Tolerance of Canine Brain to Boron Neutron Capture Therapy

Akira TAKEUCHI, Tomoko NAGATA*, Fumihito OHASHI, Nobuo SASAKI†, Yukitaka USHIO‡, and Hiroshi HATANAKA§

Department of Veterinary Surgery, and †Veterinary Hospital, Faculty of Agriculture, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan, ‡Department of Neurosurgery, University of Osaka Hospital, Fukushima-ku, Osaka 553, and §Department of Neurosurgery, Teikyo University Hospital, Kaga, Itabashi-ku, Tokyo 173, Japan

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ABSTRACT. The normal canine brain tolerance to boron neutron capture therapy (BNCT), with which selective radiotherapy of the brain tumor is expected, was demonstrated by two different series of the irradiation experiment. Normal dogs were irradiated on the skull with thermal neutrons from the reactor after intravenous infusion of $^{10}\text{B}$-enriched Na$_2$B$_4$H$_6$,SH, and the intravascular dose at the brain surface was calculated as the radiation dose. In series 1, the brains of 3 dogs were irradiated 2000 to 3000 rads by the current therapeutic regimen of BNCT and were autopsied after three years observation. In series 2, 6 dogs were given various high doses up to 9000 rads to their brain by BNCT, and autopsied after 1 month and 1 year respectively. Tolerance was excellent in all the dogs, with no unusual findings clinical, neurological, or in laboratory data during the observation period, and no pathological changes by autopsy. When the reduction of the endothelial dose to one third of the intravascular dose and the quick attenuation of thermal neutrons are taken into consideration, the actual doses to the vascular wall in both experiments might be low enough to be tolerated by the normal brain tissue.—KEY WORDS: boron, brain, neutron capture, tumor.

Poor results in the conventional irradiation therapy of cancer are due mainly to the limitation by the presence of normal tissues within the radiation field of the doses that can be delivered to tumor. In the radiation therapy of tumors it is desirable to deliver the lowest possible dose to the associated normal tissues. Boron neutron capture therapy (BNCT), which was first suggested by Locher [16], is considered to be able theoretically to give the minimum damage to the normal tissue while it destroys the tumor tissue. In principle, the use of the $^{10}\text{B}$ (n, α)$^7\text{Li}$ reaction in radiotherapy produces radiations with a short range (less than 10 μm and comparable to or less than a cell diameter), so that it should be possible to keep the dose restricted to the tumor cells. Therefore, if a tumor can be preferentially loaded with a suitable $^{10}\text{B}$ compound and irradiated with thermal neutrons from the atomic reactor which are rather weak in the biological effectiveness, malignant cells can be selectively destroyed by high-LET (linear energy transfer) radiations with both the α particle and the Li nucleus, while rendering the surrounding healthy tissue intact.

Although Sweet and Javid [19] first demonstrated that certain boron compounds would concentrate in the human brain tumor relative to the normal brain tissue, partially by taking advantage of the breakdown of the physiological blood-brain barrier, and subsequently had the first clinical trials of BNCT at the Brookhaven and Massachusetts Institute of Technology reactors [2, 20], the method lay dormant due to the failure in getting better results than other conventional irradiation therapies. The boron neutron capture therapy became successfully applicable to the brain tumor of human patients by Hatanaka and his
colleagues after atomic reactors to deliver a sufficiently large amount of thermal neutrons became available, and a suitable boron compound was found and synthesized [5, 7, 8, 9, 18]. Their preliminary clinical results by means of this revised regimen in Japan reporting the higher rate of long survivals with brain tumors have been encouraging and stimulating interest in neutron capture therapy [12].

In order to confirm the selective safeness of BNCT to the normal brain tissue, we have already reported the electronmicroscopic studies on the normal canine brain shortly after BNCT [1]. In this paper, two different series of dog irradiation are described to clarify the safeness of BNCT to the normal canine brain tissue. The first series was conducted to study the long-term effects of the current therapeutic regimen of BNCT on normal brain tissue. The second series was carried out in an attempt to estimate the maximum tolerable dose that can be safely administered to a patient’s brain undergoing BNCT.

