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CANCER Letters
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Combined use of sodium borocaptate and buthionine sulfoximine in boron neutron capture therapy enhanced tissue boron uptake and delayed tumor growth in a rat subcutaneous tumor model

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Received 10 September 2007; received in revised form 20 December 2007; accepted 3 January 2008

Abstract

We have previously reported that buthionine sulfoximine (BSO) enhances sodium borocaptate (BSH) uptake by down regulating glutathione (GSH) synthesis in cultured cells. This study investigated the influence of BSO on tissue BSH uptake in vivo and the efficacy of BSH–BSO-mediated boron neutron capture therapy (BNCT) on tumor growth using a Fisher-344 rat subcutaneous tumor model. With BSO supplementation, boron uptake in subcutaneous tumor, blood, skin, muscle, liver, and kidney was significantly enhanced and maintained for 12 h. Tumor growth was significantly delayed by using BSO. With further improvement in experimental conditions, radiation exposure time, together with radiation damage to normal tissues, could be reduced.

Keywords: Boron neutron capture therapy; Sodium borocaptate; Buthionine sulfoximine; Glutathione; Boron uptake; Rat tumor model

1. Introduction

Boron neutron capture therapy (BNCT) is a bimodal radiation therapy in which a neutron capture agent, a stable isotope of boron (^{10}B), is preferentially delivered into the malignant tissues by using a boron delivery compound, followed by irradiation with a beam of low-energy (thermal or epithermal) neutrons. Differential ^{10}B concentrations between the malignant tissues and the surrounding healthy tissues enable selective destruction of malignant tissues. But in practice, efficacy of BNCT is limited by

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doi:10.1016/j.canlet.2008.01.016

the number of available ^{10}B atoms in tumor due to lack of selectivity for tumor by boron delivery compounds [1,2].

Two known boron compounds, boronophenylalanine (BPA) and sodium borocaptate (BSH) are currently used, sometimes in combination, as boron delivery agents. BPA is preferentially taken up by growing tumor cells, whereas BSH is known to preferentially accumulate in malignant brain cells by penetration into tumor-induced disrupted blood–brain barrier [3,4]. In comparison with BPA, the total amount of BSH uptake was less and its uptake has little influence on the cell cycle [5]..

We have shown in our previous in vitro study that buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis, when given together with BSH, depletes intracellular glutathione (GSH) by inhibiting GSH biosynthetic enzyme γ-glutamylcysteine synthetase and consequently enhances BSH uptake and cytocidal effect [6].

In this study, we attempted to validate the influence of BSO on tissue uptake of BSH and to investigate the efficacy of combined BSH–BSO treatment on tumor growth by using a rat tumor model.

2. Materials and methods

All experimental animal protocols were pre-approved by the Animal Experiment Committee of University of Tsukuba and conducted following "the Regulation for Animal Experiment" guidelines.

2.1. Tumor cell line

Rat gliosarcoma cell line (9 L) was maintained in Earl's minimal essential medium, MEM, (Sigma–Aldrich, Japan) supplemented with 10% fetal bovine serum.

2.2. Reagents

Reagents used (and vendors) were as follows: BSH (Katchem, Czech Republic), BSO and tribromoethanol (Sigma–Aldrich, Japan), isoflurane (Foren, Abbott, Japan), and standard boron solution (Wako Pure Chemicals, Japan).

2.3. Animal experimentation and protocol

For determination of tissue boron concentration, 7-week-old male Fisher 344 rats (weighing ca. 150 g) were used, whereas for neutron irradiation experiments, due to size limitation by the unique design of our custom-made irradiation chamber (Fig. 1), 3-week-old male Fisher 344 rats (weighing ca. 35 g) were used.

Approximately, 10^6 of 9 L cells (100 μl) were inoculated subcutaneously into right leg of animals. Two weeks later, when a subcutaneous tumor developed into a diameter of ca. 7 mm, tumor-bearing animals were randomly divided into three groups: (a) BSO+ group received BSO (5 mmol/kg) in 3 ml phosphate buffered-saline (PBS) i.p. and BSH (100 mg/kg) in 300 μl PBS i.v. via tail vein; (b) BSO− group received BSH (100 mg/kg) in 300 μl PBS i.v. via tail vein; and (c) untreated controls. The procedure was done under isoflurane anesthesia.

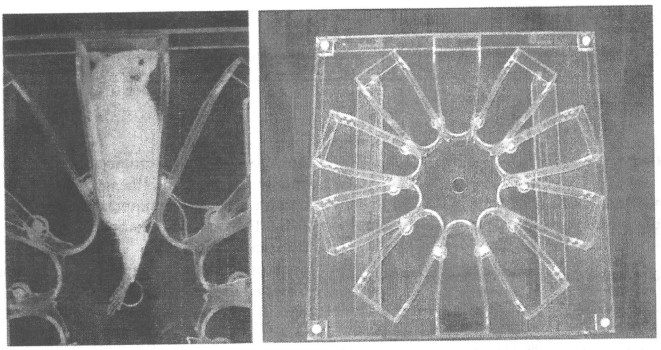

Fig. 1. Neutron irradiation of a rat with a subcutaneous tumor on the right leg using the custom-made irradiation chamber.

To examine tissue boron distribution, the animals in the BSO+ and BSO− groups ($n = 8$ each) were sacrificed at 3, 6, and 12 h after the administration of drugs, and tissue samples from subcutaneous tumor, blood, brain, skin, muscle, liver, and kidney were obtained.

