Evaluation of the Biological Effects of Boron Neutron Capture Therapy for the Human Malignant Meningioma Cell Line IOMM-Lee

Kyoko IIDA-ONISHI¹, Shinji KAWABATA¹, Shiro MIYATA¹, Takahiro MASUBUCHI¹, Atsushi DOI¹, Kunio YOKOYAMA¹, Toshihiko KUROIWA¹, Koji ONO², Hiroaki KUMADA³, Mitsunori KIRIHATA⁴ and Shin-Ichi MIYATAKE¹

¹ Department of Neurosurgery, Division of Surgery, Osaka Medical College, Takatsuki-city, Osaka 569-8686, Japan
² Particle Radiation Oncology Research Center, Kyoto University Research Reactor Institute, Kumatori, Japan
³ Research Reactor and Tandem Accelerator, Japan Atomic Energy Agency, Tohkai, Japan
⁴ Department of Agriculture, Osaka Prefectural University, Sakai, Japan

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ABSTRACT

The biological effects of boron neutron capture therapy (BNCT) for malignant meningioma (MM) were evaluated experimentally. In vitro boron uptake from boronophenylalanine (BPA) was analyzed in a human MM cell line (IOMM-Lee). The cytotoxic effect of BNCT on IOMM-Lee was also evaluated and compared with that on the human malignant glioma cell line U87Δ. In vivo boron bio-distributions following administration of BPA to mice bearing subcutaneous implants of the IOMM-Lee and U87Δ were analyzed. The tumor-bearing mice were also given BPA and neutron irradiation was performed. After irradiation, the size of the tumor was assessed. The in vitro boron concentration of IOMM-Lee cells was increased in a time- and dose-dependent manner. The surviving fraction of neutron-irradiated cells with BPA decreased exponentially along with neutron fluence. The boron concentration of tumor nodules increased dose dependently after administration of BPA, while the tumor to brain and tumor to blood ratios of the boron concentrations were almost constant. The tumor nodules treated with BNCT decreased in size significantly in comparison with the control group. BNCT strongly enhanced the growth inhibition effect for the MM compared with the malignant glioma. These findings suggest that BNCT should become a highly effective treatment for MM patients.

Address correspondence to:
Shinji Kawabata, M.D. Department of Neurosurgery, Division of surgery, Osaka Medical College, 2-7 Daigaku-machi, Takatsuki-city, Osaka 569-8686, Japan
Phone: +81-72-683-1221 Fax: +81-72-683-4064 E-mail: neu046@poh.osaka-med.ac.jp
INTRODUCTION

Patients with malignant meningiomas, relapsing at progressively shorter time intervals after radiotherapy and repeated surgical interventions have a poor prognosis for survival time and for quality of life and the options for further treatment are very limited in these cases [1-6]. Although some treatments for recurrent malignant meningioma have been reported, a standard treatment has not yet been developed [7-12]. We had been applying boron neutron capture therapy (BNCT) as a novel radiation modality for the treatment of recurrent malignant meningiomas with better local tumor control [13, 14]. BNCT is a tumor cell targeted particle irradiation approach that significantly increases the therapeutic ratio relative to conventional radiotherapeutic modalities [15-21]. However, the biological effectiveness factor for malignant meningioma is still unclear.

In the present report on the application of BNCT to malignant meningioma, we focused our analyses on the boron bio-distribution of boronophenylalanine (BPA), which has been widely used as a boron delivery agent in the treatment of malignant glioma patients, and on the biological effects of neutron capture therapy for experimental malignant meningioma both in vitro and in vivo.

MATERIALS and METHODS

Boron compounds

$^{10}$B-enriched boronophenylalanine (BPA) (>99% $^{10}$B atoms, L-isomer), which has been widely used as a boron delivery agent for the clinical treatment of patients with malignant glioma, was kindly supplied by the Stella Chemifa Corporation (Osaka, Japan). We prepared a boronophenylalanine - fructose complex solution to increase its solubility following the method reported by Coderre et al. [22].

Cell line and cell culture

The IOMM-Lee cell line was derived from the malignant meningioma tissue of a man who underwent tumor resection of the skull in 1984 [23]. This cell line was kindly supplied by Dr. Anita Lal, Department of Neurosurgery, University of California, San Francisco, USA. The U87Δ human glioma cell line was kindly provided by Dr. Ryo Nishikawa, Department of Neurosurgery, Saitama Medical College.

