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Laboratory Investigation

Pharmacokinetic study of BSH and BPA in simultaneous use for BNCT

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Summary

In order to improve the effectiveness of boron neutron capture therapy (BNCT) for malignant gliomas, we examined the optimization of the administration of boron compounds in brain tumor animal model. We analyzed the concentration of boron atoms in intracranial C6 glioma -bearing rats using inductively coupled plasma atomic emission spectrometry. Each tumor-bearing rat received one of two different amounts of sodium borocaptate (BSH) and/or 500 mg/kg of boronophenylalanine (BPA) via intraperitoneal injection. We compared the boron concentrations of the tumor, the contralateral normal brain and the blood in rats of 3 different treatment groups (BSH alone, BPA alone and a combination of both BSH and BPA). Our results show that the tumor boron concentration increased much more than 30 μg/g by the coadministration of both compounds. Additionally, the blood boron concentration remained below 30 μg/g and the boron concentration in the normal brain was low (mean 4.7 ± 1.1 μg/g). Even in comparison with the administration of BPA alone, coadministration of BPA and BSH shows an improved tumor/normal brain ratio of boron concentrations.

Introduction

Boron neutron capture therapy (BNCT) is a targeted radiation approach that has theoretical superiority to conventional radio-therapeutic modalities because of the selective irradiation to tumor cells. BNCT is a binary approach: a boron compound delivers high concentrations of ^{10}B to the target tumor, relative to the surrounding normal tissues. This is followed by irradiation of thermal or epithermal neutrons that are thermalized with depth in the tissues. The short range (less than 10 micrometers) of the alpha and ^{7}Li particles released from the ^{10}B(n,alpha) ^{7}Li reaction makes the microdistribution of ^{10}B critically important in this therapy [1]. These particles are high linear energy transfer radiation. These particles have a potential to give specific and strong cytotoxic activity to tumor cells. Therefore, if sufficient quantities of boron compounds can accumulate selectively in tumor tissues, BNCT becomes an ideal type of radiotherapy.

The estimated boron concentration for effective therapy must be higher than 20–35 μg ^{10}B per g of tumor tissue [2–4]. In addition, the boron concentration in the surrounding normal tissue should be kept low to minimize damage to the normal tissue, including the vessels. High amounts of boron in the blood induce vascular radiation damage as a cause of brain necrosis; it is therefore important to maintain the blood boron concentration below 30 μg/g [5]. To fulfill these conditions, various approaches have been employed to target boron compounds to tumors, including the use of macromolecules such as monoclonal antibodies, epidermal growth factor and dextran conjugates, and the use of microparticles such as liposomes, low density lipoprotein complexes and microcapsules [6–8]. To date, however, these attempts have never achieve practical clinical use.

Clinically, two boron compounds are available, boronophenylalanine (BPA) and sodium borocaptate (BSH). It is widely accepted that BPA is an analog of an essential amino acid and is actively taken up by tumor cells [2,9]. BPA thus accumulates greatly in the tumor but also accumulates measurably in the normal brain. BSH, on the other hand, is generally thought to penetrate tumor tissue through the destroyed blood brain barrier. That is, BSH accumulates little in the normal brain but accumulates insufficiently in the tumor [10].

We hypothesize that the simultaneous use of these compounds increases the absolute boron concentration and improves the ratio of the boron concentration in the tumor and that in the normal brain (T/N ratio), in comparison with the administration of either compound alone. Clinically, we applied neutron irradiation 12 h after the accomplishment of BSH administration, because we should avoid the irradiation when the blood boron concentration is still high, and this is the timing that the boron concentrations in tumor and blood is almost the same. To simulate clinical application, in this study, we analyzed at first the kinetics of boron concentration in the blood after BSH administration in the following animal model. In the present study, we measured the boron concentrations in the main tumors,

normal brains and blood of C6 glioma-bearing rats divided into three treatment groups (BSH alone, BPA alone and a combination of both BSH and BPA) using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Materials and Methods

C6-rat brain tumor models

The C6 rat glioma cell line (American Type Culture Collection, Rockville, MD, USA) was used for the present rat brain tumor model. Transplanted C6 cells grow rapidly in the rat brain. The histology of this rat glioma is similar to that of human malignant gliomas.

