Neutron capture therapy with a new boron-porphyrin compound in the rat 9L glioma model

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Running Title:
Neutron capture therapy with a new porphyrin in rat glioma model

KEY WORDS
Boron, BSH, glioma, neutron capture therapy, porphyrin, rat

ABSTRACT
Neutron capture therapy with a new boron-porphyrin compound was tested in a rat brain tumor model. Although the concentration of boron in the tumor was too low to elicit a therapeutic effect, prominent histopathologic changes, such as necrosis, congestion and bleeding were observed in the tumors of the rats administered the boron neutron capture therapy.

INTRODUCTION
Tumor-selective uptake of boron is essential for effective boron neutron capture therapy (BNCT). Boroncaptate sodium(BSH) is one of the boron compounds used in BNCT[1], and favorable clinical results using BSH have been reported[2]. To increase the selectivity of tumor uptake of the boron compound, a new type of boron compound composed of BSH and metalloporphyrin has been developed. This report describes the results of the treatment of rat brain tumors with BNCT using the BSH-porphyrin compound STA-BX900.

MATERIALS AND METHODS
Boron compound
STA-BX900 consists of BSH and metalloporphyrin, which tends to be uptaken by malignant tumors [3]. STA-BX900 is 2-dodecaboranyl thioethylcarbamoylmethyl-4-vinyl -deuteropophinato manganese(III). Because STA-BX900 contains manganese within the porphyrin cage, it is not photoreactive. The pharmacokinetic study of STA-BX900 revealed that this compound does not enter the normal brain tissue, and that a high tumor to blood ratio can be achieved 6 hours after intravenous injection of the compound [4]. Although the toxicity of STA-BX900 should be examined, there was no clinical side effect of STA-BX900 for rats in this experiment. STA-BX900 contains 12 boron
atoms and the molecular weight of STA-BX900 is 917.8 gm/mol. Boron accounts for 13% of the weight of the compound.

**Rat brain tumor model**

9L gliosarcoma cells suspended in conditioned medium at a concentration of 1x10^4 cells per 10 ml were stereotactically implanted 5mm deep in the right frontal lobe of Fisher 344 rats under pentobarbital anesthesia. Magnetic resonance imaging (MRI) was performed 10 days after tumor cells were implanted and those rats which did not develop brain tumors were excluded from the study. Thirty-six rats were confirmed to have developed brain tumors using MRI with Gadopentetate Dimeglumine (Gd-DTPA).

**Experimental Therapy**

All rats were transported to the Research Reactor Institute of Kyoto University (KUR). The rats were divided into three groups of 12 rats each. The control group received no radiation. The neutron group received only neutron radiation to the head without the previous injection of STA-BX900. The therapy group received neutron radiation directed to the head 6 hours after the intravenous injection of STA-BX900 solution. The injected dose was 0.05 mmol/kg. The calculated neutron flux at the center of the radiation port was 3 x 10^9 n/cm^2/sec. The neutron radiation time was 30 min. The thermal neutron fluence of each rat was measured by mean of gold foil attached to the head of each rat. The gamma dose was measured with a thermal luminescence dosimeter (TLD) attached to the head of each rat.

**Radiation dose**

The calculated tumor dose of a ^10^B(n,α) reaction was 0.789 Gy under conditions where a tumor dose of ^10^B was 1 ppm. The mean neutron fluence in the neutron group was 7.94±1.07 x 10^{12} n/cm^2. One extreme value of neutron fluence in this neutron group was abandoned with the Smirnov test. The mean neutron fluence in the therapy group was 8.91± 0.61 x 10^{12} n/cm^2 showing no statistically significant difference with the neutron group (Student's t test). The mean gamma dose in the neutron group was 3.91± 0.33 Gy. One extreme value of gamma dose in this group was abandoned with Smirnov test. The mean gamma dose in the therapy group was 4.29±0.44 Gy. The difference between the two groups was not statistically significant (Student's t test).
Survival and histopathological study

The survival times after the treatment were recorded and after death the brains were immediately removed and fixed in formaldehyde solution. Coronal sections were performed through the center of the tumor and the diameter of the tumor and maximal cross-sectional area were measured. The tumor specimens were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The extent of the tumor invasion in surrounding tissues was graded as 1: none, 2: minimum, 3: moderate, 4: extensive. Each specimen was microscopically evaluated for the presence or absence of mass effect of the tumor on normal brain tissue, subarachnoidal seeding of tumor cell, hydrocephalus or ventricular dilatation, intracerebral or intratumoral bleeding, intravascular congestion and necrosis of the tumor.