The IOMM-Lee cells and U87Δ cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO Invitrogen Corporation, Carlsbad, CA, USA) containing 100 units/ml penicillin G, 100 $\mu$g/ml streptomycin, 0.25 $\mu$g/ml amphotericin B as fungizone and 10% fetal bovine serum (GIBCO) at 37°C in a humidified atmosphere containing 5% CO₂.

In vitro boron uptake study

For the preliminary study, IOMM-Lee and U87Δ cells were washed twice in phosphate-buffered saline (PBS), resuspended in DMEM containing 20, 50 and 100 $\mu$g$^{10}$B/ml of BPA, and incubated for 2.5 and 6 hours at 37°C in an atmosphere of 5% CO₂. After the incubation, cells were washed twice in PBS, digested with trypsin with ethylene-diamine-tetraacetic acid (EDTA), and centrifuged at 1300 rpm for 5 min. Their boron concentration was determined with inductively coupled plasma atomic emission spectrometry (ICP-ABS). For ICP-ABS analysis, all samples were directly digested with nitric acid solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at room temperature overnight. The resulting solutions were diluted with distilled water, and samples were introduced into the ICP-ABS spectrometer (P-5200; HITACHI, Tokyo, Japan). The emission intensity was monitored at 249.678 nm, the boron analysis line. Boron atoms in mammalian cells should be negligible and all of the experimental condition was free from the contamination of $^{11}$B. Therefore, recovered boron by ICP-ABS analysis was approximately similar to added $^{10}$B. Standard boron solutions were prepared with distilled water by diluting a 1000-µg/ml stock standard solution (Wako). Standard curves were linear over the range of 0.1-5 $\mu$g/ml, and we prepared a calibration line during each period of operation. Each assay was done in triplicate (mean value±standard errors (SE)).

In vitro neutron irradiation study

IOMM-Lee and U87Δ cells were transferred to the atomic reactor (JRR4; Japan Atomic Energy Agency Research Reactor 4) and incubated with 500 $\mu$g$^{10}$B/ml of BPA containing DMEM for 2.5 hours, and then both types of cells were washed twice in PBS, digested with trypsin with EDTA, and centrifuged at 1300 rpm for 5 min. DMEM was then added to 1 ml of the cell suspension at a density of $3 \times 10^{6}$ cells/ml. Both types of cells were irradiated at JRR4 with reactor thermal neutron beams of 7.14 \times 10^{9} (n/cm²/sec) for 0, 5, 10, 15, and 30 min at 3.5 MW reactor power (thermal neutron mode TNB-2 at JRR4).

After neutron irradiation, colony-forming assays (CFAs) were performed. The irradiated cells were
cultured for 7 days, then fixed in methanol and stained with 1% crystal violet. Colonies composed of more than 50 cells were counted. The surviving cell fractions (SFs) were determined by dividing the number of the colonies from irradiated culture by that from unirradiated control culture.

**Tumor model**

Male nude mice (BALB/c nu/nu; Japan SLC, Inc., Shizuoka, Japan) weighing 25~30 g and aged 8~10 weeks were used. Tumor-bearing mice were prepared by subcutaneously inoculating a suspension of IOMM-Lee and U87Δ cells directly into the backs of the nude mice. After subcutaneous implantation, both kinds of cells grew as a discrete mass. During these procedures, nude mice were generally anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg). The mice were fed a regular mouse diet and water, and maintained under a standard light/dark cycle in an ambient atmosphere. Procedures to minimize discomfort, pain, and distress were performed in accordance with the Guide for the Care and Use of Laboratory Animals of Osaka Medical College.

**In vivo neutron irradiation study**

For the therapy study, neutron irradiation was performed in an atomic reactor (JRR4) 10 days after the implantation of 10³ IOMM-Lee cells. The radiation field delivered to the tissue during irradiation was composed mainly of thermal neutrons (=0.53eV), gamma photons, and heavy-charged particles (4He, 7Li, 1H, and 14C) from the 10B (n, alpha)/Li capture reaction and the 14N(n, p)14C reaction with normal tissue nitrogen. The fast neutron level (10,000 eV) was low enough to ignore. Thermal neutron flux was measured using 3-mm long gold wires placed on the surface of the tumors. The average of the thermal neutron flux was 7.14×10⁴ (n/cm²/sec) and the thermal neutron fluence became 1.29×10¹⁸ n/cm² for 30 minute irradiation. The equivalent dose was calculated using the equations below [24].

