Possible Application of Boron Neutron Capture Therapy to Canine Osteosarcoma

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ABSTRACT. Possibility for successful treatment of canine osteosarcoma by boron neutron capture therapy (BNCT) was demonstrated based upon an uptake study of the boron compound and an experimental treatment by BNCT. In the uptake study following intravenous administration of Na2B12H11SH, satisfactorily higher boron concentration with some variation between tumors is likely to be obtained 12 hours after the administration, together with significantly lower boron levels in blood and bone. Based upon these results, osteosarcoma of a mongrel dog was successfully treated by BNCT. The tumor received approximately 3800 rads with single neutron irradiation (approximately 1.4×10^13 n./cm^2) about 12 hours after intravenous infusion of Na2B12H11SH of 96% enriched ^10B in the ratio of 50 mg ^10B/kg. Clinical and radiographical improvements were remarkable and no neoplastic cell was found in any part of the original neoplastic lesion and its surrounding tissue at the time of autopsy after 30 days.—KEY WORDS: boron, dog, neutron capture, osteosarcoma, radiation.

Osteosarcoma is the most frequent bone tumor in the dog [14, 22, 23], and it’s well described characteristics are thought to be well comparable to those of human malignant bone tumors [4, 5, 17, 18], which have been treated with much frustration in prognosis. Nearly all primary bone tumors of the appendicular skeleton in the dog are malignant and highly interesting not only to veterinary clinical oncologists, but to comparative oncologists.

The treatment of dogs with osteogenic sarcoma results in relatively few long time survivals despite advances in surgical, radiotherapeutic, chemotherapeutic, and immunotherapeutic techniques together with or without amputation of the affected limb. A final goal in the treatment of primary bone tumors both in dogs and human patients is preservation of the limb or affected bony part. Attempts have been made to accomplish this goal by systemic or local perfusion of drugs and irradiation. However, these methods have generally proved unsatisfactory.

Response of canine osteogenic sarcoma to irradiation has been discouraging. In a study treating with conventional radiotherapy (X-ray or gamma ray) or fast neutrons, osteogenic sarcoma is considered nonresponsive to irradiation [3], with some cessation of tumor growth. In another study on thirty-one small animals with osteogenic sarcoma, an average of 5000 rads by various forms of radiation resulted in the percentage cure of zero [25]. In general, poor results in cancer therapy are due mainly to the limitation, by the presence of normal tissues within the radiation field, of the doses that can be delivered to the tumor. Therefore, in radiation therapy of any kind of tumors, it is desirable to deliver the lowest possible dose to the associated normal tissues.

Boron neutron capture therapy (BNCT) was first suggested by Locher in 1936 [19] and has been studied as a selective cancer therapy. Unlike conventional treatment modalities, BNCT exploits the short ranges (approximately 10 μm or less) and high energy
losses from the $^{10}$B$(n, \alpha)^{7}$Li reaction ions (mainly $\alpha$ particles) so that the radiation dose is differentially delivered on a cellular level rendering surrounding healthy tissue intact, provided the boron selectivity is appropriate and the thermal neutron exposure is sufficient. Thermal neutron itself is low enough in energy not to give any serious damages to normal tissues free from the boron compound. Although major efforts in BNCT have been directed toward the treatment of brain tumors due to the advantage of the blood-brain barrier [28], and successfully applied to the brain tumor of human patients by Hatanaka and his colleagues using Na$_2$B$_2$H$_{11}$SH as a suitable boron compound [8, 9], tumors in other parts of the body could in principle be treated also by BNCT. In view of the fact that presently accepted treatment of certain bone tumors, especially osteosarcoma, continues to yield a very poor prognosis, BNCT remains a promising technique.

The success of BNCT for the treatment of cancer is ultimately dependent upon the selective delivery of a sufficient number of $^{10}$B atoms to tumor target cells. In the present paper, as pre-clinical studies to gain further insight into the possibility of treating osteosarcoma with BNCT, uptake study of the boron compound by osteosarcoma and a preliminary experimental treatment of osteosarcoma by BNCT have been conducted.

MATERIALS AND METHODS

Animals: Five dogs bearing naturally occurring osteosarcoma, which was histologically characterized on biopsied specimen, were used in the experiments. The signalments of these dogs are described in Table 1.

