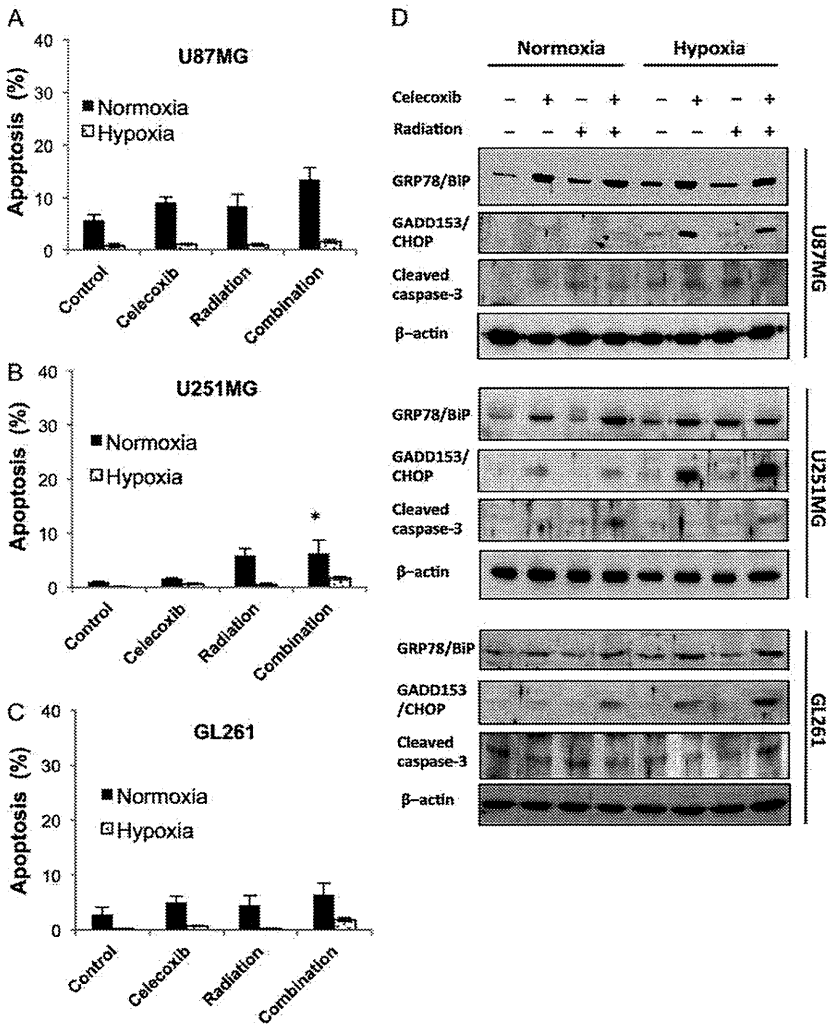

Fig. 5. Apoptosis induction. The bar graphs indicate early apoptosis fractions of U87MG (A), U251MG (B), and GL261 (C) 96 h after treatment with either radiation (6 Gy) or celecoxib (50 μM for U87MG and U251 MG and 30 μM for GL261) each alone or in combination. The black bars indicate normoxia, and dotted bars indicate hypoxia. Error bars indicate standard deviations calculated from 3 independent experiments. *$P < .05$ in comparison between celecoxib alone and combined treatment. The results of immunoblotting evaluating expression of GRP78/BiP, GADD153/CHOP, and cleaved caspase-3 6 h after each treatment in 3 cell lines are indicated in D. β-actin was used as a loading control.

U251MG and GL261 cells (Supplementary Fig. 1). Because it is known that cells in the G1 or G2/M phase are highly sensitive to radiation, cellular synchronization effects mediated through ER stress could be another mechanism that might explain the radiosensitization effects of celecoxib. Further detailed investigations are required to elucidate the effects of celecoxib on cell cycle regulation, including selective overexpression or inhibition of p53 in the cell lines used in this study.

Because celecoxib is already being used for patients as an NSAID, the hurdle before it can be applied in combination with radiotherapy to patients with GBM is not very high.[8] A limitation of this study was that the concentrations used here (30–50 μM) were significantly higher than the clinically available blood concentrations of <10 μM.[61] However, we still consider that 30 μM is feasible on the basis of the celecoxib data sheet provided by Pfizer, which noted that there was no serious toxicity after administering 2400 mg/day for 10 days (available at http://labeling.pfizer.com/ShowLabeling.aspx?id=793 [last accessed date 7 April 2013]). In addition, in a previous study, we confirmed a dominant antitumor effect by combining celecoxib with an anti-cancer drug in vivo.[9] Kardosh et al[11] also noted that ER stress could be induced at a lower concentration in vivo when used in combination with other drugs. Thus, another

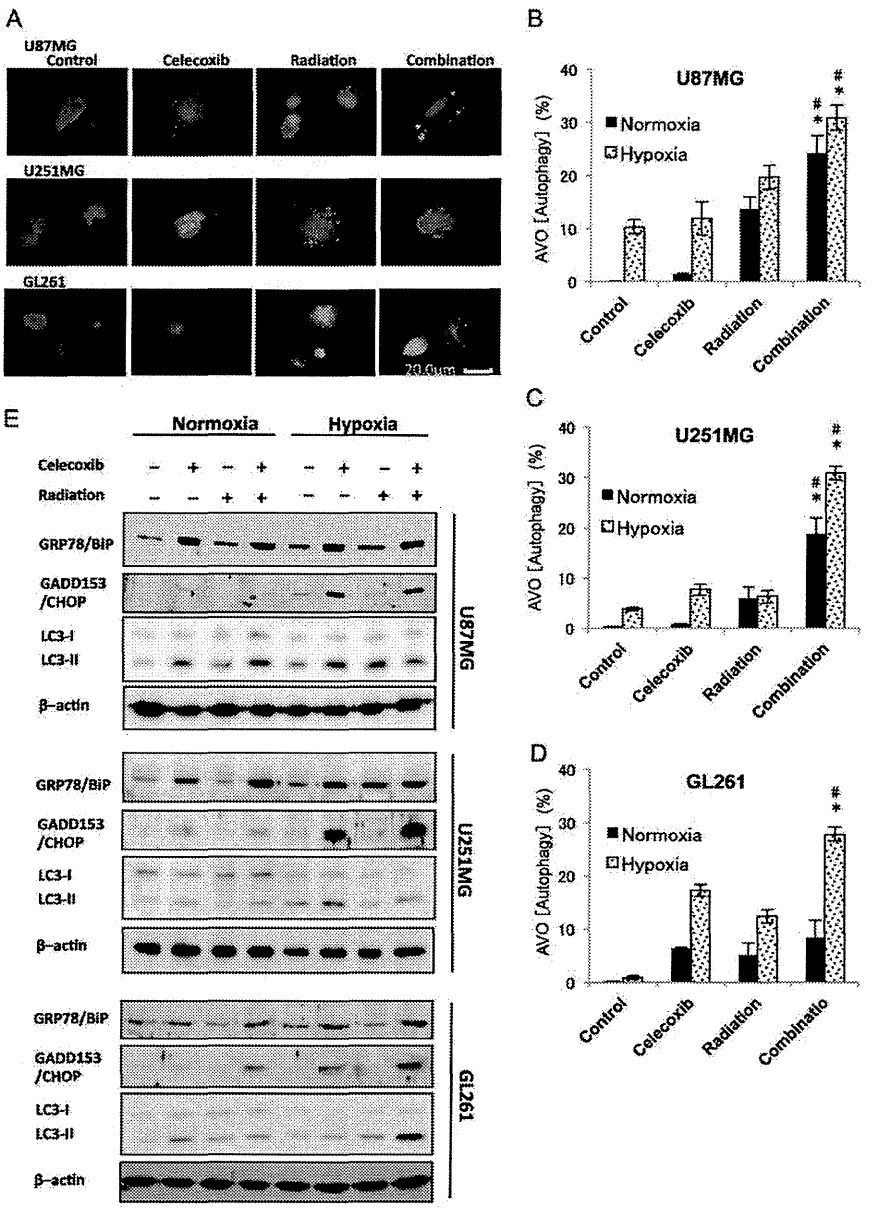

Fig. 6. Autophagy analysis. Immunofluorescent images of autophagosomes of 3 cell lines are shown in A. Autophagosomes of U87MG and U251MG were detected by Premo, and they were detected by Cyto ID in GL261. The nuclei were stained using Hoechst 33342. The images were captured 6 h after each treatment under hypoxic conditions. For quantitative analysis (B), autophagy was detected by acridine-orange staining, and the fractions were quantitated using flow cytometry 96 h after each treatment. The black bars indicate normoxia, and dotted bars indicate hypoxia. Error bars indicate standard deviations calculated from 3 independent experiments. *$P < .05$ in comparison between celecoxib alone and combined treatment. #$P < .05$ in comparison between radiation alone and combined treatment. The results of immunoblotting evaluating expression of GRP78/BiP, GADD153/CHOP, and LC3-I and LC3-II 6 h after each treatment in 3 cell lines are indicated in E. β-actin was used as a loading control. Data on GRP78/BiP, GADD153/CHOP, and β-actin are the same as those in Fig. 4.

promising approach is combination with other chemotherapeutic drugs.[9,10,17,18] To test the efficacy of these doses or combinations, an orthotopic mouse brain tumor model using GL261 cells would be suitable.