Equivalent dose (Gy-Eq) =
\[ D_{e} \times CBE_{x} + D_{n} \times RBE_{x} + D_{r} \times \text{hour} \]

Gy-Eq refers to a biologically equivalent X-ray dose that can give radiation effects totally to the tissue. \( D_{e} \) (Boron dose) = \( 7.43 \times 10^{-14} \times \text{boron concentration} \times \mu g^{10}B/g \times \Phi \) thermal neutron fluence (Gy). \( D_{n} \) (Nitrogen dose) = \( 6.78 \times 10^{-14} \times \text{nitrogen concentration} \times \text{weight percentage} \times \Phi \) thermal neutron fluence (Gy). \( D_{r} \) (Gamma-ray dose) = 0.83 Gy/hour. Here, RBE_{x} is from RBE (Relative Biological Effectiveness) for \( D_{e} \) and is 2.5. BBE_{x} is from CBE (Compound Biological Effectiveness) for \( D_{n} \) and, in the case of BPA, this value is 3.8 for the tumor tissue and 1.35 for the normal brain [16, 25, 26]. Nitrogen concentration (%) is estimated as 4% and the RBE for \( D_{r} \) is 1.0.

Nude mice were transported to the JRR4, and randomized on the basis of tumor size at 10 days after implantation into three experimental groups composed of 5-6 animals each. This experiment included untreated control and irradiated control animals without the boron compound. Two and a half hours after intraperitoneal administration of BPA (250 mg/kg b.w.), neutron irradiation was initiated. All irradiated mice were anesthetized and placed in a body and head-shield stabilizer, then irradiated at a reactor power of 3.5 MW for 30 min. After neutron irradiation, the animals remained at JRR4 for observation. The tumor volume (V) was determined by the following formula:

\[ V = LW^{2}/2 \]

Here, L is the largest length of the tumor nodule, and W is the length of the nodule perpendicular to L.

**Statistical analysis**

Values are presented as means ± SE. Statistical
analysis was performed by Mann-Whitney analysis using Statview software.

RESULTS

In vitro boron uptake studies

Boron uptake from the BPA solution was analyzed in IOMM-Lee cells by ICP-AES. The boron concentrations of IOMM-Lee cells incubated in DMEM containing 25, 50 and 100 µg¹⁰B/ml of BPA, or incubated in PBS in the case of the control cells, for 2.5 and 6 hours are shown in Figure 1. The boron concentrations were increased in a dose- and time-dependent manner; the boron concentrations obtained from incubation in 20, 50 and 100 µg¹⁰B/ml of BPA for 2.5 hours were 47.2±3.3, 84.5±1.7 and 99.1±3.3 µg/1x10⁶ cells, respectively, and those obtained from BPA incubation for 6 hours were 57.2±0.3, 104.1±6.6 and 148.8±6.0 µg/10⁶ cells, respectively. The boron concentrations obtained from PBS were 0.8±0.1 µg/10⁶ cells for 2.5 hours and 0.6±0.1 µg/10⁶ cells for 6 hours.

The boron concentrations of U87Δ cells incubated in DMEM containing 100 µg¹⁰B/ml of BPA for 2.5 and 6 hours were 289.2±16.6 µg and 400.4±63.5 µg/10⁶ cells, respectively. These results for the incubation with 100 µg¹⁰B/ml of BPA suggest that the boron concentrations of U87Δ cells were significantly higher than those of IOMM-Lee cells. In the more extensive second study, after being incubated in 500 µg¹⁰B/ml of BPA for 2.5 hours, IOMM-Lee and U87Δ cells showed boron concentrations of 1011.1±330.2 and 1068.6±198.5 µg/10⁶ cells; i.e., there was no significant difference between the two cell lines.