The C6 cells were routinely cultivated in our laboratory in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and penicillin at 37 °C in an atmosphere of 5% CO_2. All the materials for the culture medium were purchased from Gibco Invitrogen Corporation (Carlsbad, CA, USA). Cells were used for transplantation when the monolayer reached subconfluence. Each male Wistar rat (250–300 mg) was anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and placed in a stereotactic frame (Model 900, David Kopf Instruments, Tujunga, CA, USA). A midline scalp incision was made, and the bregma was identified. A 1-mm burr hole was made in the right frontal region of the skull, centered 0.5 mm anterior and 3.0 mm lateral to the bregma. A 26-gauge needle attached to a 100-μl syringe was inserted into the striatum using the above stereotactic coordinates, with the needle tip inserted 4.5 mm into the dura. The needle was left in place for 5 min before starting the infusion. An injection of 10^6 C6-glioma cells in 10 μl of phosphate-buffered saline was administered at a rate of 1 μl/minute. Upon completion of the infusion, the needle was left in place for 5 min before being withdrawn at a rate of 1 mm/min. After withdrawal of the needle, the burr hole was covered with bone wax.

Administration of BPA and BSH

BPA (99 atoms% ^{10}B, L-isomer) was kindly supplied by the Stella Chemifa Corporation (Osaka, Japan); BSH (99 atoms% ^{10}B) was purchased from Katchem, Ltd.(Czechoslovakia). Rats received the above ^{10}B-compounds intraperitoneally at 14 days after tumor transplantation. For the kinetics study, a fixed dose of BSH (100 mg/kg) was administered to the tumor-bearing rats 2.5, 4, 6, 8, 10 and 12 h before sacrifice, and the boron concentrations in the tumor tissue and blood were measured by ICP-AES, as described below.

Next, boron concentrations in the tumor tissue and blood were measured under different doses (25, 50, 100 and 300 mg/kg) of BSH. Six hours after the ^{10}B-compounds were administered intraperitoneally, tumors and blood were sampled and their boron concentrations were measured by ICP-AES.

In order to analyze the effects of the coadministration of BSH and BPA, we prepared three experimental groups. Rats were administered 500 mg/kg of BPA alone, 100 mg/kg of BSH alone, or 500 mg/kg of BPA and 100 mg/kg of BSH simultaneously. The timing of the administration was 2.5 h and 6 h prior to sampling for BPA and BSH, respectively.

Determination of boron concentration by ICP-AES

For ICP-AES analysis, all samples (0.05–0.5 g wet weight) were directly digested with 5 ml of nitric acid solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan at room temperature overnight. The resulting solutions were diluted with distilled water to 10 ml, and 0.2 ml of the filtrated samples was introduced with a carrier flow of distilled water into the ICP-AES instrument (P-5200, Hitachi, Japan). The emission intensity was monitored at 249.678 nm, the boron analysis line. Boron standard solutions were prepared with distilled water by diluting a 1000-μg/ml stock standard solution (Wako). Standard curves were linear over the range of 0.1–20 μg/ml and we prepared a calibration line during each day of operation. The limit of quantification for the rat brain was 0.5 μg/g of boron (the relative SD below 20%) [11].

Statistical analysis

Values are presented as mean ± SD. Statistical analysis was performed by the Statview software using Mann–Whitney analysis. A significance level of $P < 0.05$ was used for all analyses.