Another interesting strategy might be attributable to the effects on glioma stem-like cells, because Chen et al.[62] reported that celecoxib enhanced the radiosensitivity of cancer stem-like cells. Because hypoxia is

known to provide a niche for CD133-positive GBM stem-like cells,[63] a combination of celecoxib and radiation may be effective for tumor stem cells, which are the main culprits in local recurrence after radiotherapy.

Conclusions

The study results showed that celecoxib inhibited the growth of GBM cells and enhanced the radiosensitivity of these cells under not only normoxic but also hypoxic conditions. In addition, treatment with the celecoxib plus radiation combination caused cell cycle arrest and prominent autophagy in GBM cells under hypoxic conditions by ER stress loading. Our results suggest that celecoxib might contribute to overcoming the radioresistance of GBM cells under hypoxic conditions. These findings should be useful to further the clinical applications of celecoxib for improving outcomes in patients with GBM.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

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Reirradiation for recurrent malignant brain tumor with radiotherapy or proton beam therapy

Technical considerations based on experience at a single institution

Malignant brain tumors are a major challenge for neurosurgeons and radiation-oncologists. The incidence of malignant brain tumors was reported to be 7.2 per 100,000 from 2004 to 2006 in the United States [1]. Despite progress with standard treatment comprising maximum surgical resection, conventional external beam radiotherapy, and various kinds of chemotherapy, long-term survival without recurrence is still rare in patients with malignant brain tumors. Furthermore, there is no standard treatment for relapsed tumors after initial treatment, and treatment options after conventional radiotherapy are usually very limited because of the tolerance dose of normal brain.

Several recent reports have indicated that stereotactic radiotherapy (SRT) for recurrent brain tumor may improve survival without causing severe toxicity [2, 3, 4, 5, 6, 7]. Fogh et al. found that stereotactic reirradiation of 35 Gy in 3.5-Gy fractions was effective for recurrent high-grade glioma [2, 4, 5, 7]. Single-fraction stereotactic radiosurgery (SRS) has also been reported as a palliative salvage modality for recurrent brain tumor [3, 6]. However, Mayer et al. found an increased risk of radiation-induced normal brain necrosis with an increasing total dose and treatment volume [8], which indicates the importance of avoiding irradiation of normal brain tissue by use of the latest technology. One of the promising methods is proton beam therapy (PBT). It has been reported that PBT is superior to X-ray radiotherapy in preserving the normal tissue volume [9, 10, 11, 12, 13].

In this study, we examined the efficacy of reirradiation including conventional radiotherapy (RT), SRT, and PBT for recurrent malignant brain tumors in a retrospective analysis of patients who underwent reirradiation at our hospital.

Patients and methods

Patients

A total of 26 patients with recurrent malignant brain tumor after radiotherapy received reirradiation at our hospital between January 2005 and September 2010. All patients had a recurrent tumor according to the findings of magnetic resonance imaging (MRI) with contrast enhancement. Written informed consent was obtained from all patients prior to reirradiation and the use of a particular treatment modality (RT, SRT, PBT) was determined according to each patient's condition.

The 26 patients (12 men and 14 women) had a median age of 48 years (range, 4–76 years old). The performance status before reirradiation was 0 (n=6), 1 (n=8), 2 (n=4), 3 (n=5), and 4 (n=3). Eleven patients underwent tumor removal before reirradiation, including 8 with pathologically confirmed glioblastoma multiforme (GBM), 2 with grade 3 glioma, and 1 with anaplastic ependymoma. The other 15 patients were considered to have inoperable tumors before reirradiation, including 7 with pathologically confirmed GBM at the initial surgery, 1 with pineoblastoma (WHO grade 4), 4 with WHO grade 3 glioma, 1 with anaplastic meningioma, and 2 with atypical teratoid/rhabdoid tumors (AT/RT). The maximum tumor size before reirradiation was a medi-

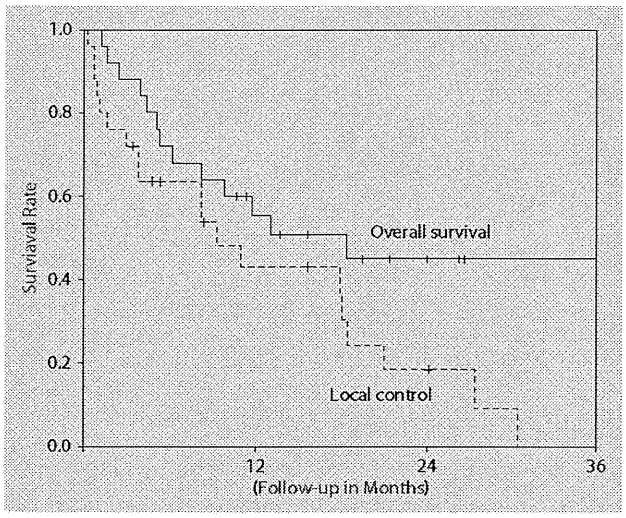

Fig. 1 ◀ Kaplan–Meier estimates of *overall survival* and *local control* for all 26 patients

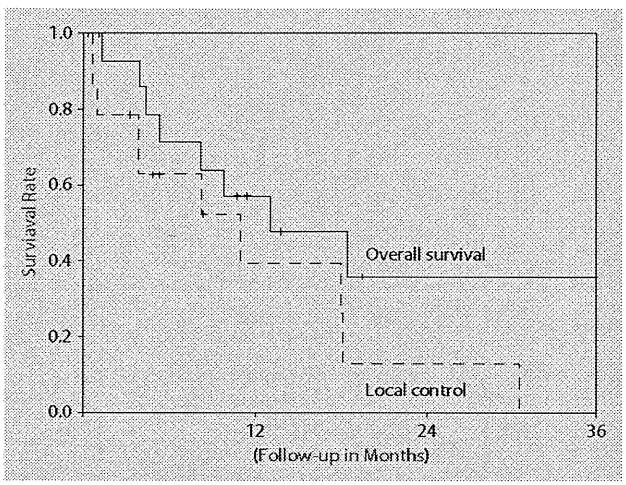

Fig. 2 ◀ Kaplan–Meier estimates of *overall survival* and *local control* for 15 patients with glioblastoma

an of 3.6 cm (range, 1.0–9.0 cm). The initial irradiation was performed using conventional RT (median dose of 60 Gy) in 21 patients, PBT in 4, and SRT in 1. The recurrent tumor was within the initial irradiation area in 21 patients and in the marginal region in 5 patients.

