

Translational Evaluation of a Dodecaborated Albumin Conjugate With Maleimide as a Boron Delivery System for a Neutron Capture Reaction to an F98 Rat Glioma Model

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Abstract

Boron neutron capture therapy (BNCT) is a biologically targeted, cell-selective particle irradiation therapy that utilizes the nuclear capture reaction of boron and neutron. Recently, accelerator neutron generators have been used in clinical settings, and expectations for developing new boron compounds are growing. In this study, we focused on serum albumin, a well-known drug delivery system, and developed maleimide-functionalized *cis*-1,2-dodecaborate albumin conjugate (MID-AC) as a boron carrying system for BNCT. Our biodistribution experiment involved F98 glioma-bearing rat brain tumor models systemically administered with MID-AC and demonstrated accumulation and long retention of boron. Our BNCT study with MID-AC observed statistically significant prolongation of the survival rate compared to the control groups, with results comparable to BNCT study with boronophenylalanine (BPA) which is the standard use of in clinical settings. Each median survival time was as follows: untreated control group; 24.5 days, neutron-irradiated control group; 24.5 days, neutron irradiation following 2.5 hours after termination of intravenous administration (i.v.) of BPA; 31.5 days, and neutron irradiation following 2.5 or 24 hours after termination of i.v. of MID-AC; 33.5 or 33.0 days, respectively. The biological effectiveness factor of MID-AC for F98 rat glioma was estimated based on these survival times and found to be higher to 12. This tendency was confirmed in BNCT 24 hours after MID-AC administration. MID-AC induces an efficient boron neutron capture reaction because the albumin contained in MID-AC is retained in the tumor and has a considerable potential to become an effective delivery system for BNCT in treating high-grade gliomas.

Introduction

High-grade gliomas are refractory to standard treatments such as chemoradiotherapy after surgical resection. Since high-grade gliomas, especially glioblastoma, diffusely infiltrates and invades normal brain parenchyma, surgery alone cannot remove all tumor cells. Thus postoperative tumor cell-selective treatment plays a very important role [1].

Recently, boron neutron capture therapy (BNCT), targetable at the cellular level with high energy particle beams, have been expected to treat high-grade gliomas. While the usefulness of radiotherapy techniques for high-grade gliomas have been widely known [2, 3], BNCT is a particle therapy that uses the reactions produced by boron-10 (^{10}B) atoms captured by thermal neutrons to destroy tumor cells selectively. When boron compounds are taken up in tumor cells, irradiation with low-energy thermal neutrons produces alpha particle and recoil lithium-7 (^7Li) nuclei as high linear energy transfer (LET) particles. Since the path length of these high-LET particles is limited to approximately 4–9 μm , the cell-killing effect is limited to the boron-containing cells. Therefore, BNCT is very suitable for diseases that require highly biologically targeted therapy, such as high-grade gliomas with a infiltrative nature to the normal brain parenchyma. BNCT using a nuclear reactor has demonstrated its applicability in several clinical trials against high-grade gliomas [4–9]. And also BNCT using an accelerator-based neutron generator has been shown to be effective in treating high-grade gliomas [10]. BNCT using an accelerator-based neutron generator will

soon become widely available in clinical settings because accelerator-based BNCT systems can easily be set up in any hospital [10, 11].

The most commonly used boron compound is boronophenylalanine (BPA), and it is the only compound that has been approved by the pharmaceutical authorities in Japan [10, 11]. However, since some clinical cases in which BNCT using BPA are not very effective to such as deep-seated tumors or multiple lesions that require long irradiation times or multi-directional irradiation or BPA-resistant tumor cells exist, further development of novel boron compounds is a key area of interest for improving the effectiveness of BNCT.

Our studies have focused on serum albumin, already an effective drug delivery system [12, 13]. We reported that maleimide-functionalized *closo*-dodecaborate (MID) and albumin conjugate (MID-AC), a serum albumin conjugate of boron compound, can selectively and highly accumulate in a mouse colon tumor 26 subcutaneous. The tumor growth obtained significant inhibition with neutron irradiation after MID-AC administration [14]. Since MID-AC has never been applied to brain tumors before, we evaluated the applicability of MID-AC to gliomas based on the biodistribution of MID-AC and the therapeutic effect of BNCT on F98 rat glioma models.

Materials And Methods

Boron compounds

Maleimide-functionalized *closo*-dodecaborate (MID), which has been successfully synthesized, was mixed with albumin from human serum lyophilized powder (Sigma-Aldrich, Tokyo, Japan) at a ratio of 1:10. The conjugate was subsequently used in several experiments (Maleimide-functionalized *closo*-dodecaborate albumin conjugate, MID-AC) [14]. BPA (L-isomer) was kindly provided by Stellar Chemifa (Osaka, Japan) and converted into a fructose complex [15]. All boron compounds used in this study were boron-10 concentrates.