Uptake study of the boron compound: A study was conducted to determine the boron concentrations in the tumor and the blood of osteosarcoma-bearing dogs which have been administered the compound Na$_2$B$_2$H$_{11}$SH, which is same organic compound as used in BNCT and synthesized with natural boron for this study. 50 mg of boron per kilogram of body weight was administered intravenously in a single dose by drip. The tumor tissue specimen were then biopsied from three to four sites at different time intervals (up to 48 hr after boron administration) together with simultaneous bleeding, and they were analyzed for boron content by a colorimetric assay [13]. Although the boron-containing tissues of primary importance are tumor and blood, the boon concentration in normal cortical bone tissue was also analyzed only at the last stage of all the study except one dog (No. 1). Based upon these data, time-dependent fluctuations of boron concentrations and tumor/blood and tumor/normal bone (only limited number of specimen) ratios were studied.

Procedures of BNCT for osteosarcoma: One dog, five-year-old female mongrel, bearing osteosarcoma on the left stifle joint, was treated by BNCT. 12 hr prior to neutron irradiation, the dog was injected intravenously with the boron compound, Na$_2$B$_2$H$_{11}$SH, enriched to 92% $^{10}$B. $^{10}$B dose administered was 50 mg/kg B.W.. The animal was anesthetized and underwent the surgical exposure of the tumor by means of skin reflection with remaining part covered with 30% boron-containing shielding rubber sheet. Since the tumor mass was too large for sufficient penetration by thermal neutron which has a half-value layer of 1.8 cm, the tumor bulk was surgically reduced prior to the irradiation and the remaining tumor was about 2 cm thick.

The irradiation facility used was a well-collimated, highly thermalized horizontal beam located in the irradiation room directly close to the MuITR (Musashi University Institute of Technology Reactor: 100 KW maximum operating power). The irradiation was carried out to a targeted fluence of $10^{13}$ n./cm$^2$ at the tumor surface which corresponded to roughly 4 hr with the reactor at full power.

During the irradiation, the anesthesia was
managed by the remotely controlling procedures using various monitors and the specially devised long and thin intravenous catheter at the separated distant room, which was perfectly radio-protected.

Dosimetry in BNCT for osteosarcoma: In order to obtain experimental dosimetry measurements during BNCT, thermoluminescence dosimeters (TLDs) and gold foils as the detectors for gamma ray and neutron flux respectively were placed at various positions on the animal’s body surface. Total surface dose to the tumor was determined by measuring the thermal neutron flux at the site of the tumor and calculating the ^10^B dose at this point. This was accomplished by sampling the blood just prior to and after irradiation and measuring the ^10^B concentrations by a colorimetric assay [13]. An average concentration was taken as a representative value. The tumor dose was estimated from the chemical analysis of boron in the tumor specimen taken from four different sites of the tumor just prior to the irradiation.

Clinical and histo-pathological evaluation of BNCT for osteosarcoma: The tumor regression together with general physical conditions was monitored periodically after the treatment clinically, radiologically and hematologically. Since this dog treated by BNCT has been suffering from lung metastasis even at the time of the first admission, the dog was sacrificed thirty days after BNCT and the histo-pathological examinations were held in the routine procedures.

RESULTS

Uptake study of the boron compound: Boron concentration in the blood, the tumor and normal bone (only at 48 hr except No. 1 dog) 12, 24, 36 and 48 hr after intravenous injection of the boron compound (50 mg B/kg B.W.) are summarized in Table 1, together with tumor/blood and tumor/bone ratios. The highest boron concentration was obtained 12 hr after the injection in both blood and tumor and was followed by progressive diminish. Concentration in the tumor was always higher than that in the blood in all specimen with some variation from 15.6 to 33.5 μg/g. Boron concentration in the bone was generally low compared with that in the blood.

The relationship between the fluctuation of boron concentration in the blood and that in the tumor was not always uniform. In No. 1 and No. 3 dogs, boron concentration in the tumor was much higher than that in the blood even at 12 hr. On the other hand, in No. 2 and No. 4 dogs, boron concentration in the tumor was only moderately higher than that in the blood. Histological studies could not clarify any significant relationship between such variations in boron uptake and the histological features of each tumor used in this study. However, the difference between tumor and blood became progressively marked due to more rapid decrease of boron concentration in the blood. Fig. 1 indicates these fluctuations of (a) No. 1 dog and (b) No. 4 dog.

