

# Suppression of Tumor Growth in a Rabbit Hepatic Cancer Model by Boron Neutron Capture Therapy With Liposomal Boron Delivery Systems

HIRONOBU YANAGIE<sup>1,2,3</sup>, MASASHI YANAGAWA<sup>4</sup>, YASUYUKI MORISHITA<sup>5</sup>, ATSUKO SHINOHARA<sup>6</sup>, NOVRIANA DEWI<sup>7</sup>, YASUMASA NONAKA<sup>8</sup>, YOSHITAKA FURUYA<sup>9</sup>, RYOUJI MIZUMACHI<sup>10</sup>, YUUJI MURATA<sup>10</sup>, HIROYUKI NAKAMURA<sup>11</sup>, MINORU SUZUKI<sup>12</sup>, YOSHINORI SAKURAI<sup>12</sup>, HIROKI TANAKA<sup>12</sup>, SHINICHIRO MASUNAGA<sup>12</sup>, KOJI ONO<sup>13</sup>, TAKUMICHI SUGIHARA<sup>7</sup>, MASAYUKI NASHIMOTO<sup>3</sup>, HARUO YAMAUCHI<sup>2,14</sup>, MINORU ONO<sup>2,14</sup>, JUN NAKAJIMA<sup>2,15</sup> and HIROYUKI TAKAHASHI<sup>1,2</sup>

<sup>1</sup>Institute of Engineering Innovation, School of Engineering, The University of Tokyo, Tokyo, Japan;

<sup>2</sup>Cooperative Unit of Medicine and Engineering, The University of Tokyo Hospital, Tokyo, Japan;

<sup>3</sup>Research Institute of Healthy Living, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan;

<sup>4</sup>Veterinary Medical Center, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan;

<sup>5</sup>Department of Human and Molecular Pathology,

Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;

<sup>6</sup>Graduate School of Humanities, Seisen University, Tokyo, Japan;

<sup>7</sup>Laboratory of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan;

<sup>8</sup>Department of Surgery, Keiai-kai Houyou Hospital, Hanamaki, Japan;

<sup>9</sup>Department of Surgery, Sodegaura Satukidai Hospital, Sodegaura, Japan;

<sup>10</sup>Department of Pharmacology, Kumamoto Institute Branch, LSI Medience Ltd. Co., Uto, Japan;

<sup>11</sup>Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan;

<sup>12</sup>Institute for Integrated Radiation and Nuclear Science, Kyoto University, Osaka, Japan;

<sup>13</sup>BNCT Joint Clinical Institute, Osaka Medical Pharmaceutical University, Osaka, Japan;

<sup>14</sup>Department of Cardiovascular Surgery, The University of Tokyo Hospital, Tokyo, Japan;

<sup>15</sup>Department of Thoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

**Abstract.** *Background/Aim:* Tumor cell destruction by boron neutron capture therapy (BNCT) is attributed to the nuclear reaction between <sup>10</sup>B and thermal neutrons. The accumulation of <sup>10</sup>B atoms in tumor cells without affecting adjacent healthy cells is crucial for effective BNCT. We previously reported that several types of liposomal boron

delivery systems (BDS) delivered effective numbers of boron atoms to cancer tissues, and showed tumor-growth suppression after thermal neutron irradiation. In the present study, we examined the effects of BNCT after intra-arterial infusion of <sup>10</sup>B-borono-dodecaborate (<sup>10</sup>B<sub>12</sub>) by liposomal BDS in rabbit hepatic cancer models. *Materials and Methods:* We prepared <sup>10</sup>B<sub>12</sub>-entrapped transferrin-conjugated polyethylene glycol liposomes constructed with distearoyl-boron lipid (TF-PEG-DSBL), and performed thermal neutron irradiation at the Kyoto University Institute for Integrated Radiation and Nuclear Science after intra-arterial infusion into rabbit VX-2 hepatic tumors. *Results:* Concentrations of <sup>10</sup>B in VX-2 tumors on delivery with TF-PEG-DSBL liposomes reached 25 ppm on day 3 after the injection. Tumor growth was suppressed by thermal neutron irradiation after intra-arterial injection of this <sup>10</sup>B<sub>12</sub>-containing liposomal BDS, without damage to normal cells. *Conclusion:* The present results demonstrate the applicability

