immediate postoperative appearance or aggravation of speech dysfunction in 67%–100% of patients.[1,6] In the present study tumor removal was guided by intraoperative CCEP recording. Unless subcortical stimulation caused speech arrest, the resection continued even in the presence of language disturbances until a prominent decrease in or disappearance of the CCEP response was observed. This strategy can be criticized since, like any neurophysiological technique based on the evaluation of an averaged response, intraoperative CCEP monitoring

may be susceptible to the temporary gap between structural injury and its detection. However, it seems that the 100 seconds required for recording the complete CCEP response is comparable with the time required for intraoperative neurophysiological methods of proven efficacy such as somatosensory evoked potentials (56.2 seconds for averaging 500 stimuli at 8.9 Hz) and auditory brainstem response (99 seconds for averaging 1000 stimuli at 10.1 Hz). It should be noted that the median resection rate in the current series was 95%, and in no cases were permanent speech production disorders remarkable after surgery, although they are encountered in approximately 2% of patients undergoing awake craniotomy with direct intraoperative brain mapping.[1,34] Therefore, intraoperative CCEP monitoring can facilitate decision making as regards the optimal resection of gliomas located in the vicinity of language-related structures, although further studies on this important issue are definitely needed.

As was pointed out by Matsumoto et al.,[21] CCEP recordings are task free and do not require any cooperation from the patient, which facilitates its use in persons who are not candidates for awake craniotomy or who cannot tolerate this procedure. In fact, in one of our patients (Case 4), removal of the neoplasm was done under general anesthesia. During surgery CCEP monitoring did not reveal any changes in the evoked response, and no postoperative language disorders were noted. While before making any solid conclusions, any beneficial outcome should be reproduced in additional cases, this observation pointed out the possibility of speech function control during brain tumor removal under general anesthesia. In such a way, the FLA and TLA can be localized with cortical mapping in patients undergoing surgery according to an awake-asleep strategy, through cortical mapping after initial implantation of the chronic subdural grid electrodes (as it was done in our patient), or by means of advanced neuroimaging. In particular, functional MRI provides 59%–100% sensitivity and 0%–97% specificity for correct identification of language-related cortical areas[12,33] and is generally considered to be fairly good for demonstrating the FLA but less effective for demonstrating the TLA.

Possible disadvantages of the proposed technique of intraoperative CCEP monitoring include an increase in operation time and the requirement of a large craniotomy to provide access to the tumor, FLA, and TLA. It has been suggested that glioma resection guided by positive cortical mapping can, by itself, be associated with a greater risk of postoperative neurological deterioration, and a "tailored" craniotomy without intentional exposure of the eloquent cortex has been advocated.[16,34,40] However, the mentioned drawbacks should not be overemphasized. While a larger craniotomy and intraoperative brain mapping have certainly led to some increase in the duration of surgery (roughly 1.5 hours per patient), the mean operation time in the present study (8.2 hours) corresponds well with our general experience with surgery for gliomas using intraoperative MRI.[23–26] Recently, Leuthardt et al.[18] reported an average operating room time of 7.9 hours (range 5.9–9.7 hours) in 12 patients treated with the combined use of awake craniotomy, intraoperative cortical mapping, and intraoperative MRI, which seems concordant with time in the present study. It should be noted that neither a more extensive surgical approach nor a relative prolongation of the surgical procedure resulted in additional morbidity in our patients.

There are several limitations to the present study. First, it was conducted at a single institution, includes a small number of highly selected cases, and does not contain a control group, which do not permit detailed statistical evaluation of the predictive value of the presented technique for postoperative speech dysfunction and its prognosis, particularly in comparison with direct intraoperative assessment of language during awake craniotomy. Second, the exact threshold of CCEP decrease, which can be used for the termination of tumor resection to avoid permanent postoperative language dysfunction, was not determined. Third, the location of stimulating and recording electrodes used for intraoperative CCEP monitoring corresponded to rather restricted sites within the cortical language-related areas, which (especially, the TLA) can be distributed rather widely.[21,29,34] Fourth, only interconnections between the FLA and the TLA were evaluated, although it is known that other cortical areas, especially the inferior parietal lobule, and their subcortical pathways are playing very important roles in speaking abilities.[1,3,6,21,22,34,35] Fifth, while diffusion tensor imaging and subcortical stimulation were routinely performed in our patients, the correspondence between identified language pathways and CCEP response was not specifically addressed. Finally, the detailed neurophysiology of the evolved response, especially as related to the direction of stimulation, should be clarified further. Therefore, despite the rather promising results presented herein, evaluation of CCEP recordings for the assessment of speech function during brain tumor surgery definitely needs additional investigation.