The survival times after the treatment were analyzed with Student's t test, the Cox-Mantel test and the Wilcoxon test of Kaplan-Meier survival curves. The maximal cross-sectional area of the brain tumor was analyzed with Student's t test. Each histopathological parameter was analyzed with Chi square test.

RESULTS

Survival and tumor size

Mean survival time was 12.8±2.7 days for the control group, 14.8±2.4 days for the neutron group and 16.2±4.4 days for the therapy group(Table 1). The difference was statistically significant between the control and the therapy groups (p<0.05, Student's t test). The analysis of survival curves obtained by the Kaplan-Meier method showed the statistically significant difference between the survival of the control and that of the neutron group by the Cox-Mantel test (p<0.05) but not by the Wilcoxon test <<Fig. 1>>. The difference between the survival of the control group and the therapy group was statistically significant by both the Cox-Mantel test (p<0.01) and the Wilcoxon test (p<0.05). No statistically significant difference was observed between the neutron group and the therapy group with either the Cox-Mantel test or the Wilcoxon test.

The maximal cross-sectional area of the brain tumor at autopsy was 43.9±16.5 mm² for the control group, 39.0±23.4 mm² for the neutron group and 36.4±22.0 mm² for the therapy group. The size of the tumor tended
to decrease with therapy, but the difference among the three groups was not statistically significant.

Histopathologic study

Twelve specimen per each group were evaluated. The numbers of cases with bleeding for the control, neutron, and therapy groups were 1, 2, and 4, respectively <<Fig. 2, Table 2>>. The numbers of cases with congestion in the tumor for the control, neutron, and therapy groups were 3, 6, and 11, respectively. The difference between the control and the therapy group was significant (p<0.01, Chi square test). The difference between the neutron-treated and therapy group was also significant (p<0.05, Chi square test). The numbers of cases with necrosis in the tumor for the control, neutron, and therapy groups were 3, 2, and 10, respectively. The differences between the therapy and the control or the neutron groups were significant (p<0.01, Chi square test). Figure 3 is a photomicrograph of the typical tumor in a control rat, showing the dense tumor tissue of the 9L gliosarcoma. Figure 4 is a photomicrograph of the typical tumor in a rat from the therapy group, showing congestion, hemorrhage, and necrosis of the tumor.

The mean scores for tumor extension for the control, neutron-treated and therapy groups were 3.4±0.7, 2.5±1.1, and 3.3±0.9, respectively (Table 2). The numbers of cases with mass effect for the control, neutron, and therapy groups were 11, 8, and 10, respectively. The numbers of cases with seeding of tumor cells for the control, neutron, and therapy groups were 10, 6, and 9, respectively. The numbers of cases with hydrocephalus for the control, neutron, and therapy groups were 3, 1, and 3, respectively. No statistically significant difference were found in tumor extension, mass effect, seeding and hydrocephalus between each study groups.

DISCUSSION

Malignant brain tumors are some of the most therapy-resistant tumors in spite of multimodality therapeutic trials[5]. BNCT is a bimodal radiotherapy that consists of tumor-selective uptake of boron compound and sufficient neutron radiation. The thermal neutron is less destructive for normal brain tissue than for tissue which contains boron compound. Tumor cells which take up the boron compound sustain lethal damage due
to the neutron capture B(n,a) reaction. Each tumor cell invading the normal brain tissue would also be damaged, so this therapy is ideal for treating infiltrative glial tumors [6].

Tumor-selective uptake of the boron compound is essential for effective BNCT. BSH does not enter normal brain tissue, however the high blood concentration relative to the tumor concentration is one of the limiting factors of BSH [7,8]. A high blood concentration of boron during BNCT leads to endothelial damage and subsequent radiation necrosis. In order to achieve a high tumor/blood ratio of boron we developed the new compound STA-BX900. In other studies, the pharmacokinetic parameters of this compound were measured using a relatively large size rat brain tumor model. STA-BX900 demonstrated a high uptake by brain tumor cells and minimal uptake by normal brain cells[4]. In the study relative high tumor/blood ratio could be achieved compared with BSH. The concentration of boron in brain tumor cells in our previous study was approximately 1 ppm 6 hours after the injection of STA-BX900. The size of the tumor in the present experiment was smaller than that of the tumor in the pharmacokinetic study. Small tumors tend to uptake more boron [9], so the concentration of boron in tumor would be higher in the present experiment.

The calculated surface dose of a 10B(n,a) reaction was 0.8 Gy. The total surface dose of the neutron group was the sum of 17O(n,a) and 14N(n,p) reactions and the contaminated g dose, which was calculated as 6.0Gy. The total surface dose of the therapy group was the sum of 10B(n,a), 17O(n,a) and 14N(n,p) reactions and the contaminated g dose, which was calculated as 7.2 Gy.