Fig. 2 shows the time-dependent fluctuation of tumor/blood ratio of boron concentration of all four cases following the administration of the boron compound. Although the ratio at 12 hr varied from 1.6 to 7.2 between dogs, the ratio was likely increasing up to 10 during 48 hr except No. 3 dog. Tumor/bone ratio was also studied in a limited number of specimen, and the ratio at 48 hr was more than 3 in 3 of 4 cases.

All tumor specimen were taken from 4 different sites of the tumor at the time of sampling and only the average value was described in this paper. In Fig. 3, boron concentration in 4 different sites of all tumor 12 hr after administration of the boron compound is shown. There are some variations in boron concentration from site to site, but all specimen had higher boron concentration than blood.
Table 1. Boron concentration and their ratio between tumor and blood or bone after administration of the boron compound (50 mg \(^{10}\)B/kg B.W.) to dogs bearing osteosarcoma

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Signalements</th>
<th>Boron concentration and ratio at different time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pointer, male, 5 y, osteosarcoma at proximal humerus</td>
<td>Blood (μg/ml) 3.7, 1.1, 0.5, 1.0</td>
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<td></td>
<td></td>
<td>Tumor (μg/g) 15.6, 5.2, 3.4, 3.1</td>
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<td>Bone (μg/g) 1.5, 1.1, —, 1.0</td>
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<td></td>
<td></td>
<td>Tumor/blood (ratio) 4.2, 4.7, 6.8, —</td>
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<tr>
<td></td>
<td></td>
<td>Tumor/bone (ratio) 10.4, 4.7, —, 3.1</td>
</tr>
<tr>
<td>2</td>
<td>Shiba Inu, male, 14 y, osteosarcoma at distal radius</td>
<td>Blood (μg/ml) 20.3, 10.7, 3.2, 2.1</td>
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<tr>
<td></td>
<td></td>
<td>Tumor (μg/g) 32.8, 24.7, 20.2, 14.5</td>
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<tr>
<td></td>
<td></td>
<td>Bone (μg/g) —, —, —, 4.1</td>
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<tr>
<td></td>
<td></td>
<td>Tumor/blood (ratio) 1.6, 2.3, 6.3, 7.0</td>
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<td></td>
<td></td>
<td>Tumor/bone (ratio) —, —, —, 3.5</td>
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<tr>
<td>3</td>
<td>Great Dane, male, 1 y, osteosarcoma at distal radius</td>
<td>Blood (μg/ml) 2.7, 2.1, 1.5, 1.3</td>
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<tr>
<td></td>
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<td>Tumor (μg/g) 19.3, 8.0, 5.3, 5.4</td>
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<td>Bone (μg/g) —, —, —, 4.3</td>
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<td></td>
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<td>Tumor/blood (ratio) 7.2, 3.8, 3.5, 4.2</td>
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<td>Tumor/bone (ratio) —, —, —, 1.3</td>
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<tr>
<td>4</td>
<td>Boxer, male, 6 y, osteosarcoma at distal tibia</td>
<td>Blood (μg/ml) 20.5, 11.0, 2.5, 1.5</td>
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<tr>
<td></td>
<td></td>
<td>Tumor (μg/g) 33.5, 25.0, 20.5, 15.0</td>
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<td></td>
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<td>Bone (μg/g) —, —, —, 4.5</td>
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<td></td>
<td></td>
<td>Tumor/blood (ratio) 1.6, 2.3, 8.2, 10.0</td>
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<td>Tumor/bone (ratio) —, —, —, 3.3</td>
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a) average concentration of 3 to 4 specimen from different sites.