In vitro neutron irradiation and colony-forming assay

CFAs were performed in IOMM-Lee and U87Δ cells after 2.5 hours of incubation with 500 µg¹⁰B/ml of BPA for 2.5 hours and irradiation with reactor thermal neutron beams. The results for each cell line are shown in Figure 2. The control groups were exposed to the neutron beam without BPA. The SFs of the control groups hardly decreased, irrespective of the thermal neutron fluence (0, 1.55x10¹⁰, 3.09 x 10¹⁰, 4.64 x 10¹¹ and 9.27 x 10¹¹ n/cm²). The SFs at each neutron fluence were 1, 1.05, 0.72, 0.59 and 0.53 in U87Δ cells, and 1, 1.44, 1.39, 1.04 and 1.04 in IOMM-Lee cells, respectively. The survival ratios of tumor cells with BPA decreased exponentially with the increase of neutron fluence in both cell lines. The SFs were 1, 0.62, 0.46, 0.38 and 0.10 in U87Δ cells, and 1, 0.32, 0.09, 0.03 and 0.02 in IOMM-Lee cells, respectively, at the same thermal neutron fluence levels as listed above. The suppressive effect was more prominent in IOMM-Lee cells than in U87Δ cells.

In vivo boron bio-distribution studies

We first focused our efforts on the differences corresponding to the injected dose following intraperitoneal injection of BPA at doses of 125, 250 and 375 mg/kg (Table 1). The boron concentrations in IOMM-Lee tumor nodules increased in a dose-dependent manner; the values were 3.20±1.00, 7.24±1.16 and 13.25±2.20 µg/g at 2.5 hours after injection of 125, 250 and 375 mg/kg of BPA, respec-

Figure 1 The time course of the boron concentration in IOMM-Lee cells (1 x 10⁶ cells). Cells were suspended in DMEM containing 20 µg¹⁰B/ml of BPA (closed triangles), 50 µg¹⁰B/ml of BPA (open circles), or 100 µg¹⁰B/ml of BPA (closed circles) or in PBS (open squares) as the control, and incubated for 2.5 or 6 hours. Each assay was done in triplicate. Bars represent the standard error.
Figure 2  The cell-survival curves for IOMM-Lee cells and U87Δ cells after irradiation with reactor thermal neutron beams with or without BPA. (A) Circles: The survival fraction of U87Δ cells. (B) Triangles: The survival fraction of IOMM-Lee cells. These figures show the sensitivity after irradiation with a thermal neutron beam in the colony-forming assay. The survival fraction is given as a function of neutron fluence. Filled symbols represent the cells which were irradiated after 2.5 hours from the incubation with 500 $\mu$g$^{10}$B/ml of BPA. Open symbols represent the cells irradiated with no boron compound. Each assay was done in triplicate.

<table>
<thead>
<tr>
<th>dose (mg/kg)</th>
<th>hrs</th>
<th>N</th>
<th>Boron concentration (µg B/g) (mean±SE)</th>
<th>Ratio</th>
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<tbody>
<tr>
<td></td>
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<td>Tumor</td>
<td>Brain</td>
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<tr>
<td>125</td>
<td>2.5</td>
<td>4</td>
<td>3.20±1.00</td>
<td>0.88±0.20</td>
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<tr>
<td>250</td>
<td>2.5</td>
<td>5</td>
<td>7.24±1.16</td>
<td>1.50±0.33</td>
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<tr>
<td>375</td>
<td>2.5</td>
<td>3</td>
<td>13.25±2.20</td>
<td>4.00±0.98</td>
</tr>
<tr>
<td>250</td>
<td>6</td>
<td>4</td>
<td>4.04±1.81</td>
<td>1.19±0.44</td>
</tr>
<tr>
<td>250</td>
<td>24</td>
<td>3</td>
<td>1.41±0.70</td>
<td>0.44±0.18</td>
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</table>

a; hours after i.p. injection of boron compound (BPA)
b; Number of animals
c; Analytic method used inductively coupled plasma atomic emission spectrometry (ICP-AES); each point represents arithmetic mean ± SE of each number of mice.
d; Calculated for each individual animal within an experimental group and the means ± SE for these were determined
e; IOMM-Lee cells
tively. The boron concentrations in the brain also increased in a dose-dependent manner; the values were 0.88±0.20, 1.50±0.33 and 4.00±0.98 μg/g at the respective doses listed above. The blood boron concentrations were 0.91±0.07, 1.60±0.28 and 2.83±0.50 μg/g, respectively. The corresponding tumor/brain ratios for the 3 doses were almost the same, namely 3.6, 4.8 and 3.3, respectively, and the tumor/blood ratios were 3.5, 4.5 and 4.7, respectively.