MATERIALS AND METHODS

Animals: Three young adult mongrel dogs and six young colony-bred adult beagles were used in Series 1 and 2 respectively. All dogs used are those found normal by physical, neurological and laboratory examinations.

10B compound: The boron compound used in the present experiments is Na2B12H11SH, which was originally developed at the Massachusetts General Hospital [18], was synthesized in a purified 10B-enriched form for clinical use by Shionogi Research Lab., Osaka, Japan and has been used by Hatanaka and his coworkers in the BNCT of brain tumors successfully [7, 8, 9, 12]. Appropriate weights of the crystalline compound were dissolved in distilled water prior to use and the volume was adjusted to give an isotonic solution, the pH was adjusted as necessary to

7.4.

Reactors: The facilities used for the neutron irradiation were the Kyoto University Research Reactor (KUR, 5000 kw) for Series 1, and the Musashi Institute of Technology Reactor (MuITR, 100 kw) for Series 1 and 2, respectively. All these reactors have been remodeled for biomedical purposes, and the well-collimated, highly thermalized horizontal beam of low-gamma neutron is available in the irradiation room directly close to the reactor core in either of these facilities.

The procedures of BNCT: Dogs of Series 1 were infused with a certain amount of Na2B12H11SH corresponding to 28 or 50 mg 10B per kilogram of body weight approximately 12 hours prior to neutron irradiation on their intact brain (about the same regimen for the brain tumor in human patients except non-performance of craniotomy), while other dogs of Series 2 were infused with varying amounts of the same boron compound at the different times prior to the neutron irradiation in order to get different boron concentration in blood at the time of the irradiation. The details of the pertinent factors for each dog of Series 1 and 2 are shown in Table 1 and 2.

Before the animals were subjected to neutron capture therapy, they were anesthetized with intravenous injection of pentobarbital sodium and the left skull was exposed by a scalp reflection together with a partial removal of the temporal muscle with aseptic surgical techniques.

Thermal neutron irradiation was limited to a circular field of 2.5 cm in diameter on the skull of the left hemisphere, and the remaining part of the skull was covered with 30% boron-containing shielding rubber sheet.

During the neutron irradiation in the completely isolated and closed irradiation room, the anesthesia was managed by the remotely controlling procedures using the various monitors and the specially deviced long and thin intravenous catheter at the separated distant room, which was perfectly radio-protected.
Neutron irradiation was carried out to a targeted fluence of $10^{13}$ n/cm$^2$ at the skull surface which corresponded to 60 to 90 minutes with KUR and 180 minutes with MulTR respectively at or near full power.

After the irradiation, the surgical wound was closed in a routine procedure and the animals were kept under careful observation. All animals subjected to BNCT were administered prednisolone before and after the irradiation to minimize the post-irradiation brain edema.

**Dosimetry:** The thermal neutron beam used imparts a background multicomponent radiation dose composed of thermal neutron and gamma ray directly to tissue, which have been measured at the surface of the irradiation area and the surrounding sites by the use of gold foil activation and thermoluminescence dosimeters (TLD). In addition, the indirect irradiation on the brain tissue is expected by neutron activation of elemental constituents present in tissue including $^{10}$B infused, the majority of which are the capture gamma ray and the alpha ray produced by the $^{10}$B(n, $\alpha$)$^7$Li reaction.

As the boron compound used can not be transported to normal brain tissue, the BNCT dose in this study was calculated by measuring the thermal neutron flux at the surface of the brain and $^{10}$B dose in the blood circulating the brain surface. This was accomplished by sampling the blood immediately before and after the irradiation, and an average concentration was taken as a representative value. The basic calculation of the BNCT dose was made according to Deutch and Murray [5], and the determination of boron concentration in blood was made by a colorimetric assay [13].

