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CLINICAL STUDY - PATIENT STUDY

Survival benefit of Boron neutron capture therapy for recurrent malignant gliomas

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Abstract We have applied boron neutron capture therapy (BNCT) to malignant brain tumors. Here we evaluated the survival benefit of BNCT for recurrent malignant glioma (MG). Since 2002, we have treated 22 cases of recurrent MG with BNCT. Survival time was analyzed with special reference to recursive partitioning analysis (RPA) classification, by Carson et al. (J Clin Oncol 25:2601–2606, 2007). Median survival times (MSTs) after BNCT for all patients and for glioblastoma as on-study histology at recurrence was 10.8 months ($n = 22$; 95% CI, 7.3–12.8 months) and 9.6 months ($n = 19$; 95% CI, 6.9–11.4 months), respectively. In our study, MST for the high-risk RPA classes was 9.1 months ($n = 11$; 95% CI, 4.4–11.0 months). By contrast, the original journal data showed that the MST of the same RPA classes was 4.4 months ($n = 129$; 95% CI, 3.6–5.4 months). BNCT showed a survival benefit for recurrent MG, especially in the high-risk group.

Keywords BNCT · BPA–PET · GBM · MG · RPA

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Introduction

We have applied a form of tumor-selective particle radiation, boron neutron capture therapy (BNCT), for malignant gliomas (MGs) [1, 2] and malignant meningiomas [3, 4]. BNCT comprises a binary approach [5]: a boron-10 (^{10}B)-labeled compound is administered that delivers high concentrations of ^{10}B to the target tumor relative to the surrounding normal tissues. This is followed by irradiation with thermal neutrons. When neutrons collide into ^{10}B atoms, high linear-energy-transfer (LET) alpha and ^{7}Li particles are released from the ^{10}B (n, alpha) ^{7}Li neutron capture reaction. The short range (5–9 micrometers) of these particles allows for relatively selective tumor killing without significant damage to the adjacent normal brain tissue.

The prognosis of recurrent MGs, especially glioblastoma multiforme (GBM) is poor [6]. We reported the effectiveness of BNCT on neuroimages for MGs [1, 2], and recently reported the survival benefit of BNCT for newly diagnosed MGs [7]. Unfortunately, the standard treatment for recurrent MG has not yet been established. Therefore, evaluation of the survival benefit of BNCT for recurrent MGs is difficult. Also with limited case numbers like this study, it is difficult to elucidate some objective assessments

of the survival benefit of BNCT. To evaluate this in low and high-risk group of recurrent MGs, we adopted the recursive partitioning analysis (RPA) classification for recurrent MG advocated by Carson et al. in a 2007 article in the *Journal of Clinical Oncology*, in which the results of 10 recent protocols of phase-1 and -2 trials applied by the new approaches to brain tumor therapy CNS consortium (NABTT) for recurrent MG were summarized [8]. They included six systemic treatment and four local treatment trials. Originally this RPA classification was not aimed at the evaluation of the effectiveness of each trial for recurrent MG; however, this RPA classification gave us a uniform background and median survival time (MST) for each recurrent MG-type patient at the time of recurrence. So we classified our recurrent MG patients treated by BNCT and compared their survival to the MSTs presented in the above journal.

Patients and methods

Patient enrollment

From 2002 to 2007 we treated a total of 22 cases of recurrent MG using BNCT. Our eligibility criteria for this trial were as follows: (1) age 15 years or older; (2) histologically proven supratentorial MG (GBM, AA, AO, or anaplastic oligodendroglioma, as on-study histology) that had proved to be progressive or recurrent after radiation therapy; (3) depth of the tumor from scalp less than 6 cm (if the lesion is deeper than 6 cm from the scalp, partial removal or cyst evacuation was applied to fit this criteria, see below); (4) no cerebrospinal fluid (CSF) dissemination at recurrence; (5) estimated life expectancy longer than 3 months, not pregnant or breast feeding, and having a KPS score of 60 or greater.

Clinical regimen of BNCT

After the confirmation of the tumor progression or recurrence of the original lesions on MRI, the patients received a BPA–PET to assess the distribution of boronophenylalanine (BPA) [9, 10]. The lesion/normal brain (L/N) ratio of BPA uptake can be estimated from this type of study, and dose planning was performed according to the L/N ratio, as described previously [1, 2]. If the lesions were deeper than 6 cm from the scalp, partial removal of the mass or cyst evacuation was applied. At this procedure, air instillation via an Ommaya reservoir was performed so that the neutron flux would penetrate to the deepest part of the tumor [11]. Within a month after the surgery, BNCT was performed.

In protocol 1, the patients were administered 100 mg/kg of sodium borocaptate (BSH) and 250 mg/kg of BPA for one hour intravenously 12 h prior and just prior to neutron irradiation, respectively. In protocol 2, the patients were administered 100 mg/kg of BSH intravenously for one hour, 12 h prior to neutron irradiation and 700 mg/kg of BPA continuously for 6 h before the irradiation. In both protocols, the neutron irradiation time was determined not to exceed 13 Gy-Eq to the normal brain by simulation. Here, Gy-Eq (Gy: Gray) corresponds to the biologically equivalent X-ray dose that would have equivalent effects on tumors and on the normal brain. For some deep tumors, air instillation was performed as stated above just prior to neutron irradiation.

Patient follow-up

Patients were followed up by bimonthly Gd-enhanced MRI. When the lesions became enlarged or new lesions appeared on the follow-up MRI, we applied BPA–PET to evaluate the tumor activity [12]. If the positron emission tomography (PET) results suggested tumor progression (TP), additional treatments were applied. If PET suggested the high possibility of radiation necrosis (RN), medical treatments for this pathology or surgical resections were applied [12, 13].

Patient characteristics

The patients' age, gross tumor volume (GTV) (Gd-enhanced lesions on MRI at relapse, use of temozolomide (TMZ) and absorbed dose by BNCT (minimum tumor dose and maximum brain dose) are summarized in Table 1. In 12 cases surgery was applied before BNCT, as a form of cyst evacuation or partial tumor removal to make a cavity to establish an Ommaya reservoir as described above. Ten cases were administered TMZ, three before the relapse and seven after BNCT. Individual information of TMZ usage is

Table 1 Patient characteristics

Description		
Age (median (range))	51	(15–67)
[a]GTV at the relapse (median (range))	42.0	(4.1–64.5) ml
[b]Reoperated cases at relapse	12	
TMZ	10	
Before BNCT	3	
After BNCT	7	

[a] GTV was measured on contrast-enhanced MRI at the relapse

[b] Cyst puncture or partial removal to make cavity for air instillation

Table 2

Case No	Age	Sex	Histology		RPA by Carson et al.	TMZ		BNCT protocol	Absorbed dose (Gy-Eq)		Survival (months)after BNCT	Cause of death
			Initial	On-study		Before	After		Min tumor	Max brain		
1	42	M	AA	GB	1	−	+	1	15.5	12.4	43.1	TP
2	57	F	AA	GB	1	−	+	2	37.3	8.3	22.0	D
3	15	F	AA	AA	2	−	+	2	56.3	10.7	33.4	A
4	53	M	Oligo	GB	2	−	−	2	73.9	13.2	6.9	D
5	51	M	AOA	AOA	2	−	−	2	27.4	8.1	32.4	D
6	33	F	G2	AA	2	−	−	1	12.7	7.1	15.0	OC
7	61	M	AA	GB	3	−	−	1	34.4	3.7	10.8	RN
8	29	F	AA	GB	3	−	−	1	25.7	5.9	9.6	B
9	62	M	AA	GB	3	−	−	1	23.4	9.9	2.5	OC
10	31	M	G2	GB	3	−	−	1	29.3	14.2	4.4	D
11	51	M	AA	GB	3	+	−	2	44.9	13.6	9.1	TP
12	48	M	GB	GB	4	−	−	1	27.2	11.1	7.8	D
13	46	F	GB	GB	4	−	+	2	49.2	12.1	12.8	D
14	41	M	GB	GB	4	+	−	2	54.3	12.7	10.3	D
15	35	M	GB	GB	5	−	−	2	37.7	13.4	6.0	D
16	45	M	GB	GB	5	+	−	2	59.0	13.8	11.4	RN
17	59	M	GB	GB	7	−	−	1	32.8	11.2	8.6	TP
18	50	M	GB	GB	7	−	−	1	32.6	13.6	15.3	RN
19	63	M	GB	GB	7	−	−	2	34.7	9.4	11.0	D
20	67	F	GB	GB	7	−	+	2	58.0	11.7	12.3	D
21	60	F	GB	GB	7	+	+	2	34.9	7.5	5.8	TP
22	54	M	GB	GB	7	−	+	2	19.7	10.7	7.4	TP

M, male; F, female; AA, anaplastic astrocytoma; Oligo, oligodendroglioma; AOA, anaplastic oligoastrocytoma; G2, grade 2 astrocytoma; GB, glioblastoma; RPA, Recursive partitioning analysis; TMZ, temozolomode; Gy-Eq, Gray equivalent; Min, minimum; Max, maximum; BNCT, boron neutron capture therapy; TP, local tumor progression; A, alive; D, CSF dissemination; RN, radiation necrosis; OC, other cause; B, both dissemination and local tumor progression

listed in Table 2. In Table 2, two histological diagnoses were prepared. One is initial histology and the other is on-study histology. Here, on-study histology means the histology that was confirmed at the last surgery for each patient, prior to BNCT.

