

## The Effects of Boron Neutron Capture Therapy on Liver Tumors and Normal Hepatocytes in Mice

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To explore the feasibility of employing boron neutron capture therapy (BNCT) to treat liver tumors, the effects of BNCT were investigated by using liver tumor models and normal hepatocytes in mice. Liver tumor models in C3H mice were developed by intrasplenic injection of SCCVII tumor cells. After borocaptate sodium (BSH) and boronophenylalanine (BPA) administration, <sup>10</sup>B concentrations were measured in tumors and liver and the liver was irradiated with thermal neutrons. The effects of BNCT on the tumor and normal hepatocytes were studied by using colony formation assay and micronucleus assay, respectively. To compare the effects of BSH-BNCT and BPA-BNCT, the compound biological effectiveness (CBE) factor was determined. The CBE factors for BSH on the tumor were 4.22 and 2.29 using  $D_{10}$  and  $D_0$  as endpoints, respectively. Those for BPA were 9.94 and 5.64. In the case of hepatocytes, the CBE factors for BSH and BPA were 0.94 and 4.25, respectively. Tumor-to-liver ratios of boron concentration following BSH and BPA administration were 0.3 and 2.8, respectively. Considering the accumulation ratios of <sup>10</sup>B, the therapeutic gain factors for BSH and BPA were 0.7–1.3 and 3.8–6.6, respectively. Therefore, it may be feasible to treat liver tumors with BPA-BNCT.

Key words: BNCT — BSH — BPA — Liver tumors

Various therapeutic options have been examined for the treatment of unresectable primary and secondary malignant tumors in the liver. These include percutaneous ethanol injection, intraarterial drug infusion, embolization, intraarterial chemoembolization and radiotherapy. However, the results of these treatments have not been satisfactory. In many cases there are multiple tumors, and the liver cannot tolerate large treatment volumes, especially in radiotherapy. Boron neutron capture therapy (BNCT) which combines an administration of a tumor-seeking boron compound with thermal neutron irradiation might be an effective treatment for malignant hepatic tumors for the following reasons. In the boron neutron capture reaction, <sup>10</sup>B absorbs a thermal neutron and releases two high linear energy transfer (LET) particles, an  $\alpha$  particle and a <sup>7</sup>Li nucleus. These particles have path lengths of 9 and 4  $\mu$ m, respectively, depositing all their energy within a range of about one cell diameter from the capture reaction site. Accordingly these high-LET particles have large relative biological effectiveness (RBE), and effective cell killing can be expected in malignant tumors, which are resistant to conventional radiotherapy using photons or electrons. The path lengths of these particles are so short that if tumor cells accumulate the boron compound selectively, only tumor cells can be killed.

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A major limitation in the application of BNCT using thermal neutrons is the tumor depth. Deep-seated tumors, such as hepatic tumors, are difficult to treat because of the poor penetration of thermal neutrons, which have energies <0.5 eV and a 15–16 mm half-value layer in water. Epithermal beams, with energies 0.5 eV to 10 keV, have better penetration in tissues.<sup>1)</sup> Although the probability of reaction of an epithermal neutron with <sup>10</sup>B is much smaller than that of a thermal neutron, as they lose energy with tissue depth, they fall into the thermal range which is so much more effective in the boron neutron capture reaction. The peak of this thermalization of epithermal neutrons is about 3 cm in depth. The heavy water facility at Kyoto University Reactor (KUR) was remodeled in 1996 for production of an epithermal neutron beam.<sup>2)</sup> We expected that the aforesaid problem (poor penetration with a thermal neutron beam) might be overcome and it might become feasible to apply BNCT to malignant hepatic tumors.

We examined the effects of BNCT on experimental liver tumors and normal hepatocytes by using two boron compounds which are being used in clinical BNCT trials for malignant glioma and malignant melanoma,<sup>3–5)</sup> to explore the feasibility of BNCT treatment of liver tumors.