Therefore, the dose, which was described as the brain dose in this study, is the total of intravascularly (intraluminally) produced neutron-capture radiation (the sum of alpha, beta, gamma and recoil proton) to be calculated as the function of neutron fluence and boron-10 concentration in the blood and core-gamma radiation at the surface of brain. Absorption by the skull was neglected.

**Clinical evaluation of the irradiated dogs:** The physical, radiological, neurological and clinico-pathological assessments were performed periodically after the neutron irradiation to determine any radiation effects due to BNCT treatment.

The dogs in Series 1 were kept alive as long as three years and then sacrificed to investigate the long-term histo-pathological effects of BNCT.

Six dogs irradiated in Series 2 were separated into two groups in such a way that three dogs irradiated by lower, moderate and higher doses will be included in both groups. One group was observed for only one month and autopsy was held to find any acute radiation effects due to BNCT, while another group was observed for one year and sacrificed to determine the delayed radiation effects.

**Histo-pathological examinations:** After autopsy, the whole brain and specimens of various organs were obtained and fixed with 10% formol. The coronal sections of the brain within the neutron radiation field were stained with hematoxylin and eosin, cresyl-violet for Nissl substance and Luxol-fast-blue for myelin sheath. The irradiated site (left hemisphere) of the brain was compared with its counterpart on the opposite side. Sections of the other organs were stained with hematoxylin and eosin.

**RESULTS**

In the first series of experiment long term effects of the regimen of BNCT simulated for brain tumor therapy on the normal canine brain were examined.

**Radiation doses given to each dog:** Thermal neutron flux at the surface of the brain, time for neutron irradiation, average $^{10}$B concentration in blood, the total radiation dose composed of BNCT dose and gamma dose,
Table 1. Pertinent dose information on BNCT studies in series 1

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>$^{10}$B dose (mg/kg)</th>
<th>Interval between injection and irradiation (hr)</th>
<th>Neutron flux (n/cm²/sec)</th>
<th>Neutron exposure time (min)</th>
<th>Neutron fluence (n/cm²)</th>
<th>Average $^{10}$B in blood ($\mu g/g$)</th>
<th>Total radiation (rads)</th>
<th>reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1-1</td>
<td>28</td>
<td>11.5</td>
<td>$2.65 \times 10^9$</td>
<td>63</td>
<td>$0.99 \times 10^{13}$</td>
<td>11.3</td>
<td>2,600</td>
<td>KUR</td>
</tr>
<tr>
<td>S1-2</td>
<td>28</td>
<td>13</td>
<td>$1.72 \times 10^9$</td>
<td>90</td>
<td>$0.93 \times 10^{13}$</td>
<td>18.7</td>
<td>3,210</td>
<td>KUR</td>
</tr>
<tr>
<td>S1-3</td>
<td>50</td>
<td>8.5</td>
<td>$8.52 \times 10^8$</td>
<td>180</td>
<td>$0.92 \times 10^{13}$</td>
<td>19.8</td>
<td>2,288</td>
<td>Mu1TR</td>
</tr>
</tbody>
</table>

a) Intravascular dose at the surface of brain in the radiation field.
b) Gamma dose rate is 60–90 rads/hr at KUR and 25 rads/hr at Mu1TR.

Fig. 1. A coronal section of a canine brain (S1–3), three years after an intravascularly produced radiation of about 2000 rads was irradiated at the surface of the left hemisphere (arrow) by BNCT: LFB stain.

and other pertinent informations for the three dogs are listed in Table 1. The total radiation dose given to the blood at the surface of the canine brain was around 2000–3000 rads and well comparable to those which will be given to the normal brain tissue at the time of BNCT for the brain tumor. Gamma dose at the surface of the brain was approximately 25 (Mu1TR) and 60–90 (KUR) rads/hour respectively, and the total gamma dose at the surface of genital organ during the neutron irradiation was less than 50 rads.