To observe tumor growth after BNCT, the animals in the BSO+, BSO−, and control groups ($n = 5$ each) were irradiated at 6 h after the drug administration.

2.4. Measurement of tissue boron concentration by inductively coupled plasma-atomic emission spectroscopy (ICP-AES)

The tissues samples were liquefied by thermolysis with 20–30% nitric acid at 115 °C for 2–3 h using a thermo-alumi-bath (ALB-121, ASAHI TECHNO GLASS, Tokyo) and water was added to a final volume of 5 ml, followed by filtration through a membrane filter (PTFE 0.5 μm, Toyo Roshi Company, Ltd., Japan). The filtrate was measured for its boron content by ICP-AES (Jarrell-Ash ICAP-575, Nippon Jarrell-Ash Co., Ltd., Kyoto, Japan). The boron concentration was determined using a calibration curve of standard boron solution (Wako Pure Chemicals, Japan).

2.5. Neutron irradiation

Neutron irradiation experiments were carried out at the JRR-4 research reactor in the Japan Atomic Energy Agency (JAEA). Tumor-bearing animals were irradiated using a custom-made irradiation chamber designed to accommodate 10 rats that can be fixed on a plexiglass board and irradiated simultaneously (Fig. 1). The irradiation was performed using a φ 10-cm collimator in a thermal neutron flux of 3.7×10^8 n/cm^2/s for 60 min. Thermal neutron fluence was measured using gold wire activation. Rat anesthesia was induced with tribromoethanol (500 mg/kg/i.p.) and maintained by isoflurane inhalation during the irradiation. The absorbed dose was calculated using the following formula described previously [7]:

$D_{total} = D_n + D_b + D_f + D_\gamma$ (where D_n = the absorbed dose due to the nitrogen capture reaction, D_b = boron capture reaction dose, D_f = the fast neutron dose, and D_γ = the γ-ray dose. The relative biological effectiveness (RBE) for nitrogen capture reaction and boron capture reaction used was 1.9 and 2.3, respectively [8].

2.6. Monitoring tumor growth

After the neutron irradiation, tumor size (i.e., tumor volume in mm) was measured for 3 weeks at days 0, 3, 6, 10, 14, 17, and 20, using the following formula:

$$\text{Tumor volume} = (\text{minor axis})^2 \times \text{major axis}/2$$

2.7. Statistical analysis

Data were analyzed with U test of Mann–Whitney and probability values of <0.05 were considered significant.

3. Results

3.1. Tissue boron concentration

Table 1 shows the effect of BSO on the boron distribution in different tissues. Regardless of BSO supplementation, tissue boron concentrations (for both the BSO+ and BSO− groups) peaked at 3 h after the administration of BSH with or without BSO and gradually decreased with time. In comparison with the BSO− group, the boron concentration in all tissue samples, except the brain sample at 6 h and 12 h, was significantly higher in the BSO+ group ($p < 0.001$–0.05).

3.2. Tumor growth after BNCT

As shown in Fig. 2, as early as day 10 after the neutron irradiation, there were obvious differences in tumor growth as determined by tumor volume. Specifically, a significant delay in tumor growth was observed in the BSO+ group as compared with the control group (p-value at days 10, 14, 17 was 0.02, 0.01, and 0.04, respectively). There were also noticeable, albeit not significant, differences in tumor growth between the BSO+ and BSO− groups as well as the BSO− and the control group.

4. Discussion

4.1. Enhanced tissue boron uptake by BSH–BSO combination

GSH, predominant intracellular non-protein thiol compound, is involved in many biological functions including antioxidation, detoxification, and protection against peroxidation, UV, heavy metals, many exogenous organic substances such as anticancer agents, and radiation damage [9,10]. The most important physiological role of GSH with regard to BNCT is radiation protection, which is considered to be through direct radical scavenging or donation of reducing equivalents to terminate a free radical reaction [11].

The dose-dependent manner of BSO-induced intracellular GSH depletion has been documented in the normal and tumor cells [6,12,13]. A range of BSO doses (0.5–5.0 mmol/kg) have been used in the treatment of various tumors [12–15]. We used 5 mmol/kg in our studies because this dose has been shown to achieve the maximum GSH depletion in

Table 1
Tissue boron concentration (μg/g) of 7-week-old Fisher-344 rats ($n = 8$)

	3 h[a]			6 h[a]			12 h[a]		
	BSO+	BSO−	p-Value[b]	BSO+	BSO−	p-Value[b]	BSO+	BSO−	p-Value[b]
Subcutaneous tumor	26.3 ± 4.3	5.0 ± 2.9	0.0027	11.9 ± 7.8	1.3 ± 0.6	0.0019	6.5 ± 9.9	1.0 ± 0.1	0.0009
Blood	55.2 ± 15.0	8.1 ± 6.2	0.0027	20.2 ± 14.0	3.4 ± 0.5	0.0055	10.8 ± 20.6	1.3 ± 0.2	0.0005
Brain	2.6 ± 1.4	0.6 ± 0.7	0.0101	0.7 ± 0.6	0.2 ± 0.1	0.1967	0.3 ± 0.4	0.1 ± 0.1	0.2004
Skin	34.2 ± 9.8	6.3 ± 4.3	0.0027	22.1 ± 13.9	3.6 ± 0.9	0.0012	9.7 ± 13.4	1.8 ± 0.3	0.0017
Muscle	7.4 ± 2.8	1.2 ± 0.9	0.0027	5.0 ± 3.6	0.8 ± 0.2	0.0321	0.3 ± 0.4	0.1 ± 0.0	0.0007
Liver	71.4 ± 22.6	12.6 ± 8.1	0.0027	30.0 ± 18.8	9.4 ± 1.9	0.0109	4.6 ± 7.6	0.8 ± 0.1	0.0005
Kidney	82.3 ± 28.5	31.6 ± 15.9	0.0043	42.2 ± 16.5	24.1 ± 4.0	0.0109	6.8 ± 7.3	2.4 ± 0.5	0.0054

Data are shown as mean ± SD.
[a] Time after the administration of BSH with or without BSO.
[b] p-Values account for the difference in the boron concentration between the BSO+ and BSO− groups.