Statistical methods

Continuous data are summarized with medians, ranges and *P*-values. Univariate analysis was performed using chi-square log-rank testing. Survival distributions (MSTs and 95% CIs) were estimated using the product limit method. The analysis was intention-to-treat and included all eligible patients. Data were entered into Microsoft Excel (Microsoft Corporation) and analyzed using JMP software version 7 (SAS Institute, Cary, NC, USA).

RPA classification

To objectively evaluate the survival benefit of BNCT for recurrent MG, we classified our BNCT cases according to the RPA classification advocated in some journals [8]. These classifications can be summarized as follows: class 1, not GBM (initial histology), KPS $\geq$ 80, frontal (tumor location); RPA class 2, not GBM, KPS $\geq$ 80, not frontal; RPA class 3, not GBM, KPS $\leq$ 70; RPA class 4, GBM, Age $\leq$ 50, KPS $\geq$ 90; RPA class 5, GBM, Age $\leq$ 50, 60 $\leq$ KPS $\leq$ 80; RPA class 6, GBM, Age $\geq$ 50, no steroid use; RPA class 7, GBM, Age $\geq$ 50, steroid use. Individual class of RPA of our cases treated by BNCT is listed in Table 2.

Analysis of the cause of death after BNCT

Unfortunately, 21 out of the 22 patients died during the observation period, as listed in Table 2. The cause of death was analyzed with the following categories: local TP, CSF dissemination, RN, and other cause of death. These classifications were based on Gd-enhanced MRI, BPA–PET, histology of the surgical specimen and autopsy. In one case, both CSF dissemination and local TP occurred simultaneously and it was impossible to determine a single pathology as the major cause of death.

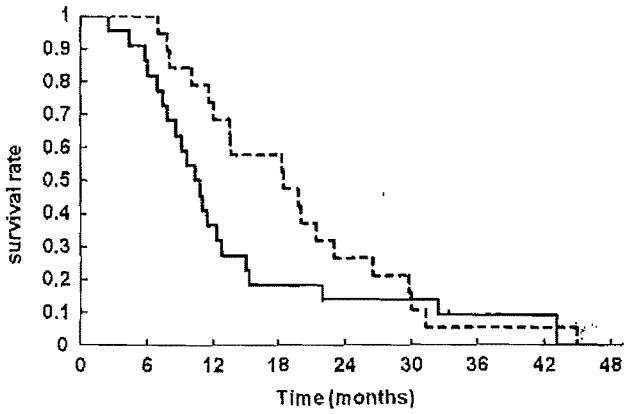

Fig. 1 Kaplan–Meier survival curves for recurrent MG cases treated by BNCT. The continuous line shows the survival of all patients after BNCT ($n = 22$). The broken line shows the survival of GBM (on-study histology) after diagnosis of GBM ($n = 19$)

Results

Survival after BNCT and after diagnosis

Individual histology (initial and on-study at relapse), RPA class, TMZ use, BNCT protocol (1 or 2), absorbed dose by BNCT, survival period after BNCT, and cause of death are summarized in Table 2. Survival after BNCT ($n = 22$) and that from initial GBM diagnosis ($n = 19$, on-study histology as GBM) are shown in Fig. 1. MST after BNCT for all patients ($n = 22$) was 10.8 months (95% CI, 7.3–12.8 months). MST after BNCT for GBM cases as on-study histology at recurrence ($n = 19$) was 9.6 months (95% CI, 6.9–11.4 months). MST after initial GBM diagnosis ($n = 19$) was 19.1 months (95% CI, 11.6–23.0 months).

Survival with special reference to RPA classes

The MSTs (months) of our BNCT cases classified according to RPA classes are shown in Table 3 and compared in each case with the values from Carson et al.: Class 1 ($n = 2$): 32.6 vs. 25.7 (Carson et al.), Class 2 ($n = 4$): 23.7 vs. 17.2, Class 3 ($n = 5$): 9.1 vs. 3.8, Class 4 ($n = 3$): 10.2 vs. 10.4, Class 5 ($n = 2$): 8.5 vs. 6.4, Class 7 ($n = 6$): 9.8 vs. 4.9. The tendencies in patient survival of our cases after BNCT were very similar to those of the original report in terms of RPA classification. Since our cases were so limited in number, we joined the worst prognosis classes (Class 3 and 7) together into one class. The MST of our cases in this combined class was 9.1 months ($n = 11$; 95% CI, 4.4–11.0 months), while that in Carson et al. was 4.4 months ($n = 129$; 95% CI, 3.6–5.4 months).

Cause of death after BNCT

We lost 21 cases out of 22. The causes of deaths were CSF dissemination (10 cases), local TP (5), both (1), RN (3), and other (2), as shown in Table 2. With regard to RN, we discuss more extensively in "Discussion".

Adverse effects of BNCT

No serious adverse effects were observed both in protocols 1 and 2 in this study of BNCT for recurrent MGs, even though all patients were applied with radiotherapy previously. Hematuria was reported in the literature using large amounts of BPA in BNCT [14]. Fortunately, we did not experience this adverse effect at all, however, three cases in protocol 2 showed transient decrease volume and turbidity of urine and fever during the first 24 h after BNCT. We concluded these side effects were caused by recrystallization of BPA in urine. Thereafter, we over hydrated the remaining patients after BNCT, and no such side effects were observed again.

Univariate analysis for the survival after BNCT

In Table 4, we analyzed factors for survival after BNCT, such as sex, age, TMZ or steroid usage, KPS, minimum absorbed dose in tumors, initial histology, GTV at the relapse, BNCT protocol (1 or 2) and RPA classes. Among them, only RPA class (RPA class 3 and 7 or others) showed a statistical significant influence on survival after BNCT.

Table 3 Comparison of NABTT trials and our BNCT series

	All patients			RPA 3 + 7		
	MST	95% CI	Number in series	MST	95% CI	Number in series
NABTT	7.0	6.2–8.0	$n = 310$	4.4	3.6–5.4	$n = 129$
BNCT	10.8	7.3–12.8	$n = 22$	9.1	4.4–11.0	$n = 11$

[a] New Approaches to brain tumor therapy CNS Consortium; 10 phase-1 and -2 trials reported by Carson et al. (J Clin Oncol 25:2601–2606, 2007)

MST, Median survival time; CI, confidence interval

Table 4 Univariate analysis of factors for survival after BNCT

Factor	Gronp	Survival (months)				*P*-Value
		Median	95% CI			
Sex	Male ($n = 15$)	9.1	6.0	–	11.0	$P = 0.2456$
	Female ($n = 7$)	12.8	5.8	–	22.0	
Age	≦50 ($n = 11$)	11.4	6.0	–	15.3	$P = 0.2482$
	>50 ($n = 11$)	9.1	5.8	–	12.3	
	≦57 ($n = 16$)	11.4	7.4	–	15.3	$P = 0.0982$
	>57 ($n = 6$)	10.8	2.5	–		
KPS	≦80 ($n = 13$)	9.6	6.0	–	11.4	$P = 0.1271$
	>80(n = 9)	12.8	5.8	–		
Initial Histology	GBM ($n = 11$)	10.3	6.0	–	12.3	$P = 0.1329$
	Not GBM ($n = 11$)	10.8	4.4	–	32.4	
TMZ	Used ($n = 10$)	12.3	5.8	–	22.0	$P = 0.1468$
	Not used ($n = 12$)	9.6	4.4	–	15.0	
Steroid	Used ($n = 13$)	9.6	6.9	–	11.4	$P = 0.1445$
	Not used ($n = 9$)	12.8	2.5	–		
GTV (ml)	≦37.2($n = 11$)	9.1	4.4	–	12.8	$P = 0.5273$
	>37.2 ($n = 11$)	10.8	7.4	–	15.3	
Minimum tumor	≦34.0($n = 12$)	9.6	2.5	–	15.3	$P = 0.9110$
Dose (Gy-Eq)	>34.0($n = 10$)	11.0	6.0	–	12.8	
	≦37.0($n = 13$)	9.6	5.8	–	15.0	$P = 0.6548$
	>37.0($n = 9$)	11.4	6.0	–	22.0	
BNCT protocol	1 ($n = 9$)	9.6	2.5	–	15.3	$P = 0.8184$
	2 ($n = 13$)	11.0	6.9	–	12.8	
RPA class	RPA 3&7 ($n = 11$)	9.1	4.4	–	11.0	$P = 0.0216$
	RPA not 3&7 ($n = 11$)	12.8	6.9	–	32.4	