Radiation dose given to osteosarcoma by BNCT: The basic calculation of BNCT dose was made according to Deuch and Murray [7] based upon the pertinent informations as follows; Neutron fluence: \(1.4 \times 10^{13} \text{n.}/\text{cm}^2\), boron concentration in the tumor: 36.19 μg/g (an average of 26.85, 33.70, 36.68, and 47.54 at 4 different sites). Including the gamma dose as high as 150 rads for 4 h irradiation at MultiTR, the total dose irradiated to this tumor was estimated to be approximately 3800 rads from the above mentioned...
NEUTRON CAPTURE THERAPY FOR OSTEOSARCOMA

Fig. 2. Time dependent fluctuation of tumor/blood ratio of boron concentration in each case after administration of the boron compound.

Fig. 3. Boron concentration in four different sites of each case 12 hours after administration of the boron compound. Dotted line represents level of boron concentration in blood.

calculation.

The radiation dose measured at the surface of the dog during the irradiation was less than 50 rads as gamma dose and $1 \times 10^{10} \text{n./cm}^2$ as neutron fluence respectively.

Clinical findings of osteosarcoma treated by BNCT: As shown in a lateral radiograph taken before BNCT (Fig. 4), the destructive process was rather restricted to the medulla partially involving the cortex in this case. The tumor looked to have arisen within the metaphysis and broken through the cortex. Cortical penetration and subperiosteal extension have caused a large soft tissue mass contigu-
Fig. 4. Lateral radiograph of osteosarcoma originating from the tibial crest of a mongrel dog; before BNCT.

Fig. 5. Microscopic picture of the biopsied specimen from the case shown in Figure 4; before BNCT. HE. ×100.

Fig. 6. Radiograph of the case shown in Figure 4; 8 days after BNCT.

Fig. 7. Radiograph of the case shown in Figure 4; 20 days after BNCT.

ous to the bone, in which perpendicular striations of new bone formation is seen within the subperiosteal cuff. Beside these characteristic radiographical findings as osteosarcoma, this was histopathologically designated as osteosarcoma on a biopsied specimen (Fig.
5).

Clinical differences after BNCT was quite evident. The dog before treatment was showing a clinical course of limping without any articular movement of the stifle and weight bearing on the affected limb. However, these manifestations were markedly improved as early as a week after BNCT. Time sequential radiographies showing the course of tumor regression after BNCT also demonstrated a positive effect. A radiograph taken 8 days after the irradiation (Fig. 6) shows that the tumor size had dramatically decreased. This improvement was followed by a nearly complete remission of the clinical manifestations and a steady shrinking of tumor size on radiograph for the next two weeks. After 20 days, the tumor mass could hardly be seen radiographically (Fig. 7).

No adverse effects following the irradiation have been observed clinically, and the blood counts and the liver functions have been found to be within the normal range after the treatment.

**Histopathological findings of osteosarcoma treated by BNCT**: Since long-term survival could not be expected in this case due to lung metastasis found at the time of the first admission, this dog was sacrificed 30 days after BNCT, and autopsy and histopathological examination were held to confirm the therapeutic effect of the treatment. No neoplastic cell was found in any part of the original neoplastic lesion and its surrounding tissue (Figs. 8; a and b). Furthermore, no significant damage to the bone matrix was seen in the adjacent bone tissue.

**DISCUSSION**

In order to take advantage of boron neutron capture therapy as an selective cancer therapy, selection and synthesis of boron compounds are most crucial problems. Such compounds should penetrate into the malignant tissue, but should not remain in the blood [6] and the normal cells. Since BNCT has been mainly focused to the brain tumor, much of the effort to produce boron compounds with improved tissue distribution
properties has relied on the blood-brain barrier phenomenon in the normal brain to achieve high tumor-to-brain boron concentration ratios. An alternative approach involves the use of boron compounds with a strong affinity to tumor cells including tumor-specific antibodies [10, 12, 20, 21, 26, 32, 33, 34]. As the trials of this kind are still in process now and encountering many problems to be overcome, it appears at the present time that the compound Na₂B₁₂H₁₁SH [9, 27] remains the first choice for our study in view of its availability, ¹⁰B enrichment, low toxicity, and pharmacological properties.

In the present study, we have shown that the boron compound can be selectively taken by canine osteosarcoma with individual variations in higher concentration compared with blood and normal bone.