Cell Culture

In this study, we used F98 rat glioma cells because they can infiltrate and invade the normal brain parenchyma of a Fischer rat and are reportedly refractory to various treatments, including radiotherapy. Moreover, F98 rat glioma models progress histologically to undifferentiated or anaplastic gliomas in *in vivo* pathological histology [16]. They often serve as brain tumor models for evaluating the BNCT therapeutic effects [17–20]. F98 rat glioma cells were kindly provided by Dr. Rolf Barth (The Ohio State University, Columbus, Ohio, USA). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 10% fetal bovine serum and penicillin, streptomycin, and amphotericin B at 37 °C in an atmosphere of 5% CO₂. All materials were purchased from Gibco Invitrogen (Grand Island, NY, USA).

F98 rat glioma model

Each 10-week-old male Fischer rat used in this study weighed between 200 and 250 g. The rats were anesthetized by intraperitoneal injection of a mixed anesthetics containing medetomidine (ZENOAQ,

Fukushima, Japan) (0.4 mg/kg), midazolam (SANDOZ, Yamagata, Japan) (2.0 mg/kg), and butorphanol (Meiji Seika, Tokyo, Japan) (5.0 mg/kg). Each head was fixed with a stereotactic frame (Model 900; David Kopf Instruments, Tujunga, CA, USA), and F98 rat glioma cells were implanted into each rat brain. 10^3 F98 rat glioma cells diluted in a 10 μ L solution of DMEM containing 1.4% agarose (Wako Pure Chemical Industries, Osaka, Japan) for therapeutic experiments or 10^5 F98 rat glioma cells for biodistribution experiments were injected at a rate of 20 μ L/min by an automated infusion pump, respectively. Our research groups use these surgical procedures routinely [17–20].

Biodistribution of boron compounds in F98 rat glioma models

Approximately 12 days post-implantation of 10^5 F98 rat glioma cells, when the tumor was expected to have grown sufficiently, each boron compound was administered at the following bodyweight (b.w.) doses: 12 mg boron (B)/kg b.w. for BPA and 20 mg B/kg b.w. for MID-AC, respectively. All rats were euthanized at a fixed time, and the tumor, brain, blood, heart, lung, liver, kidney, spleen, skin, and muscle were removed. Consequently, each organ was weighed and digested with 1N nitric acid solution. After each organ dissolved sufficiently, the boron concentration was measured by using inductively coupled plasma atomic emission spectroscopy (ICP-AES; iCAP6300 emission spectrometer, Hitachi, Tokyo, Japan). Results were normalized as μ g B/g.

Survival analysis of a neutron irradiation experiment for F98 rat glioma models

This study aimed to evaluate the therapeutic effects of MID-AC on brain tumors. A neutron irradiation experiment was conducted at the nuclear reactor (Institute for Integrated Radiation and Nuclear Science, Kyoto University, Kumatori, Osaka, Japan) for 14 days post-implantation of 10^3 F98 rat glioma cells into the cells the brains of Fischer rats. Thirty-five F98 rat glioma models were randomly divided into the following five groups: untreated control group (Untreated), neutron-irradiated control group (Irradiation only), neutron irradiation following 2.5 hours after termination of intravenous administration (i.v.) of BPA (BNCT using BPA 2.5 h), and neutron irradiation following 2.5 or 24 hours after termination of i.v. of MID-AC (BNCT using MID-AC 2.5 h or 24 h). Regarding MID-AC, this neutron irradiation experiment was conducted not only following 2.5 h after termination of i.v. of MID-AC, but also at 24 h, in order to evaluate the effect of the long retention of boron in the tumor. In other words, it was confirmed whether the administration of a boron compound with a long retention time in the tumor would have sufficient BNCT effect even after a long time after administration.

All rats were anesthetized by intraperitoneal injection of a mix of anesthetics described above, and the study groups received BPA or MID-AC. Excluding their heads, all rat bodies were attached on a plate lined with ^6LiF ceramic tiles to shield and reduce neutron irradiation, and then neutron irradiation was performed. F98 rat glioma models were irradiated at a reactor power of 5 MW with the Heavy Water Irradiation Facility for 20 minutes. After neutron irradiation, all rats remained under the same experimental conditions as the control groups, and observations continued until their time of death or euthanasia. Finally, the therapeutic effects of BNCT were evaluated by Kaplan–Meier survival curves, and

the percent increased life span (%ILS) was determined based on the median survival times (MST). %ILS was calculated by the equation $(\text{MST of each BNCT group} - \text{MST of Untreated group}) \times 100 / (\text{MST of Untreated group})$.