Results

Figure 1 shows the quantitative kinetic analysis of the boron concentration of the tumor and blood after intraperitoneal administration of BSH (100 mg/kg) in

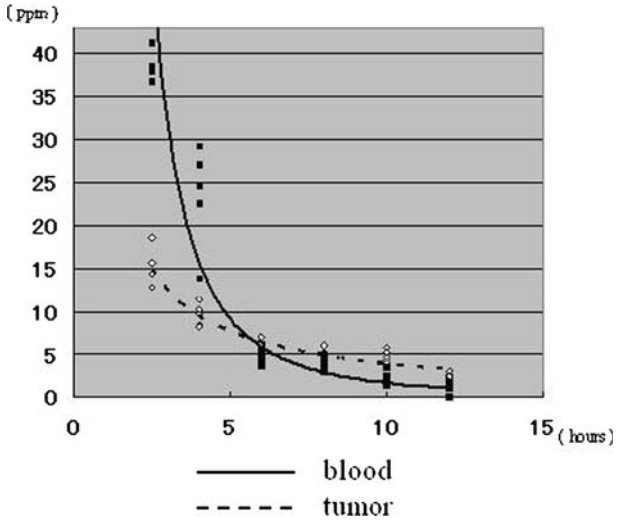

Figure 1. Quantitative boron concentration after intraperitoneal administration of BSH (100 mg/kg) in rat brain tumor models. Six hours after BSH administration, the boron concentrations in the tumor and blood were equal.

rat brain tumor models. The dosage of BSH used in the present experiment is that same as that used in clinical BNCT for malignant gliomas in humans [12–14]. Six hours after BSH administration, the boron concentration in the tumor and blood were almost equal ($n \geq 6$; minimum number of experiments was 6), and the boron concentration in the blood was 4.4 ± 2.1 μg/g, a value was far below the putative critical level of 30 μg/g [5]. In Figure 2, we present our data on the boron concentration of the tumor, blood and normal brain of the contralateral side as well as the tumor/blood (T/B) ratio of boron concentration and the T/N ratio by intraperitoneal administration of BSH alone. These data were obtained at 2.5 and 6 h after the administration of 100 mg/kg BSH. The boron concentration of the tumor tissue obtained 2.5 h after BSH administration was higher than that obtained at 6 h after administration (13.9 ± 2.5 μg/g vs. 5.4 ± 0.9 μg/g). The higher the boron concentration in tumor tissue, the stronger the anti-tumor effect by neutron irradiation. Nevertheless, the boron concentration in blood obtained 2.5 h after BSH administration was 36 ± 4.1 μg/g, which is considered to be risky for vessels in the radiation field, while that obtained at 6 h after administration was 4.4 ± 2.1 μg/g, which is believed to be safe. At both time points, the boron concentrations of the contralateral brain (normal brain) were very low. Additionally, we obtained relatively high T/N and T/B ratios at 6 h after BSH administration compared with those at 2.5 h (T/B: 1.47 ± 0.6 vs. 0.39 ± 0.06, $P < 0.01$;T/N: 81.7 ± 69.8 vs. 18.3 ± 12.0, $P < 0.01$).

If the boron concentration in the blood and normal brain is within the safety limit (held to be less than 30 μg/g), a high boron concentration in the tumor tissue is ideal for efficient BNCT. Therefore, we analyzed the boron concentration in tumor tissue and in blood with dose escalation of BSH administration. We fixed the timing of BSH administration at 6 h prior to sampling. Figure 3a, b show the boron concentration of tumor tissue and blood, respectively. There was no significant difference in the both of tumor tissue and blood boron concentration at doses of 25, 50 and 100 mg/kg of BSH alone. Note however, that when BSH was administered at a dose of 300 mg/kg, the blood boron concentration increased abruptly to 32.1 ± 3.8 μg/g, which is deemed to be risky for vessels. With BSH administration at 300 mg/kg, the boron concentration in tumor tissue was highest (17.3 ± 2.2 μg/g), however it is still lower than the putative minimum effective boron concentration (more than 20–35 μg/g) and as stated above, even at this dose, the boron concentration in the blood was much higher than the putative safety limit.