### MATERIALS AND METHODS

**Mice and liver tumor model** Eight- to twelve-week-old male C3H/He mice were used for this study. Six to eight mice were used for each data point. The liver tumor model

was prepared as follows. SCCVII tumor cells (mouse squamous cell carcinoma), were maintained in Eagle's minimum essential medium supplemented with 292 mg/liter glutamine and 12.5% fetal calf serum. Cells were collected from monolayer cultures, and suspended in phosphate-buffered saline (PBS) at a concentration of  $2 \times 10^7$ /ml. Mice were anesthetized by intraperitoneal injection of Nembutal (50 mg/kg body weight) and tumor cells ( $1 \times 10^6/50 \mu\text{l}$ ) were injected into the surgically exposed spleen, which was resected 3 min later. In 80–90% of all transplanted mice, a few to several hundred liver tumor nodules developed 10–14 days after transplantation. Tumor size ranged from 1 mm to 1–2 cm. Only liver tumors larger than 1 cm were subjected to experiments in the present work.

**Boron compound administration** We employed two boron compounds, borocaptate sodium (BSH) and *p*-boronophenylalanine (BPA). These compounds have been used in clinical trials for treatment of malignant glioma and malignant melanoma, respectively.<sup>3–5</sup> Because of the difference in the solubility of the compounds in water at physiological pH, they were administered by different routes. BSH was dissolved in physiological saline at a concentration of 6 mg/ml, and was administered intraperitoneally at a dose of 75 mg/kg body weight. Because BPA was relatively insoluble at physiological pH, it was prepared as an aqueous suspension at a concentration of 100 mg/ml, and administered intragastrically at a dose of 1500 mg/kg body weight. Both compounds were purchased from BBI (Boron Biologicals, Inc., Raleigh, NC).

**Measurement of boron concentrations in tumors and liver** Mice were killed by cervical dislocation 15 to 240 min after compound administration, and tumor and liver samples were collected for  $^{10}\text{B}$  measurement. The  $^{10}\text{B}$  concentrations in these tissues were measured by prompt  $\gamma$ -ray spectrometry using a thermal neutron guide tube installed at KUR.

**Thermal neutron irradiation and dose measurement** In the case of epithermal neutron irradiation, the incident neutrons lose energy due to scattering by hydrogen and fall into the thermal neutron range in the body tissues. Within a few centimeters below the body surface, the thermal neutrons flux derived from epithermal neutrons increases steeply. On the other hand, in the case of a thermal neutron beam, we can get more homogeneous thermal neutrons, especially within 1 cm below the body surface.<sup>2</sup> Since typical depths for mouse liver are about 2 cm, we used a thermal neutron beam for irradiation in this study, and not an epithermal neutron beam.

Thermal neutrons containing negligible amounts of epithermal and fast neutrons were delivered from the right side of the mouse body at the heavy water facility of KUR. During the irradiation, each mouse was held stationary in a LiF box (6×4×3 cm) with a 3×3 cm window over

the upper abdomen, including the whole liver. Since LiF blocks thermal neutrons, the remainder of the body was shielded from the thermal neutron irradiation. The timing of neutron irradiation was based upon the data on  $^{10}\text{B}$  concentrations in the tumor and liver. All irradiations were carried out in mice anesthetized with Nembutal (50 mg/kg body weight).

Neutron fluences were measured by radioactivation of gold foils (3 mm diameter; 0.05 mm thick) on the body surface and in the liver parenchyma 0.5 cm below the liver surface of a dead mouse in a preliminary study to determine the ratio of the neutron fluxes (liver/body surface). The thermal neutron fluence in the liver parenchyma of each mouse was calculated from the ratio of the neutron fluxes obtained in the preliminary study and the actually measured neutron fluence at the body surface of each mouse. The average flux of thermal neutrons on the body surface was  $2.0 \times 10^9$  n/cm<sup>2</sup>/s. Thermoluminescent dosimeters were used for  $\gamma$ -ray dosimetry. Total and core  $\gamma$ -ray dose rates were 0.0003 Gy/s and 0.00006 Gy/s at a reactor power level of 5 MW, respectively.

Thermal neutron fluence was converted to physical dose using Eq. 1:

$$D = \phi[(6.933 \times 10^{-14}B) + (6.782 \times 10^{-14}N)] + G \quad (1)$$

where  $D$ =physical dose (Gy),  $B$ = $^{10}\text{B}$  concentration (ppm),  $N$ =nitrogen concentration (weight %),  $\phi$ =thermal neutron fluence (n/cm<sup>2</sup>),  $G$ = $\gamma$ -ray dose (Gy). The first term in the parenthesis is the dose (Gy) from the  $^{10}\text{B}(n, \alpha)^7\text{Li}$  reaction per neutron. The second term is the dose (Gy) from the nitrogen neutron capture reaction ( $^{14}\text{N}(n, p)^{14}\text{C}$  reaction) per neutron. A value of 3.483% was used as  $N$  (concentration of the nitrogen in the hepatic tissues).