Representative case

A 48-year-old man with a right temporal mass was operated emergently for consciousness disturbance in a hospital. The operation was partial tumor removal and histological diagnosis was GBM. He received fractionated X-ray radiation therapy (XRT) with a total dose of 80 Gy and chemotherapy consisting of nimustine and vincristine. Even during the radiotherapy, the tumor continued to enlarge, and the patient was referred to our institute for BNCT (Fig. 2 a, a′). He was classified as RPA class 4. The BNCT was performed with the minimum tumor absorbed dose of 27.2 Gy-Eq, and maximum brain absorbed dose of 11.1 Gy-Eq. One week after BNCT the mass shrunk rapidly (Fig. 2 b, b′). Three months after BNCT, the original mass became enlarged in Gd-MRI. He was operated on again. The histology was mainly necrosis with small pocket of residual tumor cells. He was well for another 4 months. We lost this case 7.8 months after BNCT and 13.5 months after initial surgery, due to CSF dissemination (Fig. 2 c, c′). This is a representative case of recurrent MG treated by BNCT, with regard to the rapid tumor shrinkage after BNCT and the occurrence of radiation necrosis and CSF dissemination as the cause of death.

Discussion

Here we reported the survival benefit of BNCT for recurrent MG cases, mainly GBM. The MST after BNCT for GBM cases as on-study histology at recurrence ($n = 19$) was 9.6 months (95% CI, 6.9–11.4 months). In the literature, we found a summary of a large series of eight phase-2 trials of chemotherapies for recurrent GBM cases [15]. In this report, the authors mentioned the MST of GBM after relapse as 25 weeks (5.8 months; 95% CI, 21–28 weeks, 4.9–6.5 months; $n = 225$). In comparison with this result, our data for the survival benefit of BNCT in recurrent GBM was not bad.

As to BNCT for recurrent GBM, two small series have been reported in the literature. A Swedish group and a Finnish group reported that MSTs for recurrent GBM after BNCT were 8.7 ($n = 12$) [16] and 7.5 months ($n = 7$) [17], respectively. Our data in the current report is almost equal to/somewbat better than the findings in these reports.

Kaplan–Meyer analysis in Fig. 1 showed that MST after BNCT for all patients ($n = 22$) was 10.8 months (95% CI, 7.3–12.8 months). We are not sure whether this result is reliable, as this is the result of a small series from a single institute. To evaluate the survival benefit of BNCT in low

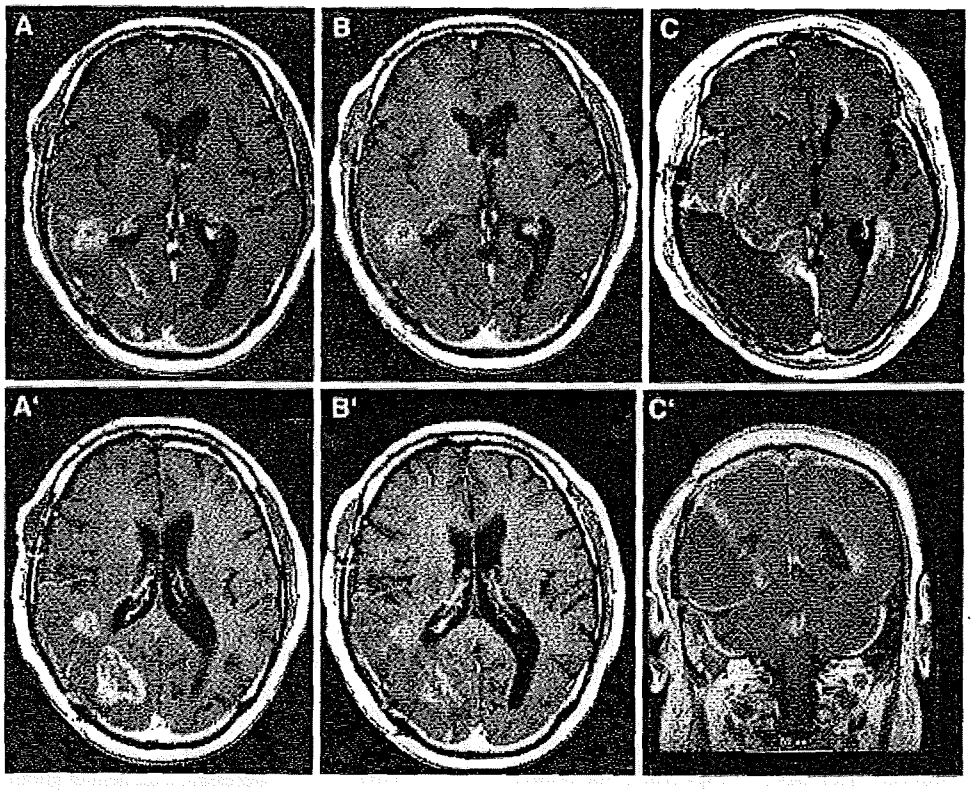

Fig. 2 A representative case of recurrent GBM treated by BNCT. (a, a') MRI, prior to BNCT. Gd-enhanced lesions were at the right temporo-occipital lobe; (b, b') MRI, 48 h after BNCT. Marked shrinkage of the lesions was reeognized; (c, c') MRI, 7 months after BNCT. CSF dissemination was prominent

and high-risk group of recurrent MGs, we applied RPA to our cases as advocated in the literature [8]. Inclusion criteria for our trial and the 10 NABTT phase-1 and -2 trials reported in Carson et al. were not very different. Our case numbers for each RPA class were so limited, however, that the MST of our cases in each RPA class were relatively better in comparison with original NABTT results, as listed above. In the original article, RPA class 3 (Not GBM, KPS $\leq$ 70) and class 7 (GBM, Age $\geq$ 50, steroid use) showed extremely poor prognosis (supplementary Table 1). The MST of our combined class 3 and class 7 cases was 9.1 months (n = 11; 95% CI, 4.4–11.0 months), while that in the original article was 4.4 months (n = 129; 95% CI, 3.6–5.4 months). We cannot know whether our current MST data is significantly better than that of each NABTT trial because their raw data were not available. But at least, BNCT showed a good survival benefit even for the highest-risk group, RPA class 3 and 7.

TMZ is the sole promising drug for GBM so far. A Swedish BNCT group reported potential TMZ effects with combination of BNCT at the relapse of GBM [16]. However, in our univariate analysis, TMZ did not contribute prominently to the prolongation of survival in our series (Table 4). In our 22 cases, we used TMZ in 10 cases, before BNCT in 3 cases (Cases 11, 14 and 16) and after BNCT in 7 (Cases 1, 2, 3, 13, 20, 21 and 22). For the former three cases, TMZ could not control the tumor growth and methylation-specific PCR showed an unmethylated O6-methylguanine DNA methyltransferase (MGMT) promoter [18] (data not shown). We stopped the administration of TMZ after BNCT as we judged TMZ was not efficacious for these three cases. Among the latter seven cases, only two (Cases 1 and 2, both classified as RPA class 1) showed methylated promoter status for MGMT, with good prognoses. For the other five cases, we were not sure of the MGMT expression status of the tumor. In the high-risk group in our series (RPA class 3 and 7), three cases were administered TMZ after BNCT (Cases 20, 21 and 22). Among them, Case 21 and 22 showed a relatively short survival after BNCT. We do not deny the meaning of TMZ use at relapse; however, in our series for this high-risk group, the survival benefit of TMZ was limited. In the literature, TMZ has actually shown modest survival benefit at relapse of recurrent GBM [19]. Brada et al. reported only 5.4 months prolongation as MST with TMZ at relapse in the report.

There are several reports with relatively good results for recurrent MG, with an MST of around 10 months after the stereotactic radiosurgery (SRS) [20] or stereotactic radiotherapy (SRT) [21] at relapse. However, there was big difference in GTV at the relapse between these SRS or SRT cases and ours. The median GTV of the former two was 10.1 and 12.7 ml, while the median GTV of our cases was 42.0 ml. There might also be a difference as to performance status or age between the SRS or SRT reports and our cases. The result of re-irradiation for recurrent

GBM was poor [22]. The MST of this report was 26 weeks after the treatment. In addition, BNCT can be applied in only one day. Taken together, BNCT could be one of the promising radiation treatment options for recurrent MG at relapse.