Conclusions

Results of the present study demonstrate the feasibility of CCEP monitoring during the resection of intracranial tumors affecting language-related cerebral structures. This neurophysiological method can be useful in the objective evaluation of speech function as well as its direct assessment during awake craniotomy. Moreover, it can be helpful in predicting the prognosis of intraoperative and postoperative deteriorations in language and for deciding on the optimal resection of a neoplasm. Further experience with intraoperative CCEP recordings may open the possibility of monitoring speech function during neurosurgical procedures performed under general anesthesia.

Disclosure

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Author contributions to the study and manuscript preparation include the following. Conception and design: Muragaki, Saito, Ku-

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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

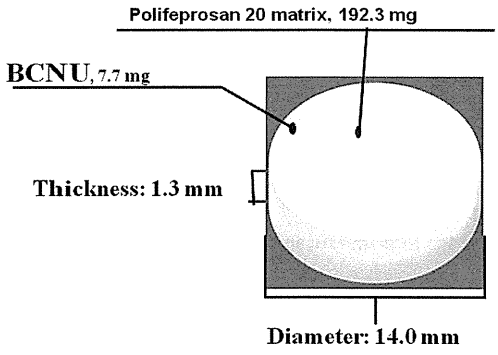

Fig. 1 BCNU implant configuration.

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 $mg \cdot m^{-2} day^{-1}$) 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 $mg \cdot m^{-2} day^{-1}$) 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:

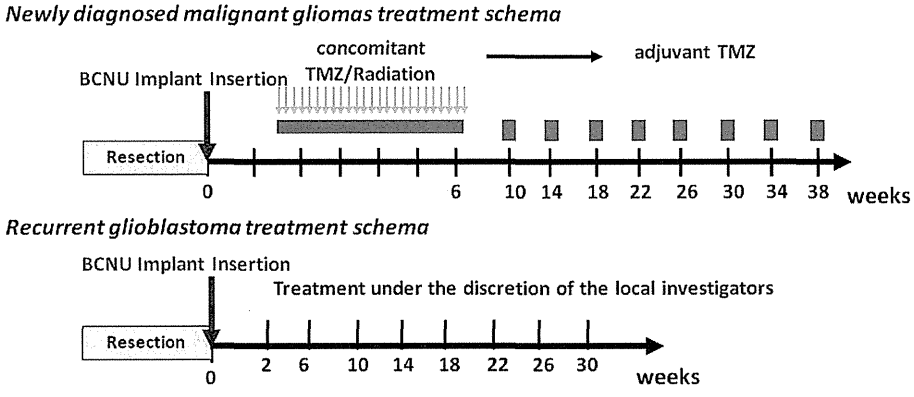

Fig. 2 Treatment schema. TMZ: temozolomide.

(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 (cm^2)	Mean	23.0	16.9
	SD	15.0	10.5
	Min	2.0	3.5
	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 (sheets)	Mean	7.7	7.9
	SD	0.87	0.35
	Min	5.0	7.0
	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 4 cases 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 cm^2, 23.0 ± 15.0 cm^2, and 16.9 ± 10.5 cm^2 in the entire population, patients with newly diagnosed MGs, and patients with recurrent MGs, respectively. The median tumor sizes were 22.6 cm^2, 22.5 cm^2 and 22.6 cm^2 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 that at 12 months was 62.5% (95% CI: 34.9–81.1%)

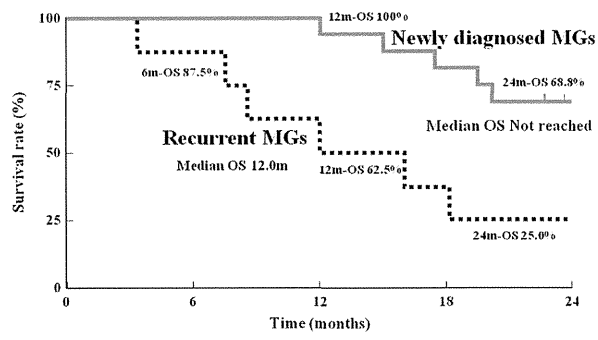

Fig. 3 Kaplan-Meier curve of survival period/rate. MGs: malignant gliomas, OS: overall survival rate, m: months, 6m-OS: the overall survival rates at 6 months, 12m-OS: the overall survival rates at 12 months, 24m-OS: the overall survival rates at 24 months.

