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Abstract

To determine the influence of buthionine sulfoximine (BSO) on boron biodistribution after sulfhydryl borane (BSH) administration for boron neutron capture therapy, the effectiveness of the combination of BSO with sulfhydril- (BSH) and non-sulfhydril ($B_{12}H_{12}$, BNH_3) boron compounds, and the interval between BSO and BSH administration, the retention of boron in tissues have been evaluated using a 9L rat tumor model. Simultaneous administration of BSH and BSO showed significantly higher boron accumulation compared to that without BSO, however there was no difference in tissue boron level between $B_{12}H_{12}$ and BNH_3 administration with BSO or without BSO. The longer interval (6 h) between BSH and BSO administration related to the highest boron concentration in the brain and subcutaneous tumors compared to shorter intervals (0.5, 3 h). Boron concentration in subcutaneous and brain tumor was maintained 6 and 12 h after the administration of BSH following BSO pretreatment.

Keywords: sulfhydryl borane, buthionine sulfoximine, neutron capture therapy.

1. Introduction

The success of boron neutron capture therapy (BNCT) highly depends on the selective and sufficient accumulation of ^{10}B atoms in tumor cells to achieve at least 10 - 30 μg / g of ^{10}B in tumor tissue and high tumor/brain and tumor/blood ratios of boron concentration. There are only two boron delivery agents available for clinical BNCT trials for malignant glioma: ^{10}B-enriched boronophenyl alanine (BPA) and sulfhydryl borane (BSH). Of these, BSH passively distributes through the disrupted blood-brain barrier (BBB) from blood to tumor tissues. The boron concentration in the normal brain with an intact BBB remains minimal, whereas the tumor ^{10}B concentration is related to both the tumor vessel density and the serum ^{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 (Yamamoto et al, 2013).

To determine the influence of BSO on boron biodistribution after BSH administration, the differences between the combination of BSO with sulfhydril- (BSH) and non-sulfhydril ($B_{12}H_{12}$, BNH_3) boron compounds, the interval between BSO and BSH administration, and the retention of boron in the normal brain, brain tumor, subcutaneous tumor and other normal organs have been evaluated.

2. Materials and methods

Reagents used or vended were BSH (Katchem, Prague, Czech Republic), buthionine sulfpximine (BSO), pentobarbital sodium (Kyoritsu Seiyaku, Japan) and tribromoethanol (Sigma-Aldrich, MO, USA), isoflurane (Escain, Mylan, Osaka, Japan), and standard boron solution (Wako Pure Chemicals, Tokyo, Japan). The boron compounds without sulfhydryl group, $B_{12}H_{12}$ and $B_{12}H_{11}NH_2$-Na_2 (BNH_3) were kindly provided by Prof. Nakamura from Gakushuin University, Tokyo, Japan.

All protocols of animal experiments were pre-approved by the Animal Experiment Ethic Committee of the University of Tsukuba and conducted following the ‘‘Regulations for Animal Experiments”. The preparation of 9L glioma cells for the subcutaneous tumor and the brain tumor model was described in our previous work (Yoshida et al., 2008). Briefly, rat gliosarcoma cell line (9 L) was maintained in Earl’s minimal essential medium (MEM, Sigma-Aldrich, MO, USA) supplemented with 10% fetal bovine serum (JRH Nitirei Bioscience, Japan) and 1% penicillin-streptomycin (Sigma-Aldrich, MO, USA). To determine the tissue boron concentration, 7-week-old

male Fisher (F344) rats, weighing ca. 150 g, were used. 2×10^6 of 9L cells were subcutaneously inoculated in the right leg, and $1x10^5$ of the cells were simultaneously injected in the rat brain. When the flank tumor grew up to 10 mm in diameter, BSO (5 mmol/kg in phosphate buffer saline i.p.) and boron compound containing sulfhydryl group (BSH) or boron compounds without sulfhydryl group ($B_{12}H_{12}$ or BNH_3) in PBS at a dose of 100 mg/kg each were administered via tail vein.