We lost many cases of recurrent MGs after BNCT by CSF dissemination, as we reported (in preparation) and as shown in Table 2 and Fig. 2. In other words, local control by BNCT for even recurrent MG was fairly good. There was a tendency for CSF dissemination to occur in relatively long-term survivors from diagnosis (data not shown). On the other hand, a major problem in BNCT for recurrent MG was the occurrence of RN. We experienced RN by BNCT especially for recurrent MG, because the patients had been treated by radiotherapy prior to BNCT. Although BNCT is cell-selective particle radiation, some particle dose is inevitably absorbed by the normal brain tissue as shown in Table 2. The diagnosis of this pathology is difficult; however, amino acid PET may give us good clue for it, as stated above [12]. Most of RN could be controlled with medical or surgical treatments as above; however, we lost three cases by RN in our series. Preventive medical treatments such as by anticoagulants or by vitamin E must be considered after BNCT, especially for recurrent cases. This is not mentioned in other BNCT reports for recurrent MG [16, 17]; however, it should be seriously considered. In Swedish reports of BNCT for recurrent GBM, the authors mentioned a median time to tumor progression of 6 months after BNCT, but there was no statement as to how TP was judged in their report. It is very difficult to differentiate RN and TP on MRI, especially with high-dose radiation treatment. So we did not apply the analysis of time to tumor progression in our series. In univariate analysis (Table 4), there was no correlation of minimum tumor dose by BNCT and survival after BNCT. Especially for recurrent cases, if we increase the minimum tumor dose by BNCT, the incidence of RN probably increases, as discussed here. Therefore, it is very difficult to elucidate the most suitable dose of BNCT at relapse. Regardless, RN is a serious problem to be overcome in the field of BNCT.

XRT plus concomitant TMZ (Stupp's regimen) has been the global standard so far for newly diagnosed GBM [23]. Pellettieri et al. reported that BNCT at relapse after Stupp's regimen might be the best treatment of GBM [16]. Also in our series BNCT at relapse showed a good MST after the initial GBM diagnosis of 19.1 months ($n = 19$; 95% CI, 11.6–23.0 months). But it cannot be concluded so easily that BNCT at relapse after Stupp's regimen is the best for the treatment of GBM because 19 cases in our series were referred to our institute at relapse with a significant interval after initial treatments. This interval might prolong the survival after initial GBM diagnosis at a glance.

In summary, the RPA classification advocated by Carson et al. predicted the patient survival trends of our BNCT series; however, BNCT showed the most prominent survival benefit in the high-risk group (RPA classes 3 and 7).

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Pseudoprogression in boron neutron capture therapy for malignant gliomas and meningiomas

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Pseudoprogression has been recognized and widely accepted in the treatment of malignant gliomas, as transient increases in the volume of the enhanced area just after chemoradiotherapy, especially using temozolomide. We experienced a similar phenomenon in the treatment of malignant gliomas and meningiomas using boron neutron capture therapy (BNCT), a cell-selective form of particle radiation. Here, we introduce representative cases and analyze the pathogenesis. Fifty-two cases of malignant glioma and 13 cases of malignant meningioma who were treated by BNCT were reviewed retrospectively mainly via MR images. Eleven of 52 malignant gliomas and 3 of 13 malignant meningiomas showed transient increases of enhanced volume in MR images within 3 months after BNCT. Among these cases, five patients with glioma underwent surgery because of suspicion of relapse. In histology, most of the specimens showed necrosis with small amounts of residual tumor cells. Ki-67 labeling showed decreased positivity compared with previous samples from the individuals. Fluoride-labeled boronophenylalanine PET was applied in four and two cases of malignant gliomas and meningiomas, respectively, at the time of transient increase of lesions. These PET scans showed decreased lesion:normal brain ratios in all cases compared with scans obtained prior to BNCT. With or without surgery, all lesions were decreased or stable in size during observation. Transient increases in enhanced volume in malignant gliomas and meningiomas immediately after BNCT seemed to be pseudoprogression. This pathogenesis was considered as treatment-related intratumoral necrosis in the subacute phase after BNCT. *Neuro-Oncology 11, 430–436, 2009 (Posted to Neuro-Oncology [serial online], Doc. D08-00183, March 16, 2009. URL http://neuro-oncology.dukejournals.org; DOI: 10.1215/15228517-2008-107)*

Keywords: boron neutron capture therapy (BNCT), glioma, malignant meningioma, positron emission tomography (PET), pseudoprogression

With the advent of temozolomide (TMZ), concomitant chemoradiation and maintenance chemotherapy with TMZ has become the worldwide standard of care for malignant gliomas (MGs), especially glioblastoma multiforme (GBM).[1] With the spread of this chemoradiotherapy, pseudoprogression (psPD) has become a main topic in neurooncology, since it was reported by Chamberlain et al.[2] In their report, surgery confirmed necrosis without evidence of recurrent tumor in 7 (14%) of 51 patients with MG within 6 months after TMZ chemoradiotherapy. Because the definition of psPD has not been established universally, the incidence is difficult to estimate, but a high percentage has been reported, up to 21% for chemoradiotherapy using TMZ.[3] The main part of surgically resected samples showed necrosis, but the pathogenesis of psPD has not been fully elucidated.

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We have applied a form of tumor-selective particle radiation, boron neutron capture therapy (BNCT), to MGs[4,5] and malignant meningiomas (MMs).[6,7] BNCT comprises a binary approach:[8] A boron-10 (^{10}B)-labeled compound is administered that delivers high concentrations of ^{10}B to the target tumor relative to the surrounding normal tissues. This is followed by irradiation with thermal neutrons. When neutrons collide into ^{10}B atoms, high–linear-energy-transfer (LET) alpha and ^{7}Li particles are released from the ^{10}B (n, alpha) ^{7}Li neutron capture reaction. The short range (5–9 μm) of these particles allows for relatively selective tumor killing with minimum damage to the adjacent normal brain tissue. These high-LET particles exert rapid and distinctive shrinkage of the mass not only in MG[4,5] but also in MM.[6,7] We noticed that in some cases of MG and in some cases of MM treated by BNCT, transient increases in enhanced volume in MR images[8] appeared just after BNCT.

Here we retrospectively review those cases that showed transient increases of enhanced volume in MR images within 3 months after BNCT. We analyzed these cases with histology and fluoride-labeled boronophenylalanine (F-BPA) PET data.

Materials and Methods

Patients

From 2002 to 2007, we used BNCT to treat 52 cases of MG (29 were newly diagnosed and 23 were recurrent cases) and 13 cases of recurrent MM. All the gadolinium (Gd)-enhanced MR images were retrospectively reviewed. The cases that showed transient increases of enhanced volume in MR images within 3 months after BNCT were picked up, and the characteristics were investigated as shown in Table 1. Case numbers were assigned sequentially for BNCT. Some cases underwent surgery for the suspicion of relapse, and for some cases tissue samples were analyzed with Ki-67 labeling.[9] Most cases underwent F-BPA-PET[4–7,10,11] before neutron irradiation, as described below, and some cases entered this study during the observation period when the enhanced area increased.

PET Scan

All F-BPA-PET scans were performed at Nishijin Hospital, Kyoto, Japan. BPA was originally synthesized as described previously,[12,13] and the protocol for PET measurements using a Headtome III tomograph (Shimadzu Co., Kyoto, Japan) has also been described elsewhere.[14,15] Briefly, regional BPA incorporation into tumors and contralateral brain tissue (as a nontumorous control area) was measured on PET images after an intravenous injection of F-BPA at a dose of 37–55.5 MBq (1–1.5 mCi) per 10 kg of body weight. PET images were collected continuously for a 60-min period, for a total of 15 periods. The lesions on the PET images were confirmed using contrast-enhanced MRI performed at levels equivalent to those used for the PET imaging studies. To obtain quantitative measurements using Amide software (SourceForge, Inc., Mountain View, CA, USA), oval regions of interest (ROIs) were placed on the tumors, including peak values in tumors of various sizes. At the corresponding level, the contralateral brain area was also chosen for ROI analysis. All of the macroscopically necrotic tumor areas observed on MR images were excluded when the ROIs were designated. We designated several ROIs from tumor-affected areas and adopted regions with the highest values as representative ROIs.