Second, the boron concentrations following intraperitoneal BPA administration to the tumor-bearing mice at a dose of 250 mg/kg b.w. were observed at different time-points. The tumor boron concentrations decreased in a time-dependent manner in IOMM-Lee tumor-bearing mice; the boron concentrations at 2, 6 and 24 hours were 7.24±1.16, 4.04±1.81 and 1.41±0.70 μg/g, respectively. Low concentrations were observed in the brain and blood at the 3 time-points: 1.50±0.33, 1.19±0.44 and 0.44±0.18 μg/g in the brain, and 1.60±0.28, 1.42±0.54 and 0.43±0.08 μg/g in the blood, respectively. Tumor/brain ratios and tumor/blood ratios of boron concentration at the 3 time-points were roughly equivalent at 3.2-4.8 and 2.9-4.5.

**In vivo neutron irradiation studies**

In the bio-distribution studies, the boron concentration in the tumor 2.5 hours after intraperitoneal administration of BPA (250 mg/kg b.w.) was 7.24 μg^{10}B/g, and that in the brain was 1.50 μg^{10}B/g. Using mean \(^{10}\text{B}\) concentrations, the total X-ray equivalent dose for tumors was calculated to be 3.91 Gy-Eq, whereas the value for the irradiated group with the neutron beam only was found to be 1.98 Gy-Eq. The total X-ray equivalent dose for the brain was calculated to be 2.22 Gy-Eq. Because it is currently impossible to qualify tumor or brain boron concentrations on a real-time basis in either animals or humans undergoing BNCT, these equivalent dose estimates must be considered approximations at best and should be interpreted with caution.

Neutron irradiation was performed 2.5 hours after intraperitoneal bolus injection of BPA (250 mg/kg b.w.). Nude mice were randomized into three experimental groups of 5-6 animals each: a treatment group (250 mg/kg b.w. of BPA), a neutron-in-

![Figure 3 Tumor growth inhibition after BNCT. BNCT was performed at JRR4 10 days after implantation of 10^5 IOMM-Lee human malignant meningioma cells. Nude mice were transported to the JRR4 and randomized on the basis of tumor size into three experimental groups of 5 animals each: the treatment group (250 mg/kg of BPA) (closed triangles); the neutron-irradiated group without boron compound (open circles); and the untreated control group (closed circles). BNCT was initiated 2.5 hours after intraperitoneal administration of BPA. Values shown represent the mean tumor volumes±SE for the 5 animals in each group.](image-url)
radiated group (without BPA) and an untreated control (Figure 3). In the untreated group, the tumor and brain were not irradiated; thus the absorbed dose was 0 Gy-Eq.

Thirty-five days after implantation, the mean tumor volume ± SE in the BNCT group was 445 ± 168 mm³. In contrast, that of the neutron-irradiated group without BPA was 2652 ± 419 mm³, and that of the untreated group was 2286 ± 364 mm³ (Figure 3). BNCT inhibits IOMM-Lee growth. IOMM-Lee-bearing nude mice, which had been administered BPA intraperitoneally, followed by BNCT, showed an almost 6-fold inhibition in growth of the tumor volume in comparison with the untreated control group and the neutron-irradiated group without BPA.

DISCUSSION

Meningiomas are common intracranial neoplasms derived from arachnoidal (meningothelial) cells. Most meningiomas are benign (WHO Grade 1), well-circumscribed, slow-growing, and curable by surgery depending on location. However, some meningiomas are clinically aggressive and can lead to significant complications and even death. Many, but not all, of these aggressive tumors are histological Grade II (atypical) or Grade III (anaplastic or malignant) tumors. Anaplastic or malignant meningioma makes up 1.0-2.8% of all meningiomas [1,2].

With atypical, anaplastic or malignant meningiomas, it is difficult to prevent tumor recurrence, even after complete resection [3,4]. Jaaskelainen et al. reported a 5-year recurrence rate of 78% for malignant meningioma [5]. Mahmood et al. found that malignant meningiomas had 5- and 10-year survival rates of 60% and 30%, respectively [6]. Although some treatments have been reported for recurrent malignant meningioma, a standard therapy has not yet been developed. At the present time, radical resection is the primary therapy, and the adjuvant treatment strategy after surgical resection is radiotherapy in the form of fractionated X-ray radiation or stereotactic radiosurgery for focal residual and recurrent meningioma [7,8,14]. However, radiotherapy is associated with neurotoxicity due to damage to normal structures adjacent to the tumor [7]. Chemotherapy including temozolomide [9], hydroxyurea [10-12], and others has been reported, although the efficacy of these treatments has not been clearly established.