Despite the small difference in radiation dose between the neutron and the therapy group, the histopathological findings in these groups were remarkably different. The therapy group demonstrated frequent necrosis, venous congestion and hemorrhage which suggest a disturbance of blood circulation in the tumor. Hemorrhage may be caused by endothelial damage due to BNCT. Radiation necrosis normally occurs some months after the radiation therapy[10]. Because the mean survival in our study was less than 28 days, we may assume that the disturbance in blood circulation in our tumor model was not caused by usual radiation necrosis or fibrinoid necrosis of cerebral vessels. Histopathologic examination of
our tumor model after the BNCT did not demonstrate any pathologic change such as fibrinoid necrosis. Goodman et al. reported progressive edema and necrosis in the peritumoral region after BNCT in a rat glioma model, although the concentration of boron in tumor was too low to obtain a lethal B(n,a) reaction [11]. They concluded that this histologic change indicated a pathologic endothelial response. However in their model, bleeding, congestion or necrosis were not as frequently seen as in our present study. These findings indicate that STA-BX900 may induce more damage to the endothelium than BSH. This may be due to the difference in the intracellular distribution of boron which has to be further investigated. In future studies we will investigate the long-term pathophysiologic processes of the brain tumor and blood vessels after BNCT.

The pharmacokinetics of other boron porphyrin derivative such as BOPP have been investigated [12,13]. BOPP reached high concentration levels of boron in tumor tissue and the tumor-to-blood ratio was high. However this compound induces some degree of clinical toxicity and is photoreactive [14], and in vitro it is more toxic to 9L gliosarcoma cell lines than BSH or BPA[15]. These problems should be solved in the future. STA-BX900 is not photoreactive, but it clinical toxicity was not been evaluated as yet.

The results of the study demonstrated that survival time tended to increase and the size of the tumor to decrease after BNCT using this boron-porphyrin compound, although the concentration of boron in the tumor was too low to elicit a therapeutic effect. Future pharmacologic improvement in STA-BX900 may involve increasing the content of boron in the molecule. Moreover the improvement in the dose application should be considered to attain a more significant therapeutic effect.

CONCLUSION

A neutron capture therapy experiment using a rat brain tumor model with a boron-porphyrin compound is reported. Prominent histopathologic changes such as congestion, necrosis and hemorrhage, were observed in the tumors of the therapy group. These findings would be caused by endothelial damage caused by this boron-porphyrin compound.
ACKNOWLEDGMENTS
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REFERENCES
8. Stragliotto G., Fankhauser H.: Biodistribution of boron sulfhydryl for

Figure legends
Fig. 1 Kaplan-Meier survival curve of control, neutron and therapy group rats
Fig. 2 Number of rats with intratumoral bleeding, congestion, and necrosis.
Fig. 3 Photomicrograph of the typical tumor in a control rat, showing the dense tumor tissue of the 9L gliosarcoma.
Fig. 4 Photomicrograph of the typical tumor in a rat from the therapy group, showing congestion, hemorrhage, and necrosis within the tumor.
Table I  Mean survival time and maximal cross-sectional area of each group of rats

<table>
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<th>Mean survival time (days)</th>
<th>Max cross area (mm²)</th>
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<tbody>
<tr>
<td>Control</td>
<td>12.8±2.7 *</td>
<td>43.9±16.5</td>
</tr>
<tr>
<td>Neutron</td>
<td>14.8±2.4</td>
<td>39.0±23.4</td>
</tr>
<tr>
<td>Therapy</td>
<td>16.2±4.4 *</td>
<td>36.4±22.0</td>
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* The difference between control and therapy is statistically significant (p<0.05, Student's t test).

Table II  Histopathologic findings of each group of rats

<table>
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<td>tumor extension</td>
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<td>2.5±1.1</td>
<td>3.3±0.9</td>
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<td>10</td>
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<tr>
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<td>2</td>
<td>4</td>
</tr>
<tr>
<td>congestion</td>
<td>3 *</td>
<td>6 #</td>
<td>11*##</td>
</tr>
<tr>
<td>necrosis</td>
<td>3*</td>
<td>2*</td>
<td>10*</td>
</tr>
</tbody>
</table>

* The difference of number of rats with congestion between control and therapy and the difference of number of rats with necrosis between therapy and control or neutron are statistically significant (p<0.01, Chi square test).
# The difference of number of rats with necrosis within tumor between neutron and therapy is statistically significant (p<0.05, Chi square test).