Treatment methods

The gross tumor volume (GTV) was defined as the area of contrast enhancement on MRI or the tumor bed. The planning target volume (PTV) was defined as the GTV plus a margin of 1–10 mm; edema was not included in the treatment area. The prescribed irradiated dose was chosen based on the organs at risk, such as the optic nerve, optic chiasma, and brain stem. Treatment planning for PBT was based on computed tomography (CT) images taken at 3-mm intervals in the treatment location. Proton beams with an energy of 250 MeV were generated by a booster synchrotron at the Proton Medical Research Center (PMRC). The treatment planning system provides dose distributions and settings for the collimator configuration, bolus, and range-shifter thickness. The relative biological effectiveness (RBE) for PBT was assumed to be 1.1 [14, 15]. Treatment planning for SRT was based on CT images taken at 1-mm intervals in the treatment location. SRT was delivered with an energy of 6 or 10 MV using photons from a linear accelerator with a multileaf collimator. The irradiation dose was calculated based on coverage of at least 95% of the PTV. Different radiation schedules were compared using the biologically effective dose (BED) [16, 17], which was calculated with a linear quadratic model according to the following equation: BED = nd (1+d/[α/β]), where n is the number of fractions, d is the fraction dose (Gy), and α/β is the tissue repair capacity (Gy). The actual radiation dose was converted to the equivalent dose in 2-Gy fractions (EQD2).

Follow-up procedures and evaluation criteria

Acute treatment-related toxicities were assessed weekly during treatment in all patients. After completion of reirradiation, physical examination, MRI, and blood tests were performed every 3 months for the first 2 years and every 6 months thereafter. The Kaplan–Meier method was used for calculation of local control and overall survival rates, and a log-rank test was performed for evaluation of differences between groups. Acute and late treatment-related toxicities were assessed using the National Cancer Institute Common Criteria, v.3.0, and the RTOG/EORTC late radiation morbidity scoring scheme [19].

Results

Reirradiation was completed in all patients at doses of 30–60 Gy (median, 42.3 Gy, EQD2). The dose of initial radiotherapy ranged from 34.5 to 94.4 Gy (median, 60 Gy, EQD2), and thus the total dose of all radiotherapy was 64.5–150.4 Gy (median, 100.0 Gy, EQD2). The total dose of all radiotherapy for cases with recurrence inside the area of the first radiotherapy ranged from 64.5 to 106.2 Gy (median, 100.0 Gy, EQD2).

The treatment modality of conventional RT (n=8), SRT (n=10), or PBT (n=8) for reirradiation was chosen based on the location, distribution, and size of the recurrent tumor. Treatment periods were 14–43 days (median, 29 days), 2–47 days (median, 19 days), and 14–51 days (median, 35 days) for RT, SRT, and PBT, respectively. The period between initial radiotherapy and reirradiation was 2.7–320 months (median, 16.2 months). The GTVs were 14.3–135.3 ml (median, 77.2 ml), 0.2–46.2 ml (7.4 ml), and 3.9–

217.3 ml (30.2 ml) for RT, SRT, and PBT, respectively.

Of the 21 patients with glioma, 13 received concurrent chemotherapy, including 8 treated with temozolomide (75 mg/m^2 daily or 150–200 mg/m^2 for 5 days), 4 who received nimustine hydrochloride (80 mg/m^2), and 1 treated with cisplatin and etoposide.

Toxicity

Acute treatment-related toxicity was generally mild. Headache, dermatitis, and nausea were all of grade 1 or 0. Ten patients needed temporary steroid therapy during reirradiation. Performance status remained unchanged from before to after reirradiation. Two patients showed radiation necrosis. One was treated by surgery and the other was treated by conservative therapy. Both are still alive and radiation necrosis was well controlled 13.7 and 19.4 months after reirradiation.

Survival and local control

The outcomes of reirradiation are summarized in ◘ **Tab. 1**. Eleven of the 26 patients died within 1 year and 13 died within 2 years. The 1- and 2-year overall survival rates of all patients after reirradiation were 55.4 and 45.1%, respectively (◘ **Fig. 1**), and the median survival period after reirradiation was 18.3 months (95% CI, 0.0–38.3 months). At the time of analysis, 11 patients were alive and 15 patients were dead; the median follow-up period for survivors was 19.4 months. The cause of death in all patients was tumor recurrence, including 11 with local recurrence and 4 with recurrence outside the reirradiated area as the initial recurrence after reirradiation. The 1- and 2- year local control rates after reirradiation were 43.0 and 18.4%, respectively, and the median local control period after reirradiation was 9.3 months (95% CI, 5.5–13.1 months). Six of the 15 patients with GBM (n=15) died within 1 year and 8 died within 2 years. The 1- and 2- year overall survival rates for patients with GBM (n=15) after reirradiation were 57.1 and 35.7%, respectively (◘ **Fig. 2**), and the median survival period was 13.1 months (95% CI,

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Reirradiation for recurrent malignant brain tumor with radiotherapy or proton beam therapy. Technical considerations based on experience at a single institution

Abstract

Background and purpose. Radiotherapy for recurrent malignant brain tumors is usually limited because of the dose tolerance of the normal brain tissue. The goal of the study was to evaluate the efficacy and feasibility of reirradiation for patients with recurrent malignant brain tumors.

Patients and methods. The subjects comprised 26 patients with recurrent malignant brain tumors treated with conventional radiotherapy (RT, n=8), stereotactic radiotherapy (SRT, n=10), and proton beam therapy (PBT, n=8) at our institute. Fifteen patients had glioblastoma, 6 had WHO grade 3 glioma, and 5 had other tumors. The dose of initial radiotherapy was 34.5–94.4 Gy. Different radiation schedules were compared using the equivalent dose in 2-Gy fractions.

Results. Reirradiation was completed in all patients without a severe acute reaction. The reirradiation doses were 30–60 Gy (median, 42.3 Gy) and the total doses for the initial and second treatments were 64.5–150.4 Gy (median, 100.0 Gy). Currently, 11 patients are alive (median follow-up period, 19.4 months) and 15 are dead. The median survival and local control periods after reirradiation of the 26 patients were 18.3 and 9.3 months, respectively. For the 15 patients with glioblastoma, these periods were 13.1 and 11.0 months, respectively. Two patients showed radiation necrosis that was treated by surgery or conservative therapy.

Conclusion. Reirradiation for recurrent malignant brain tumor using conventional RT, SRT, or PBT was feasible and effective in selected cases. Further investigation is needed for treatment optimization for a given patient and tumor condition.

Keywords
Glioblastoma · Proton beam therapy · Radiotherapy · Reirradiation · Recurrent

Erneute Bestrahlung mit üblicher Strahlen- oder Protonentherapie bei rezidivierendem bösartigem Hirntumor. Technische Aspekte basierend auf an einer Einrichtung gesammelten Erfahrungen

Zusammenfassung

Hintergrund und Zielsetzung. Bei bösartigen Hirntumoren ist eine operative Behandlung schwierig und bei rezidivierenden Tumoren schränkt die Dosistoleranz des normalen Hirngewebes eine Strahlentherapie häufig ein. Ziel der Studie war es, die Durchführbarkeit und Wirksamkeit einer erneuten Bestrahlung (Rebestrahlung) bei Patienten mit einem rezidivierenden bösartigen intrakraniellen Tumor zu bewerten.

Patienten und Methoden. Bei den Probanden handelte es sich um 26 Patienten, die mit konventioneller Strahlentherapie (RT, n=8), stereotaktischer Strahlentherapie (SRT, n=10) und Protonentherapie (PBT, n=8) in unserer Einrichtung behandelt wurden. Die Behandlung wurde in Abhängigkeit vom Tumorzustand ausgewählt. Ein Glioblastom hatten 15 Patienten, darunter 6 Patienten mit einem Gliom vom WHO-Grad III. Die Dosis der anfänglichen Strahlentherapie lag bei 34,5–94,4 Gy. Es wurden verschiedene Bestrahlungspläne anhand der Äquivalentdosis in 2-Gy-Fraktionen verglichen.

Ergebnisse. Bei allen Patienten wurde die Rebestrahlung ohne schwere akute Reaktion abgeschlossen. Die Rebestrahlungsdosen betrugen 30–60 Gy (Median 42,3 Gy) und die Gesamtdosen der ersten und zweiten Behandlung 64,5–150,4 Gy (Median 100,0 Gy). Gegenwärtig leben noch 11 Patienten (medianer Nachuntersuchungszeitraum 19,4 Monate), 15 Patienten sind bereits verstorben. Die mediane Überlebenszeit betrug 18,3 Monate und der Zeitraum für die lokale Kontrolle 9,3 Monate bezogen auf alle Patienten sowie 13,1 bzw. 11,0 Monate bezogen auf die Glioblastompatienten. Eine beherrschbare Strahlennekrose hatten 2 der 26 Patienten.