Boron neutron capture therapy (BNCT) is based on a nuclear reaction. Non-radioactive isotope 10B atoms absorb low-energy (<0.5 eV) neutrons (thermal neutrons) and disintegrate into alpha (4He) particles and a recoiled lithium nucleus (7Li). These particles deposit high energy along their very short path (9 and 4 µm) [15,16]. A 10B-labeled compound is administered that delivers high concentrations of 10B to the target tumor. Only tumor cells with 10B are destroyed after irradiation with thermal neutrons. Thus BNCT is a tumor cell-selective particle radiation therapy. To perform BNCT effectively, it is essential to convey the 10B atoms efficiently and selectively into the tumor tissue but not into normal tissue. BNCT has been performed as an adjuvant therapy for malignant glioma. In our institute, we have used BNCT for malignant glioma in combination with surgery, and have reported good results [17-21,27]. We have also used BNCT as a treatment for recurrent malignant meningioma [13,14], which is also difficult to cure, as mentioned above. Stenstam et al. have also published clinical case reports on the use of BNCT as a treatment for malignant meningioma [28].

The present studies were performed in order to confirm that BNCT is a promising therapy for malignant meningioma. As described previously, BNCT is a unique cell-specific particle radiation therapy for tumor cells that can uptake boron compounds effectively, irrespective of the photon sensitivity of the target tumor cells. Generally, meningioma is considered to be radio-resistant [29]. In addition, malignant meningioma has the characteristic of infiltration into the surrounding normal tissues. Therefore BNCT has a theoretical advantage as a treatment for this aggressive infiltrative tumor.

It is well known that IOMM-Lee cells were established as a human cell line of malignant meningioma and that their pathology is highly compatible to malignant meningioma cells in actual patients [23]. For the present study, we chose the IOMM-Lee cell line from among the stock of available malignant meningioma cell lines because of its stable cell proliferation both in vitro and in vivo [30]. In addition, IOMM-Lee cells showed good BPA accumulation in vitro, and good tumor/blood and tumor/normal brain ratio of BPA accumulation were confirmed in vivo by preliminary experiments. These findings were similar to those made in human cases [13].

With neutron irradiation in the absence of 10B, the colony-forming ability decreased only slightly in both IOMM-Lee cells and U87Δ cells. In the presence of 10B, neutron irradiation clearly inhibited the colony-forming ability both in IOMM-Lee cells and in U87Δ cells. With the administration of neutron irradiation in the same tumor boron con-
centration, the colony-forming ability in IOMM-Lee cells was inhibited more than in U87Δ cells (Figure 2). In our in vitro experimental study, the cells were cultured in a monolayer fashion, and all tumor cells were uniformly exposed to BPA. Therefore the tumor cells could receive a high boron concentration, and the colony-forming ability could be clearly inhibited. As BPA was administered through the blood after the intraperitoneal injection in the in vivo study, it was necessary to investigate whether a high enough tumor boron concentration could be attained in vivo as well as in vitro. In this experimental study using a mouse subcutaneous tumor xenograft model, the boron concentrations in the IOMM-Lee tumor nodules increased in a boron dose-dependent manner and decreased in a time-dependent manner (Table 1). The tumor/blood and tumor/brain ratios were 2.8~4.7 and 3.2~4.8, respectively, and a dose and time escalation study showed no significant improvements in these ratios (Table 1). These results were similar to the data that we obtained from subcutaneous glioma models in nude mice (data not shown).

Coderre et al. reported that the tumor/blood ratio of BPA was fixed at about 3, even in a dose escalation study in the nude mice subcutaneous Harding-Passner murine melanoma model after intraperitoneal injection of BPA [31]. In addition, Miura et al. reported that the tumor/blood ratio of BPA was about 2 in the nude mice subcutaneous murine Mammary Carcinoma EMT-6 model [32]. Other researchers have found the tumor/blood ratios to be constant in the range of 2-5 independent of tumor model, boron dose, duration of administration, and route of administration [33-35].