Table 1. Characteristics of cases that showed transient increases of enhanced volume in MR images within 3 months after BNCT

Case	Histology	New or Recurrent	RT Pre-BNCT	RT Post-BNCT	First PET L/N Ratio	Second PET L/N Ratio	Maximum BNCT (Gy-Eq)	Minimum BNCT (Gy-Eq)	Exploratory Surgery
Case 1	GBM	Recurrent	60 Gy	—	7	—	64.1	34.4	–
Case 4	GBM	New		SRS	—	—	50.6	23.8	+
Case 5	GBM	New		BNCT[a]	7.8	—	108.7	47.4	+
Case 10	GBM	Recurrent	80 Gy		2.8	—	48.3	27.2	+
Case 14	GBM	New		BNCT[b]	5.1	—	141	37.1	+
Case 15	AA	Recurrent	60 Gy		3.1	—	55.9	33.7	+
Case 35	GBM	New		30 Gy	—	—	90.6	61.4	–
Case 46	GBM	New		30 Gy	4.8	2	115	63	–
Case 48	GBM	Recurrent	60 Gy		3.3	1.7	50.5	49.2	–
Case 51	GBM	New		20 Gy	2.6	1.5	64	44.6	–
Case 57	AA	Recurrent	60 Gy		4.7	2.1	104.2	44.9	–
Case 33	MM	Recurrent	60 Gy + SRS		2.8	1.8	55.1	29.8	–
Case 50	MM	Recurrent	50 Gy		3.2	1.9	75.8	18.8	–
Case 56	MM	Recurrent	50 Gy		4.4	—	111.5	50.7	–

Abbreviations: BNCT, boron neutron capture therapy; RT, radiotherapy; L/N, lesion:normal brain; Gy-Eq, gray-equivalent; GBM, glioblastoma multiforme; SRS, stereotactic radiosurgery; AA, anaplastic astrocytoma; MM, malignant meningioma.

[a]BNCT was applied twice, because patient moved during the neutron irradiation during the first BNCT.

[b]BNCT was intentionally applied twice.

Clinical Regimen of BNCT

Candidates for BNCT routinely received F-BPA-PET to assess the distribution of boronophenylalanine (BPA).[10,11] The lesion:normal brain (L/N) ratio of BPA uptake can be estimated from this type of study, and dose planning was performed according to the L/N ratio, as described previously.[4,5]

The patients were administered 100 mg/kg sodium borocaptate for 1 h intravenously 12 h prior to neutron irradiation and 250–700 mg/kg BPA was administered for 1–6 h just prior to neutron irradiation. Amounts of BPA were decided by the disease and protocols as described previously.[4–6] The neutron irradiation time was determined not to exceed 15 Gy-Eq (gray-equivalents) to the normal brain by simulation. Here, Gy-Eq corresponds to the biologically equivalent x-ray dose that would have equivalent effects on tumors and on the normal brain.

Dose estimation of BNCT was done as follows. Blood was sampled every 2 h after sodium borocaptate administration until neutron irradiation was completed, to monitor the boron concentration in the blood. The boron concentration from sodium borocaptate in the blood during neutron irradiation was estimated from the measured ^{10}B concentration–time relationship. From previous BNCT experience, which was performed with craniotomy, we hypothesized that the boron concentrations in tumor and blood contributed from sodium borocaptate were equal just prior to neutron irradiation. We confirmed that BPA concentration in blood is equal to that in normal brain. Therefore, the boron concentrations from BPA in the tumor and normal brain were estimated from BPA concentration in blood by the L/N ratio of BPA-PET, as described above. Judging from these boron concentrations contributed from each boron compound, neutron fluence rate simulated by a dose-planning program, and the factors of relative biological effectiveness of neutron beam and each compound,[4] total doses to tumor and normal brain could be estimated, as in the following formula:[16]

$$\text{Equivalent dose (Gy-Eq)} = D_B \times CBE_B + D_N \times RBE_N + D_\gamma \times \text{hour},$$

where D_B is the boron dose (Gy) = $7.43 \times 10^{-14} \times$ boron concentration (μg ^{10}B/g) × thermal neutron fluence (Φ, calculated as the thermal neutron fluence rate [n/cm^2/s] × radiation time); D_N is the nitrogen dose (Gy) = $6.78 \times 10^{-14} \times$ nitrogen concentration (weight %) × Φ; and D_γ is the gamma-ray dose = 0.83 Gy/h.

Briefly, boron dose correlates to the ^{10}B concentration in the tissue and neutron fluence. Neutron fluence decays more in the deeper part of the tissue. Therefore, even in the same tumor, boron dose decreases in the deeper part compared with the superficial part of the tumor.

Results

Eleven of 52 cases of MG and 3 of 13 cases of MM showed transient increases of enhanced volume in MR images within 3 months after BNCT (Table 1). Among the 11 MG cases, five were recurrent and had been treated with fractionated x-ray treatment (XRT) prior to BNCT. The other six were newly diagnosed gliomas and were treated with two sessions of BNCT or with BNCT followed by fractionated XRT with 20–30 Gy. All MM cases listed in Table 1 were recurrent cases and had been treated with fractionated XRT with and without stereotactic radiosurgery.

Among the 11 cases of MG, five underwent surgery for suspicion of relapse when the mass increased in size after BNCT. The other six cases of MG and three cases of MM were followed up without additional surgery.

F-BPA-PET was applied in four and two cases of MGs and MMs, respectively, at the time of transient increase of lesions. Of these four MGs, two were newly diagnosed and two were recurrent cases. These PET scans showed decreased L/N ratios in all cases compared with scans obtained prior to BNCT (Table 1).

Representative Case Presentation: Case 5

Case 5 was a 70-year-old male with the manifestation of motor-dominant aphasia. He received surgery at first with the histological diagnosis of GBM. The tumor was partially removed. He was treated with BNCT twice with a 1-month interval. The enhanced mass on MR images shrank rapidly 5 weeks after the first BNCT (Fig. 1A-2); however, the enhanced lesion and perilesional edema became enlarged 2 months after initial BNCT (Fig. 1A-3). He received no chemotherapy. With this increase in lesion size, motor aphasia became slightly aggravated. The patient received recraniotomy 3 months after initial BNCT because tumor recurrence was suspected. Hematoxylin and eosin (H&E) staining and Ki-67 immunohistochemistry were applied to samples obtained at the first and second craniotomy (Fig. 2). H&E staining of the sample obtained at the second craniotomy showed necrosis in the main part, with ambiguously viable tissue. Ki-67 staining showed decreased positivity in the second surgical specimen compared with the first surgical specimen. Three months after the second craniotomy, the original lesion was stable in MR images, but tumor progression (TP) was recognized as cerebrospinal fluid (CSF) dissemination (Fig. 1A-4).

Representative Case Presentation: Case 35

Case 35 was a patient with newly diagnosed left temporal GBM. He received surgery with partial tumor removal (Fig. 1B-1) and was treated with BNCT followed by 20 Gy XRT without chemotherapy. This combination of radiotherapy resulted in drastic shrinkage of the mass within a month (Fig. 1B-2). However, 2 months after BNCT, motor aphasia was aggravated and MR images showed an increase of the enhanced area (Fig.

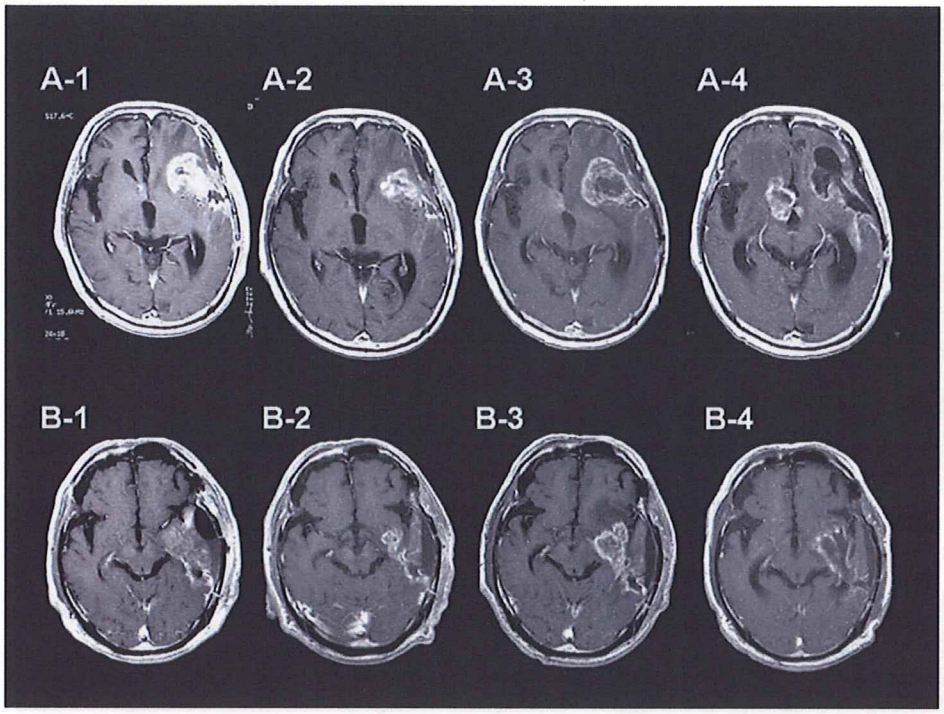

Fig. 1. Two representative cases of pseudoprogression of newly diagnosed glioblastoma multiforme treated by boron neutron capture therapy (BNCT). (A-1–A-4) Gadolinium (Gd)-enhanced MR images of case 5: prior to BNCT (A-1), 5 weeks after BNCT (A-2), 2 months after BNCT (A-3), and 6 months after BNCT and 3 months after second craniotomy (A-4). Case 5 was operated on 3 months after BNCT for the suspicion of tumor progression (judged from A-3). The operated lesion was stable for more than 3 months without chemotherapy. (B-1–B-4) Gd-enhanced MR images of case 35: prior to BNCT (B-1), 1 month after BNCT (B-2), 2 months after BNCT (B-3), and 7 months after BNCT (B-4).