Clinical findings during and after BNCT: During the neutron irradiation of the left parietal region of the brain, the vital signs of the animals remained uneventful under the general anesthesia managed by means of the remote control procedures. All dogs receiving BNCT have recovered from anesthesia without any particular problems and returned to normal activity soon after the irradiation. No unusual findings have been observed in the laboratory data of hematology, urine analysis and blood chemistry throughout their survival time as long as three years, except a very temporary post-surgical changes in hematology. Neurological and behavioral examinations were also held periodically, but no abnormal changes were found throughout their lives. All dogs were found in the excellent physical conditions at the time of autopsy.

Histo-pathological findings: All brains showed no macroscopic abnormality. The dura had no adhesion to the cortical surface, and the operated and irradiated site had been completely healed. Fig. 1 shows the coronal section of the irradiated area of dog S1–3, in which no difference is found between left hemisphere (irradiated side shown by arrow) and right hemisphere (non-irradiated intact side).

Histological examination of all brains showed no abnormality. Any of these pathological findings as destruction of brain tissue or neuronal loss, vascular changes, inflammatory cell infiltration, or others has never been observed. Fig. 2 (a and b) may show the normal structure of brain tissue at the site of irradiation of dog S1–3.

In the second series, the maximum dose,
that can be safely administered to the normal brain by BNCT, was estimated

Radiation doses given to each dog: Thermal neutron flux at the surface of the brain, time for neutron irradiation, average $^{10}$B concentration in blood, total radiation dose composed of BNCT dose and gamma dose, and other pertinent informations for six dogs are listed in Table 2.

By means of various combinations of the different intervals between $^{10}$B injection and irradiation and $^{10}$B dose administered, average $^{10}$B concentrations in blood varying from 33 to 102 $\mu$g/g blood were obtained, which have produced different total irradiation doses from 3800 to 9300 rads by the irradiation of

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**Table 2. Pertinent dose information on BNCT studies in series 2 at MulTR**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>$^{10}$B dose (mg/kg)</th>
<th>Interval between injection and irradiation (hr)</th>
<th>Neutron flux ($n/cm^2/sec$)</th>
<th>Neutron exposure time (min)</th>
<th>Neutron fluence ($n/cm^2$)</th>
<th>Average $^{10}$B in blood ($\mu$g/g)</th>
<th>Total radiation (rads)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2-1</td>
<td>30</td>
<td>30</td>
<td>$1.28 \times 10^9$</td>
<td>180</td>
<td>$1.38 \times 10^{18}$</td>
<td>33.25</td>
<td>5.040</td>
<td>sacrificed</td>
</tr>
<tr>
<td>S2-2</td>
<td>30</td>
<td>5</td>
<td>$1.12 \times 10^9$</td>
<td>180</td>
<td>$1.21 \times 10^{18}$</td>
<td>48.05</td>
<td>6.020</td>
<td>one month after BNCT</td>
</tr>
<tr>
<td>S2-3</td>
<td>73</td>
<td>10</td>
<td>$1.13 \times 10^9$</td>
<td>180</td>
<td>$1.22 \times 10^{18}$</td>
<td>66.03</td>
<td>8.021</td>
<td></td>
</tr>
<tr>
<td>S2-4</td>
<td>50</td>
<td>90</td>
<td>$8.70 \times 10^8$</td>
<td>150</td>
<td>$0.78 \times 10^{18}$</td>
<td>47.17</td>
<td>3.864</td>
<td>sacrificed</td>
</tr>
<tr>
<td>S2-5</td>
<td>75</td>
<td>15</td>
<td>$8.04 \times 10^8$</td>
<td>180</td>
<td>$0.86 \times 10^{18}$</td>
<td>82.43</td>
<td>7.017</td>
<td>one year after BNCT</td>
</tr>
<tr>
<td>S2-6</td>
<td>50</td>
<td>30</td>
<td>$8.82 \times 10^8$</td>
<td>180</td>
<td>$0.95 \times 10^{18}$</td>
<td>102.0</td>
<td>9.338</td>
<td></td>
</tr>
</tbody>
</table>

a) Gamma rate is 25 rads/hr.
b) Intravascular dose at the surface of brain in the radiation field.
Fig. 3. A coronal section of canine brain (S2–3), one month after an intravascularly produced radiation of 9300 rads was irradiated at the surface of the left hemisphere (arrow) by BNCT: LFB stain.