Determining the physical dose, estimated photon-equivalent doses, and the compound biological effectiveness based on the in vivo neutron irradiation experiment

The physical dose was calculated from thermal, epithermal, fast neutrons, and gamma rays contained in the irradiated neutrons. This is calculated by the equation $D_B + D_N + D_H + D_\gamma$, which correspond to $^{10}\text{B}(n,\alpha)^7\text{Li}$, $^{14}\text{N}(n,p)^{14}\text{C}$, and $^1\text{H}(n,n)^1\text{H}$ capture reactions and γ -ray, respectively. More specifically, D_B is the physical dose of boron derived from the equation of $7.43 \times 10^{-14} (\text{Gy cm}^2/\mu\text{g } ^{10}\text{B/g}) \times \text{boron concentration } (\mu\text{g } ^{10}\text{B/g}) \times \text{thermal neutron fluence } (1/\text{cm}^2)$. D_N is the physical dose of nitrogen derived from the equation of $6.78 \times 10^{-14} (\text{Gy cm}^2/\text{weight } \%) \times \text{nitrogen concentration } (\text{weight } \%) \times \text{thermal neutron fluence } (1/\text{cm}^2)$. D_H is the elastic scattering between epithermal or fast neutrons and the hydrogen nucleus, and D_γ is the measured value of gamma rays mixed in the neutron beam [20]. Based on the results of the biodistribution experiments for F98 rat glioma models, the physical doses to the brain and the tumor of the F98 rat glioma model were calculated in BNCT using BPA 2.5 h, MID-AC 2.5 h, and MID-AC 24 h. The estimated photon-equivalent dose for BNCT using BPA 2.5 h was calculated by the equation $D_B \times \text{compound biological effectiveness (CBE)} + D_N \times \text{relative biological effectiveness of nitrogen (RBE}_N) + D_H \times \text{relative biological effectiveness of hydrogen (RBE}_H) + D_\gamma$ [20]. CBE is a biological effectiveness factor specific for each irradiated tissue and each boron compound at the neutron capture reaction. Based on the survival time of each group and the estimated photon-equivalent doses in BNCT using BPA 2.5 h, the CBE of MID-AC was calculated by the estimated photon-equivalent doses acquired in BNCT using MID-AC.

Statistical Analysis

The Kaplan–Meier curve evaluated the MST from the implantation date of F98 rat glioma cells, and log-rank tests determined the potentially significant differences among the groups. A p -value less than 0.05 were considered statistically significant. Statistical analyses were performed by the JMP® Pro version 15.1.0. software (SAS, Cary, NC, USA).

Results

Biodistribution of boron compounds in F98 rat glioma models

Boron concentrations in the tumor, brain, and blood at 2.5, 12, and 24 hours after termination of i.v. of MID-AC were 6.1 ± 2.0 (0.4 ± 0.1 in brain and 19.7 ± 9.8 in blood), 8.5 ± 1.5 (0.4 ± 0.1 in brain and 15.3 ± 1.0 in blood), and 6.1 ± 0.2 (0.6 ± 0.4 in brain and 9.9 ± 1.5 in blood) $\mu\text{g B/g}$, respectively. In the case of MID-AC, boron concentrations were gradually decreased in almost organs, but it tended to be retained and slowly metabolized in the tumor, kidney, and spleen. Boron concentrations in the tumor, brain, and blood

after 2.5, 6, 12, and 24 hours after termination of i.v. of BPA were 20.6 ± 2.6 (5.5 ± 0.6 in brain and 7.7 ± 0.5 in blood), 15.0 ± 3.4 (3.7 ± 0.6 in brain and 4.1 ± 0.4 in blood), 9.1 ± 3.3 (2.5 ± 0.6 in brain and 2.9 ± 0.4 in blood), 8.2 ± 0.8 (2.3 ± 0.3 in brain and 2.9 ± 0.4 in blood) μg boron (B)/g, respectively. The boron concentrations in all organs were highest at 2.5 hours after termination of i.v. of BPA and then immediately decreased due to metabolism by the kidney. Table 1 presents a summary of our results, and Fig. 1 demonstrates boron concentration uptake in each organ.

