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

Y. Shibata[1] M.D., A. Matsumura[1] M.D., T. Yamamoto[1] M.D., K. Nakagawa[1] M.D., Y. Yoshii[1] M.D., T. Nose[1] M.D., I. Sakata[2] Ph.D., S. Nakajima[3] M.D., Y. Hayakawa[4] M.D., K. Ono[5] M.D.

Department of Neurosurgery, University of Tsukuba[1], Toyo Hakka Co. Ltd.[2], Department of Operative Surgery, Asahikawa Medical College[3], Department of Radiation Physics, University of Tsukuba[4], Kyoto University Research Reactor Institute[5]

Address for correspondence and reprint requests :
Yasushi Shibata M.D.
Department of Neurosurgery, University of Tsukuba,
Tsukuba, Ibaraki, 305, Japan
Telephone: +81-298-53-3220
Fax: +81-298-53-3214

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KEY WORDS

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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). Borocaptate 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 1x104 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 $3 \times 10^9 \text{n/cm}^2$/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 $^{10}B(n,\alpha)$ 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 $7.94 \pm 1.07 \times 10^{12} \text{n/cm}^2$. 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 $8.91 \pm 0.61 \times 10^{12} \text{n/cm}^2$ showing no statistically significant difference with the neutron group (Student's t test). The mean gamma dose in the neutron group was 3.91 ± 0.33Gy. 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 ± 0.44Gy. 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 mm^2 for the control group, 39.0 ± 23.4 mm^2 for the neutron group and 36.4 ± 22.0 mm^2 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.

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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

	Mean survival time (days)	Max cross area (mm^2)
Control	12.8$\pm$2.7 *	43.9$\pm$16.5
Neutron	14.8$\pm$2.4	39.0$\pm$23.4
Therapy	16.2+4.4 *	36.4+22.0

* 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

	control	neutron	therapy
tumor extension	3.4$\pm$0.7	2.5$\pm$1.1	3.3$\pm$0.9
Mass effect	11	8	10
seeding	10	6	9
hydrocephalus	3	1	3
bleeding	1	2	4
congestion	3 *	6 #	11*#
necrosis	3*	2*	10*

* 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).