Fig. 4. A coronal section of a canine brain (S2–6), one year after an intravascularly produced radiation of 8000 rads was irradiated at the surface of the left hemisphere (arrow) by BNCT: LFB stain.

Fig. 5. Microscopic picture of the irradiated site of same brain (S2–3) shown in Fig. 3: (a) HE stain (×40), (b) Folzer stain (×200).

nearly constant neutron fluence. All these dogs were separated into two groups based upon the total dose calculated in the manner so that the doses of three dogs in both groups will distribute in a similar dose range.

Clinical evaluation of the irradiated dogs: None of the dogs receiving the higher doses up to 9000 rads to their brain by BNCT has shown any clinical, neurological, hematological or other laboratory alterations. All these dogs were sacrificed on a certain time after the irradiation and underwent autopsy in the excellent physical conditions.

Histo-pathological findings: Three dogs from S2–1 to S2–3 were sacrificed one month after the irradiation for the investigation of
the acute effects of the irradiation, while other dogs from S2–4 to S2–6 were sacrificed one year after the irradiation for the investigation of the delayed effects of the irradiation. All brains in both groups showed no macroscopic alterations. Fig. 3 and 4 show the coronal sections of the irradiated brain of dog S2–3 and S2–6, both of which have received the highest dose in each group. No difference was found between left hemisphere (irradiated side shown by arrow) and right hemisphere (non-irradiated intact side).

Histological examinations of the brains in both groups have revealed none of pathological changes suggesting radiation damages, such as neuronal loss, vascular lesions and inflammatory changes. Fig. 5 (a and b) and 6 (a and b) show the normal histological structure of brain tissue at the site of the irradiation in dogs S2–3 and S2–6 respectively.

**DISCUSSION**

It is easily realized that, in principle, BNCT can render the radiation damage to normal tissue involved in the irradiation field minimal. As the fact, the high performance status scale of the patients with brain tumor successfully treated with BNCT [11] may be a good proof of less brain damage by this type of treatment. Performance status scale is an index to be used for the evaluation of quality of post-therapeutic life in tumor patients. However, it would be difficult to evaluate the pure effects of BNCT alone to the normal brain tissue in such patients, since most of these brains may have been injured by preceding conventional radiotherapy and/or surgery.

A controlled animal experiment should be essential to know the accurate effects of BNCT on the normal brain tissue, but only very little study has been made ever for the revised regimen of BNCT, which has been successfully used to treat the brain tumor [12] recently.

In the series of our previous experiments to give 2000 to 3000 rads to the normal canine
brain by BNCT, neither electron microscopic changes of the brain immediately after the irradiation [1] nor light-microscopic lesions in the brain within a year after the irradiation [7] have been revealed as yet. However, one of the major concerns regarding the safety of BNCT should be to make clear the long-term effects to the normal brain tissue, which may cause any neurologic and functional disorders as delayed complications.

In Series 1 of the present study, three normal canine brains were irradiated with BNCT in a similar regimen as that for human patients with brain tumor, and subjected to the histo-pathological examination three years after the BNCT to know the long-term effects of BNCT on the normal brain tissue. In none of these brain tissues, loss of neurons, gliosis, demyelination or any other pathological changes suggesting delayed radiation damage was observed.

From these findings, together with no unusual findings in clinic, neurological and laboratory data during the observation period for three years, it was revealed that the normal canine brain can well tolerate the irradiation by revised regimen of BNCT for the brain tumor even from the stand point of long-term post-irradiation effects. In addition, the fact that the diameter of the circular irradiation field is as long as about half of longitudinal length of the brain may be enough to stress further safety to the normal brain. Since craniotomy, which is generally accompanied in the revised regimen for the human brain tumor, has not been performed both in Series 1 and 2, the actual radiation dose at the surface of brain can be estimated a little less than that measured at the surface of the skull. However, this should be small enough to be neglected in this study due to the thin canine skull and low attenuation of the gamma ray contaminated, comparing with big advantages to enable the evaluation of BNCT with minimum effects of infection and mechanical injury on the brain by the craniotomy.