Survival analysis of a neutron irradiation experiment for F98 rat glioma models

Neutron irradiation experiments were conducted after administration of each boron compound to the F98 rat glioma models. The treatment effect was evaluated by using Kaplan–Meier survival curves. Each MST was as follows: Untreated; 24.5 days [95% confidence interval (CI); 23–27 days], Irradiation only; 24.5 days [95% CI; 23–27 days], BNCT using BPA 2.5 h; 31.5 days [95% CI; 28–40 days], BNCT using MID-AC 2.5 h; 33.5 days [95% CI; 23–40 days], and BNCT using MID-AC 24 h; 33.0 days [95% CI; 27–36 days]. Statistically significant differences were observed between Untreated and BNCT using MID-AC groups (vs. BNCT using MID-AC 2.5 h, $p = 0.0019$; vs. BNCT using MID-AC 24 h, $p = 0.0005$, respectively, log-rank test). BPA, used as a capture compound, also showed a significant survival gain (vs. BNCT using BPA 2.5 h, $p < 0.0001$). MSTs did not demonstrate any significant differences in BNCT using MID-AC 2.5 h or 24 h compared to BNCT using BPA 2.5 h (vs. BNCT using MID-AC 2.5 h, $p = 0.94$; vs. BNCT using MID-AC 24 h, $p = 0.67$, respectively, log-rank test). Each %LS value was as follows: BNCT using BPA 2.5 h; 28.6%, BNCT using MID-AC 2.5 h; 36.7%, and BNCT using MID-AC 24 h; 34.7%. Table 2 and Fig. 2 show the detailed results and the Kaplan–Meier survival curves.

Determining the physical dose, estimated photon-equivalent doses, and the CBE based on the in vivo neutron irradiation experiment

RBE_N and RBE_H have been adapted to 3.0 [21]. The CBE for the normal brain tissue from BPA was 1.35 [4], and the CBE for the brain tumor from BPA was 3.8 [4, 22]. There was no statistically significant difference in the respective MSTs in BNCT groups. Therefore, we assumed the estimated photon-equivalent doses of brain tumor were also equivalent between BNCT using BPA 2.5 h and BNCT using MID-AC at 2.5 h or 24 h after the neutron irradiation experiment for F98 rat glioma models. Based on this assumption, the estimated photon-equivalent dose of the brain tumor obtained by BNCT using BPA 2.5 h was 10.8 Gy-Eq. Consequently, CBE of MID-AC was estimated from in vivo survival to be 13.4 at MID-AC 2.5 h and 12.3 at MID-AC 24 h, respectively. Table 3 presents a summary of these results.

Discussion

We investigated a promising albumin conjugate of maleimide-functionalized *closo*-dodecaborate, named MID-AC, as a boron delivery compound to improve the efficacy of BNCT against high-grade gliomas. When assessing boron compounds in clinical and pre-clinical trials, it is essential to consider the biological effects of BNCT, which can be represented by the CBE value specific for each combination of

irradiated tissue and boron compound at the neutron capture reaction. Previous clinical investigations on BNCT for high-grade gliomas determined that the CBE value of BPA was 3.8 [15]. In the *in vivo* neutron irradiation study, BNCT groups obtained statistically significant prolongation compared to Untreated (Table 2). Each BNCT using a MID-AC group demonstrated higher values in both MST and %ILS than BNCT using BPA 2.5 h; however, these differences were not significant (Fig. 2). Therefore, based on the neutron irradiation experiments of F98 rat glioma models, the estimated photon-equivalent doses were approximated to calculate the compound-specific CBE factor of MID-AC for brain tumors. Briefly, the estimated photon-equivalent dose acquired by BNCT using BPA 2.5 h was regarded as equivalent to the doses acquired by BNCT using MID-AC 2.5 h or 24 h. The compound-specific CBE factors were calculated to be 13.4 (MID-AC 2.5 h) and 12.3 (MID-AC 24 h), respectively. Our study identified that the efficient cell-killing effect of MID-AC in BNCT might contribute to the CBE value, which is approximately 3–3.5 times higher compared to BPA. While MID-AC provides an efficient boron neutron capture reaction, the boron concentration delivered to the brain tumor cells is still of low level. Improving MID-AC to provide more boron to brain tumor cells would further enhance the efficacy of BNCT against high-grade gliomas.

Serum albumin, contained in MID-AC, has been frequently used as a drug delivery system [12, 13]. The delivery method of serum albumin to tumor tissues is passive as a result of the enhanced permeability and retention (EPR) effect [23, 24], but also active due to secreted protein acidic and rich in cysteine (SPARC) and gp60 receptors [12]. These receptors are known to be highly expressed in high-grade glioma cells, and treatment with albumin-containing compounds using SPARC, which is a target of active transport [25]. Some albumin-containing compounds have allowed the fluorescent diagnosis of high-grade gliomas [13, 26]. Moreover, the conjugate of boron-dipyrrromethene dyes (BODIPY), which has been used as a fluorescent imaging probe for cancer diagnosis [27], and human serum albumin have proved their efficacy as suitable imaging probes and boron compounds for BNCT [28]. Therefore, applying these visualization techniques may be able to allow clinicians to evaluate the eligibility of BNCT-related compounds in treating cancerous lesions. While exploiting albumin's ability to deliver boron compounds to high-grade gliomas, we have focused on a maleimide with a high binding affinity to albumin. Consequently, we have synthesized maleimide-functionalized *closo*-dodecaborate (MID) as a boron compound and conjugated it to the serum albumin at Cys34 [14]. Since fluorescent-labeled MID-conjugated bovine serum albumin has been also found to accumulate in the cytosol of HeLa cells [29], visualization techniques using MID are bound to be established in the future.