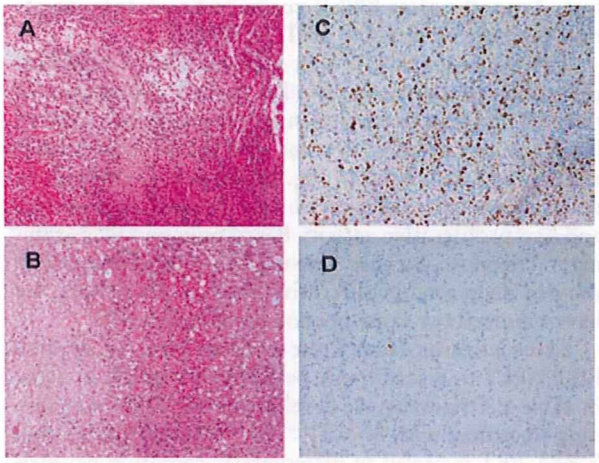

Fig. 2. Hematoxylin and eosin (H&E) and Ki-67 staining of specimens obtained from the first and second craniotomy of case 5. (A and B) H&E staining of the specimen of first craniotomy (A) and second craniotomy (3 months after boron neutron capture therapy [BNCT]; B). (C and D) Ki-67 staining of the specimen of the first craniotomy (C) and second craniotomy (3 months after BNCT; D). Original magnification, ×200 for A–D.

1B-3). The lesion was controlled well by steroids, and the enhanced area decreased in size on follow-up MR images (Fig. 1B-4). We lost this case due to uncontrollable hydrocephalus by CSF dissemination.

Representative Case Presentation: Cases 50 and 56

Both patients had recurrent MMs and had been treated with repetitive surgery and fractionated XRT with 50 Gy. Case 50 had rhabdoid meningioma. BNCT also showed shrinkage of the mass (Fig. 3A-2) with transient increase of enhanced volume (Fig. 3A-3). The enhanced mass gradually shrank, again without any treatment (Fig. 3A-4). Case 56 had anaplastic meningioma. In this case, BNCT did not show prominent reduction of mass size but showed decreased enhancement in tumor mass just after BNCT (Fig. 3B-2). However, the mass became voluminous 2.5 months after BNCT and spontaneously decreased again in size and in enhancement of the core of the mass, with decreased perilesional edema (Fig. 3B-4). In both cases, BNCT could well control the mass locally, but we lost these cases by uncontrollable, shunt-ineffective hydrocephalus due to CSF dissemination.

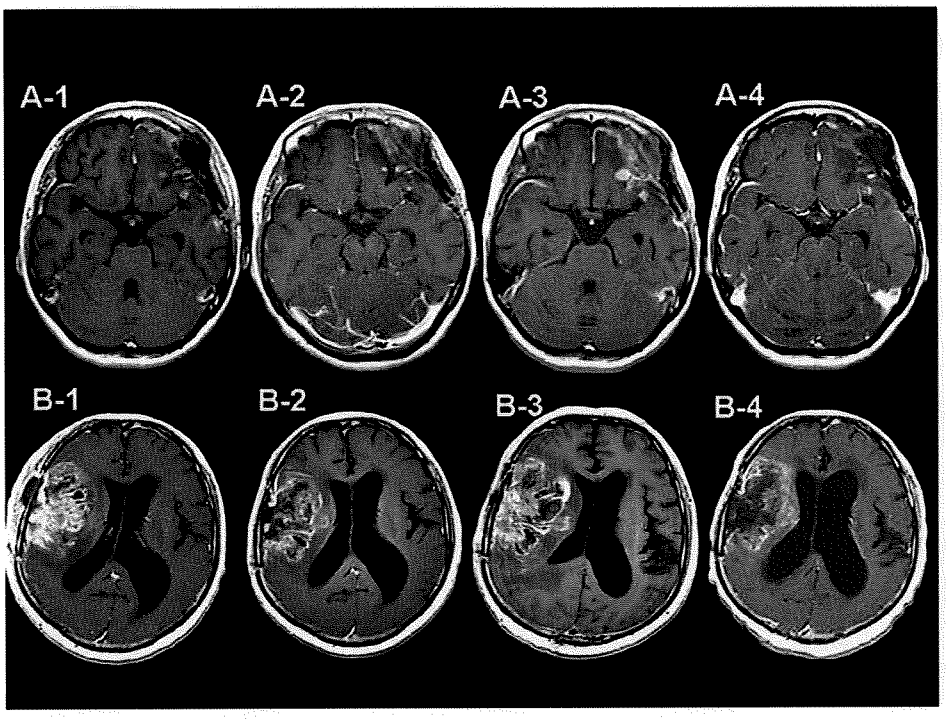

Fig. 3. Two representative cases of pseudoprogression of recurrent malignant meningiomas treated by boron neutron capture therapy (BNCT). (A-1–A-4) Gadolinium (Gd)-enhanced MR images of case 50: prior to BNCT (A-1), 48 h after BNCT (A-2) 1 month after BNCT (A-3), and 2 months after BNCT (A-4). (B-1–B-4) Gd-enhanced MR images of case 56: prior to BNCT (B-1), 1.5 months after BNCT (B-2), 2.5 months after BNCT (B-3), and 6 months after BNCT (B-4).

Discussion

psPD does not have a definitive definition. If we tentatively define psPD as a transient increase of enhanced volume in relatively early phases after some treatments without TP, a high incidence of psPD is reported.[3] psPD in MG has been recognized widely with the advent of TMZ treatment, and XRT alone has been reported to cause psPD.[17–20] However, combining some chemotherapeutic agents such as TMZ with XRT causes psPD with higher frequency and earlier compared with XRT alone.[2,3]

With BNCT, 11 of 52 MG and 3 of 13 MM cases experienced psPD, based on the above tentative definition. Six of 29 newly diagnosed MG cases and 5 of 23 recurrent MG cases showed psPD. We do not know the exact incidence of psPD with BNCT, because we could not review all MR images of all patients, and not all the patients received MRI at the same schedule in each hospital before being referred to our institute for BNCT. However, we do know that psPD occurred in these cases only by BNCT without chemotherapy, because we did not apply any chemotherapeutic agents until TP definitively occurred. In this article, we reviewed the cases that showed increased enhanced volume within 3 months after BNCT. In these cases, there was no true TP; however, we experienced two cases in whom true TP was proven by surgical specimen. They showed increased enhanced volume 4 months and 5 months after BNCT, respectively. We lost these two cases by local TP. Therefore, 3 months after BNCT seems to be an appropriate time period to review for psPD in BNCT.

The five cases of MG that received BNCT relatively early after diagnosis received surgery for suspicion of true TP. In these five cases, surgical specimens showed large necrotic areas with some viable cells with bizarre appearance, as shown in Fig. 2B. It is very difficult to determine whether these cells were derived from tumors and had proliferative activity based only on H&E staining.[21] Therefore, for case 5 we used Ki-67 staining, which showed decreased proliferative activity. Elsewhere, we have reported the same phenomenon in case 4.[5]

After learning from these earlier cases, we applied F-BPA-PET to discriminate psPD from true TP, as listed in Table 1. Originally, we developed F-BPA-PET for planning treatment with BNCT.[4–6,10,11] We then noticed that F-BPA-PET is useful to differentiate radiation necrosis (RN) and true TP, especially with repetitive analysis.[22] In our limited experience, pure RN always showed L/N ratios less than 1.9, and RN with small number of viable tumor cells showed L/N ratios less than 2.2.[22] Generally speaking, amino acid tracer has been used and is suitable for analyzing metabolism in malignant brain tumors[23,24] as well as for differentiating between RN and true TP,[24] because of low background activity compared with fluorodeoxyglucose-PET. We therefore applied F-BPA-PET and obtained reliable results, as shown here. As stated above, we almost always applied F-BPA-PET prior to

BNCT, so it was easy to compare the L/N ratio between the first PET obtained before BNCT and the second PET obtained when psPD, RN, or true TP was suspected.[22] In our BPA-PET series of recurrent GBM, all L/N ratios prior to BNCT were greater than 2.5, and L/N ratios of GBM at recurrence after BNCT were also greater than 2.5. Therefore, at least regarding GBM, when the tumor shows increase of enhancement in MRI within 3 months after BNCT, we may wait and see if the L/N ratio is less than 2.2.[22] In any case, early discrimination between psPD and true TP is important to apply potent alternative treatment for true TP patients without time delay.