Schlussfolgerungen. Die Rebestrahlung bei rezidivierendem bösartigem Hirntumor anhand konventioneller RT, SRT oder PBT war durchführbar und wirksam. Weitere Untersuchungen sind notwendig, um die optimale Behandlung für einzelne Patienten bzw. einen bestimmten Tumorzustand herauszufinden.

Schlüsselwörter
Glioblastom · Protonentherapie · Strahlentherapie · Rebestrahlung · Rezidivierend

Tab. 1 Background and clinical characteristics of the patients

No.	Age (years)	Sex	Tumor	Initial dose (Gy)	Initial BED_2 (Gy)	Recurrence area	Tumor volume (cc)	Re-RT dose (Gy)	Re-RT BED_2 (Gy)	Cumulative BED_2 (Gy)	Reirradiation technique	Overall survival (months) [a]	Follow-up and status
1	58	M	Plneoblastoma	56.0	112.0	In-field	14.3	49.6	95.8	207.8	Conventional RT	15.6	Alive, extrafield recurrence
2	36	M	Glioma (WHO grade III)	50.0	100.0	In-field	116.1	49.6	95.8	195.8	Conventional RT	26.6	Alive, local recurrence
3	41	M	Glioblastoma	60.0	120.0	In-field	26.8	42.3	97.5	217.5	SRT	10.6	Alive, local recurrence
4	73	M	Glioma (WHO grade III)	60.0	120.0	In-field	30.1	40.0	80.0	200.0	Conventional RT	1.6	Death due to cancer
5	66	F	Glioblastoma	60.0	120.0	In-field	0.6	42.3	97.5	217.5	SRT	19.4	Alive, radiation necrosis
6	62	M	Glioblastoma	60.0	120.0	In-field	46.2	42.3	97.5	217.5	Conventional RT	1.1	Lost to follow up, local recurrence
7	67	M	Glioblastoma	56.0	168.0	Extrafield	No data	60.0	120.0	288.0	PBT	18.3	Death due to cancer
8	64	F	Glioblastoma	60.0	120.0	In-field	0.2	44.0	168.0	288.0	SRT	4.4	Death due to cancer
9	48	M	Glioblastoma	46.0	92.0	In-field	33.4	42.3	97.5	189.5	SRT	13.7	Alive, radiation necrosis
10	29	F	Glioma (WHO grade III)	54.0	108.0	In-field	73.7	32.5	75.0	183.0	Conventional RT	5.0	Death due to cancer
11	65	F	Glioma (WHO grade III)	60.2	116.3	In-field	11.5	30.0	60.0	176.3	SRT	40.3	Death due to cancer
12	73	F	Glioblastoma	60.2	116.3	Border	7.4	40.0	80.0	196.3	SRT	11.4	Alive, local recurrence
13	43	F	Glioma (WHO grade III)	63.8	123.1	In-field	No data	42.3	97.5	220.6	Conventional RT	2.5	Death due to cancer
14	39	F	Glioblastoma	53.1	102.6	In-field	80.6	53.1	102.6	205.2	Conventional RT	9.7	Death due to cancer
15	32	M	Glioblastoma	60.0	120.0	In-field	135.3	40.0	80.0	200.0	Conventional RT	5.3	Death due to cancer
16	72	F	Glioblastoma	63.8	123.1	In-field	17.4	40.0	120.0	243.1	SRT	1.3	Death due to cancer
17	6	M	AT/RT	34.5	63.0	In-field	6.5	30.0	60.0	123.0	SRT	11.8	Death due to cancer

Tab. 1 Background and clinical characteristics of the patients (continued)

No.	Age (years)	Sex	Tumor	Initial dose (Gy)	Initial BED_2 (Gy)	Recurrence area	Tumor volume (cc)	Re-RT dose (Gy)	Re-RT BED_2 (Gy)	Cumulative BED_2 (Gy)	Reirradiation technique	Overall survival (months) [a]	Follow-up and status
18	4	M	AT/RT	49.5	92.2	In-field	6.2	49.6	95.8	188.0	SRT	19.9	Alive, local recurrence
19	46	F	Glioblastoma	94.4	180.1	Border	3.9	30.0	60.0	240.1	PBT	36.2	Death due to cancer
20	56	M	Glioblastoma	94.4	180.1	Extra-field	30.2	50.0	100.0	280.1	PBT	3.9	Death due to cancer
21	76	F	Glioblastoma	94.4	180.1	Extra-field	5.0	56.0	168.0	348.1	SRT	8.2	Death due to cancer
22	69	F	Glioblastoma	52.0	104.0	In-field	29.7	30.0	60.0	164.0	PBT	13.1	Death due to cancer
23	36	F	Glioblastoma	60.0	120.0	In-field	107.7	30.0	60.0	180.0	PBT	48.0	Alive, local recurrence
24	34	F	Glioma (WHO grade III)	53.1	102.6	In-field	88.9	30.1	58.1	160.7	PBT	26.2	Alive, local recurrence
25	68	F	Anaplastic meningioma	53.1	102.6	In-field	217.3	36.6	87.5	190.1	PBT	3.0	Death due to cancer
26	6	M	Ependymoma (WHO grade III)	49.6	95.8	In-field	5.4	49.6	95.8	191.6	PBT	20.5	Alive

AT/RT atypical teratoid/rhabdoid tumor, *Re-RT* reirradiation [a]*Overall survival* since reirradiation

2.1–24.1 months). The 1- and 2-year local control rates after reirradiation were 26.2 and 0.0%, respectively, and the median local control period was 11.0 months (95% CI, 1.7–20.2 months).

Typical cases

A typical clinical course of a patient treated with conventional RT is shown in **Fig. 3**. The patient was a 59-year-old man with pineoblastoma at the pineal region. Eighteen years before reirradiation, he received conventional RT of 56 Gy in 28 fractions for the pineal tumor. Two years before reirradiation, he had recurrence in the right occipital lobe, right cerebellar hemisphere, and corpus callosum. Although the lesions of the right occipital lobe and right cerebellar hemisphere were surgically treated, the lesion of the corpus callosum tumor, which was partly in the first irradiated area, was difficult to remove by surgery (**Fig. 3a**). In this case, we selected conventional RT because the tumor was larger than 3 cm and there was a small volume of normal brain tissue around the recurrent tumor. The GTV for reirradiation included only the Gd-enhanced area on MRI and the PTV included the GTV plus a 5-mm margin (**Fig. 3b**). The tumor was significantly shrunk at 10 months after reirradiation (**Fig. 3c**) and was well controlled at 16 months.

A typical clinical course of a patient treated with SRT is shown in **Fig. 4**. The patient was a 66-year-old woman with right occipital lobe glioblastoma. Nine months before reirradiation, she received conventional postoperative RT of 60 Gy in 30 fractions for the right occipital lobe glioblastoma. Two small recurrent tumors appeared in the right occipital lobe and right temporal lobe, and the tumor in the right occipital lobe was in the field of the initial irradiation (**Fig. 4a**). In this case, we selected SRT because the tumor was small. The GTV for reirradiation included only the Gd-enhanced area on MRI and the PTV included the GTV plus a 5-mm margin (**Fig. 4b**). The reirradiation dose was 39 Gy in 13 fractions to cover 95% of the PTV. Five months after reirradiation, radiation necrosis occurred and necrotomy was performed (**Fig. 4c**). At 15 months after reirradiation, the tumor was well controlled.