In terms of boron distribution, the boron concentrations of the tumor were almost equal at 2.5 and 24 hours after systemic administration of MID-AC, while those of the tumor were decreased with time after systemic administration of BPA (Table 1 and Fig. 1). This result suggests that MID-AC is transferred from the blood vessel to the tumor tissue, and MID-AC is retained in tumor cells for an extended period. At the same time, the maximum concentration of boron in each organ was approximately 20 $\mu\text{g B/g}$ suggests that toxicity to the body was not high (Fig. 1). As a result, in the case of MID-AC, the long time boron retention in the tumor renders it possible to continuously and steadily acquire boron neutron capture reactions for a long time while neutrons are irradiated. In addition, BPA contains one boron atom, whereas MID-AC contains twelve boron atoms per molecule obtained from dodecaborate. Considering that one

molecule from each compound is taken up by one tumor cell, the probability of a capture reaction is higher for compounds containing more boron atoms per molecule, thus facilitating a higher antitumor effect.

Suitable requirements for BNCT boron compounds are low intrinsic toxicity, high boron accumulation for target lesions, low uptake into normal tissues, and water solubility [17, 30]. In addition, it is also important to consider the method of delivering compounds into the tumor cells because both the blood-brain barrier (BBB) and the blood-brain tumor barrier (BBTB) prevent the uptake of compounds [31–33]. In high-grade gliomas, since the BBB or BBTB is locally disrupted at the core of the tumor bulk, chemical compounds containing boron could be transported to the tumor cells. However, glioma cells tend to infiltrate into the normal brain parenchyma where the BBB is still intact, and thus delivery of these compounds can be very challenging. Apart from these requirements and provided that boron compounds can remain in the tumor for a substantial period of time, irradiation times can be easily adjusted, thus expanding the respective therapeutic range. As a result, novel boron compounds must meet as many of these requirements as possible. Only two compounds, BPA and sodium borocaptate (BSH), are currently used in clinical BNCT [30, 34]. For a long time now, a wide variety of boron delivery compounds, amino acids, liposomes, porphyrins, and serum albumin conjugates, etc., have been developed and studied as they could be more effective in BNCT. But still, the most commonly used boron compound is BPA, and it is the only compound that has been approved by the pharmaceutical authorities in Japan [10, 11].

BPA almost meets the requirements above however BPA-resistant brain tumor cells sometimes exist. Therefore, the development of tumor-targeting boron compounds generated by different approaches should address and treat BPA-resistant brain tumor cells in biologically targeted therapy, BNCT. In BNCT for high-grade gliomas, BSH utilizes BBB breakdown and is not cell-selective [30]. Although many boron compounds were developed and studied, only a few of them can be as effective as or even more effective than BPA when administered intravenously in the BNCT animal model of high-grade glioma [17]. MID-AC targets tumors with a different mechanism than BPA and has a very attractive and promising compound delivery capacity in BNCT of high-grade gliomas, showing comparable results to BPA even by intravenous administration. In addition, since the efficacy of BNCT was similar at 2.5 hours and 24 hours after MID-AC administration, variable neutron irradiation times can be available without the necessity of continuously administering boron compounds. Since MID-AC is deemed to be very safe, it is expected to be useful in clinical practice when establishing protocols that involve larger doses, longer doses, and repeated administration. Such modification approaches are difficult to achieve with a compound that has a very rapid clearance rate, although this may also be advantageous for BPA. MID-AC properties such as long retention in tumor cells and high tumor cell selectivity can enable treatment of deep-seated tumors that require long irradiation times or multi-directional irradiation.

Conclusion

MID-AC, a dodecaborated albumin conjugate with maleimide, can efficiently induce boron neutron capture reactions into the tumor cell nucleus and, thus, be effective against brain tumor cells. The long

time retention of MID-AC in tumor cells renders it possible to continuously acquire stable boron neutron capture reactions for a long time while neutrons are irradiated. Therefore, MID-AC has a considerable potential to become an effective delivery system for BNCT in treating high-grade gliomas.