The absorbed dose by BNCT, listed in Table 1, is applied only once. By BNCT we can deliver an enormous absorbed dose to tumor tissue. The range of maximum tumor dose by BNCT was 48.3–141 Gy-Eq, and the minimum dose range was 18.8–63.0 Gy-Eq (Table 1). These doses were applied at once. These values can be estimated approximately as 234.7–1,774.2 Gy and 45.1–383.3 Gy as given in daily doses of 2 Gy by fractionated XRT, with the assumption of α/β value of 10 Gy in the linear-quadratic model. We are not certain of the threshold of BNCT dose to cause psPD. The recurrent cases listed here received especially high doses overall, considering the addition of previous XRT, so psPD may frequently occur after BNCT in recurrent MG cases, because reirradiation is known as a risk factor for RN.[25,26] The latter half of the cases of newly diagnosed MG treated by BNCT in our protocol received fractionated XRT boosts with 20–30 Gy to decrease the possibility of recurrence.[16] Therefore, these high amounts of radiation might be a cause of marked psPD in BNCT cases. Because we did not apply any chemotherapy after BNCT until TP was confirmed by histology or PET, psPD described here may be caused only by radiation effects. Among the GBM patients treated with chemoradiation using TMZ, a high incidence of psPD was observed in methylated O^6-methylguanine-DNA methyltransferase (MGMT) promoter status, and those cases that exhibited psPD showed good prognosis compared with the cases of unmethylated MGMT promoter.[27] Taken together, intensive treatments may be the primary factor of psPD in MG, as Brandsma et al. reported.[28]

So far, we are not sure of the true mechanism of psPD. If psPD occurs by the same mechanism as the acute and subacute phase of radiation injury, psPD may be presumably caused by vasodilatation, disruption of the blood–brain barrier, and edema, due to endothelial cell damage.[29] Also, vascular endothelial growth factor (VEGF) may play an important role in psPD as it does in RN. By this speculation, anti-VEGF antibody may be applicable for symptomatic psPD.[30]

As stated above, more effective treatments may also result in higher incidences of psPD.[28] If this is true, high-LET and high relative-biological-effectiveness particle radiation, such as BNCT, may cause psPD frequently. Also, it is difficult to discriminate RN and psPD, especially with high-LET radiotherapy such as BNCT. We actually omitted one recurrent GBM case with possible psPD because it was difficult to discriminate the case from RN. We will probably observe the same phenomenon by these modalities. We would like to stress that psPD can occur not only with MG but also with MM by BNCT. Because all the incidences of psPD in our BNCT series occurred within the original tumor, psPD by BNCT can be defined as intratumoral treatment-related necrosis in the subacute phase after BNCT.

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Case Report
Head and Neck Oncology

Boron neutron capture therapy for papillary cystadenocarcinoma in the upper lip: A case report

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Abstract. Boron neutron capture therapy (BNCT) is a tumor-selective radiation therapy using α and ^{7}Li particles, which are produced by the reaction of neutron with boron (^{10}B), and taken up by the tumor. The authors report their first experience of BNCT on a patient with no history of surgery, chemotherapy or conventional radiotherapy for papillary cystadenocarcinoma in the upper lip.

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Boron neutron capture therapy (BNCT) is a tumor-selective radiation therapy that destroys cancer cells using α and ^{7}Li particles, which are generated by the reaction of thermal neutron with boron (^{10}B), and taken up by the tumor[4]. Clinical trials have studied the use of BNCT for brain tumors (cutaneous primary or cerebral metastasis from melanoma) and for head and neck cancer[3,7]. The authors have used BNCT to treat recurrent oral cancer and metastasis of lymph nodes after conventional treatment.

We report our first experience of using BNCT on a patient with no history of surgery (except biopsy), chemotherapy or conventional radiotherapy for papillary cystadenocarcinoma in the upper lip.

Case Report

An 78-year-old woman was referred with a 3-month history of mass formation in the upper lip. An extraoral examination revealed swelling in the left subnasal area. Intraorally, an irregularly shaped tumor mass was observed in the mucolabial fold from the upper left central incisor to the canine. The tumor mass was hard, with pitting ulceration on the surface, possibly due to contact with the edge of the upper denture. An incisional biopsy was performed, and papillary cystadenocarcinoma diagnosed. The patient refused surgical treatment, chemotherapy and radiotherapy. As a result, periodic follow-up was carried out on an outpatient basis.

The tumor gradually enlarged over three and a half years and surgery was recommended again; the patient refused. The possible course and prognosis, if observation was the only treatment, was explained to the patient. As a result, the patient consented to BNCT. The treatment was approved by the ethical committee of Osaka Medical College and by the BNCT committee of the Kyoto University Research Reactor Institute (KURRI).

At that time, the well-defined tumor was 40 × 30 × 25 mm in size, covering the area from the upper left lip mucosa to the buccal fold. Owing to pain and tenderness, the patient had difficulty using her dentures and eating. Magnetic resonance imaging (MRI) revealed a large space-occupying

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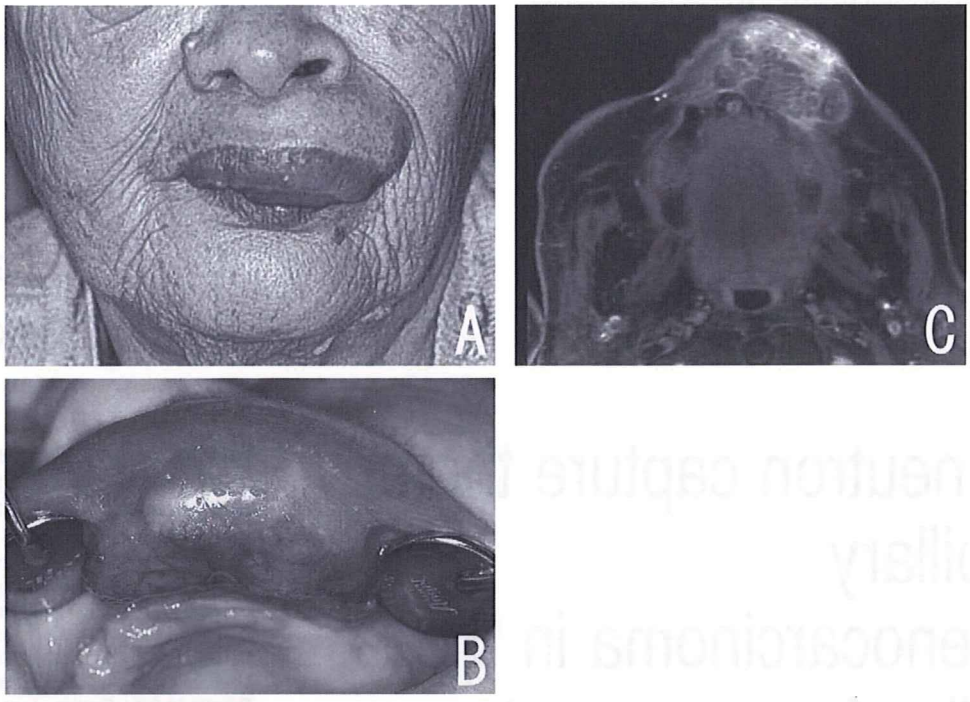

Fig. 1. (A, B) A large tumor mass that induced pain and difficulty in eating was seen in the left upper lip. (C) MRI (Gd-enhanced fat-suppressed image) revealed a large space-occupying mass lesion located in the left upper lip.

mass lesion located in the left upper lip (Fig. 1).

An ^{18}F-boronophenylalanine positron emission tomography (BPA-PET) examination[6] was performed on 15 December 2005 to investigate the uptake of BPA to the tumor. The ratio of boron uptake in the tumor to that in normal tissue (T/N ratio) was 3.2 (Fig. 2).

The first BNCT was performed under BPA (500 mg/kg) as an intravenous drip infusion at the KURRI[2,10] The authors applied the BNCT intentionally in 2 fractions at an interval of 4 weeks. The total dose was 63.4 Gy-Eq at the tumor peak and 59.8 Gy-Eq for the deepest portion (Table 1). Gy-Eq (Gy:Gray) is the biologically equivalent X-ray dose that would give an effect equivalent to that of total BNCT radiation.