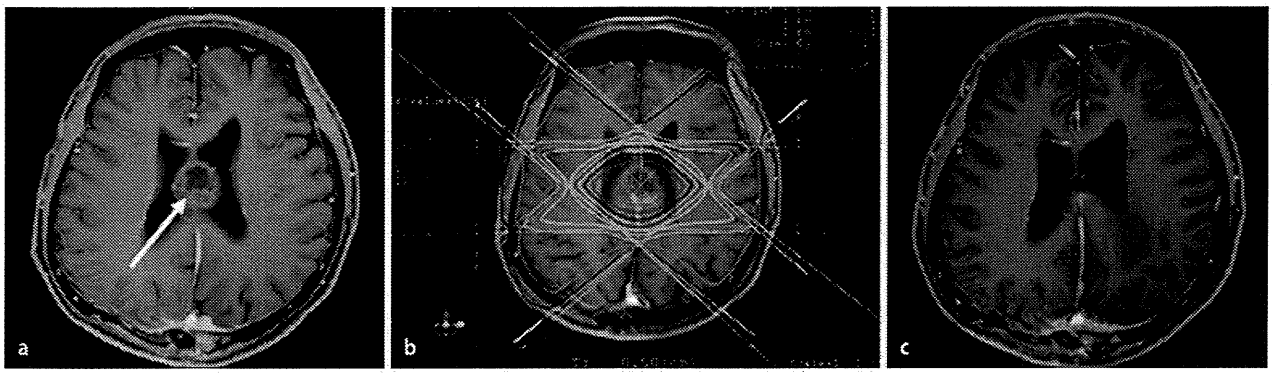

Fig. 3 ▲ Imaging for a patient treated with reirradiation with conventional RT. **a** Postcontrast T1-weighted MRI before reirradiation. **b** *Isodose curves* for conventional RT representing 100-10% of the prescribed dose at 10% intervals. **c** Postcontrast T1-weighted MRI at 10 months after reirradiation

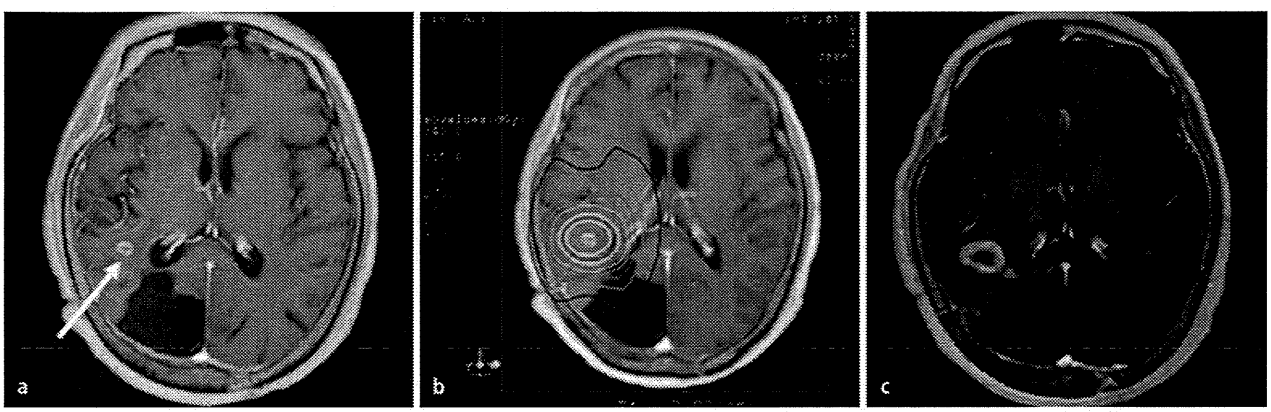

Fig. 4 ▲ Imaging for a patient treated with reirradiation with SRT. **a** Postcontrast T1-weighted MRI before reirradiation. **b** *Isodose curves for SRT representing 100-10% of the prescribed dose at 10% intervals.* **c** Postcontrast T1-weighted MRI at 5 months after reirradiation

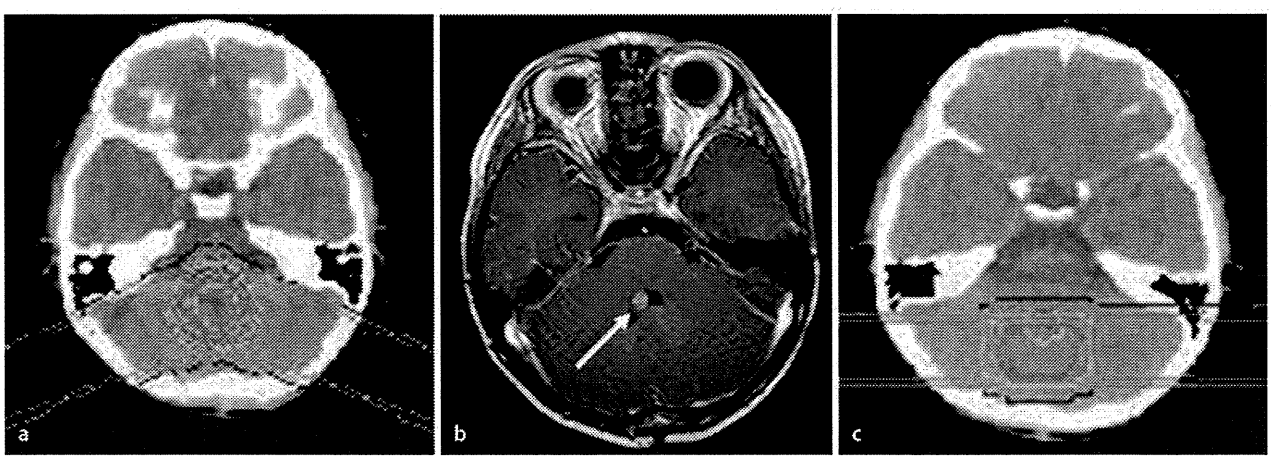

Fig. 5 ▲ Imaging for a patient treated with reirradiation with PBT. **a** *Isodose curves* for initial PBT representing 100-10% of the prescribed dose at 10% intervals. **b** *Postcontrast T1-weighted MRI before reirradiation.* **c** *Isodose curves for the second treatment with PBT representing 100-10% of the prescribed dose at 10% intervals*

A typical clinical course of a patient treated with PBT is shown in Fig. 5. The patient was a 6-year-old boy with a tumor in the fourth ventricle. Tumor resection was performed 7 months before reirradiation and pathological findings indicated ependymoma. Five months before reirradiation, initial PBT of 50.4 GyE in 28 fractions was performed (Fig. 5a). Three months before reirradiation, local relapse occurred (Fig. 5b) and re-resection was performed. In this case, we selected PBT because the brain stem was close to the recurrent tumor. The GTV for reirradiation was the tumor bed on MRI and the PTV included the GTV plus a 5-mm margin (Fig. 5c). The margin on the brain stem side was 0 mm at reirradiation. The reirradiation dose was 50.4 GyE in 28 fractions. At 24 months after reirradiation, the tumor was well controlled and no late toxicity had occurred.

Discussion

Several recent reports have indicated that reirradiation for recurrent glioma is a feasible and effective treatment option [2, 3, 4, 5, 6, 7]. In many cases, SRT or SRS was used for recurrent glioma after radiotherapy. In general, the dose of initial radiotherapy is about 60 Gy in 30 fractions for high-grade glioma and about 30–40 Gy in several fractions was frequently used as the reirradiation dose. Thus, the cumulative dose sometimes reached 100 Gy or more. Our results support the feasibility of reirradiation for recurrent malignant brain tumor using modern treatment modalities and depending on the dose concentration.

Although SRT or SRS is often used for treatment of recurrent brain tumors, these methods are usually not applicable to large or irregularly shaped tumors, since an increase in the treatment volume exposes large areas of normal brain tissue to the detrimental effects of a high-dose irradiation. In our hospital, PBT is available for various kinds of tumors, including large or irregularly shaped tumors [9, 10, 11, 12, 13, 18, 20, 21]; however, definitive selection criteria for the reirradiation method to brain tumors have not been established yet. Therefore, we select SRT, which is low in cost compared to PBT, for small tumors and conventional RT or PBT for larger tumors that are difficult to treat with SRT. Comparison of the outcomes of these methods was difficult at this point because of the small number of patients in this study. Several reports have shown overall survival of approximately 10 months after reirradiation for GBM [2, 3, 4, 5, 6, 7], and Combs et al. found a median survival period of 16 months for WHO grade 3 tumors [2] after reirradiation with SRT. In this study, the median survival after reirradiation for GBM was 13.1 months, indicating that the outcome using RT, SRT, or PBT was similar to that in patients treated with SRT or SRS.