Finally, we analyzed the effectiveness of the simultaneous use of both BSH and BPA. Figure 4a, b and c show the boron concentration in tumor tissue, in the normal brain and in blood, respectively, with the administration of 100 mg/kg BSH alone at 6 h prior to sampling, 250 mg/kg BPA alone at 2.5 h prior to sampling, and the simultaneous administration of both compounds. Tumor boron uptake was found to be greatly increased by the simultaneous administration of BPA and BSH compared to that of the administration of each boron compound alone (30.8 ± 7.3 μg/g vs. 19.7 ± 5.2 μg/g (BPA) and 5.4 ± 0.9 μg/g (BSH)). Additionally, the blood boron concentration in the group which received both reagents was 19.2 ± 3.1 μg/g, which is within putative safety limits for blood boron concentration.

Figure 4d shows that the T/N ratio of the coadministration group increased significantly compared to that of the BPA-only group (6.7 ± 1.5 vs. 4.4 ± 0.7, $P < 0.01$).

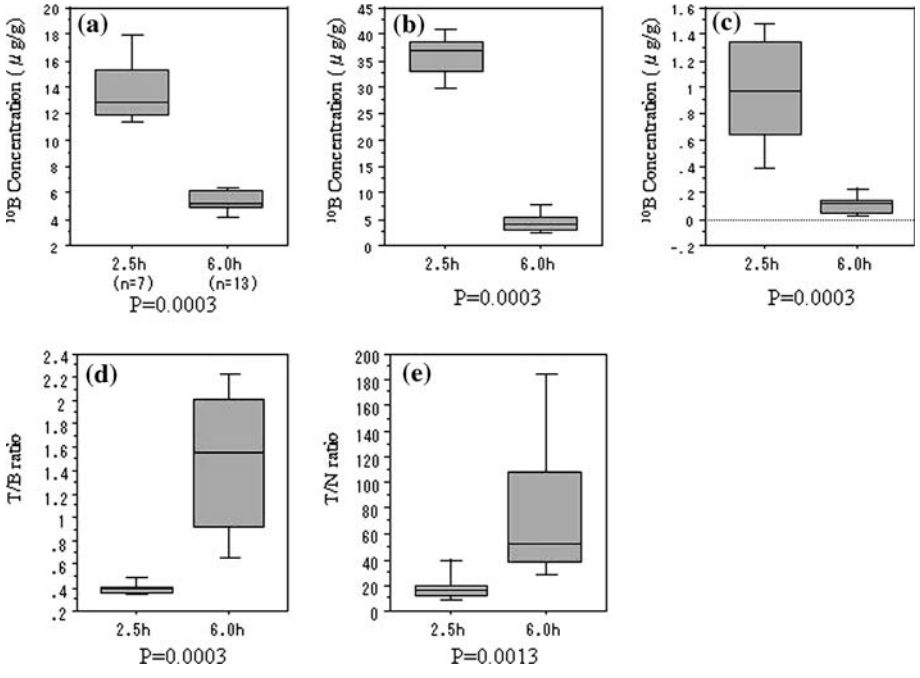

Figure 2. Quantitative boron concentration and T/B and T/N ratios after intraperitoneal administration of BSH alone. The data shown are those at 2.5 h and 6 h after BSH injection. Frames a, b and c show the boron concentrations of the tumor, blood and normal brain, respectively; frames d and e are the T/B and T/N ratios.