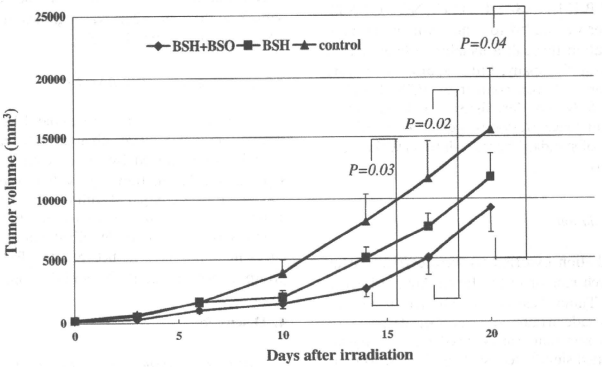

Fig. 2. Tumor growth curve of Fisher-344 rats after the BSH-mediated BNCT with and without BSO ($n = 5$, error bars = SE). p-Values account for the differences between the BSO+ and the control groups at specific time points. See Section 2 for experimental details.

tumor cells with no apparent side effects in mice [12,13].

We showed in our previous study that by depleting GSH, BSO resulted in an increased BSH uptake with associated radiosensitization and reduction of radiation damage to the cultured cells [6]. In this in vivo study, boron concentrations in tumor tissue, blood, skin, muscle, liver, and kidney were enhanced significantly (e.g., nine times in the subcutaneous tumor at 6 h) and maintained at high level for as long as 12 h after the administration of BSH and BSO. The observed sustained and high tissue boron levels is likely due to the formation of borocaptate dimer (BSSB), because the retention of the BSSB in cultured cells has been shown longer compared to that of the monomer BSH [16–20].

Clendenon et al. [21] previously demonstrated that the blood boron concentration in non-tumor-bearing rats decreased 4.8 times from 38.8 μg/ml at 0.5 h to 8.0 μg/ml at 3 h after the administration of BSH. In this study, the blood boron concentration in the BSO− group of 8.1 μg/ml at 3 h, which is parallel to Clendenon's data, was enhanced 6.8 times to 55.2 μg/ml in the BSO+ group. It is therefore conceivable that in the presence of cellular GSH, the significant loss of the administered BSH occurs within 3 h and depletion of cellular GSH by BSO averts the loss. Besides, as shown in Table 1, large SD values for the tissue boron concentrations indicate great variation within the group. In the BSO+ group, the animals with higher blood boron concentration

had higher tissue boron concentration (data not shown). Therefore, the observed large SD values within a group of animals could be due to the difference in the blood clearance of the administered BSH among the animals contributed by the differences in their sensitivity to BSO and thus the level of GSH depletion.

Although the mechanism behind the aforementioned phenomenon is not known, all these findings indicate a close association between GSH and BSH metabolism. Metabolic transformation of BSH in vivo produces BSH metabolites, such as BSH sulfenic acid (BSOH), BSH sulfinic acid (BSO(2)H), BSH disulfide (BSSB), BSH thiosulfinate (BSOSB), and BSH–*S*-cysteine conjugate (BSH–CYS) [22]; it is therefore of our interest to examine if one or more of these metabolites are involved in GSH depletion process.

4.2. Delayed tumor growth by BSH–BSO-mediated BNCT

Although there were apparent differences in tumor growth between the BSO+ and the BSO− groups or the BSO− and the control groups, the significant difference was observed only between the BSO+ and the control groups. This unexpected finding, despite significantly enhanced boron concentration in all tissues examined, could be partially explained by insufficient radiation dose.

The respective tissue boron concentration for the BSO+ and the BSO− groups was 11.9 ± 7.8 ppm and 1.3 ± 0.6 ppm. With thermal neutron fluence of 1.33×10^{12} n/cm^2, D_b for the BSO+ and the BSO− groups was calculated to be 1.19 Gy and 0.13 Gy, respectively. Since $D_n = 0.20$, $D_f = 0.017$, and $D_\gamma = 570.8 \pm 31.0$ mGy, the calculated gamma ray equivalent dosage was 3.72 Gy-eq in the BSO+ group and 1.28 Gy-eq in the BSO− group. In comparison with 20–100 Gy-eq used in the clinical practice, the therapeutic radiological dosage in our animal experiments was significantly lower. It may therefore be necessary to raise the dosage by increasing the BSH administered or irradiation time in forthcoming experiments.

In conclusion, this study denotes that the combined use of BSH and BSO in BNCT can induce sustained and high level of boron in different tissues. With further improvement, BSH–BSO-mediated BNCT should allow shorter radiation exposure time and consequently reduction in irradiation damage to normal tissues. Since this study was the first partial body irradiation experiment of rats at the JRR-4 reactor, all-around improvement in the experimental conditions is in progress for our on-going research.