To compare the effects of thermal neutrons and photons,  $^{60}\text{Co}$   $\gamma$ -ray irradiation was also employed. Mice were fixed in the box and irradiated over the upper abdomen at the dose rate of 0.18 Gy/min through a 3 cm slit between 5 cm thick lead shields which protected the remainder of the body.

**Evaluation of the effects on tumors** After thermal neutron irradiation, the liver tumors were excised from mice for colony formation assay. After excision, the tumors were minced with scissors. A single cell suspension was then obtained by digesting tumor fragments using a mixture of 0.05% trypsin and 0.02% EDTA at 37°C for 15 min. The suspension was diluted and plated into culture dishes in appropriate numbers to yield between 20–200 colonies per dish. After incubation for 10 days, the dishes were fixed with ethanol, stained with crystal violet, and colonies were counted to determine the survival fraction (SF). SF was calculated by dividing the colony formation rate of irradiated tumors by that of the unirradiated tumors. The average colony formation rate of these control tumors was 50%.

**Evaluation of the effects on the normal hepatocytes** In order to investigate the radiation response of hepatocytes, we used the micronucleus method. The procedure of MN assay was reported previously.<sup>6)</sup> Briefly, a partial hepatectomy (resection of 2/3 of the liver) was performed immediately after irradiation of the mice to stimulate hepatocytes to divide and express radiation-induced micronuclei. Five days later, a laparotomy was performed, the inferior vena cava was cut, and 2 ml of a solution of 0.25% trypsin was infused into the liver through a fine catheter inserted into the portal vein. The right lateral portion of the liver, which had received the highest radiation dose, was removed, minced and filtered with nylon mesh. The

cells were washed by low-speed centrifugation with PBS, fixed with Carnoy's solution, dropped onto a glass microscope slide, dried, and stained with Hoechst 33342 before observation. The micronuclei in about 500 hepatocytes per mouse were examined by a fluorescence microscope. The fraction of cells not expressing micronuclei was determined to compare the effects under various BNCT conditions and  $\gamma$ -ray irradiation.

**RESULTS**

**Pharmacokinetics of boron compounds** The clearance curve of <sup>10</sup>B following BSH administration was biphasic in the tumors and liver with a  $T_{1/2}$  of  $36.3 \pm 0.6$  and  $18.2 \pm 1.1$  min in the initial phase and  $103.5 \pm 10.2$  and  $118 \pm 14.1$  min in the second phase, respectively (Fig. 1). The <sup>10</sup>B concentration in the tumors after BSH administration decreased very rapidly. Average <sup>10</sup>B concentrations in tumors and liver at 30 min after BSH administration were 11 and 35 ppm, respectively (Fig. 1). On the other hand, in the case of BPA, <sup>10</sup>B concentration in tumors reached a maximum (11 ppm) at 3 h, and decreased very gradually thereafter. The <sup>10</sup>B concentration in the liver after BPA administration was nearly constant between 2 and 4 h, being 3.9 ppm at 3 h after BPA administration (Fig. 1).

**Dosimetry of thermal neutron irradiation** Based on the data of <sup>10</sup>B concentrations in tumors after BSH and BPA administration, the timing of neutron irradiation was determined. In order to irradiate tumors with the same physical dose and dose rate, thermal neutron irradiation was started 3 h after BPA administration, and in the case of BSH, neutron exposure was started so that the middle of the irradiation time would be 30 min after BSH injection, in view of the rapid clearance of <sup>10</sup>B from tumors. The <sup>10</sup>B concentration in the tumor during the irradiation averaged 11 ppm for BSH and BPA. The physical dose rates of each radiation component to tumor and liver are shown in Table I. The contribution of the <sup>10</sup>B(n,  $\alpha$ )<sup>7</sup>Li component to the total dose of tumors was 65%. We irradiated normal liver at the same timing when tumors were irradiated in order to evaluate the damage to normal liver during neutron irradiation of the tumors. The <sup>10</sup>B concentration in the liver during the irradiation averaged 35 ppm for BSH and 3.9 ppm for BPA. The contribution of the <sup>10</sup>B(n,  $\alpha$ )<sup>7</sup>Li component to the total dose was 85% in the case of BSH+neutrons, which was greater than that for BPA+neutrons (40%). The dose rate from BSH+neutrons was 4.2 times higher than that from BPA+neutrons (0.341/0.082).