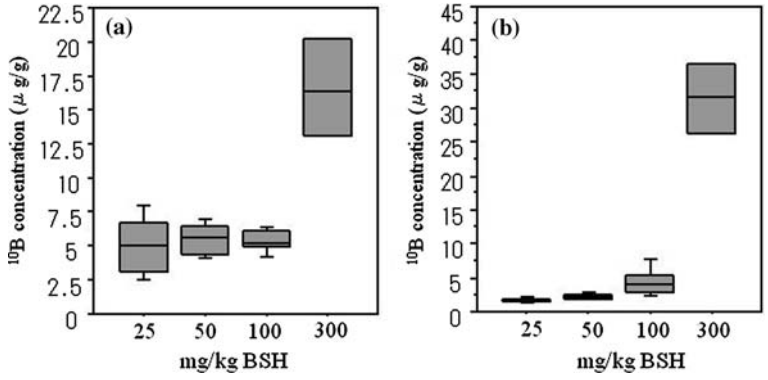

Figure 3. Quantitative boron concentration of the rat brain tumor and blood six hours after different doses of BSH alone. Frame a shows boron concentrations of the tumor tissue. Frame b shows boron concentrations of the blood. Differences in boron concentrations of tumor tissue between the doses of 25, 50 and 100 mg/kg of BSH are not significantly different, however, the boron concentration at a dose of 300 mg/kg BSH is much higher than that of the other groups. The blood boron concentration increases little by little with dose from 25 to 100 mg/kg of BSH. However, the concentration at a dose of 300 mg/kg of BSH is extremely high compared to that of the other groups.

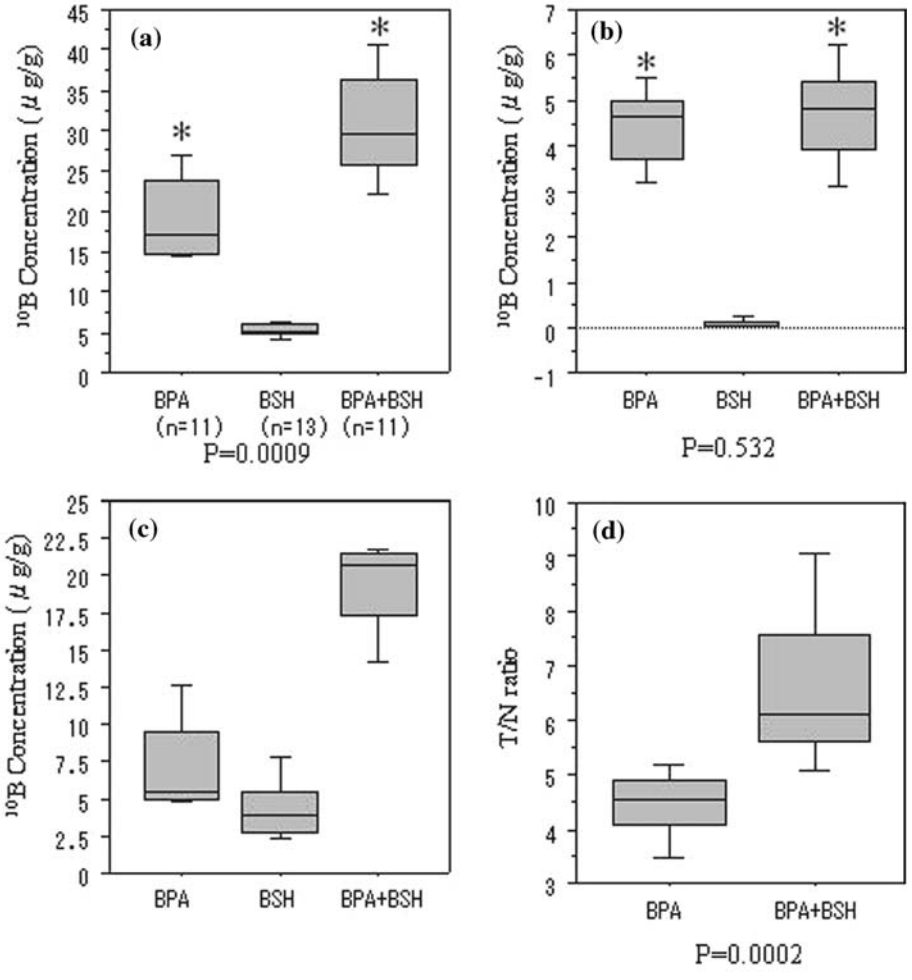

Figure 4. Quantitative analysis of boron concentrations in C6 glioma-bearing rats. The tumor tissue, brain tissue and blood of rats were sampled 2.5 h after the administration of BPA (500 mg/kg) and 6.0 hours after the administration of BSH (100 mg/kg). Frames a, b and c show the boron concentrations of tumor tissue, normal (contralateral) brain tissue and blood, respectively; frame d shows the T/N ratio.