To compare the efficacy of boron compounds with/without the sulfhydryl group, the animals were sacrificed 3 h after the administration of boron reagents (n= 5). In other group, BSH was injected 0, 0.5, 3, and 6 h after the administration of BSO, and the animals were sacrificed at 3, 6 and 12 hours after the administration of boron reagents (n= 5). Blood, liver, kidney, muscle, subcutaneous tumor, brain and brain tumor samples were obtained and analyzed for boron concentration using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (ICPS-8100, Shimadzu, Tokyo, Japan). The data were statistically analyzed by the paired t-test using JMP statistical software (ver.10, SAS, Marlow, UK) and difference in comparison was regarded as significant with $p < 0.05$.

3. Results

Simultaneous administration of BSH and BSO showed significantly higher boron concentration compared to that without BSO. There was no difference in tissue boron levels between BSO-pretreatment (BSO+) and no-BSO (BSO –) groups after $B_{12}H_{12}$ or BNH_3 administration (Table 1).

The longer interval (6 h) between BSH and BSO administration related to the highest boron concentration in the brain and subcutaneous tumor, compared to the shorter intervals (0.5, 3 h) (Fig.1). The boron concentration in subcutaneous tumor with the 6 h interval between BSO and BSH injection was significantly higher compared to that after 0.5 and 0 h intervals ($p = 0.007$ and 0.007, respectively). Moreover, the boron concentration in the brain tumor with the 6 h interval was significantly higher compared to that with 0, 0.5, and 3 h intervals ($p= 0.003$, 0.0045, and 0.0356, respectively).

The boron concentration in blood, kidney, liver and subcutaneous tumor samples was maintained 6 and 12 h after administration of BSH following BSO pretreatment (6 h interval) (Fig. 2). The blood boron concentration using BSO pretreatment was up to 20 times higher at 6 h (47.6 μg/ml) and 12 h (55.7 μg/ml)

compared to that without BSO, leading to the retention of boron level in the liver, kidney, subcutaneous tumor and brain tumor.

4. Discussion

Buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis, depletes intracellular sulfhydryl-conjugates such as glutathione (GSH) by inhibiting its biosynthetic enzyme c-glutamylcysteine synthetase (Griffith and Meister, 1979). We previously reported that BSH, a boron-containing sulfhydryl-conjugate, shows higher *in vitro* and *in vivo* boron uptake in tumor cells by simultaneous administration with BSO, leading to the improvement of tumor control probability (Yoshida 2004, 2008). In the previously used Fisher-344 rat subcutaneous tumor model, boron accumulation in subcutaneous tumor, blood, skin, muscle, liver, and kidney was significantly enhanced after administration of BSH (100 mg/kg) with BSO (5 mmol/kg) and maintained for 12 h, and the *in vivo* tumor growth was significantly delayed by BSH-BSO-mediated BNCT (Yoshida 2004, 2008). However, it is still unclear whether such enhancement effect is caused by the sulfhydryl group of BSH, and if it is similar in brain and brain tumors which the intact BBB. Metabolic transformation of one or more BSH metabolites, such as BSH sulfenic acid (BSOH), BSH sulfinic acid (BSO(2)H), BSH disulfide (BSSB), BSH thiosulfinate (BSOSB),and BSH–S-cysteine conjugate (BSH–CYS) (Gibson et al, 2001) can be affected by GSH depletion, or directly influenced by BSO. In this study, simultaneous administration of BSH and BSO showed significantly higher boron concentration compared to that without BSO. There was no difference in tissue boron level between BSO-pretreatment and no-BSO groups after $B_{12}H_{12}$ or BNH_3 administration.

We suggest that time-dependent increase in tumor boron concentration might be related to the increasing level of redox regulating agents in tumor tissue, though, under limited conditions of this study, high boron level in subcutaneous and brain tumor samples could be associated with the retention of boron in the blood. Thus, higher boron concentration after BSO-pretreatment might have a potential risk of normal tissue damage by BNCT. Further evaluation should be focused on more ideal intervals between BSO and BSH administration and the timing of neutron irradiation. Hypervascular extraaxial tumor, such as meningioma, might be a candidate for this treatment method.