The tumor tended to decrease in size after the first BNCT, and the second BNCT brought pain relief. An MRI after 5 months showed the tumor had shrunk by 86%. At present, 12 months after the second BNCT, the patient can wear full dentures, eat more easily and the pain has disappeared. MRI reveals shrinkage of the mass lesion (Fig. 3). Although mild stomatitis was observed after the first BNCT fraction, the patient was able to eat. Following the second fraction, extensive erosion developed in the oral mucosa associated with marked contact pain. After 2 weeks, the extensive erosion was cured. At the time of writing, the tumor has nearly disappeared macroscopically.

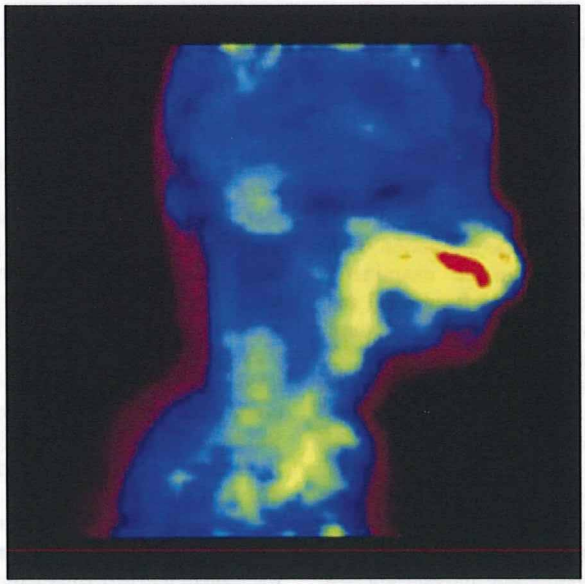

Fig. 2. Ratios of 18F-BPA accumulation in the area responsible for the tumor (upper lip) and normal tissues were assessed, and the T/N ratio was 3.2.

Discussion

A stable isotope of boron, ^{10}B divides to become an alpha and lithium particle when it captures a neutron. The sizes of those particles are about 9 μm and 4 μm, respectively, roughly equivalent to the size of cancer cells. In BNCT, ^{10}B is taken up by cancer cells where it reacts with neutrons during irradiation to destroy cancer cells selectively[3]. Farr et al.[5] were the first to use BNCT to treat a glioblastoma multiforme in 1954, since when the therapy has been carried out mainly for brain tumors. Mishima et al.[9] reported the results of using BNCT for a cutaneous malignant melanoma in 1989, and Kato et al.[7] reported using it for head and neck cancer in 2001. In the authors' hospital, BNCT

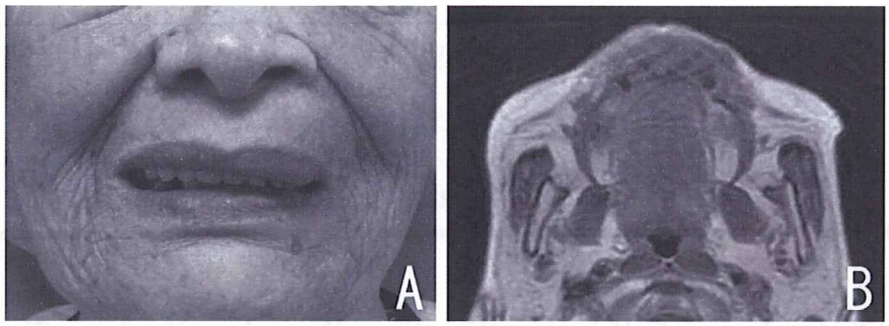

Fig. 3. (A) 12 months after treatment, the tumor mass had markedly decreased in size and the patient could wear full dentures and eat without difficulty. (B) There were no apparent high-signal areas on T2-weighted (fat-suppressed) images.

Table 1. BNCT parameters.

Dose (Gy-Eq)				
Fraction	Tumor peak (depth from skin surface, cm)	Minimum tumor (depth from skin surface, cm)	Oral mucosa	Irradiation time (min)
1	34.6 (2.5)	31.9 (3.5)	15.4	60
2	28.8 (2.5)	27.9 (3.5)	12.9	49
Total	63.4	59.8	28.3	109

has been used since 2005 for recurrent and/or advanced oral cancers that are difficult to treat using other therapies.

In general, malignant salivary gland tumors are considered to be radio-resistant, and radical surgery is the first treatment of choice[11]. BNCT is a tumor-selective, high LET radiation therapy with relatively high biological effect, therefore it should work well even for radio-insensitive tumors, compared with low LET radiation therapy using photons[8]. For head and neck cancer, Kato et al.[7] described the merits of BNCT as: the tissues and organs can be preserved; the therapy is effective for cases in which surgery is impossible; and it should be effective in squamous cell carcinoma, which is commonly involved in the head and neck, because the T/N ratio is high. Aihara et al.[1] reported a case of recurrent mucoepidermoid carcinoma (high-grade malignancy) that responded well to BNCT. In the present case, as in the Aihara case, the T/N ratio in ^{18}F-BPA PET results had a relatively high value. The choice of this therapy should depend on the uptake of the boronic compound by the tumor not on the histologic type, including radiosensitivity, of the tumor.

The authors applied BNCT in 2 fractions at an interval of 4 weeks. The irradiation time for single-fraction BNCT would have been too long for the authors' elderly patient and the oral mucosa might have been damaged irreversibly by absorbing such a large dose.

One of the advantages of applying BNCT to head and neck tumors is its superficial location from the skin surface, making it possible to administer curative doses to the target using epithermal neutrons[7].

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Delivery of sodium borocaptate to glioma cells using immunoliposome conjugated with anti-EGFR antibodies by ZZ-His

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ABSTRACT

Nanoparticles are effective of delivering cargo into cells. Here, sodium borocaptate (BSH) was encapsulated in liposomes composed of nickel lipid, and anti-epidermal growth factor receptor (EGFR) antibodies were conjugated to the liposomes using the antibody affinity motif of protein A (ZZ) as an adaptor (immunoliposomes). The immunoliposomes were used to deliver BSH into EGFR-overexpressing glioma cells. Immunohistochemical analysis using an anti-BSH monoclonal antibody revealed that BSH was delivered effectively into the cells but not into EGFR-deficient glioma or primary astrocytes. In an animal model of brain tumors, both the liposomes and the BSH were only observed in the tumor. Moreover, the efficiency of ^{10}B's delivery into glioma cells was confirmed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) both *in vitro* and *in vivo*. The results suggest that this system utilizing immunoliposomes provides an effective means of delivering ^{10}B into glioma cells in boron neutron capture therapy (BNCT).

1. Introduction

Glioblastoma multiforme (GBM) is one of the most malignant and aggressive brain tumors. Although at present, treatment mainly consists of surgery and radiotherapy [1], it is difficult to remove all tumor tissues without severe damage to the brain, and healthy brain tissue is less tolerant of conventional radiotherapy than tumor tissue.

Boron neutron capture therapy (BNCT) provides a way to selectively destroy malignant cells and spare normal cells. BNCT is a binary method for the treatment of cancer based on the tumor-selective delivery of ^{10}B followed by radiation with low energy thermal neutrons [2]. It is assumed that the selective accumulation of ^{10}B in tumors will cause the killing of cancer cells and induce a therapeutic effect [3]. BNCT has been applied clinically for the treatment of malignant brain tumors, malignant melanoma, head and neck cancer, and hepatoma [4]. For effective BNCT, enough ^{10}B must be encapsulated in the tumor and a high ratio of tumor to blood is needed. Different approaches have been proposed for delivering ^{10}B into tumor cells, including the use of boronated macromolecules such as monoclonal antibodies and microparticles such as liposomes, etc. [2]. However, these methods have several disadvantages, such as low delivery efficiency, a lack of specificity and stability *in vivo*, and the high cost of preparing nanoparticles. Two boron compounds, sodium borocaptate (BSH) and boronophenylalanine (BPA), are undergoing clinical trials [5]. BPA is an analog of an essential amino acid and actively carried to brain tumors. However, it also accumulates in normal brain tissue. By contrast, BSH accumulates little in normal tissue but accumulates insufficiently in tumors compared with BPA [6]. Therefore, an easy-to-prepare and universal ^{10}B delivery system is still awaited.

In recent years, drug delivery research has increasingly focused on antibody-targeting liposomes in the treatment of cancers including glioblastoma [7]. Liposomes are unilamellar phospholipid vesicles with high interior encapsulation for water-soluble compounds such as borane ions [8]. Targeted liposomes provide an advantage over untargeted liposomes not because of increased localization to tumor sites but because of increased interaction with

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