It was assumed that there would be no dose rate effect on hepatocytes arising from the high LET component of the BNCT reaction, because survival curves of cells irradiated with high LET irradiations are linear and have no shoulder,<sup>6,7)</sup> indicating that these cells are unable to repair sublethal damage.

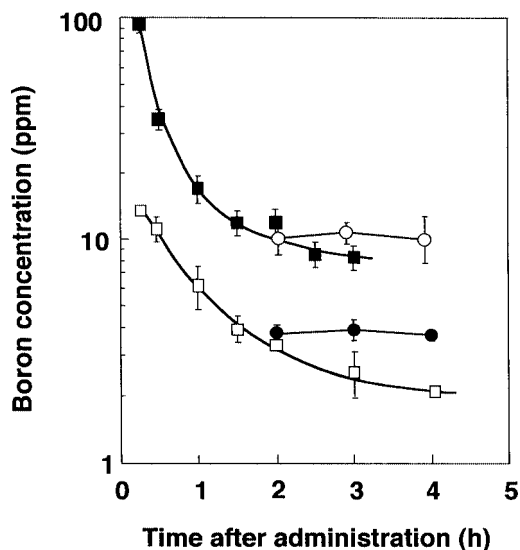


Fig. 1. <sup>10</sup>B concentrations (ppm) in the liver and tumor after intraperitoneal injection of BSH at 75 mg/kg body weight and intragastric administration of BPA at 1500 mg/kg body weight. Liver after BSH, ■; tumor after BSH, □; liver after BPA, ●; tumor after BPA, ○. Each data point with vertical line represents the mean value and standard deviation.

Table I. Dose Rate of Each Radiation Component to the Tumor and Liver

Type of radiation	Absorbed physical dose rate (Gy/min)
<sup>10</sup> B(n, $\alpha$ ) <sup>7</sup> Li component	0.092 <sup>a)</sup> (tumor)
	0.29 <sup>b)</sup> (liver, BSH)
	0.032 <sup>c)</sup> (liver, BPA)
<sup>14</sup> N(n, p) <sup>14</sup> C component	0.028
$\gamma$ -ray component	0.022

a) <sup>10</sup>B concentration of 11 ppm in tumor.

b) <sup>10</sup>B concentration of 35 ppm in liver.

c) <sup>10</sup>B concentration of 3.9 ppm in liver.

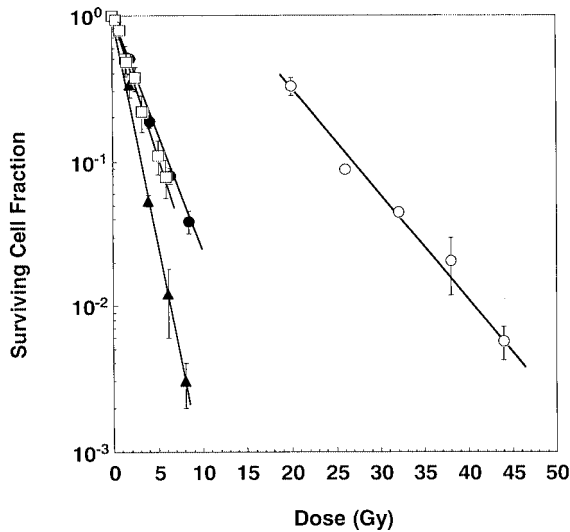


Fig. 2. Surviving cell fractions of SCCVII tumor cells transplanted in the liver. BPA+Beam,  $\blacktriangle$ ; BSH+Beam,  $\bullet$ ; Beam alone,  $\square$ ;  $\gamma$ -ray,  $\circ$ . Each data point with vertical line represents the mean value and standard deviation.

**Response of liver tumors** Fig. 2 shows the survival curves of tumor cells derived from tumors that were treated with BNCT as a function of dose (in Gy). The exponential parts of cell survival curves were fitted to the following equation:  $-\ln SF = \alpha_1 D + b$ . The  $\alpha_1$  values for  $\gamma$ -rays, Beam alone, BSH+Beam and BPA+Beam were 0.159, 0.444, 0.392 and 0.737, respectively (Table II). Thermal neutron irradiation either in the presence or absence of boron compound was more effective than  $^{60}\text{Co}$   $\gamma$ -ray irradiation. BPA-BNCT was most effective, and, interestingly, at the same physical dose to the tumor, BSH-BNCT was less effective than neutron irradiation alone.

