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EFFECTIVENESS OF DIFFERENT BNCT-DRUG INJECTION METHODS

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Abstract. *The BNCT-drugs (BSH and BPA) toxicity for 8-10-week-old severe combined immunodeficiency (SCID) male mice outbred with SPF-status was studied. The possibility of increasing BPA (L-p-boron phenylalanine) and BSH (sodium salt of borocaptate) therapeutic dose was shown. The relationship between a safe therapeutic dose and the administration method of BPA was found. The intraperitoneal injection allows one to increase the dose of BPA at least twice – 700 mg/kg b.w, BSH – at least 8 times, 800 mg/kg b.w with intraperitoneal injections. The BPA intraperitoneal and intratumoral injections demonstrate higher results in comparison with intravenous administration. The highest and statistically significant concentration of ^{10}B in the tumor was found for intraperitoneal injection of BPA for intratumoral injection of BPA – 27±5 μg/g (heterotopic tumors, P=0.95). The highest tumor/blood ratio for BPA was 7.5 for intratumoral administration in the point of 1h (heterotopic tumors). The maximum concentration of ^{10}B with the introduction of BSH was with intravenous administration and was 8±3 μg/g (orthotopic tumors). The highest tumor/blood ratio was 9.5 for intravenous injection in 1h point (heterotopic tumors).*

Keywords: *BNCT, BSH, BPA, ISP-AES, therapeutic dose, toxic effect*

1. INTRODUCTION

It is known that selective accumulation of boron-10 in cancer cells followed by epithermal neutron irradiation leads to death of tumor cells without affecting surrounding healthy cells. One of the problems of boron neutron capture therapy (BNCT) is the targeted delivery of boron to tumor cells. The effectiveness of the BNCT depends on many factors, including ^{10}B drugs. These substances should satisfy the following conditions:

- non toxicity;
- accumulate mainly in a tumor with a tumor/normal tissue difference> 3:1 at doses of ≈ 20–35 μg/g ^{10}B;
- quickly removed from the blood and normal tissues, but remaining in the tumor for several hours during neutron irradiation.

Currently, two low-molecular weight substances are used: BPA (L-p-boron phenylalanine) and BSH (sodium salt of borocaptate). Their therapeutic efficacy has been demonstrated in patients with high-grade gliomas, recurrent tumors of the head and neck, as well as melanoma. These BNCT-drugs meet the formulated requirements to a limited extent [1].

It has been previously shown that the oral method does not provide the necessary concentration gradient of the tumor/normal tissue [2]. Therefore, BPA in the fructose complex (BPA-f) intravenously were administered. In addition, it was found that prolonged BPA infusion increases the effectiveness of BNCT [3]. It should be noted that intravenous administration is the most commonly used method for BPA, BSH and other BNCT-drugs [4, 5, 6, 7]. In some studies, the intra-arterial administration was used, for example, a suspension of BSH with lipiodol in a rat model [8]. Along with intravenous administration, researchers often use intraperitoneal administration of BPA and BSH [7, 9, 10]. It is known that direct BNCT-drug injection into the tumor allows getting a high gradient of the tumor/normal tissue [11, 12, 13].

The method of BNCT-drug injection is an important factor that can significantly change the tumor/normal tissue gradient. However, there are no systematic data on the effect of BNCT-drug administration methods on the tumor/normal tissue ratio. There is also no ordered data on the relationship of the administration methods with the possibility of increasing the BNCT-drug dose. All of this shows the necessity of further studies in this direction.

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2. Materials and methods

All the experiments were carried out in compliance with the principles of humane treatment of animals in accordance with the directive of the European Union (86/609/EEC).

2.1. Model heterotopic and orthotopic xenograft human glioblastoma: tumor induction

The research has been done on 8-10-week-old severe combined immunodeficiency (SCID) male mice outbred with SPF-status. U87 human glioblastoma cell culture was obtained in the Center for Genetic Resources of Laboratory Animals, SPF Animal Farm, FRC IC&G SB RAS. Cells were cultured on DMEM/F12 medium (1:1) (Biolot, Russia), 10% fetal bovine serum (Gybco, USA), gentamicin 50 μg/ml (Dalchimpharm, Russia) at a temperature of 37° C and 5% CO_2. 18-21 days before the experiment. U87 human suspension of glioblastoma cells was prepared with the concentration of 100,000 cells per 1 μl.