5. Acknowledgments

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6. Reference

Gibson CR., Staubus AE., Barth RF., et al., 2001. Boron neutron capture therapy of brain tumors: investigation of urinary metabolites and oxidation products of sodium borocaptate by electrospray ionization mass spectrometry. Drug Metab. Dispos. 29,1588-1598

Griffith OW, Meister A., 1979. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (S-n-butylhomocysteine sulfoximine). J. Biol. Chem. 254, 7558-7560

Yamamoto T, Tsuboi K, Nakai K, et al. 2013. Boron neutron capture therapy for brain tumors. Transl Cancer Res 2:80-86

Yoshida F, Yamamoto T, Nakai K, et al., 2008. Combined use of sodium borocaptate and buthionine sulfoximine in boron neutron capture therapy enhanced tissue boron uptake and delayed tumor growth in a rat subcutaneous tumor model. Cancer Lett. 263, 253-258

Yoshida F, Matsumura A, Yamamoto T, et al., 2004. Enhancement of sodium borocaptate (BSH) uptake by tumor cells induced by glutathione depletion and its radiobiological effect. Cancer Lett. 215, 61-67

Table 1

	BSH		$B_{12}H_{12}$		BNH_3	
	BSO+	BSO-	BSO+	BSO-	BSO+	BSO-
Subcutaneous tumor	26.3± 4.3	5.0± 2.9	3.1± 0.6	2.5± 0.3	2.8± 0.8	2.4 ± 0.4
Blood	55.2±15.0	8.1± 6.2	6.9± 1.1	6.0± 1.0	5.8± 0.9	5.5± 1.0
Brain	2.6± 1.4	0.6± 0.7	0.5± 0.05	0.5 ± 0.1	0.6± 0.2	0.5 ± 0.2
Skin	34.2± 9.8	6.3± 4.3	4.8 ± 1.1	4.0 ± 0.5	2.5 ± 1.6	3.0± 1.1
Muscle	7.4 ± 2.8	1.2± 0.9	1.5 ± 0.2	1.3 ± 0.3	1.1 ± 0.2	1.0 ± 0.2
Liver	71.4± 22.6	12.6±8.1	5.2 ± 0.3	4.6 ± 0.4	3.1 ± 1.2	2.4 ± 0.5
Kidney	82.3± 28.5	31.6±15.9	10.6± 1.0	9.6± 1.5	4.4± 2.1	4.4± 1.3

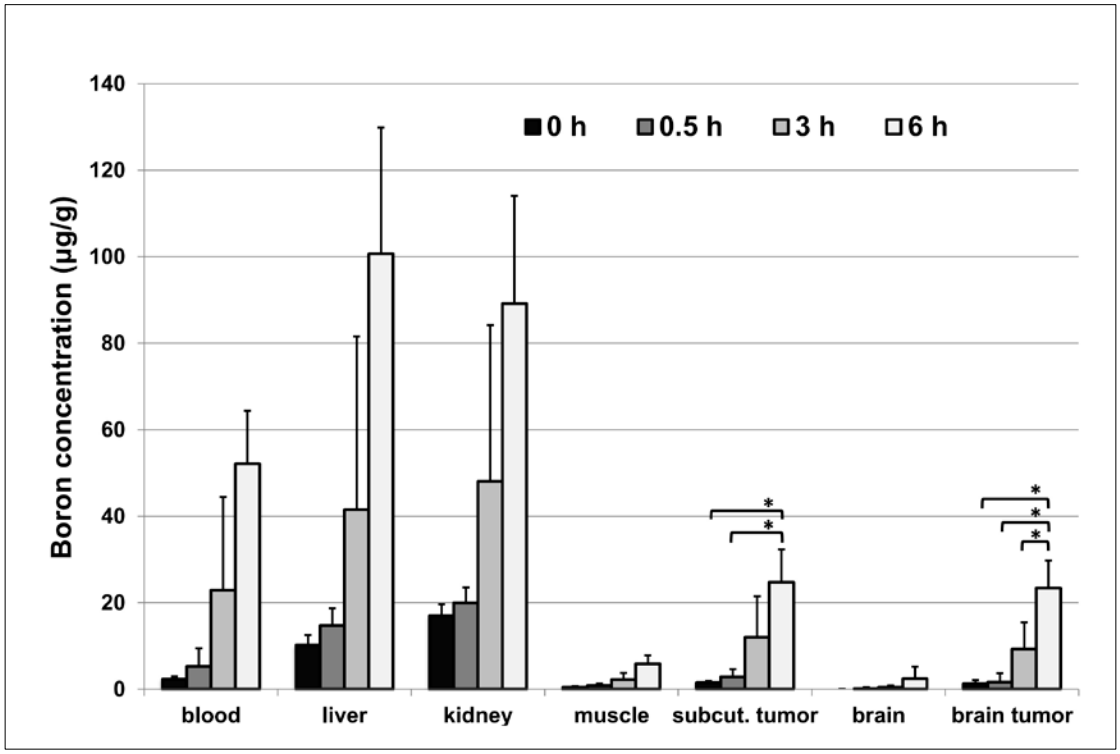
140
120
100
80
60
40
20
0
Boron concentration (µg/g)
0 h
0.5 h
3 h
6 h
*
*
*
*
*
blood
liver
kidney
muscle
subcut. tumor
brain
brain tumor