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*Correspondence to:* Hironobu Yanagie, MD, Ph.D., Institute of Engineering Innovation, School of Engineering, The University of Tokyo, 2-11-16 Yayoi, Bunkyo-ku, Tokyo 113-8656, Japan. Tel: +81 358009194, Fax: +81 358009195, e-mail: h.yanagie@gmail.com

*Key Words:* Boron neutron capture therapy, BNCT, transferrin-conjugated polyethylene glycol, PEG, liposome, <sup>10</sup>B-borono-dodecaborate <sup>10</sup>B<sub>12</sub>, distearoylboron lipid, DSBL, hepatocellular carcinoma, HCC.

of  $^{10}\text{B}$ -containing TF-PEG-DSBL liposomes as a novel intra-arterial boron carrier in BNCT for cancer.

Hepatocellular carcinoma (HCC) is one of the most difficult types of tumor to cure by surgery, chemotherapy, or radiotherapy (1, 2). Surgery is only indicated for 30% of patients with HCC due to the complications of liver cirrhosis and multiple intrahepatic tumors. In clinical settings, anticancer agents are generally administered intra-arterially after mixing with iodized poppy-seed oil (3). However, since anticancer agents easily separate from iodized poppy-seed oil within a short time period, they do not effectively accumulate in tumor cells. Higashi and co-workers previously reported the preparation of a long-term inseparable water-in-oil-in-water (WOW) emulsion containing 8-60 mg of epirubicin for use in arterial injection therapy for patients with HCC, and showed reductions in tumor sizes in patients with HCC (4-6).

Nanoscale liposomes have been extensively investigated as carriers for anticancer drugs in attempts to direct active agents to tumors or protect sensitive tissues from toxicity. Since the size of the WOW emulsion is in the micron ( $\mu\text{m}$ ) scale, liposomes are able to deliver drugs to cancer cells in tumors via the enhanced permeability and retention effect.

Boron neutron capture therapy (BNCT) has been used to inhibit the growth of various cancer types, such as malignant brain tumors, melanoma, and head and neck tumors (7-9). The cytotoxic effects of BNCT are due to a nuclear reaction between  $^{10}\text{B}$  and thermal neutrons, which induces high linear-energy transfer of  $\alpha$  particles and lithium recoil. These particles ( $\alpha$  and  $^7\text{Li}$ ) destroy cells within an approximately 10- $\mu\text{m}$  path length from the site of the capture reaction. Therefore, the development of selective boron delivery systems for effective BNCT is of importance (10-15). As one of these drug delivery systems, we reported that immunoliposomes carrying  $^{10}\text{B}$ -boronododecaborate ( $^{10}\text{BSH}$ ) exerted cytotoxic effects against human pancreatic carcinoma cells *in vitro* by BNCT (16), and an intra-tumoral injection of boronated immunoliposomes suppressed tumor growth *in vivo* by BNCT (17). We also previously prepared polyethylene glycol (PEG) liposomes as an effective  $^{10}\text{B}$  carrier (18, 19).

The accumulation of  $^{10}\text{B}$  atoms in tumor cells without affecting adjacent healthy cells is crucial for effective BNCT. Transferrin (TF)-conjugated PEG liposomes may be useful for *in vivo* cytoplasmic targeting by chemotherapeutic agents or plasmid DNAs that target cells. TF-PEG liposomes readily bind to cancer cells and are internalized by receptor-mediated endocytosis, which enhances the extravasation of liposomes into solid tumor tissue (19, 20).