For heterotopic xenograft cells, the suspension was injected subcutaneously into the right hind paw in a volume of 100 μl (10 million cells per animal). For the orthotopic xenotransplantation of cells, intracranial inoculation of 500 thousand cells was performed using a stereotactic setup followed by the MRI. To confirm the presence of a tumor and measure its size, a BioSpec 117/16USR 11.7 T high-field tomograph was used. Using this technology tumor in mice survival are more than 80%.

2.2. Biodistribution studies

Commercially available substances (previously enriched with ^{10}B), such as BPA ($C_8H_7NH_2COOH$—$B(HO)_2$) and BSH ($Na_2B_{12}H_{11}SH$), were used as a boron monoisotopic ^{10}B targeting delivery substance. Three administration protocols with different concentrations of BPA and BSH were assessed:

- intravenously (into the retro-orbital sinus);
- intraperitoneally;
- intratumorally.

To estimate BPA and BSH biodistribution the animals were euthanized, organs of interest were taken out and later frozen and kept at T= -20°C.

Samples of blood, tumor, skin, brain, liver, kidney and spleen were processed for the total boron measurement by the Atomic Emission Spectrometry with Inductively Coupled Plasma (ICP-AES iCap-6500, Thermo). Boron determination in the organs is described in detail in [14].

2.3. End-points

The total boron concentration in tumor, blood and clinically relevant normal tissues were evaluated for each of the time-points. Individual tumor/blood and tumor/normal pouch boron concentration tissue ratios were calculated for each animal and then averaged for the animals in each group using formulas in [15].

3. Results

3.1. Toxic doses of the drugs

The toxicity of standard drugs for BNCT was studied. The possibility of using BPA and BSH in the recommended maximum therapeutic doses on small laboratory animals was confirmed. They are 350 and 100 mg/kg body weight (b.w), accordingly. For the statistical reliability of the results, data obtained from five animals for the same concentration of the drug and injection method were used.

Intravenous injection (into the retro-orbital sinus) of BPA at a concentration from 700 and more mg/kg resulted in the animal's death. At the same time, an intraperitoneal injection of a BPA dose of 700 mg/kg b.w, and 1050 mg/kg b.w did not cause toxic effects.

For the BSH, we also used the intravenous and intraperitoneal injection method. According to the results of a long observation, the BSH in doses of 400, 600, 800 mg/kg b.w (which exceeds the recommended therapeutic dose 4, 6 and 8 times, respectively) did not cause obvious toxic effects in the studied animals.

Thus, to ensure a higher concentration of the drugs in the tumor, the maximum therapeutic doses can be exceeded 3 times for BPA and 8 times for BSH.

3.2. Comparison of the accumulation efficiency of BPA and BSH in orthotopic and heterotopic tumors with intravenous injection

This experiment was carried out under the conditions of recommended therapeutic drug doses – 350 mg/g for BPA and 100 mg/g for BSH. The administration into the retro-orbital sinus was performed.

Table 1 shows a comparison of the results of ^{10}B determination by ICP-AES in the orthotopic tumor for BPA and BSH for all time points.

Table 1. The presence of ^{10}B in the orthotopic tumor. Intravenous injection, μg/g

Time point, h	BPA	BSH
0.5	10.9±0.4	8±13
1	**14.1±0.9#**	**8±3#**
2	9.8*	3.4±0.4
4	7.2±1.5	0.9±0.7
8	4.3*	0.2±0.2
12	3.4±0.1	not detected**
24	0.33±0.09	not detected**

* – the result for one animal; ** – ^{10}B concentration is less than the limit of detection method; # – a statistically significant (P=0.95).

It should be noted that the accumulation efficiency of ^{10}B in the tumor for BPA is higher than for BSH. The 1h point is highlighted in bold, the concentration ^{10}B is the highest for it. Results for BPA and BSH 1h have a statistically significant difference (P=0.95). For BPA, the tumor/brain ratio at this point is 4.4; tumor/blood – 3.0. For BSH, the tumor/brain ratio at this point is 3.5; tumor/blood – 0.42.

Table 2 shows a comparison of the results of ^{10}B determination by ICP-AES in the heterotopic tumor for BPA and BSH for all time points. For a heterotopic tumor, the efficiency of ^{10}B accumulation in a tumor for BPA is higher than for BSH as well as for orthotopic tumors. The 1h point is highlighted in bolt, the concentration of ^{10}B is the highest for it. BSH-result for 1h has a statistically significant difference (P=0.95). For BPA, the tumor/brain ratio at this point is 2.4; tumor/blood – 1.4. For BSH, the tumor/brain ratio at this point is 9.5; tumor/blood – 0.47.

Table 2. The presence of ^{10}B in the heterotopic tumor. Intravenous injection, μg/g.