Acute adverse events were generally mild in our patients. Although comparison with other reports is difficult because of the small number of events, all patients completed the planned reirradiation without change in the Karnofsky performance score suggesting that reirradiation is feasible at least in the short term. As for late adverse event, 2 patients demonstrated radiation necrosis. Although they were controllable in our series, these cases indicate that reirradiation to the recurrence at or close to critical regions such as the brain stem or the optic chiasma is very difficult to achieve while preserving functions. It is fundamental that the irradiated volume of normal brain must be minimized to reduce toxicity [8]. In this regard, PBT has an advantage of preserving normal brain tissue around the tumor. However, PBT is generally expensive, and compared with other radiation modalities such as SRT or SRS, significant clinical benefits of PBT in recurrent brain tumors have not been proved yet. Therefore, we currently consider that SRT as the first treatment option for a recurrent malignant brain tumor is acceptable.

Conclusion

In conclusion, reirradiation for recurrent malignant brain tumors using conventional RT, SRT, or PBT is feasible and effective in selective cases. Further investigation is needed for optimizing treatment modalities for each patient and tumor condition.

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Conflict of interest. On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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Review Article

Boron neutron capture therapy for brain tumors

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Abstract: Boron neutron capture therapy (BNCT) is a unique method that can provide the delivery of tumor cell-selective high-linear energy transfer (LET) particle radiotherapy to tumor mass and the microscopic invasion while avoiding radiation damage to the surrounding normal brain tissue. The rationale of BNCT is based on the nuclear interaction of ^{10}B with thermal neutrons with the release of high LET α and ^{7}Li particles through the boron neutron capture reaction, $^{10}B(n, \alpha)\ ^{7}Li$. The very short path length (<9 μm) of α-particles and ^{7}Li enables high-LET irradiation of tumor cells without undesirable damage to ^{10}B-unloaded normal cells. Eight non-randomized prospective external beam BNCT trials for glioblastoma (GBM) have been performed over the past 15 years using two available boron drugs and neutron beams at the nuclear reactor. The reported median time to progression and the median survival time (MST) are 6-12 and 12-27 mos, respectively. Optimization of dosage and boron delivery agents, the combined use of different boron agents, the combination of BNCT with other therapeutic modalities, and the development of in-hospital accelerator-based neutron sources are underway for the improvement of BNCT. In light of the existing clinical reports, there is a clear need for more evidence-based data.

Key Words: Boron neutron capture therapy; glioblastoma; accelerator

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Introduction

Glioblastoma (GBM) is a common malignant brain tumor in adults, and many recur within several months and show fatal progression within 2 years after the initial treatment. Extensive resection of the contrast-enhancing part of a tumor under image-guided surgery using fluorescence with 5-aminolevulinic acid, neuronavigation, and intraoperative magnetic resonance imaging (MRI) is shown to be beneficial for prolongation of the post-operative survival time (1,2). Aggressive cyto-reductive surgery is not indicated for the tumor in the eloquent brain. Invading cells are evident at distances of 2 to 3 cm or even further from the main tumor mass of GBM, which can be clinically identified by the contrast enhancement area on a magnetic resonance image (MRI). Thus, post-operative adjuvant therapies are essential for the treatment of post-surgical residual tumor mass and microscopic invading tumor cells in the patients with GBM.

Among several chemotherapeutic agents for malignant glioma (3,4), the effectiveness of temozolomide or carmustine wafers has been shown. For example, the EORTC clinical trial provided Class I evidence that the concomitant and adjuvant use of temozolomide with the conventional radiotherapy leads to a modest but significant survival advantage (median survival time, or MST: 14.6 mos) compared to the conventional radiotherapy alone (MST 12.1 mos), approximately with 25% of the patients surviving longer than 24 mos (5).

Two prospective studies provided Class II evidence and also showed modest benefits of carmustine wafers for GBM patients (4). In the report of Westphal *et al.* (6), a subanalysis of 207 GBM patients showed that the carmustine wafer group had a longer mean survival (13.5 mos) than the placebo group (11.4 mos). In a study by Valtonen *et al.* (7), regarding the survival of the 27 GBM patients among the whole series of 32 patients, the group that received bis-chloroethylnitrosourea (BCNU) wafers had a longer mean survival (53.3 wks) than the placebo wafer group (39.9 wks). Because of the limited benefits produced by

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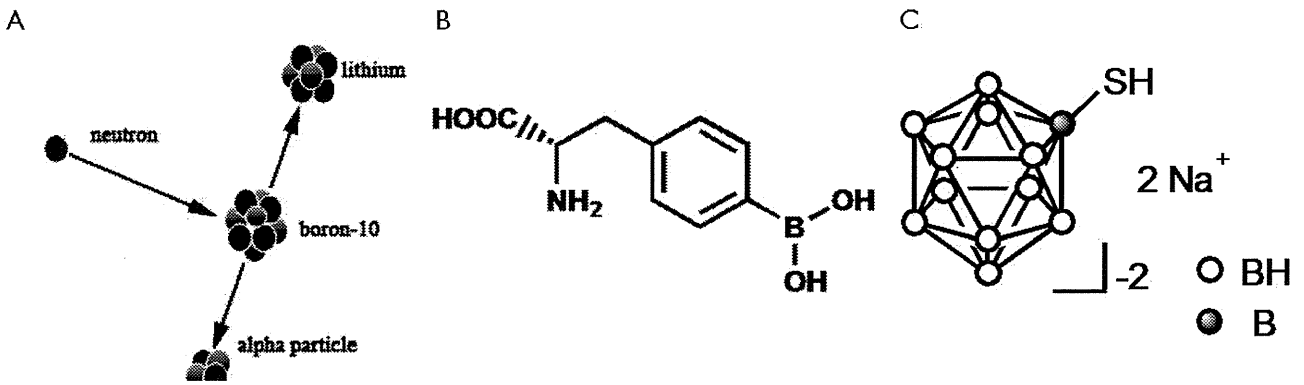

Figure 1 Neutron capture reaction of ^{10}B (A) and currently available boron delivery agents: boronophenylalanine *p*-dihydroxyboryl-phenylalanine (BPA, B) and borocaptate sodium sulfhydryl borane $Na_2B_{12}H_{11}SH$ (BSH, C)

standard (conventional) radiotherapy and chemotherapy to date, there has been also significant interest in new entity of radiotherapy and targeted molecular agents for the treatment of GBM.

Dose escalation studies using conventional X-ray fractionation, stereotactic radiosurgery, fractionated proton beam radiation, or other conformal radiotherapies have shown median survival times which vary from 9.5 to 26 mos (8,9). These studies and their failure analyses imply that at least 90 Gy must be delivered to achieve local control of GBM. Such a high-dose of radiation exceeds the accepted tolerance of normal brain tissue. Thus, high-dose radiation must be delivered with the upmost selectivity for tumor cells, to minimize radiation damage to the surrounding normal brain. Tumor-cell selectivity at the microscopic level is thus desirable. BNCT has been indicated primarily for GBM because of the theoretical selective sterilization of microscopic invading cells in the brain.

Boron neutron capture therapy (BNCT)

Boron neutron capture therapy (BNCT) has been proposed to provide tumor cell-selective high-linear energy transfer particle radiotherapy. The nuclear reaction between boron-10 (^{10}B) and thermal neutrons releases high LET α and ^{7}Li particles through the boron neutron capture reaction, $^{10}B(n, \alpha)\ ^{7}Li$ (*Figure 1*). The very short path length (<9 μm) of α-particles and ^{7}Li enables high-LET irradiation of ^{10}B-loaded tumor cells, minimizing undesirable damage to ^{10}B-unloaded normal cells. The effectiveness of BNCT is highly dependent on the amount of these particles and the selectivity of the boron compound in tumor cells. In BNCT clinical study, the minimum tumor dose of gross tumor volume (GTV) was around 30 Gy (10).

Although low-energy thermal neutrons (<0.53 eV) are captured most efficiently by ^{10}B nuclei, the shallow penetration limits their usefulness. For external beam BNCT, it is essential to use epithermal neutrons, which lose energy during the penetration of normal tissue (e.g., skin, cranium) and convert to thermal neutrons. Most commonly in BNCT for brain tumors, epithermal neutron beam irradiation is performed at a research reactor, and in a single fraction (*Figure 2*).