Declarations

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Conflicts of interest/Competing interests

All authors declare that they have no conflicts of interest.

Availability of data and material

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Code availability

The JMP® Pro version 15.1.0. software (SAS, Cary, NC, USA) was used for statistical analysis.

Authors' Contributions

H.K., H.N., M.S., and S.K. made substantial contributions to the study conception and design. H.K. K.Y., Y.F., T.K., K.T., R.H., and S.K established this study's methodology. K.N., K.K., and H.N. conceived the concept of manufacturing MID and established the manufacturing process. H.K. and H.N. conducted formal analysis, investigation, and data curation. H.K. and S.K. wrote the original draft preparation. T.T., H.T., T.W., and M.S. wrote, reviewed, and edited the manuscript and supervised the neutron irradiation experiments. S.I.M. and M.W. supervised this project. S.K. and T.K. acquired funding. All authors read and approved the submitted version.

Ethics approval

All animal experiments complied with the guide for the care and use of laboratory animals approved by the Animal Use Review Board and Ethical Committee of Osaka Medical and Pharmaceutical University (License No. 2019-029) and the Institute for Integrated Radiation and Nuclear Science, Kyoto University (KURNS; Kumatori, Osaka, Japan) (License No. 2019-9).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Tables

Table 1

Summary of the boron concentrations after intravenous administration of each boron compound on the F98 rat glioma model.

Boron compound ^a	Time (h) ^b	n ^c	Boron concentrations \pm SD ($\mu\text{g B/g}$) ^d			Ratio	
			Tumor	Brain	Blood	T/Br ^e	T/BI ^f
MID-AC	2.5	5	6.1 \pm 2.0	0.4 \pm 0.1	19.7 \pm 9.8	17.6	0.3
	12	4	8.5 \pm 1.5	0.4 \pm 0.1	15.3 \pm 1.0	19.7	0.6
	24	3	6.1 \pm 0.2	0.6 \pm 0.4	9.9 \pm 1.5	10.4	0.6
BPA	2.5	4	20.6 \pm 2.2	5.5 \pm 0.6	7.7 \pm 0.5	3.8	2.7
	6	4	15.0 \pm 3.4	3.7 \pm 0.6	4.1 \pm 0.4	4.1	3.7
	12	4	9.1 \pm 3.3	2.5 \pm 0.6	2.9 \pm 0.4	3.7	3.2
	24	4	8.2 \pm 0.8	2.3 \pm 0.3	2.9 \pm 0.4	3.6	2.8
^a Each boron compound used was administered at the following body weight (b.w.) doses; 20 mg B/kg b.w. for maleimide-functionalized closo-dodecaborate albumin conjugate (MID-AC), 12 mg boron (B)/kg b.w. for boronophenylalanine (BPA), respectively.							
^b Time indicates the period from the time of intravenous administration of each boron compound to the time of euthanasia of F98 rat glioma models.							
^c n indicates Number of Fischer rats.							
^d Boron concentration \pm SD ($\mu\text{g B/g}$) is the value measured by ICP-AES for the acquired boron concentration of each organ listed in the table, and is expressed as the mean boron values ($\mu\text{g B / gram: weight of organ}$) \pm standard deviation.							
^e T/Br indicates the tumor to brain ratio.							
^f T/BI indicates the tumor to blood ratio.							

Table 2

Survival times of the F98 rat glioma model after the neutron irradiation experiment.

Group	n ^a	Survival times (days)			%ILS ^c	p-value ^d
		Mean ± SD	Median	95% CI ^b		
Untreated	6	24.8 ± 1.3	24.5	23–27	-	-
Irradiation only	6	24.7 ± 1.3	24.5	23–27	-	0.90
BNCT using BPA 2.5 h	8	33.1 ± 4.8	31.5	28–40	28.6	< 0.0001
BNCT using MID-AC 2.5 h	8	33.0 ± 6.2	33.5	23–40	36.7	0.0019
BNCT using MID-AC 24 h	7	33.0 ± 3.3	33.0	27–36	34.7	0.0005
^a n indicates Number of Fischer rats.						
^b CI is confidence interval.						
^c The percent increased life span (%ILS) was defined relative to the median survival times (MST) of Untreated. %ILS was calculated by the equation (MST of each BNCT group - MST of Untreated group) × 100 / (MST of Untreated group).						
^d p-values were calculated using the log-rank test compared to Untreated based on the results obtained from the Kaplan-Meier curves in the neutron irradiation experiment for F98 rat glioma model.						

Table 3

Summary of the physical dose and the estimated photon-equivalent dose for the brain or the tumor in the F98 rat glioma model.