Time point, h	BPA	BSH
1	**12±5**	**6±1#**
2	**11±4**	1±2
3	8±2	3±1
4	4±2	1.1±0.6

– a statistically significant (P=0.95).

A high gradient tumour/normal tissue boron distribution for BSH for heterotopic tumors is noteworthy. However, in both cases (orthotopic and heterotopic tumor) the error in determining the BSH is higher than for BPA. Figure 1 presents a comparison of the found ^{10}B concentration in tumor for a point of 1 h.

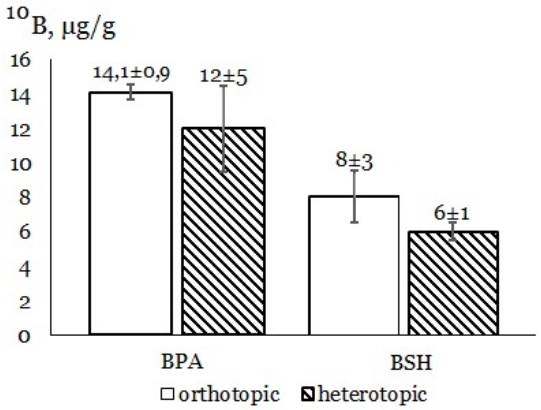

Figure 1. Comparison of the ^{10}B concentration in the tumor for a point of 1h, μg/g (n=3, P=0.95)

The difference in boron concentration values has statistically significant for orthotopic tumors at injection BPA (14.1±0.9 μg/g) and BSH (8±3 μg/g); (P=0.95). Also the difference in boron concentration values has statistically significant for heterotopic tumors at injection BPA (12±5 μg/g) and BSH (6±1 μg/g); (P=0.95). The tumor/brain ratio at this point for BPA is 4.4 (ortho) and 2.4 (hetero). Then, for BSH tumor/brain the ratio is 3.5 and 9.5 (ortho and hetero, respectively).

3.3. Comparison of the accumulation efficiency of BPA and BSH in heterotopic tumors with intraperitoneally injection

This experiment was carried out under the conditions of recommended therapeutic drugs doses – 350 mg/g b.w for BPA and 100 mg/g b.w for BSH. The intraperitoneal administration was performed.

Table 3. The presence of ^{10}B in the heterotopic tumor. Intraperitoneal injection, μg/g.

Time point, h	BPA*	BSH
1	26	**6.9±2.5#**
2	**28**	**4.3±4.9**
3	12	2.2±0.8
4	6.1	0.96±0.58

* – the result for one animal; # – a statistically significant (P=0.95).

Table 3 shows a comparison of the results of ^{10}B determination by ICP-AES in the heterotopic tumor for BPA and BSH for all time points – 1, 2, 3 and 4 h. For heterotopic, the accumulation efficiency of ^{10}B for BPA is higher than for BSH. The 2h point for BPA is highlighted in bolt; the concentration ^{10}B is the highest for it. The tumor/blood ratio in 1h point is 3.4. The 1h and 2h points for BSH are highlighted in bolt. The tumor/blood ratio at this point is 0.28.

3.4. Comparison of the accumulation efficiency of BPA and BSH in heterotopic tumors with intratumoral injection

This experiment was carried out under the conditions of recommended therapeutic drug doses – 350 mg/g b.w for BPA and 100 mg/g b.w for BSH. The intratumoral administration was performed.

Table 4 shows a comparison of the results of ^{10}B determination by ICP-AES in the heterotopic tumor for BPA and BSH for all time points – 1, 2, 3 and 4 h. For heterotopic, the accumulation efficiency of ^{10}B for BPA is higher than for BSH. The 2h point for BPA is highlighted in bolt; the concentration of ^{10}B is the highest for it. The tumor/blood ratio in 1h point is 7.5. The 1h point for BSH is highlighted in bolt. The tumor/blood ratio at this point is 0.57.

Table 4. The presence of ^{10}B in the heterotopic tumor. Intratumoral injection, μg/g

Time point, h	BPA	BSH
1	16±10	6±3
2	**27±5#**	**9±5**
3	9.9±0.4	6±3
4	10.1±0.5	3.2±0.5

– a statistically significant (P=0.95).

4. Discussion

We showed that the use of BPA and BSH for human glioblastoma U87 can be promising if it is possible to increase the concentration of ^{10}B in the brain. It is known that the prolonged administration of the drugs is used in BNCT [16]. However, our studies have shown that the highest concentration of boron in the tumor accumulates for 1-2 hours after drug administration (see tables 1-4), while the type of drugs and administration method have an effect on the ratio of tumor/normal tissue.