To deliver ^{10}B, two boron drugs, p-dihydroxyboryl-phenylalanine (BPA) and sulfhydryl borane $Na_2B_{12}H_{11}SH$ (BSH), are currently available for BNCT clinical studies (*Figure 1*). Positron emission tomography (PET) is used to estimate the ^{10}B concentration and to determine the eligibility of a patient for BNCT, by calculating the lesion-to-normal (L/N) ratio of ^{18}F-labeled BPA. The uptake in ^{11}C-methionine-PET, which has been more extensively studied for cancer diagnoses, is shown to have a linear correlation with that of ^{18}F-BPA-PET (*Figure 3*), indicating the potential application of ^{11}C-methionine-PET for BNCT dose planning and candidate selection (11). Before neutron irradiation, boron compounds (BSH and/or BPA) are administered intravenously, and then blood samples are drawn serially after the intravenous injection of the boron agent to measure its level in the blood.

Neutron source for BNCT: from reactor to accelerator

The major issues in BNCT research concern the neutron

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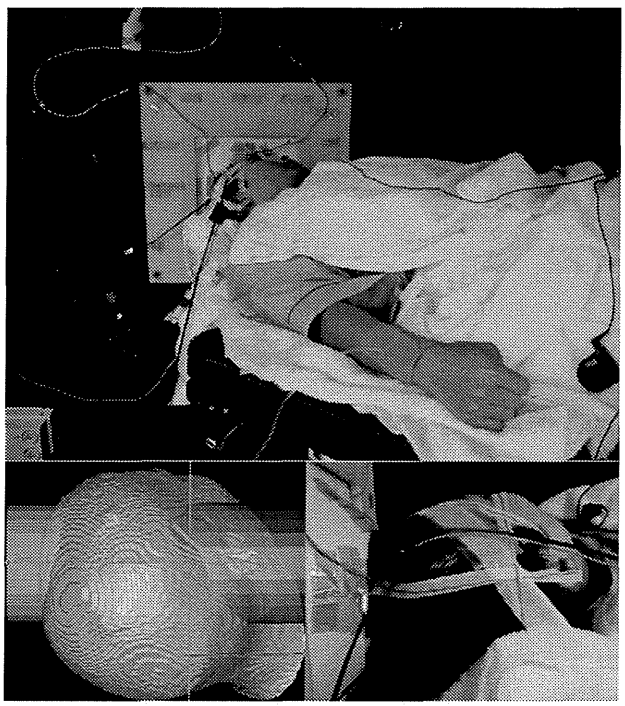

Figure 2 Neutron irradiation room and head positioning at Japan Research Reactor No. 4 (JRR-4). The patient's head position is fixed under the laser-guided positioning device in the neutron irradiation room. The relation between the beam direction and the patient's head position is also shown

sources, boron compounds, and clinical applications. BNCT research has been conducted for more than 60 years using nuclear research reactors. The first clinical studies for malignant brain tumors were performed at Brookhaven National Laboratory (BNL) and Massachusetts Institute of Technology (MIT) in 1950s and 1960s. In these early BNCT trials, low-energy thermal neutron beams were used for irradiation; however, because of shallow penetration, BNCT with thermal neutrons required craniotomy, to allow the neutrons to reach deeper regions of the brain.

In the 1990s, external beam BNCT using higher-energy (0.53-10 keV) epithermal neutrons was initiated using the Brookhaven Medical Research Reactor (BMRR) at BNL and a High Flux Reactor (HFR) at Petten, the Netherlands. This extended the therapeutic range deeper into the brain from 4 to 8 cm, and allowed the application of nonoperative external beam irradiation (12).

A typical research reactor for BNCT has only one irradiation port fixed in the side wall of the irradiation room, and this limits achieving desirable dose distribution compared to the current multiple field irradiation or conformation radiotherapy. The locations of research reactors usually require the transfer of the patient from a hospital, and this is unusually not possible until a few weeks after surgery. In Japan, the availability of machine time is limited by research projects, maintenance, and inspections. To resolve these nuclear reactor limitations, in-hospital

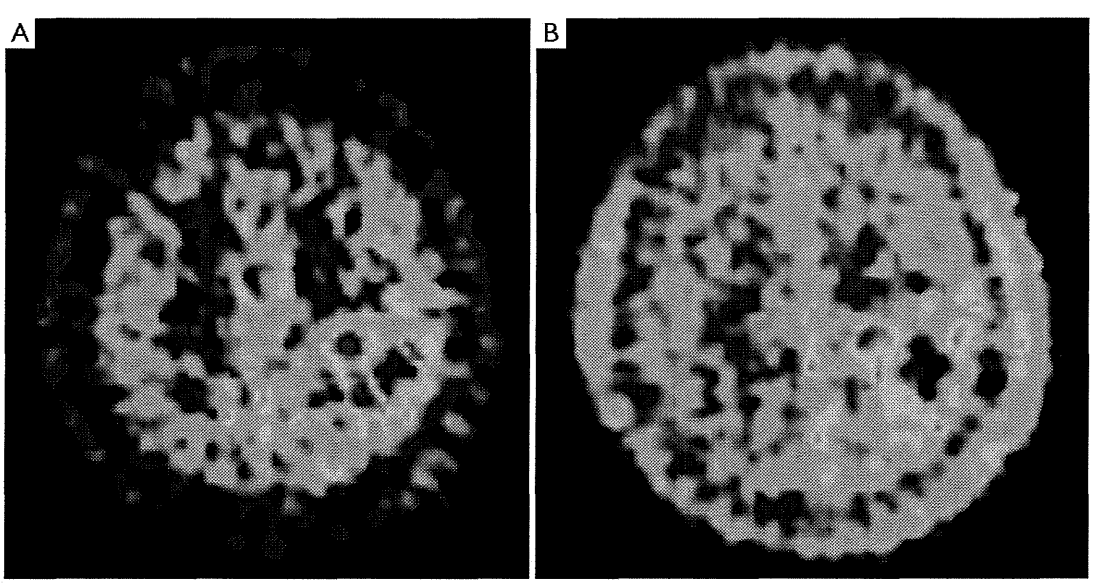

Figure 3 ^{11}C-Methionine-PET (A) and ^{18}F-BPA-PET (B) of a left frontal glioblastoma (GBM). Similar uptake is shown at the posteromedial wall of the surgical cavity

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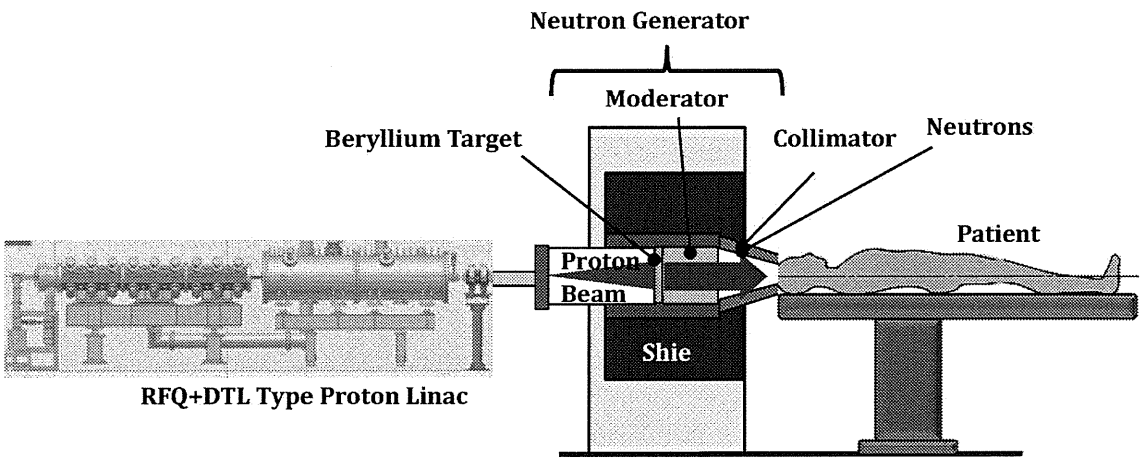

Figure 4 Schematic drawing of the linac-based beryllium target accelerator BNCT system which is under development in Tsukuba, Japan. Reduced proton energy (8 MeV) and current (10 mA) of a radio-frequency quadrupole (RFQ) linac and a drift-tube-linac (DTL) as proton accelerators minimize activation of the target and other materials of the neutron generator device, but are high enough to generate an epithermal neutron flux >1.0^9 n/cm^2/s. A schematic drawing of the linac-based BNCT device is also shown

accelerator-based neutron sources have been developed and are now providing neutron beams for clinical study of BNCT. The accelerator BNCT system consists of a proton accelerator, target, moderator, collimator, and irradiation room, and neutrons are provided by the reaction of the target material (Be, Li, etc.) and the accelerated protons (*Figure 4*). The first clinical trial of BNCT for brain tumors using the beryllium target accelerator system was initiated at KURRI in Japan in late 2012.