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 days (Fig. 4). When this group was subdivided, the PFS rates at 6 months and 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 second recurrence at 7.3 months after insertion of

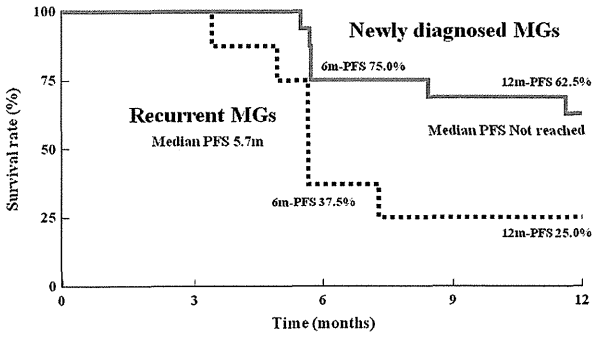

Fig. 4 Kaplan-Meier curve of progression-free survival period/rate (judged by the efficacy and safety evaluation committee). MGs: malignant gliomas, PFS: progression-free survival rate, m: months, 6m-PFS: progression-free survival rate at 6 months, 12m-PFS: progression-free survival rate at 12 months.

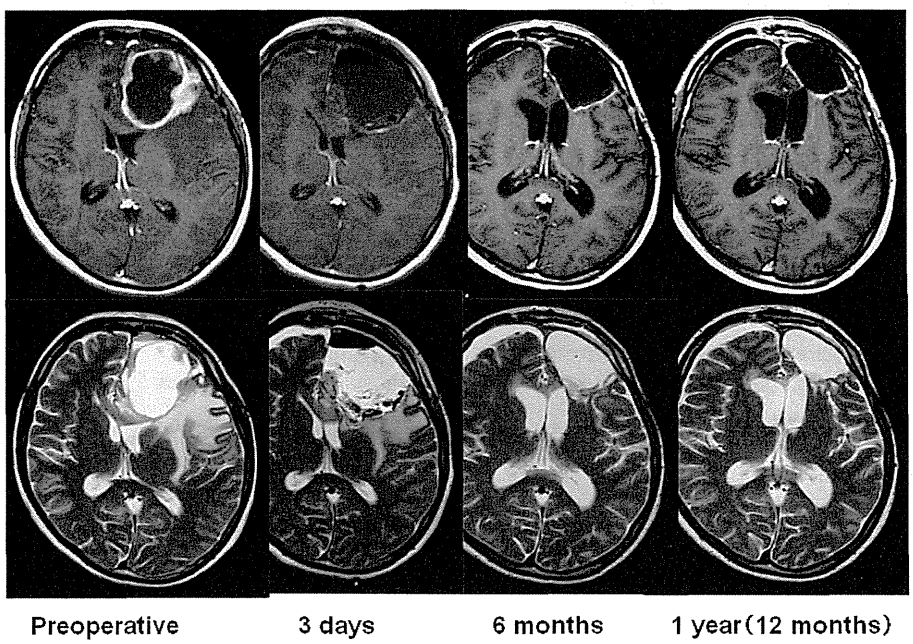

Fig. 5 Time course of magnetic resonance (MR) imaging findings (1st relapse of recurrent glioblastoma), axial gadolinium contrast-enhanced T_1-weighted MR images (*upper row*), and T_2-weighted MR images (*lower row*).

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 and who received BEV therapy (1 newly diagnosed

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	5	(20.8)		–
Facial swelling	5	(20.8)		–
Alopecia	16	(66.7)		–
Radiation-induced skin 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.

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 MGs was 100.0% at 12 months and 68.8% at 24

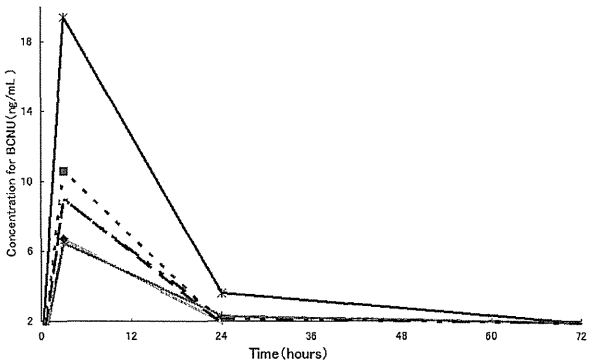

Fig. 6 Time course of BCNU levels in whole blood. Six Japanese patients with malignant gliomas received the maximum blood concentration of BCNU at about 3 hours after implant placement was 19.4 ng/mL. The lower limit of quantitation (2.00 ng/mL).

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 be careful. Infection and meningitis were not observed

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.