Acknowledgement

This research was supported by Grant-in-Aid for Scientific Research (No. 17591245) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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The British Journal of Radiology, 81 (2008), 749–752

SHORT COMMUNICATION

Treatment results of boron neutron capture therapy using intra-arterial administration of boron compounds for recurrent head and neck cancer

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ABSTRACT. The effect of boron neutron capture therapy (BNCT) is correlated with the density of boron in the tumour. BNCT using intra-arterial administration of boron compounds was performed for recurrent head and neck cancer. Of the five patients treated, one achieved a complete response and four achieved a partial response. There was one case of transient headache but no severe adverse effects were observed. The advantages of using an intra-arterial administration route for BNCT, which causes the selective killing of tumour cells, might offer a new option in the treatment of recurrent head and neck malignancies. These promising results require further verification and optimization of the BNCT schedule; however, dose escalation would appear to be justified because the toxicity appears to be very low.

Received 26 January 2007
Revised 1 August 2007
Accepted 9 August 2007

DOI: 10.1259/bjr/65306248

Recurrent head and neck malignancies after radical radiotherapy are often radio- and chemoresistant, and show extensive infiltration, thus necessitating a wide resection of the surrounding tissue, although many cases are inoperable. Retreatment by conventional radiotherapy carries a high risk of acute and late morbidities, although regressions will inevitably occur. To avoid severe impairment of the orofacial structures and functions, it is therefore necessary to explore new treatments for recurrent head and neck malignancies.

Boron neutron capture therapy (BNCT) is based on the mechanism of a nuclear reaction: non-radioactive isotope ^{10}B atoms that absorb low energy (0.5 eV) neutrons (thermal neutrons) disintegrate into an alpha (^{4}He) particle and a recoiled lithium nucleus (^{7}Li). These particles deposit high energy along their very short path (<10 μm) [1]. As a result, only cells with ^{10}B are destroyed preferentially following thermal neutron irradiation. Therefore, BNCT is tumour-cell-targeted radiotherapy that is considered to have significant superiority over conventional external beam radiotherapy. In our group, recurrent head and neck tumours after standard therapy have been treated with BNCT in ongoing clinical trials since 2001 [2]. In order to accumulate boron in the tumour selectively, intra-arterial administration has been chosen as the drug delivery system since June 2004 [3]. We compared the dose–volume histogram (DVH) data for recurrent head and neck malignancies in an intra-arterial group (IA group) with DVH data for an intravenous group (IV group) to demonstrate the advantages of the IA route over the IV route as a drug delivery system for BNCT [4]. Thus, the purpose of the study was to investigate the treatment results, advantages and problems of IA administration as a drug delivery system for BNCT in recurrent head and neck tumours.

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Methods and materials

Patients

A total of five patients were included in the present study from June 2004 to December 2004. The extent of the tumour and the general condition of the patients are summarized in Table 1.

In all cases, X-ray radiotherapy of more than 70 Gy had been previously administered, and there was no possibility of surgery because of the area of the tumour and the condition of the surrounding tissue. In addition, sufficient healing could not be expected following retreatment by conventional radiotherapy or chemoradiotherapy.

Written informed consent was obtained from all patients. In addition, approval was obtained from the

Table 1. Patient characteristics

Case no.	Gender (age (years))	Primary sites	Pathology	Previous RT dose	Treated sites	Tumour volume (cm^3)u
1	Male (78)	Hard palate	Sq.c.cq	86.0 Gy	Hard palate	NE
2	Male (50)	Nasopharynx	Sq.c.cq	110.0 Gy	Nasopharynx	47.4
3	Female (51)	Tongue	Sq.c.cq	70.2 Gy	Tongue	18.6
4	Female (30)	Maxillary sinus	Adenoid cyctic cancer	80.0 Gy	Maxillary sinus, skull base	103.2
5	Female (69)	Hard palate	Sq.c.cq	96.0 Gy	Nasal cavity, skull base	131.5

Sq.c.cq, squamous cell carcinoma; RT, radiation therapy; NE, no evaluation.

ethical committees of Aichi Cancer Center and Research Reactor Institute of Kyoto University.

Methods of BNCT

An overview of BNCT has previously been published by our research group [2]. Boronophenylalanine-fructose (BPA-f) solution was administered at a dose of 250 mg kg^{-1} for 1–1.5 h through the IA route. For IA administration, catheters were inserted in either the superficial temporal artery or the occipital artery, and the tips of the catheters were located in either the external carotid artery or its branches feeding the tumour.

As ^{10}B concentration in the tumour was expected to decrease rapidly after administration of the BPA-f solution, immediate neutron irradiation was required and carried out within 15 min after the administration of the BPA-f solution. The irradiation time was determined so that the maximum dose to the surrounding normal tissue (oral mucosa) would be less than 12 Gy-Eq [2, 5], because all of the cases enrolled in the present study had already received a dose >70 Gy based on conventional radiotherapy with photons.

Accumulation of BPA in the tumour and normal tissue was imaged and quantified as a tumour/normal tissue ratio (T/N ratio) in an ^{18}F-BPA-positron emission tomography (PET) study prior to BNCT, as we have previously described [6]. In the ^{18}F-BPA PET study performed before BNCT, the ^{18}F-BPA was injected through the same route as in the BNCT on the treatment day.