Boron compound and delivery system

A variety of boron delivery agents have been investigated to date, including amino acids, porphyrins, nanoparticles, polyamines, biochemical precursors, DNA-binding agents, sugars, antisense agents, peptides, proteins, monoclonal antibodies, and liposomes. However, there are only two boron delivery agents available for clinical BNCT trials for malignant glioma: ^{10}B-enriched BPA and BSH (*Figure 1*). ^{10}B constructs 20% of natural nonradioactive boron and has high efficiency in capturing thermal neutrons to generate boron neutron capture reaction, ^{10}B(n, α) ^{7}Li (13). Successful BNCT is dependent on the selective accumulation and absolute level of ^{10}B atoms in tumor cells.

These boron delivery agents must be as safe as glucose, and drug administration of gram-order is commonly needed to achieve a high enough intracellular boron level to sterilize tumor cells. A boron delivery agent should be non-toxic at the clinically effective doses, achieve at least 10-30 μg ^{10}B/g of tumor, have high tumor/brain and tumor/blood concentration ratios, and show rapid clearance from the blood circulation and normal tissues (but persist in the tumor). They should also be water soluble and chemically stable (14).

BPA has structural characteristics similar to those of a melanin precursor, and promising clinical results were shown in a pilot study of BNCT for skin melanoma (14). BPA is usually administered intravenously as a soluble fructose complex, BPA-F, at doses ranging from 250 to 900 mg BPA/kg. BPA can penetrate across the blood-brain barrier into the normal brain, and is actively transported through the tumor cell membrane due to the elevated rate of amino acid transport in proliferating cells. Although the uptake of BPA depends highly on individual tumors, high tumor-to-normal-BPA-uptake ratios (2.1-7.1) were demonstrated in an ^{18}F-BPA-PET study of newly diagnosed GBMs (15).

BSH biodistribution studies have suggested that BSH is distributed through passive diffusion from the blood to tumor tissues via the disrupted blood-brain barrier. The boron concentration in the normal brain with an intact blood-brain barrier remains minimal, whereas the tumor ^{10}B concentration is related to both the tumor vessel density and the blood ^{10}B level. Tumor-to-blood boron concentration ratios ranging from 0.5 to 1.0 have been reported in human patients treated with BSH-mediated BNCT (12). Vascular

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irritation, fever, skin reaction (erythema), and peripheral vasoconstriction have been reported as probable adverse effects of BSH injection (10). Japanese clinical trials have used a combination of BPA and BSH based on experimental data which showed these different compounds accumulate in different subpopulations of tumor cells (12).

Clinical studies of BNCT

In a clinical trial using epithermal neutrons at the BNL in which 53 GBMs were irradiated to evaluate the safety and effectiveness of external beam BNCT (16,17), no major adverse events were found following the 2-h intravenous injection of BPA-F at a dose of 250 to 330 mg/kg. However, patients who received 330 mg/kg BPA showed precipitates in the urine. MST following one, two and three field (one fraction each) BNCT were 14.8, 12.1 and 11.9 mos, respectively. Two of the seven subjects received an average brain dose (ABD) of 8 Gy-Eq or above, using three fields, and had grade 3 CNS toxicity. An ABD of 6.2 Gy-Eq was associated with 50% incidence of somnolence. Other grade 3 radiation toxicity was ototoxicity (17,18).

In the clinical trial at Harvard/MIT (19), no adverse event was found in relation to the intravenous injection of 250 mg/kg over 1 h, 300 mg/kg over 1.5 h, and 350 mg/kg over 1.5 h. The tumors with volumes <60 cm^3 and >60 cm^3 were associated with a 19% and 67% incidence of developing grade 3 or higher toxicity, respectively. Experimental data suggest that a longer infusion time up to 6 hours may improve the homogeneity of boron accumulation in tumors in BPA-mediated BNCT (20,21). This method was applied to the phase II clinical trial at the Studsvik BNCT facility for 29 patients suffering from GBM, who received 900 mg/kg BPA-F in a 6-h infusion, where the average boron concentration in the blood was 24.7 μg/g (22,23). Four patients developed grade 3-4 toxic events including epileptic seizures, hematuria, thrombosis, and erythema. These events except for seizures may relate to BPA administration. The median progression free survival and median MST were 5.8 and 17.7 mos, respectively.

The Finnish phase I/II trial showed that the BPA dose level of 450 mg/kg was the optimal dose for further BNCT studies of newly diagnosed GBM (24,25). In that study, 290 mg/kg of BPA was infused over 2 h in the first 12 patients suffering from GBM using two fields, and the BPA dose to subsequent patients was escalated from 330 mg/kg (n=1) to 360 mg/kg (n=3), 400 mg/kg (n=3), 450 mg/kg (n=3), and 500 mg/kg (n=8). The maximum tolerated dose was reached at the BPA dose level of 500 mg/kg, where grade 3 (n=2) and grade 4 (n=1) CNS toxicity was found. Kankaanranta *et al.* (26) also reported a phase I dose escalation study for recurrent malignant glioma after initial treatment using X-ray fractionated radiotherapy at a dose of 50 to 61Gy, and they recommended up to 400 mg/kg L-BPA as a 2-h infusion. The MST values for the dose groups of 290, 330/360, 400, 450, and 500 mg BPA/kg were 13.4, 11.0, 16.9, 21.9 and 14.7 mos, respectively. The other studies' protocol using long-term infusions showed that the median time from BNCT treatment to tumor progression was 5.8 mos, and the MST after BNCT was 14.2 mos (22,23).

The longer perfusion method was also employed in a trial at Osaka Medical College (700 mg/kg for 6 h) (15). Experimental data also suggest that the combination of BNCT and photon radiation leads to significant gains in survival (21). In the trial conducted at Osaka Medical College, the first 10 patients suffering from GBM were administered 100 mg/kg of BSH and 250 mg/kg of BPA in a 1-h infusion (protocol 1), and the latter 11 patients were administered 100 mg/kg of BSH and 700 mg/kg of BPA in a 6-h infusion (protocol 2). A 2 Gy daily fraction of X-ray irradiation was added in protocol 2 for a total dose of 20 to 30 Gy. The MST for all patients and for protocol 2 patients were 15.6 and 23.5 mos, respectively (15).

In a trial at the University of Tsukuba and Tokushima University at Japan Research Reactor No. 4 (JRR-4) of the Japan Atomic Energy Agency (JAEA) (10), the low dose (250 mg/kg) of BPA was administered over 1 h and 5 g BSH /kg was infused over 1 h in 8 patients with a single irradiation field. These patients received additional photon radiation defining the signal abnormality in T2-weighted MRI after the completion of BNCT. The MST and the time to progression were 27.1 and 11.9 mos, respectively. The 1-year and 2-year survival rates were 87.5% and 62.5%, respectively. This small number of patients showed the most favorable outcome with BNCT to date and treatment was well tolerated without severe acute or subacute adverse events. Four of 15 patients showed delayed radiation necrosis and median survival time of 4 patients including 1 alive patient was 43.4 mos (15.1-76.0). Although it is not certain whether the additional photon irradiation has a role in the clinical response to BNCT, the survival of the small cohort seemed to be favorable.

The clinical studies for newly diagnosed GBM revealed that the median time to progression varies from 6 to 12 mos and the MST varies from 12 to 27 mos after BNCT as an initial

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treatment (10). More clinical data are needed to confirm the effectiveness of this modality, although the existing results appear promising, and warrant further investigation. Future areas of research include clinical applications, the development of new boron delivery agents, and accelerator neutron sources.

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