The tolerance of the normal brain to BNCT has been attributed to the fact that the radiation doses to the normal brain tissue are mainly only from the adjacent blood vessels, since the boron compound is difficult to pass from blood to cell through the blood-brain barrier existing in the normal brain tissue. Therefore the radiation doses as high as 3000 rads in our experiments are those which can be expected from the blood in the vascular lumen at the brain surface. Assuming the $^{10}\text{B}$ to be distributed uniformly throughout the lumen of a capillary of the brain, it is clear that a significant fraction of the $^{10}\text{B} \rightarrow ^7\text{Li}$ energy released within the lumen will not be absorbed by the capillary wall. Analytical and Monte Carlo calculations have shown that the BNCT dose to the vasculature lining is considerately low and is estimated as approximately one third of the dose delivered to the blood [14, 15, 17]. According to this calculation, it can be easily understood that the actual dose to the vascular wall at the surface of the normal brain in our experiments may have been less than 1000 rads, which must be low enough not to have caused any clinical and histopathological changes of the normal brain tissue. In general, the normal brain tissue is thought to tolerate a single radiation dose of at least 2000 rads [10].

These characteristic low radiation doses to the normal brain tissue and its vascular wall with BNCT seemed to have substantiated the feasibility of irradiating higher doses to the normal brain tissue without causing damages, therefore of giving higher doses to tumor cells also.

In Series 2 of the present study, the cerebral vessels of the dogs were exposed to varying amounts of intraluminal radiations ranging from 3800 to 9300 rads at the surface of their brains. None of the dogs receiving these high doses by BNCT procedure has shown any clinical, neurological, hematological or other laboratory alterations. Histological studies of brain tissue at one month and at
one year after irradiation, could not uncover any signs suggesting radiation damage even in the dogs which received the highest doses. Meanwhile, in X-ray irradiation by similar experimental design [4], serious histological changes have been reported to occur in the irradiated site of the brain receiving 3800 rads.

The histological brain lesions by neutron irradiation with the excessive $^{10}$B in the circulating blood, which is a similar design as that for the present experiment, have been documented in the pathological studies on the patients under early trials of BNCT, which failed due to unexpectedly large radiation doses to the brain vasculature [2, 3]. The most common histo-pathological findings in these cases were massive cerebral edema and acute necrotizing lesion of vessels of all sizes in cases died within a month, and the intense radiation necrosis with extreme thickening and fibrosis of blood vessel walls in cases died in a year respectively. In our experiments, although post-irradiation brain edema was intended to be controlled by large doses of steroid administered, any findings suggesting radiation necrosis with damage to the walls of capillaries and small arterioles within the irradiated cerebral tissue have never been observed.

As far as we can judge on the basis of our one-year observation, the normal canine brain seems to tolerate as high as 7000 to 9000 rads of radiation produced in the cerebral vascular lumen by $^{10}$B(n, α)$^7$Li reaction. When we think that only one third of this intravascular dose will be irradiated to the vascular endothelial lining as described before and taking into consideration of the quick attenuation of thermal neutrons, the dose to be actually irradiated to the vascular wall of the normal brain might be less than 2000 rads, which is the generally accepted maximum tolerable single dose to the normal brain. These facts may be enough to rationally support the advantage of BNCT, in which more effective therapeutic dose can be irradiated to the tumor with minimum damage to the surrounding normal tissue.

The minimal effects of BNCT on the normal brain tissue have been proved by these two series of experiments on the canine brain in the present paper. These results not only demonstrate the safety of the current regimen of boron neutron capture therapy, but also suggest a possible expansion of the safety limit for the range of the therapy.

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