The concentration of ^{10}B in the tumour during irradiation was estimated by multiplying the T/N ratio by the ^{10}B concentration in the normal tissue during irradiation. In the present study, the ^{10}B concentration in the normal tissue was assumed to be equal to the blood ^{10}B concentration during irradiation, which was calculated as the mean of the ^{10}B concentrations in the blood when sampled just before and after the irradiation (as the ^{10}B concentration in the blood should have decreased after the injection of BPA).

The thermal neutron fluence was measured by the radioactivation of gold wires (0.25 mm in diameter and 1.0 cm long) placed onto the skin surface of the lesion.

Table 2. Treatment results

Case no.	Root of administration	T/N ratio of ^{18}F-BPA	No. of BNCT treatments	IR time (min)	Maximum tumour dose (Gy-Eq)	Minimum tumour dose (Gy-Eq)	Mean tumour dose (Gy-Eq)	Tumour effect	Adverse effect	Outcome (months after BNCT)
1	Bilateral maxillary arteries	4.1	1	60	NE	NE	30.0	CR	Slight pain	Local recurrence (3) Death (10)
2	Bilateral external carotid arteries	10.0 12.5[a]	2	30 90[a]	31.1 97.1[a]	6.2 1.7[a]	15.5 45.0[a]	PR	None	Local progression (5) Death (12)
3	External carotid artery	10.0	1	80	68.7	26.8	48.9	PR	None	Local progression (2) Death (12)
4	External carotid artery and IV	7.0 7.0[a]	2	75 60[a]	71.2 52.7[a]	25.0 18.5[a]	53.4 39.5[a]	PR	Headache Nausea	Local progression (12) Death (24)
5	External carotid artery and IV	4.0	1	60	95.2	24.8	66.3	PR	Slight pain	Local progression (18) Alive with disease (26)

BNCT, boron neutron capture therapy; BPA, boronophenylalanine; T/N ratio, tumour/normal ratio; IR time, irradiation time; IV, intravenous; CR, complete response; PR, partial response.
[a]Second course of BNCT.

The compound biological effectiveness (CBE) factor was calculated using the following equation:

$$\text{CBE factor} = (D_{\text{X-ray}} - D_{\text{beam}} \times \text{RBE}_{\text{beam}})/D_{\text{BNCR}} \quad (1)$$

where $D_{\text{X-ray}}$, D_{beam} and D_{BNCR} are the doses of reference X-ray, the epithermal neutron beam and the ^{4}He nuclei and ^{7}Li particles derived from the $^{10}B(n,\alpha)^{7}Li$ reaction required for equal biological effect, respectively, and RBE_{beam} is the relative biological effectiveness (RBE) for the epithermal neutron beam alone in the absence of ^{10}B (see [2, 7]).

Clinical response

Within 6 weeks after BNCT, the responses were assessed. The definition of the antitumour response using visual examination, palpation, MRI and fluorodeoxyglucose (FDG)-PET was based on the standard definitions established by the World Health Organization in 1979 [8].

Results

Two patients received BNCT twice because of insufficient dose after one treatment. BPA was administered through both the IA and IV routes in two patients because their whole tumours were not supplied by the external carotid artery.

Of the five patients, one achieved a complete response (CR) and four achieved a partial response (PR). The outcome is summarized in Table 2, but all patients, with the exception of one, developed disease recurrence and died. Two patients complained of transient slight pain on the treated sites, and there was one case of headache and nausea immediately after treatment. However, no severe adverse effects caused by this treatment were observed. The post-treatment observation period was 4–20 months (with a median of 12 months).

Discussion

One of the problems when adminstering BNCT to many sites of malignancy is the poor penetration of thermal neutrons into the body, thus resulting in the delivery of insufficient doses to deep-seated tumours. In current BNCT clinical trials, epithermal neutron beams have been used; these lose energy within the body and are thermalized at a peak of 2–3 cm from the body surface, thus improving the distribution of thermal neutron fluence in deep-seated tumours [9]. However, the problem regarding the deficit of radiation dosage caused by the decrease of neutron fluence remains when BNCT is applied in a clinical setting.

In an attempt to overcome this problem, we used IA administration of BPA. As previously reported, IA administration of BPA enables an increase in ^{10}B concentration in the tumour, which is beneficial as a higher dose is delivered to the tumour [4]. Consequently, IA administration of BPA improved the dose distribution in head and neck tumours treated with BNCT when compared with IV administration. Of the six patients in the IV group [4], two had a PR, while three had no change (NC) and one had progressive disease (PD). Conversely, of the five patients in the IA group, one achieved a CR and four achieved a PR. However, one patient with CR developed a recurrence of the disease 3 months after undergoing this treatment, and four cases of PR also eventually developed recurrence of the disease. Minimum doses ranging from 1.7–26.8 Gy-Eq provided by IA administration of BPA may therefore be inadequate for controlling recurrent head and neck malignancies.

To overcome this, many preclinical studies using new boron compounds or a new drug delivery system, such as the use of liposomes, have succeeded in augmenting the ^{10}B concentration in the tumour [10–13]. From a clinical viewpoint, fractionated BNCT involving IA administration of BPA and/or BNCT in combination with intra-arterial chemotherapy may be a practical and potentially effective approach. Indeed, BNCT using intra-arterial administration of boron compounds and cisplatin has been used since 2005.

Few treatment options have so far been proposed for recurrent head and neck malignancies following standard treatments such as surgery, radiotherapy and chemotherapy. The advantages of BNCT, which causes selective killing of tumour cells, might therefore offer a new option in the treatment of recurrent head and neck malignancies. In addition, supplementary IA BNCT might be used as a boost with conventional therapy to prevent recurrence.