Fig. 1

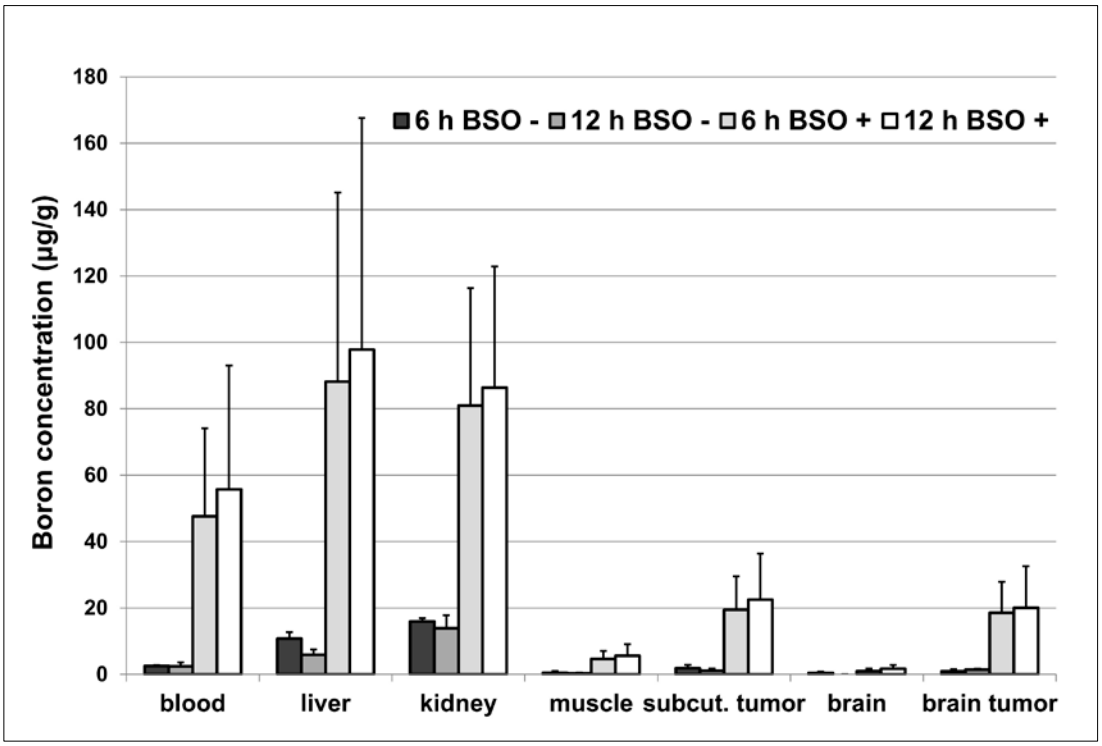
180
160
140
120
100
80
60
40
20
0
Boron concentration (µg/g)
6 h BSO -
12 h BSO -
6 h BSO +
12 h BSO +
blood
liver
kidney
muscle
subcut. tumor
brain
brain tumor

Fig. 2

Figure and table legend

Table 1: Boron concentration in samples (μg/g) after administration of sulfhydril- (BSH) and non-sulfhydril- ($B_{12}H_{12}$ and BNH_3) boron compounds. Each sulfhydril- or non-sulfhydril-boron compound was administrated with BSO simultaneously. All samples were collected 3 h after the compound administration. Data shown as means ± SDs.

Fig.1 Boron concentration in samples (μg/g) depending of the interval (h) between BSO and BSH administration.*$p < 0.05$

Fig.2 Retention of ^{10}B in the tissues (boron concentration, μg/g) 6 and 12 h following BSH administration with/without BSO.