Group	Physical dose ^a (Gy)		Photon-equivalent dose ^b (Gy-Eq)	
	Brain	Tumor	Brain	Tumor
Untreated	0.0	0.0	0.0	0.0
Irradiation only	0.8	0.8	1.3	1.3
BNCT using BPA 2.5 h	1.6	3.3	2.5	10.8
BNCT using MID-AC 2.5 h	0.9	1.6	-	-
BNCT using MID-AC 24 h	1.0	1.7	-	-
<p>^a The physical dose is attributed to the $^{10}\text{B} (n,\alpha) ^7\text{Li}$, $^{14}\text{N} (n,p) ^{14}\text{C}$, and $^1\text{H} (n,n) ^1\text{H}$ reactions produced by the thermal, epithermal, and fast neutron fluxes, and gamma rays in the irradiated neutrons. It is calculated by using following equation: Physical radiation dose (Gy) = $D_B + D_N + D_H + D_\gamma$. D_B is boron dose. D_N: is neutron dose. D_H is hydrogen dose. D_γ is gamma-ray dose.</p>				
<p>^b The estimated photon-equivalent dose is calculated by using following equation: $D_B \times \text{compound biological effectiveness (CBE)} + D_N \times \text{relative biological effect of nitrogen (RBE}_N) + D_H \times \text{relative biological effect of hydrogen (RBE}_H) + D_\gamma$. RBE_N and RBE_H are 3.0. In the case of BPA, the CBE for the normal brain tissue is 1.35 and the CBE for the brain tumor is 3.8 in BPA.</p>				

Figures

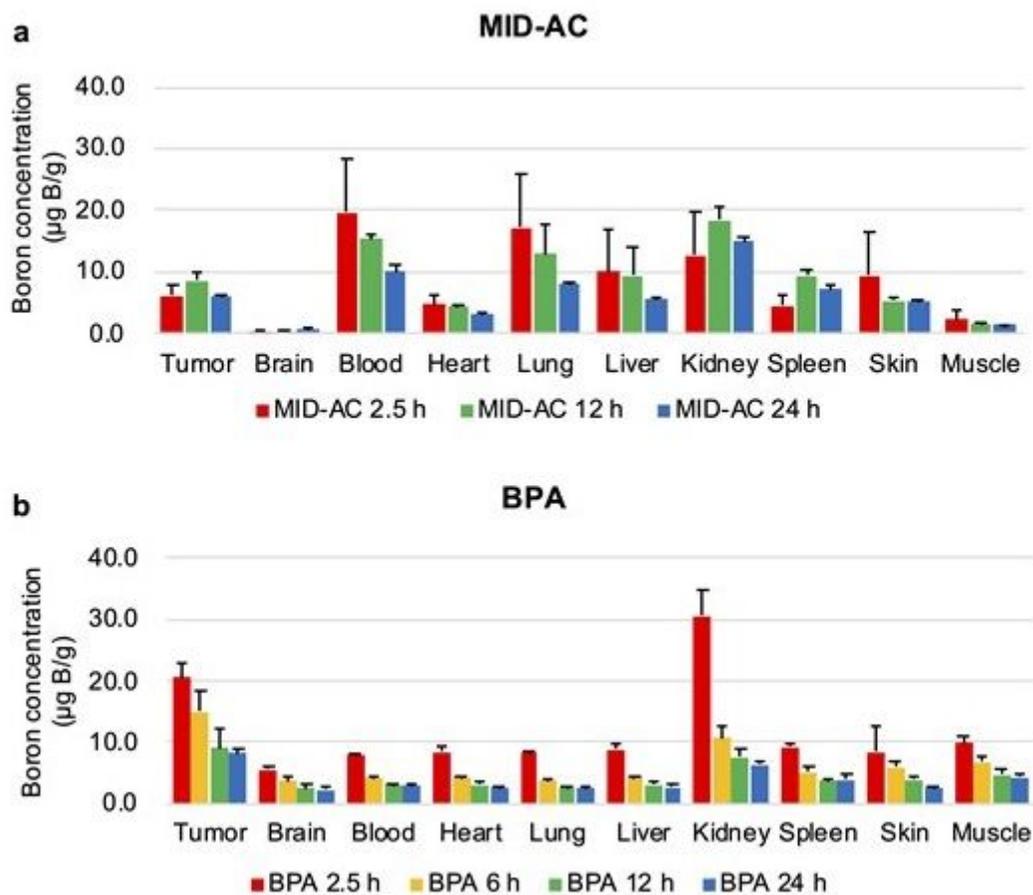


Figure 1

Transition of boron concentrations in each organ in the F98 glioma model after each boron compound administration. Each boron compound was administered to a Fischer rat bearing intracranial implants of 105 F98 rat glioma cells at the following body weight (b.w.) doses. (a) 20 mg boron (B)/kg b.w. for maleimide-functionalized closo-dodecaborate albumin conjugate (MID-AC). (b) 12 mg B/kg b.w. for boronophenylalanine (BPA). At some predetermined times (2.5, 12, and 24 hours for MID-AC and 2.5, 6, 12, and 24 hours for BPA), the animals were euthanized and each organ was removed. The concentration of boron in each organ was determined by ICP-AES. The mean concentration of boron values in each organ ($\mu\text{g B/gram}$) and standard deviation are shown for each group of 3-5 rats.