Previous experiences in BNCT for the brain tumor [8, 9] have shown that at least 30 μg ¹⁰B/g of tumor is essential to get more than 3000 rads with low-gamma thermal neutron fluence (1×10¹³ n./cm²). However, beside the absolute ¹⁰B concentration in tumor cells, the potential therapeutic efficacy of a boron compound is strongly dependent on the tumor/blood and tumor/normal bone boron concentration ratio. The higher this ratio, the more favorable will be the subsequent dose distribution between the tumor cells and the microvascular endothelium [35] and the bone cells in the normal bone tissues. In our studies on canine osteosarcoma, boron concentration higher than 30 μg/g was found to be obtained in a half of cases 12 hr after boron administration with tumor/blood ratio less than 2. Although the relatively small number of the specimen for boron analysis may make conclusive interpretation of the results difficult, low uptake of the boron compound by the normal bone is strongly suspected from our data. If this concept could be accepted, the main radiation dose by ¹⁰B(n, α)⁷Li reaction to the normal bone would be the intravascular radiation, only one third of which has been thought to be irradiated to the vascular wall [15, 16, 24]. Safeness of the present regimen of BNCT to the brain vasculature and its damage due to over dose by BNCT have been well described [1, 2]. This has led us to conduct a preclinical study to treat canine osteosarcoma by BNCT with the neutron irradiation 12 hr after the intravenous administration of the boron compound. The effectiveness of BNCT in this limited study of canine osteosarcoma was strongly demonstrated, clinically, radiographically and histologically, although only one experiment was arranged and long-term survival was not achieved. In general, it is thought that more than 10000 rads of X-ray should be irradiated to get approximately 80 percent death of osteosarcoma cells, while 2000 rads of fast neutron can cause 100 percent death of the cells [29, 30]. Our present experience obtaining nearly complete disappearance of tumor cells from the lesion together with clinical and radiographical improvement may suggest rather high relative biological effectiveness (RBE) of BNCT as far as the therapeutic effect on osteosarcoma is used as a criterion.

In most of bone tumors, the transition from zones of marked lysis to zones of uninvolved bone is gradual, making the borders of the lesion indistinct. Therefore, the amputation of the affected limb has been thought to be only the choice and the topical surgery has been discarded. Recently, a new trend to combine chemotherapy and radiotherapy including fast neutron irradiation has focused on osteosarcoma [11, 30, 31], but there should be a certain limitation as non-selective cancer therapy to such procedures also.

The successful demonstration of selective boron uptake and therapeutic effectiveness in a case of osteosarcoma presented in this paper, although the follow-up period was not long enough, would guarantee the clinical success. For further development of BNCT, efforts to develop new compounds, to investi-
igate more sophisticated mechanisms of boron delivery, to expand the target of applications, and to develop improved neutron beams will all contribute to the ultimate success of neutron capture therapy in the treatment of osteosarcoma without performing amputation of the affected extremity not only in animals but in human patients.

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REFERENCES

要約

硼素中性子捕捉療法による犬の骨肉腫治療の可能性に関する検討：竹内 寛(東京大学農学部家畜外科学教室)—犬の骨肉腫が硼素中性子捕捉療法（BNCT）で治癒する可能性を、硼素化合物の取り込み試験ならびに1例の治療実験の結果から示唆することができた。取り込み試験では、NaB_{13}H_{13}SHを50mB/kgの割合で静注し、腫瘍・血液・骨のB濃度の経時の変化を調べた。各症例ごとに病期差があり、必ずしも均一な傾向は得られなかったが、注入12時間後には、硼素の摂取濃度が有効なBNCTに十分なレベルであり、かつ血液および骨中の濃度が明らかに低い傾向が認められた。これらの検討結果に基づいて、1例の骨肉腫例に対してBNCTを実施した。^{10}Bを用いて合成した上記化合物を、50mB/^{10}B/kgの割合で静注し、12時間後に1.4×10^{14}n/cm²の中性子照射を武蔵工大原子炉で実施した。腫瘍表面の受けた合計線量は約3800レドと考えられるが、照射後は臨床所見およびX線所見の改善が目覚ましく、20日後のX線写真上には、腫瘍塊がほぼ認められなかった。30日後に安楽死させ、病理組織学的検査を行った。腫瘍原発部位およびその周辺には腫瘍細胞は検出されず、正常骨組織の著明な変性も認められなかった。