Discussion

Theoretically, the higher the absolute boron concentration in the tumor and the higher the T/N ratio, the more effective and the safer the clinical results that can be expected from BNCT. Despite a long clinical history of BNCT using BSH and BPA throughout the world, published data on the precise boron atom distribution are limited. Furthermore, no report has yet been published on boron concentrations in rat brain tumor

models with the coadministration of BPA and BSH at the optimal timing when the blood boron concentration decrease sufficiently within the safety range.

ICP-AES data indicate that BSH accumulates very little in the normal brain, which explains the higher T/N ratio obtained with the administration of BSH alone compared to the administration of either BPA alone or of both BSH and BPA. Needless to say, a high T/N ratio provides an advantage for BNCT. From the point of view of the T/N ratio, the administration of BSH alone is the best method, but only if it is possible to achieve a sufficient boron concentration in the tumor tissue while maintaining a low and safe blood boron concentration.

In the present study, we were unable to achieve an effective concentration of boron in the tumor tissue at BSH doses of 25–100 mg/kg. Even at a dose of 300 mg/kg BSH, the boron concentration in the tumor tissue remained insufficient, while the concentration in the blood was too high and risky. At this high dosage, it was found that when the blood boron concentration decreased to a safe level, boron concentration in the tumor tissue also decreased (data not shown). We thus conclude that the administration of BSH alone can not produce effective conditions for BNCT in the present experimental model.

BPA, on the other hand, was found to accumulate well in the tissues at a dose of 500 mg/kg (sampling at 2.5 h after administration). When we increased the dose of BPA, the boron concentration in the tumor tissue increased as well while maintaining a safe level of concentration in the blood, however, the T/N ratio remained constant even with dose escalation of BPA (data not shown). This is consistent with the data presented by Joel DD et al. [15]. We therefore investigated the coadministration of BPA and BSH for the purpose of obtaining a higher T/N ratio.

The data presented in Figure 4 show that the coadministration of BSH and BPA achieved a sufficient boron concentration in the tumor tissue while maintaining a safe level of boron concentration in the normal brain and blood. Furthermore, coadministration showed a higher T/N ratio than the administration of BPA alone. Interestingly, the coadministration of BSH and BPA provided a relatively higher boron concentration both in tumor tissue and in blood than expected, that is, the boron concentration obtained by coadministration produced higher levels than the mere summation of each compound. These results are consistent with the observations reported by Barth [16], however, the mechanism of this phenomenon remains unclear and it has not yet been determined whether these results occur only in rodent experimental models or whether they apply to human models as well.

In the present study, we analyzed only boron concentration, as determined by ICP-AES. There are many issues that remain to be resolved before our results may appropriately be applied to human BNCT trials. First, the compound biological effect (CBE) factors of BSH and BPA are different; we assume that the CBE factors of BPA and BSH for tumors are 3.8 and 2.5, respectively [12]. Second, we did not analyze boron distribution at the cellular or subcellular level. Some investigators have reported that BPA accumulation in experimental tumor models is inhomogeneous by secondary ion mass spectroscopy (SIMS) [17–19], which has been observed by ourselves. Additionally, our original SIMS analysis revealed that BSH is distributed more diffusely in the tumor mass in a rodent brain tumor model (manuscript in preparation). BPA accumulates preferentially in the growth-phase cells G2/M phase than in the quiescent cells of the tumor and accumulates especially in the G2/M phase than in the G0/G1 phase of the tumor cells as previously described [20–22]; this may be the reason for the inhomogeneous distribution of boron atoms under the administration of BPA alone. The heterogeneity of boron uptake in tumor cells under BPA administration may be overcome by the coadministration of both compounds simultaneously. Actually, an improved rate of tumor cure is achieved by the simultaneous combination of BPA and BSH in BNCT for squamous cell carcinoma model in mice [20].

In clinical studies, we have treated malignant brain tumors with BNCT using BSH and BPA simultaneously with favorable results in radiographic response [12]. Longer observation periods and greater numbers of subjects are necessary to assess the overall survival rates for the clinical use of both compounds simultaneously.

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