**Response of normal hepatocytes** Proportions of cells without micronuclei as a function of dose (in Gy) are shown in Fig. 3. The curves decreased exponentially with increasing radiation dose. The data were fitted to the following equation:  $-\ln PMN0 = \alpha_2 D + c$ , where PMN0 is the proportion of cells without micronuclei and  $D$  is the radiation dose in Gy. The  $\alpha_2$  values for  $\gamma$ -rays, Beam alone, BSH+Beam and BPA+Beam were 0.107, 0.147, 0.107 and 0.267, respectively (Table II). At equal physical doses, BPA-BNCT caused the most damage to normal hepatocytes. On the other hand, BSH-BNCT and  $\gamma$ -ray irradiation caused almost the same damage to normal hepatocytes.

**Compound biological effectiveness (CBE) factors for individual beam** Boron neutron capture irradiation modalities consist of a mixture of low LET radiation ( $\gamma$ -rays) and high LET radiations ( $\alpha$  and Li particles, and induced protons), so definition of the biological effects of the  $^{10}\text{B}(n, \alpha)^7\text{Li}$  reaction relative to photons is compli-

Table II. Parameters of Cell Survival Curves and Dose-response Coefficients for Proportion of Cells without Micronuclei for  $\gamma$ -rays and for the Different BNCT Irradiations

Radiation group	$-\ln SF = \alpha_1 D + b$	$-\ln PMN0 = \alpha_2 D + c$
	$D$ : radiation dose (Gy)	
	$\alpha_1$ ( $\text{Gy}^{-1}$ )	$\alpha_2$ ( $\text{Gy}^{-1}$ )
BSH+Beam	0.392	0.107
BPA+Beam	0.737	0.267
Beam alone	0.444	0.147
$\gamma$ -ray	0.159	0.107

SF, survival fraction; PMN0, proportion of cells without micronucleus.

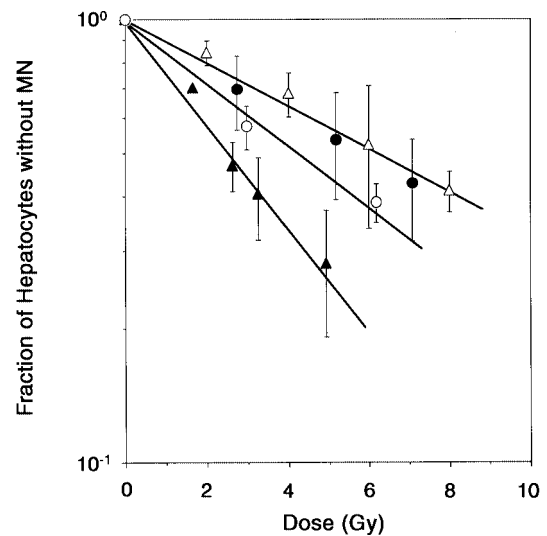


Fig. 3. Proportion of cells without micronuclei as a function of radiation dose. BPA+Beam,  $\blacktriangle$ ; BSH+Beam,  $\bullet$ ; Beam alone,  $\circ$ ;  $\gamma$ -ray,  $\Delta$ . Each data point with vertical line represents the mean value and standard deviation.

cated. The tracks of the  $\alpha$  particle and  $^7\text{Li}$  nucleus are so short that the distance between the boron compound and the cell nucleus is a very important factor determining the effect on the nucleus for  $^{10}\text{B}(n, \alpha)^7\text{Li}$  reaction, and different boron compounds are generally expected to take different microdistributions in tissues or cells. To compare the effects of the  $^{10}\text{B}(n, \alpha)^7\text{Li}$  reaction by different boron compounds relative to photons, the term compound biological effectiveness (CBE, below) has been defined as an alternative to the RBE.<sup>8)</sup>

$$\text{CBE} = \frac{\{\text{X-ray} - (\text{Thermal beam component} \times \text{RBE})\}}{^{10}\text{B}(n, \alpha)^7\text{Li component}} \quad (2)$$

The sum of the  $^{14}\text{N}(n, p)^{14}\text{C}$  and  $\gamma$ -ray components is defined as the thermal beam component. The RBE for the