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Radiotherapy and Oncology 88 (2008) 192–195
www.thegreenjournal.com

BNCT in mesothelioma

A novel concept of treatment of diffuse or multiple pleural tumors by boron neutron capture therapy (BNCT)

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Abstract

Two patients, one with malignant pleural mesothelioma and one with a malignant short spindle cell tumor, received boron neutron capture therapy (BNCT). In each case, the tumors regressed or remained stable in size for 3–6 months following BNCT. No acute or late adverse events higher than grade 2 were observed.
 Radiotherapy and Oncology 88 (2008) 192–195.

Keywords: Boron neutron capture therapy; Thoracic tumor; Malignant pleura mesothelioma

Boron neutron capture therapy (BNCT) is based on the following nuclear reaction: non-radioactive isotope ^{10}B atoms that have absorbed low energy (<0.5 eV) neutrons (thermal neutrons) disintegrate into alpha (^{4}He) particles and recoiled lithium nuclei (^{7}Li), [$^{10}B(n,\alpha)\ ^{7}Li$] [1]. These particles deposit large amounts of energy along their very short paths (<10 μm). In BNCT, patients are irradiated with thermal neutrons following administration of ^{10}B-containing agents, which have the characteristics of accumulating selectively in tumors. If a sufficient number of ^{10}B atoms accumulate in tumor cells with a large gradient of ^{10}B concentration between the tumor cells and normal tissue cells, subsequent thermal neutron irradiation provides selective killing of tumor cells with the sparing of normal tissue cells. Therefore, BNCT has the possibility to deliver a curative dose to tumors diffusely spreading in radiosensitive organs, such as lung or liver, without causing fatal adverse effects [2–4]. In this article, we describe the treatment procedure used, its feasibility and the clinical results in two patients with diffuse or multiple pleural tumors treated with BNCT.

Case reports

The BNCT procedures were as follows: boronophenylalanine (BPA), which has been used as a boron compound in clinical trials, was administered at a dose of 250 or 500 mg/kg in a BPA-fructose (BPA-f) solution for 1.5–3.0 h through an intravenous route. Immediate neutron irradiation was applied within 15 min of finishing administration of the BPA-f solution. The details of the BNCT procedure on the treatment day are described in the presentation of each case below.

Treatment plans were constructed using the Simulation Environment for Radiotherapy Applications (SERA) system and JAERI Computational Dosimetry System (JCDS), which are currently available BNCT treatment planning systems [5,6]. The total biologically absorbed dose (Gy-Eq) was calculated as the sum of physical dose components multiplied by the relative biological effectiveness (RBE) and compound biological effectiveness (CBE) of each dose component using the following equation:

$$D_{\text{total}}(\text{Gy-Eq}) = D_{B10}(\text{Gy}) \times \text{CBE}_{\text{BPA}} + D_{\text{proton}}(\text{Gy}) \times \text{RBE}_{\text{proton}} + D_{\gamma\text{-ray}}(\text{Gy}) \times \text{RBE}_{\gamma\text{-ray}}$$

where D is the physical absorbed dose (Gy), $\text{CBE}_{\text{BPA}} = 3.8$ for tumor, $\text{CBE}_{\text{BPA}} = 1.35$ for lung, $\text{RBE}_{\text{proton}} = 2.5$ and $\text{RBE}_{\gamma\text{-ray}} = 1.0$.

To evaluate the D_{B10}, the ^{10}B concentrations in normal and tumor tissues were estimated. The ^{10}B concentrations in normal tissue were assumed to be equal to blood ^{10}B concentrations during irradiation. The ^{10}B concentration in tumors during irradiation was estimated by multiplying the ^{10}B concentration in blood by the ratio of tumor to blood

0167-8140/$ - see front matter doi:10.1016/j.radonc.2008.06.009

concentrations (T/B ratio). The ^{10}B concentrations in blood during irradiation were calculated as the mean of ^{10}B concentrations in the blood sampled just before and after irradiation, since ^{10}B concentrations in blood should decrease after the finish of injection of BPA. The T/B ratio was quantified by analyzing *p*-boronophenylalanine positron emission tomography (^{18}F-BPA PET) images. The procedure used for the 18F-BPA PET study was described in our previous report [7].

The doses reported in the following sections included some uncertainties inevitable in BNCT, ascribed to the following reasons: (1) the blood was not sampled during irradiation to estimate the ^{10}B concentration in the blood; and (2) T/B ratios estimated on the basis of ^{18}F-BPA PET studies performed before BNCT are not guaranteed to be applicable to dose estimation in BNCT, since the schedule for the administration of ^{18}F-BPA in the PET study was different from that in BNCT on the treatment day.

The feasibility of the treatment in both cases was reviewed and approved by the Institutional Review Board, Kyoto University Research Reactor Institute (KURRI) or Japan Atomic Energy Agency (JAEA). The patients gave written informed consent to all the activities performed at the KURRI and JAEA.

Patient 1

A 59-year-old man was presented with a left pleural effusion in March 2002. He had an experience of occupational exposure to asbestos. In January 2005, he was represented with a large left pleural effusion and a subcutaneous tumor at the drainage site of the left chest wall and was admitted to hospital for further study. Biopsy of the subcutaneous tumor revealed malignant pleural mesothelioma (MPM; epithelial type). He had received radiotherapy (RT) (2 Gy × 25) for the chest wall tumor. However, a repeat CT scan showed progressive disease spreading in the left pleural cavity, and he suffered from left chest pain. Since he had received renal dialysis, application of chemotherapy was not planned. He was referred to our center for further treatment of MPM by BNCT. A ^{18}F-BPA PET study performed before BNCT showed good accumulation of BPA in the tumor with a T/B ratio of 3.0.