In our clinical BNCT for malignant meningioma, we estimated the tumor/normal brain ratio of BPA with the use of BPA-PET [13, 14]. Using the blood boron concentration just prior to neutron irradiation and the tumor/brain ratio determined from the BPA-PET, we can estimate the boron concentrations of the tumor and the normal brain. Therefore, in our clinical BNCT, surgical sampling is not required during neutron irradiation. In other words, we do not determine the real boron concentration in the tumor during neutron irradiation. With respect to glioma cases, several bio-distribution studies of boron compounds from clinical samples were previously reported [36-38], while only one report was published regarding BNCT for malignant meningioma. Stenstrom et al. reported three malignant meningioma patients who were evaluated for BPA concentrations in the tumor and in surrounding tissue [28]. This uptake study showed levels of boron accumulation in the tumor, and the tumor/blood ratios were comparable with those observed in cases of malignant glioma. This report suggested that BNCT is a potentially adequate treatment for malignant meningioma. Our current study also suggests the suitability of BNCT as a treatment for human malignant meningioma on the basis of the bio-distribution of BPA.

For successful BNCT, there must be selective accumulation of 10B in the tumor, and low levels in the blood and normal brain tissue. The biological effects of BNCT thus depend on the microdistribution of 10B. Measured biological effectiveness factors for the components of the dose from 10B(n,α)7Li reaction have been termed compound biological effectiveness (CBE) factors and are drug (e.g., BPA) dependent [15]. CBE factors are calculated using either experimental or clinical data [24]. However, the term CBE remains somewhat undefined. CBE factors should include both the micro and macro level of 10B distributions, and should account for the character of each type of tumor cell and the structure of each type of tumor tissue. CBE factors are should be based not only on the cellular level of boron uptake but also on the bio-distribution of boron in the tumor tissue.

For clinical BNCT, the overall calculation of photon-equivalent doses requires several assumptions about RBES, CBE factors, and the boron concentrations in various tissues, based on currently available human or experimental data [15]. However, the sensitivity of BPA-based BNCT as a treatment for malignant meningioma has never been reported. The CBE factors for the boron neutron capture reaction from BPA are still unclear. The current study showed that the sensitivity to thermal neutrons without boron in IOMM-Lee cells was almost the same as in a malignant glioma cell line, U87Δ, and that the sensitivity of IOMM-Lee cells to the BPA-BNCT was higher than that of U87Δ cells. Therefore, the IOMM-Lee cell line is an appropriate candidate for BPA-based BNCT. In our clinical BNCT, we adopted 3.8 as the CBE value of BPA, not only for malignant glioma but also for malignant meningioma for dose estimation. As mentioned above, if the sensitivity of malignant meningioma to this neutron capture reaction is higher than that of human malignant glioma, as is that of the IOMM-Lee cell line, the CBE for malignant meningioma should become higher than 3.8, which is the CBE used for malignant glioma. If this hypothesis is true, the neutron irradiation time adopted for malignant meningioma can be shortened to give the same absorbed tumor dose as that administered to hu-
man glioma, which might result in a reduced radiation dose administered to the normal brain.

In the current study carried out in IOMM-Lee-bearing mice, the tumor volume on day 35 of the group which received BPA systemic injection followed by neutron irradiation was 445 mm³ and showed significant growth delay, compared to both untreated control and irradiated control animals, which showed tumor volumes of 2286 and 2652 mm³, respectively (P<0.01). The growth inhibition rate of treated tumors was 85% on day 28. The growth rate of the irradiated control group was similar to that of the untreated control group. These findings definitively support the effect of clinical BNCT on malignant meningioma. When the same subcutaneous malignant meningioma (IOMM-Lee) model was exposed to 4 Gy of photon irradiation, the growth inhibition was only 52% on day 28 (data not shown). In this study, 3.91 Gy-Eq was estimated as the total absorbed dose for the treated group, and the growth inhibition effect was higher than that by 4 Gy photon irradiation. Therefore, the estimated biological effect of treating malignant meningioma with BPA following neutron irradiation, here the CBE factor, should be higher — i.e., it should be simply calculated as at least 5.6 instead of 3.8, the value for malignant glioma.

The biological effects of boron neutron capture reaction using BPA as a 10B delivery drug for malignant meningioma are higher than those for malignant glioma. Therefore, this type of tumor can be considered well indicated for BPA-based BNCT. In deciding whether other malignancies are well-indicated for BNCT or not, it will be important to consider various factors, including the micro- and macro-scopical distributions of 10B and their biological effects on neutron irradiation. CBE factors experimentally derived from animal models must be used in BNCT clinical dosimetry protocols with extreme caution [15, 39]. However, for clinical treatment, the limiting factor is the dose absorbed by the normal brain or other normal tissues (e.g., skin, mucosa), and it is still uncertain which is better, dose escalation for the tumor or dose reduction for the normal tissue.

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