Table III. RBE and CBE Factors for Tumor and Hepatocytes following Thermal Neutron Irradiation in Combination with BSH and BPA and Thermal Neutron Beam Alone

	Tumor		Hepatocytes
	$D_{10}$	$D_0$	$D_0$
BSH+Beam	4.22	2.29	0.94
BPA+Beam	9.94	5.64	4.25
Beam alone	5.07 (RBE)	2.79 (RBE)	1.37 (RBE)

KUR thermal beam component (in the absence of  $^{10}\text{B}$ ) and the CBE factors for BSH and BPA on liver tumors were determined using the physical dose  $D_{10}$  that result in SF=0.1 and  $D_0$  values as endpoints. CBE factor is calculated in similar way to the RBE. Because boron neutron capture irradiation consists of mixed irradiation modes, subtraction of the products of doses of  $\gamma$ -ray and induced protons by the RBE from X-ray doses required for equal biological effect is required in the numerator of Eq. 2. The values are listed in Table III. The CBE factors for BSH on the tumors were 4.22 and 2.29 and that for BPA was 9.94 and 5.64. The CBE factors for BPA were higher than those for BSH.

We determined the RBE or CBE factors on normal hepatocytes using MN assay. Using the  $D_0$  value as the endpoint, the CBE factor for BSH was 0.94 and that for BPA was 4.25 (Table III).

## DISCUSSION

**Pharmacokinetics of boron compounds** The concentration of  $^{10}\text{B}$  from BSH in the liver is higher than that in the tumor and blood. The reason for the high accumulation of BSH in the liver is uncertain. One possibility is that BSH may bind glutathione (GSH) by forming a covalent disulfide bond. GSH is the substrate of the glutathione conjugation reaction (detoxification reaction), and occurs at high levels in hepatocytes.<sup>9,10</sup> Joel *et al.* reported that administration of GSH monoesters (GSH-ME) significantly increased cellular GSH and boron uptake and retention in tumors.<sup>11</sup> This suggests that the higher accumulation of BSH in the liver may be related to the high GSH level in hepatocytes and result in a low tumor-to-liver ratio. However, the CBE factor for BSH on hepatocytes was not so high compared with that for BPA or the tumors. If BSH binds intracellular GSH, intracellular  $^{10}\text{B}$  from BSH would be expected to result in more damage to the hepatocytes. So, there may be another reason for the high accumulation of BSH in the liver.

Coderre *et al.* reported that BPA selectively delivered boron to areas of actively dividing tumor cells. Because almost all normal hepatocytes are resting cells in the G0

phase, accumulation of BPA in hepatocytes is low, and the tumor-to-liver ratio of BPA is higher than that of BSH.<sup>12</sup>

**Response of liver tumors** The microdistribution of  $^{10}\text{B}$  in tissue and cells varies with the boronated compound and even the same compound shows different microdistributions in different tissues. The macroscopic radiation doses to tumors were identical between tumors given BPA-BNCT and BSH-BNCT because tumor  $^{10}\text{B}$  concentrations in both compound groups were the same (11 ppm). Therefore, these data suggest that the actual radiation dose to the tumor cell nucleus from BSH-BNCT might be smaller than that from BPA-BNCT. Because BPA is incorporated into tumor cells via the metabolic pathway of amino acids<sup>13</sup> and BSH hardly enters the cells,<sup>14,15</sup> the higher CBE factor for BPA can be ascribed to intracellular localization.<sup>12</sup>

**Response of hepatocytes** Jirtle *et al.* reported the clonogenicity of hepatocytes following irradiation using a transplantation system.<sup>16</sup> This assay might be suitable to compare the radiation response of hepatocytes with that of hepatic tumors, because the effects on hepatic tumors were evaluated with colony formation assay. Although this hepatocyte clonogenic assay is very reliable and elegant, it is very laborious. Therefore, we used the micronucleus assay, which can estimate chromosomal damage, and is much easier and more rapid than clonogenic assay using a transplantation system.

The CBE factors for BSH and BPA on hepatocytes were 0.94 and 4.25, respectively, and these values are lower than those on the tumor. A possible reason for this is that normal hepatocytes are larger in size than tumor cells (SCCVII in this study) and have rich cytoplasm and a relatively small nucleus. Since high-LET particles from  $^{10}\text{B}(n, \alpha)^7\text{Li}$  reaction have very short path length, if a boron compound is distributed homogeneously in the tumor and liver, the tumor cells with their large nucleus/cytoplasm ratio would be more damaged.