In the first BNCT, the upper portion of the tumor was treated with anterior and posterior epithermal neutron beams at KUR in November, 2005. A 20-cm circle collimator, which encompassed the upper thoracic portion, was used to collimate both beams. Following the administration of BPA at a dose of 250 mg/kg, 40-min irradiation with each beam was performed. The dose was calculated under the assumption that the ^{10}B concentration in the blood during irradiation was 16 ppm and that in the tumor was 48 ppm, according to the T/B ratio of 3.0. The doses delivered to the tumor volume, which was encompassed in the treatment field, ranged from 10 to 30 Gy-Eq. The maximum dose delivered to the left lung was 6.5 Gy-Eq.

One month after the 1st BNCT, a 2nd BNCT was performed to treat the lower portion of the tumor. In the 2nd BNCT, three-port irradiations with anterior, posterior and left epithermal neutron beams collimated using a 20-cm collimator were carried out following the administration of BPA at a dose of 500 mg/kg. The ^{10}B concentration in the blood was estimated at 29 ppm. The dose delivered to the tumor volume, which was encompassed in the treatment field, ranged from 10 to 60 Gy. The left lung volumes receiving $\geqslant$7 Gy-Eq (V_7) and $\geqslant$10 Gy-Eq (V_{10}) were 29.0% and <1.0%, respectively.

In the post-treatment course, chest pain disappeared the day after BNCT. Follow-up computed tomography (CT) at 1 and 6 months after the 2nd BNCT confirmed partial regression (PR) of the tumor (Fig. 1). No adverse effect was observed during irradiation. As grade 1 acute adverse effects, non-hematotoxic adverse effects, including chill, fatigue, anorexia and diarrhea, and a decrease in platelets (89,000/mm^3), were observed. No acute adverse effect higher than grade 2 was experienced. Consolidation in the left lower lung irradiated with the 2nd BNCT appeared 1 month after the 2nd BNCT and lasted for 6 months. The patient had fever and chest pain, which were evaluated as grade 2 toxicity according to the Radiation Therapy Oncology Group (RTOG) scoring system [8]. Radiographic findings suggested radiation pneumonitis in the region where a dose greater than 4 Gy-Eq was delivered. No steroid therapy was applied to treat the radiation pneumonitis. In the upper portion of the left lung field treated with the 1st BNCT, no sign of radiation pneumonitis was observed. The patient died of local extension of MPM 12 months after the 1st BNCT.

Patient 2

A 43-year-old man was presented with a 7-cm diameter lung tumor in the left lung in August 2003. Since, in 2000, he had received treatment for left seminoma, the lung tumor was suspected to be a metastatic lung tumor from the seminoma. He received surgical resection of the tumor and histological examination revealed a malignant short spindle cell tumor. After the operation, he experienced local recurrences of tumors, which were treated with surgical resection and chemotherapy. In February, 2007, he was referred to our center for further treatment by BNCT for recurrent multiple tumors spreading in the left plural space. A ^{18}F-BPA PET study before BNCT revealed a T/B ratio of 2.0.

The first BNCT was performed at the JRR-4 research reactor in JAEA in June, 2006. Three tumors located in the left lower lung were defined as gross tumor volume (GTV) and treated with BNCT. Since multi-port irradiation is not possible during a single session of BNCT, due to a limitation of the equipment in the irradiation room at JRR4, a single posterior beam collimated by a 15-cm collimator was applied to the patient following the administration of BPA at a dose of 500 mg/kg. The irradiation time was 24 min. The ^{10}B concentration in the tumor during irradiation was estimated to be 33 ppm. The mean and maximum doses of radiation delivered to the tumors ranged from 16.2 to 32.7 Gy-Eq, and from 25.1 to 44.7 Gy-Eq, respectively. The maximum dose delivered to the left lung was 11.4 Gy-Eq. The left lung volumes receiving $\geqslant$7 Gy-Eq (V_7) and $\geqslant$10 Gy-Eq (V_{10}) were 3.4% and <1.0%, respectively.

In August, 2006, three tumors located in the upper portion of the left lung were treated with a 2nd round of BNCT. According to the positions of the tumors, a posterior beam

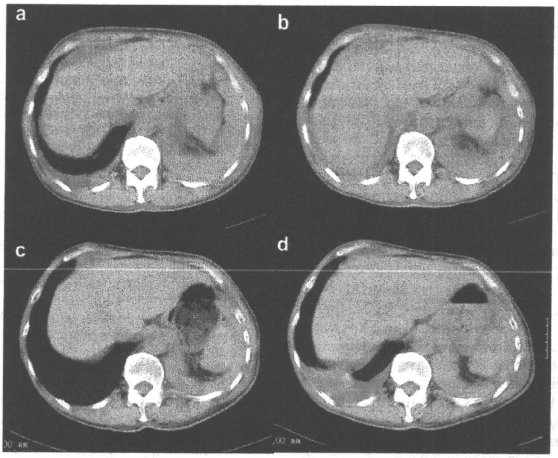

Fig. 1. (a) Pretreatment CT images. (b) Follow-up CT after 1 month showing slight regression of tumors. (c) Follow-up CT after 6 months showing regression of tumors judged as PR. (d) Follow-up CT after 7 months showing enlargement of the tumors abutting the thoracic spine.

collimated with a 15-cm collimator was selected. Twenty minute irradiation was carried out following administration of BPA at a dose of 500 mg/kg. The ^{10}B concentrations in the blood and tumor during irradiation were estimated to be 25.3 and 51 ppm, respectively. The mean and maximum doses in the tumors ranged from 5.8 to 13.3 Gy-Eq, and from 8.2 to 32.7 Gy-Eq, respectively. The maximum dose delivered to the left lung was 5.6 Gy-Eq.

