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). With the spread of this chemoradiotherapy, pseudoprogression (psPD) has become a main topic in neurooncology, since it was reported by Chamberlain et al. 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. The main part of surgically resected samples showed necrosis, but the pathogenesis of psPD has not been fully elucidated.

Received July 13, 2008; accepted November 4, 2008.

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We have applied a form of tumor-selective particle radiation, boron neutron capture therapy (BNCT), to MGs and malignant meningiomas (MMs). BNCT comprises a binary approach: 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 but also in MM. 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 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. Most cases underwent F-BPA-PET 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 Nishijinn Hospital, Kyoto, Japan. BPA was originally synthesized as described previously and the protocol for PET measurements using a Headtome III tomograph (Shimadzu Co., Kyoto, Japan) has also been described elsewhere. 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

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

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.

*aBNCT was applied twice, because patient moved during the neutron irradiation during the first BNCT.*

*bBNCT 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

$$\text{Equivalent dose (Gy-Eq)} = D_b \times \text{CBE}_B + D_N \times \text{RBE}_N + D_\gamma \times \text{hour},$$

where $D_b$ is the boron dose (Gy) = $7.43 \times 10^{-14} \times$ boron concentration ($\mu$g $^{10}$B/g) $\times$ thermal neutron fluence ($\Phi_t$, calculated as the thermal neutron fluence rate [n/cm$^2$/s] $\times$ radiation time); $D_N$ is the nitrogen dose (Gy) = $6.78 \times 10^{-14} \times$ nitrogen concentration (weight %) $\times$ $\Phi_t$; and $D_\gamma$ 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 MM, 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. 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.

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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.
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. psPD in MG has been recognized widely with the advent of TMZ treatment, and XRT alone has been reported to cause psPD. However, combining some chemotherapeutic agents such as TMZ with XRT causes psPD with higher frequency and earlier compared with XRT alone.

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. Therefore, for case 5 we used Ki-67 staining, which showed decreased proliferative activity. Elsewhere, we have reported the same phenomenon in case 4.

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. We then noticed that F-BPA-PET is useful to differentiate radiation necrosis (RN) and true TP, especially with repetitive analysis. 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. Generally speaking, amino acid tracer has been used and is suitable for analyzing metabolism in malignant brain tumors as well as for differentiating between RN and true TP, 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

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).
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. 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. 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. 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. Among the GBM patients treated with chemoradiation using TMZ, a high incidence of psPD was observed in methylated O6-methylguanine-DNA methyltransferase (MGMT) promoter status, and those cases that exhibited psPD showed good prognosis compared with the cases of unmethylated MGMT promoter. Taken together, intensive treatments may be the primary factor of psPD in MG, as Brandsma et al. reported.

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. 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.

As stated above, more effective treatments may also result in higher incidences of psPD. 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.

Acknowledgments

This work was partly supported by Grants-in-Aid for Scientific Research (B) (16390422 and 19390385) from the Ministry of Education, Culture Sports, Science, and Technology (MEXT) of Japan; a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare of Japan to S.-I.M.; the Regional Science Promotion Program of the Japan Science and Technology Corporation; and the “Second-Term Comprehensive 10-Year Strategy for Cancer Control” of the Ministry of Health, Labour, and Welfare of Japan to S.-I.M. This work was also supported in part by the Takeda Science Foundation for Osaka Medical College; a Grant-in-Aid for Cancer Research (12217065) from MEXT to K.O.; and a Grant-in-Aid for Scientific Research for Young Researchers (B) (18791030) from the Ministry of Education, Science, and Culture of Japan to S.K.
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