**Clinical prospect** To provide therapeutic benefit for the treatment of hepatic tumors, the boron neutron capture reaction should cause more damage to the tumors than to the surrounding hepatocytes. The ratio of CBE factors on liver tumors and on hepatocytes was 2.44 (2.29/0.94)–4.49 (4.22/0.94) for BSH and 1.33 (5.64/4.25)–2.34 (9.94/4.25) for BPA. Thus, the CBE factors for both BSH and BPA on liver tumors are higher than those on hepatocytes. However, BSH has the serious disadvantage that it accumulated more in liver than in liver tumors (the tumor:liver ratio was 0.3). In the clinical situation, the tumors and surrounding liver are irradiated simultaneously, so the physical dose delivered to the liver is greater than to the tumors. This situation is not acceptable. On the other hand, the  $^{10}\text{B}$  concentration after BPA administration in the tumors is higher than in the liver (the tumor:liver ratio was 2.8). Therapeutic gain factors are obtained by multi-

plying the ratio of CBE factors between liver tumors and hepatocytes by the tumor-to-hepatocyte boron accumulation ratio. These factors are 0.7–1.3 for BSH and 3.8–6.6 for BPA. This means that BPA is preferable to BSH for clinical application.

We must overcome the problem of poor penetration of thermal neutrons in the application of BNCT to liver tumors. The high therapeutic gain factor for BPA and the use of epithermal neutrons might solve this problem. The probability of reaction of an epithermal neutron with  $^{10}\text{B}$  is much smaller than that of a thermal neutron. For boron neutron capture reaction, the epithermal neutron must lose energy in the body. The peak of thermalization of epithermal neutrons is about 2–3 cm in depth and the neutron fluence rate decreased steeply in the body to one-fourth to one-seventh of the peak at around 8–10 cm in depth in the case of the KUR beam. Since the therapeutic gain factor for BPA is 3.8–6.6, liver tumors within 8–10 cm below the body surface are more irradiated with high LET particles from the boron neutron reaction than surrounding normal hepatocytes. Therefore, tumors located below the liver surface may be treated with BPA-BNCT without causing severe damage to normal hepatocytes. In order to treat liver tumors, however, another point that we have to consider is the absolute boron concentration in the tumors. The  $^{10}\text{B}$  concentration in hepatic tumors after BPA administration was not high enough in the present study. In our study, a squamous cell carcinoma model of mouse was employed for convenience of *in vivo-in vitro* assay of the effects, but others have reported a larger accumulation ratio of BPA in adenocarcinoma of rat ( $>4$ ).<sup>17)</sup> Similar results were reported using an intra-hepatic nude mouse xenograft model by Mallesch *et al.*<sup>18)</sup> They found that after

intraperitoneal injection of 12 mg of BPA, the  $^{10}\text{B}$  concentration was 16 ppm and the tumor:normal liver ratio was in the 3–5 range. On the other hand, the  $^{10}\text{B}$  concentration in soft tissue sarcoma in rats after intraperitoneal injection of 600 mg/kg of BPA was reported to be 36 ppm.<sup>19)</sup> This concentration in soft tissue sarcoma is about 3 times higher than that in hepatic tumors. The low concentration in the hepatic tumors requires a higher thermal neutron fluence and leads to an elevated background dose produced by the beam alone. Since liver tissues are radiosensitive, it is very important to reduce the background dose. Both higher absolute boron concentrations in the tumors and higher tumor-to-liver ratio are prerequisite for treating hepatic tumors with BNCT. Transcatheter arterial administration of boron compounds may enable both higher  $^{10}\text{B}$  concentration and tumor-to-liver ratio to be achieved. This technique of intraarterial administration of an anticancer drug or embolizing material has been well-established and is widely used to treat hepatocellular carcinoma (HCC) or liver metastases. So, we intend to investigate the  $^{10}\text{B}$  concentration in hepatic tumors of rat or rabbit after intraarterial administration of BPA.

#### ACKNOWLEDGMENTS

The authors would like to thank Dr. H. B. Stone for fruitful discussion of the data and for linguistic advice. This study was supported by Grants-in-Aid for Cancer Research (08266108, 09255228, 10153233) from the Ministry of Education, Science, Sports and Culture of Japan.

(Received April 19, 2000/Revised July 21, 2000/Accepted July 27, 2000)

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