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

Dr. Tomokazu Aoki is a member of the medical advisory committee of NPC-08 study and received consulting fees from Nobelpharma Co. Ltd. and an honoraria for speaking from Eisai Co. Ltd. Dr. Ryo Nishikawa is a member of the medical advisory committee of NPC-08 study and received consulting fees from Nobelpharma Co. Ltd. Dr. Nishikawa is also a member of the Avaglio study steering committee (funded by F. Hoffmann-La Roche, Ltd) and has received research funding and speaking fees from MSD KK as well as honoraria for speaking from Eisai Co. Ltd. Dr. Kazuhiko Sugiyama is a member of the medical advisory committee of NPC-08 study and received consulting fees from Nobelpharma Co. Ltd. and honoraria for speaking from Eisai Co. Ltd. Dr. Masao Matsutani is a coordinating investigator of NPC-08 study, a member of the advisory committee of MSD KK, and a coordinating investigator for Chugai Pharmaceutical Co. Ltd. Dr. Matsutani also received consulting fees from Nobelpharma Co. Ltd.

The authors declare no other conflicts of interest.

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Comparison of Multiple Parameters Obtained on 3T Pulsed Arterial Spin-Labeling, Diffusion Tensor Imaging, and MRS and the Ki-67 Labeling Index in Evaluating Glioma Grading

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ABSTRACT

BACKGROUND AND PURPOSE: Pulsed arterial spin-labeling, DTI, and MR spectroscopy provide useful data for tumor evaluation. We evaluated multiple parameters by using these pulse sequences and the Ki-67 labeling index in newly diagnosed supratentorial gliomas.

MATERIALS AND METHODS: All 32 patients, with grade II (3 each of diffuse astrocytoma, oligodendroglioma, and oligoastrocytoma), grade III (3 anaplastic astrocytomas, 4 anaplastic oligodendrogliomas, and 1 anaplastic oligoastrocytoma), and grade IV (14 glioblastomas and 1 glioblastoma with an oligodendroglioma component) cases underwent pulsed arterial spin-labeling, DTI, and MR spectroscopy studies by using 3T MR imaging. The following variables were used to compare the tumors: relative cerebral blood flow, fractional anisotropy; ADC tumor/normal ratios; and the Cho/Cr, NAA/Cho, NAA/Cr, and lactate/Cr ratios. A logistic regression and receiver operating characteristic analysis were used to assess parameters with a high sensitivity and specificity to identify the threshold values for separate grading. We compared the Ki-67 index with various MR imaging parameters in tumor specimens.

RESULTS: Significant correlations were observed between the Ki-67 index and the mean, maximum, and minimum ADC, Cho/Cr, and lactate/Cr ratios. The receiver operating characteristic analysis showed that the combination of the minimum ADC and Cho/Cr ratios could differentiate low-grade and high-grade gliomas, with a sensitivity and specificity of 87.0% and 88.9%, respectively. The mean and maximum relative cerebral blood flow ratios were used to classify glioblastomas from other-grade astrocytomas, with a sensitivity and specificity of 92.9% and 83.3%, respectively.

CONCLUSIONS: Our findings indicate that pulsed arterial spin-labeling, DTI, and MR spectroscopy are useful for predicting glioma grade. Additionally, the parameters obtained on DTI and MR spectroscopy closely correlated with the proliferative potential of gliomas.

ABBREVIATIONS: ASL = arterial spin-labeling; C1 error = average observed sensitivity and specificity; C2 error = observed number of instances of tumor-grade misclassification; FA = fractional anisotropy; Lac = lactate; NPV = negative predictive value; PASL = pulsed arterial spin-labeling; PPV = positive predictive value; rCBF = relative cerebral blood flow; ROC = receiver operating characteristic

Grading gliomas is necessary to determine the appropriate treatment strategy and assess prognosis. Classifying lesions into 4 grades based on histologic analyses requires tumor specimens obtained via biopsy or surgical resection.[1]

On conventional MR imaging with gadolinium contrast, the presence of FLAIR abnormalities or gadolinium enhancement reveals the appearance of new lesions. Advanced MR imaging, pulsed arterial spin-labeling (PASL), DTI, and MR spectroscopy provide useful data for evaluating tumors preoperatively. The PASL technique allows cerebral blood flow to be measured noninvasively without exogenous contrast agents. The usefulness of perfusion MR imaging with arterial spin-labeling (ASL) for assessing brain tumor angiogenesis and grading gliomas has been evaluated.[2-7] DTI provides information on anisotropy, including fractional anisotropy (FA), and ADC. A recent study investigating DTI of gliomas showed that the FA and ADC tumor/normal tissue ratios are possible indicators of glioma proliferation and/or grading.[8-10] MR spectroscopy is also a noninvasive method that allows the measurement of various metabolites in vivo, such as Cho, Cr, NAA, and the pathologic levels of lactate (Lac), and has been reported useful for investigating gliomas.[11-13] The use of a combination of these noninvasive parameters has been reported to increase the diagnostic accuracy of glioma grading.[7,9,11,12,14-18]

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