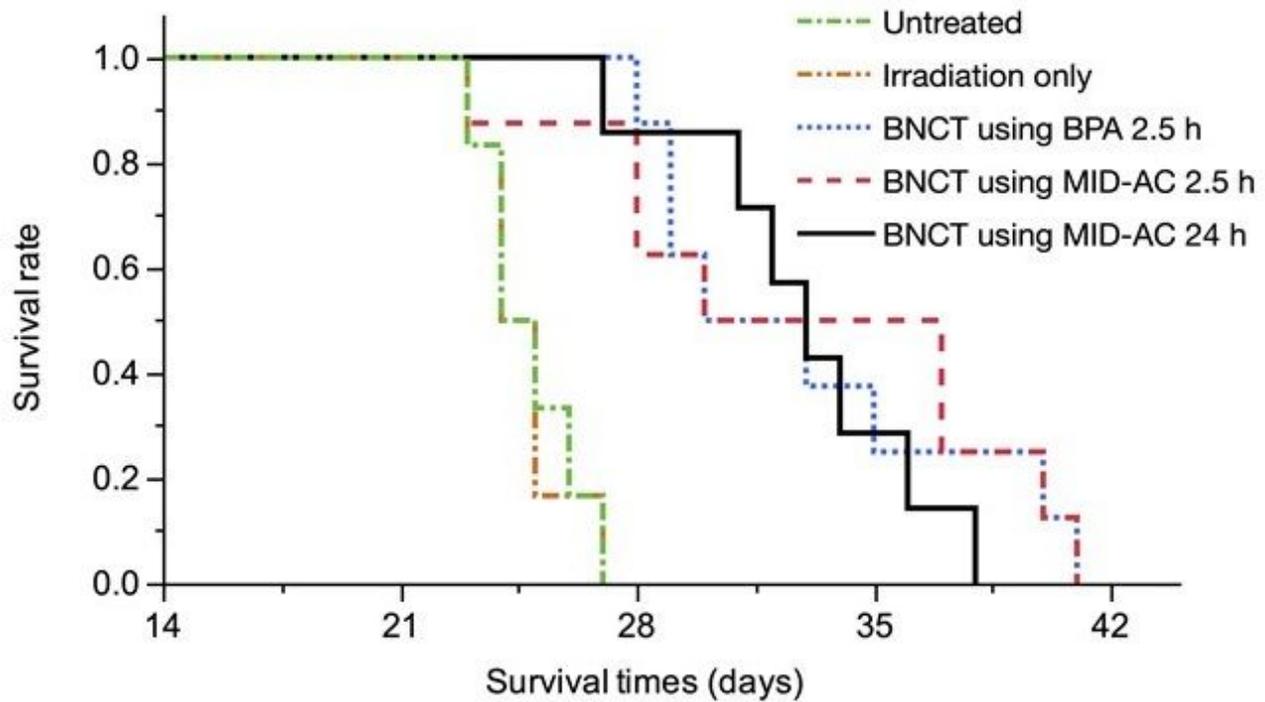


Figure 2

Kaplan-Meier survival curves for F98 rat glioma models after the neutron irradiation experiment. Survival times in days after implantation of F98 rat glioma cells have been plotted for following 5 groups; Untreated, Irradiation only, BNCT using boronophenylalanine (BPA) 2.5 h, BNCT using maleimide-functionalized closo-dodecaborate albumin conjugate (MID-AC) 2.5 h, and BNCT using MID-AC 24 h. The results showed that there were statistically significant differences between the 3 neutron irradiation groups following boron compounds administration and Untreated (vs. BNCT using BPA 2.5 h, $p < 0.0001$; vs. BNCT using MID-AC 2.5h, $p = 0.0019$; vs. BNCT using MID-AC 24 h, $p = 0.0005$, respectively, log-rank test). There was no significant difference between BNCT using MID-AC 2.5 h or 24 h and BNCT using BPA (vs. BNCT using MID-AC 2.5h, $p = 0.94$; vs. BNCT using MID-AC 24h, $p = 0.67$, respectively, log-rank test).