Left back pain, a chief complaint before BNCT, disappeared within a few days of the 1st BNCT. Follow-up CTs at 1 and 3 months after the 1st BNCT revealed regression of the tumors located in the lower portion of the left lung (Fig. 2). The tumors in the upper portion, treated during the 2nd BNCT, remained stable in size at 3 months after the 2nd BNCT. All tumors were enlarged within 7 months of BNCT. One month after the 1st BNCT, Grade 1 radiation dermatitis was detected on the left back skin. No radiation pneumonitis or other late adverse effects were observed during the post-treatment course for 16 months. Although he received proton therapy and chemotherapy for recurrent tumors, he died of extension of the tumors 18 months after the 1st BNCT.

Discussion

In this pilot study, our main concern was the occurrence of lung toxicity. The second BNCT in Patient 1 caused Grade 2 lung toxicity. A focal lung reaction in the lower lung field was observed 1 month after the 2nd BNCT. Since V_{20}, the lung volume irradiated with greater than 20 Gy, has been adapted as the parameter for normal tissue complication probability in many studies using conventional fractionate RT [8,9], V_7 was assessed as the equivalent parameter for evaluating lung toxicity in a single-dose treatment in this

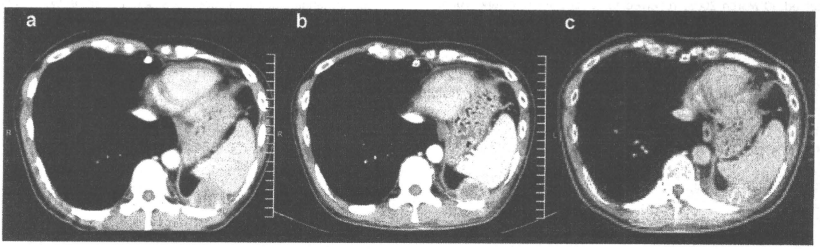

Fig. 2. (a) Pretreatment CT images. (b) Follow-up CT after 1 month showing slight regression of the tumors behind the spleen. (c) Follow-up CT after 3 months showing regression of the tumors judged as PR.

pilot study, assuming an α/β coefficient of 3.0 for normal lung tissues according to the linear–quadratic model. In the 2nd BNCT in Patient 1, the V_7 was 29.0%. In the other three BNCTs, in which radiation pneumonitis was not experienced, the V_7 ranged from 0 to 3.4%. It is impossible to draw any conclusions on the provability of lung toxicity in BNCT from only two cases. Therefore, to reveal the relationship between lung dose delivered by BNCT and the probability of lung toxicity, we are preparing for a phase 1 study treating MPM with BNCT.

The prognosis of MPM has been dismal, and the median survival length is 9–12 months without intervention [10]. Trimodality therapy, surgical resection followed by chemotherapy and radiotherapy, has been applied to MPM patients with curative intent [11,12]. However, unfortunately, the proportion of patients capable of surgery is assessed as fewer than 25% due to medical illness or advanced stage of MPM [10]. Recently, a new chemotherapeutic regimen for MPM, pemetrexed plus cisplatin, provided a 41.3% response rate [13]. However, patients are required to receive chemotherapy every 21 days until the tumor shows regrowth. Although it is not certain that BNCT surpasses the response rate of the new chemotherapy regimen, BNCT has the possibility to be a very effective treatment modality to palliate the symptoms of MPM patients, as suggested in the present cases. In the two cases in this pilot study, palliation of the symptoms, chest or back pain, disappeared within a few days of BNCT. According to the report by de Graaf-Strukowska et al. [14], RT provides local palliation in at least 50% of patients with MPM who were treated using a 4-Gy/fraction scheme to a median dose of 36 Gy. BNCT can deliver an adequate dose for palliation in single or two-fractionated BNCT on 1 or 2 days as demonstrated in the present cases, which seems more beneficial for patients suffering from various symptoms compared with chemotherapy or conventional radiotherapy. Since no severe adverse effects were observed in the two cases, dose escalation may be possible for controlling the tumor. Further clinical study of BNCT is warranted to shed new light on the best way to treat inoperable diffuse pleural tumors such as MPM or pleuritis carcinomatosis.

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Received 10 March 2008; received in revised form 9 June 2008; accepted 18 June 2008; Available online 22 July 2008

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J Neurooncol (2009) 91:199–206
DOI 10.1007/s11060-008-9699-x

CLINICAL STUDY - PATIENT STUDY

Survival benefit of Boron neutron capture therapy for recurrent malignant gliomas

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Received: 18 June 2008 / Accepted: 8 September 2008 / Published online: 24 September 2008

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

Electronic supplementary material The online version of this article (doi:10.1007/s11060-008-9699-x) contains supplementary material, which is available to authorized users.

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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 $<$ 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.