For intravenous injections in case of BPA, the difference for the found concentration of ^{10}B for various types of tumors is not significant – 14.1±0.9 and 12±5 μg/g. For BSH, we see the same results: within the confidence interval, the results coincide – 8±3 and 6±1 μg/g.

When comparing the effectiveness of various BPA administration methods, it should be noted:

- the highest concentration of ^{10}B in the tumor was found for intraperitoneal injection – 28 μg/g (heterotopic tumors) and for intratumoral injection – 27±5 μg/g (heterotopic tumors), see Figure 2;
- the highest tumor/blood ratio is 7.5 for intratumoral administration in 1h point (heterotopic tumors).

The difference in boron concentration values has statistically significant for heterotopic tumors at

intravenous (12±1 μg/g) and intratumorally (27±5 μg/g) injection (P=0.95).

In addition, an intraperitoneal injection of a dose of BPA of 700 mg/kg and 1050 mg/kg b.w did not cause toxic effects. Therefore, when using higher doses of BPA, an increase in the accumulated concentration of ^{10}B in the tumor can be expected up to 36 μg/g at least.

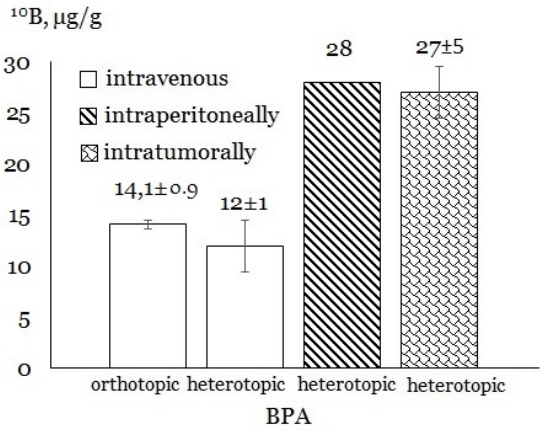

Figure 2. Comparison of the ^{10}B concentration in the tumor for various injection methods, μg/g (n=1-3, P=0.95)

When comparing the effectiveness of various BSH administration methods, it should be noted:

- the tumor/blood ratio is less than 1.0 with any method of administering the drugs;
- the highest concentration of ^{10}B in the tumor was found for intravenous injection – 8±3 μg/g (orthotopic tumors), see Figure 3;
- the highest tumor/blood ratio is 9.5 for intravenous injection in 1h point (heterotopic tumors).

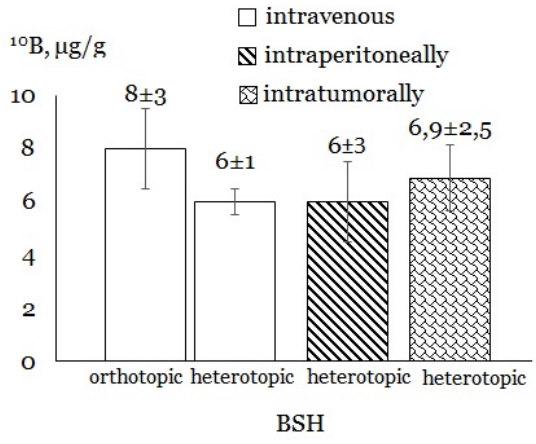

Figure 3. Comparison of the ^{10}B concentration in the tumor for various injection methods, μg/g (n=3, P=0.95)

It can be seen that the ^{10}B concentration in the tumor coincides within the confidence interval for three injection methods and does not exceed the value of 8 μg/g (the difference in boron concentration values is statistically insignificant). As in the case of BPA for BSH, the therapeutic dose can be increased to 800 μg/g. Therefore, an increase in the accumulated concentration of ^{10}B in the tumor can also be expected when using higher drug doses of up to 48 μg/g at least.

5. Conclusion

To ensure a higher concentration of drugs in the tumor, the maximum therapeutic doses can be exceeded 3 times for BPA and 8 times for BSH. This is possible with intraperitoneal or intratumoral administration of BPA and with intraperitoneal administration of BSH.

The highest and statistically significant concentration of ^{10}B in the tumor was found for intraperitoneal injection of BPA for intratumoral injection of BPA – 27±5 μg/g (heterotopic tumors, P=0.95). The maximum concentration of ^{10}B with the introduction of BSH was with intravenous administration and it was 8±3 μg/g (orthotopic tumors).

Using the increased doses in combination with the intratumoral or intraperitoneal method of drug administration, we can increase the effectiveness of BNCT for glioblastoma U87.

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