We are interested in applying BNCT to the treatment of HCC. Therefore, in the present study, we developed  $^{10}\text{BSH}$ -entrapped TF-PEG liposomes with  $^{10}\text{B}$ -distearoyl-boron lipid (DSBL) (21, 22). These liposomes were used as a selective boron delivery system to cancer tissues in a VX-2

hepatic tumor model in order to investigate the application of BNCT to the treatment of HCC with the aim of increasing the selection of therapies available for patients (Figure 1).

## Materials and Methods

**Preparation of PEG and TF-PEG liposomes containing  $^{10}\text{B}$ -compound.** Boron-entrapped liposomes were prepared by the reverse-phase evaporation method and extrusion method (18, 19).

The composition ratio of liposomes in uniting-type 25% DSBL stealth liposomes (PEG-DSBL) was as follows: distearoylphosphatidylcholine (DSPC):DSBL:cholesterol:PEG-2000=0.75:0.25:1.0:0.11 (mol ratio). The concentration of boron entrapped in PEG-DSBL liposomes was 2,700 ppm.

The composition element ratio of entrapping-type  $^{10}\text{BSH}$ -entrapped stealth (PEG) liposomes was as follows: DSPC:cholesterol:PEG-2000=1.0:1.0:0.11 (mol ratio) +  $^{10}\text{BSH}$  (125 mmol/l). The enclosed boron concentration entrapped in PEG liposomes was 4700 ppm.

$^{10}\text{BSH}$ -entrapped TF-PEG-DSBL liposomes were prepared by the coupling of TF to the PEG-COOH moieties of PEG-DSBL liposomes according to the protocol described by Ishida *et al.* [DSPC:DSBL:cholesterol:PEG-2000=2840:336:1546:928 (/mg)] (20) (Figure 2).

Boron concentrations in prepared liposomes were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (24).

**Target tumor cells and rabbits.** Rabbit VX-2 cells (a Shope virus-derived squamous cell carcinoma cell line) were cultured *in vitro* and supplemented with 10% fetal bovine serum and 500  $\mu\text{g}$  streptomycin/penicillin under 5%  $\text{CO}_2$  conditions. Female New Zealand white rabbits with VX-2 cells inoculated into the left lobe of the liver were obtained from Nihon SLC Ltd. (Shizuoka, Japan) and used at a mean body weight of 2 kg (23). The procedures for tumor implantation and animal sacrifice were in accordance with the approved guidelines of the Institution's Animal Ethics Committee and with the Declaration of Helsinki (approval number; LSI Medience: P090818 & P100455, KUR: 2010-18 & 25).

**Evaluation of tumor-growth suppression by experimental BNCT.** Rabbit VX-2 cells were inoculated into the left lobe of the liver, and 2 weeks after tumor cell inoculation,  $^{10}\text{BSH}$ -TF-PEG-DSBL liposomes were administered by an intra-arterial injection via the proper hepatic artery into the VX-2 rabbit hepatic tumor model. Tumor-bearing rabbits were irradiated with thermal neutrons (fluence:  $2 \times 10^{12}$  n/cm $^{-2}$ ) at the Kyoto University Institute for Integrated Radiation and Nuclear Science 48 h after the intra-arterial injection of liposomes. Neutron fluence was measured by gold foil at two points on the frontal side of the abdomen and rear side of the rabbit holder, while the gamma ray dose was assessed using a thermoluminescent dosimeter at the same points. After irradiation, the size of the tumor and the status of the abdomen were investigated on day 14 after BNCT by sacrificing rabbits for morphological observations. Pathological analyses were also performed on tumor samples resected after neutron irradiation, fixed in Optimal Cutting Temperature compound, and frozen at  $-80^\circ\text{C}$ . Harvested tumor samples were sliced into 6- $\mu\text{m}$ -thick sections using a cryostat and deposited on glass slides before being stained with hematoxylin and eosin.

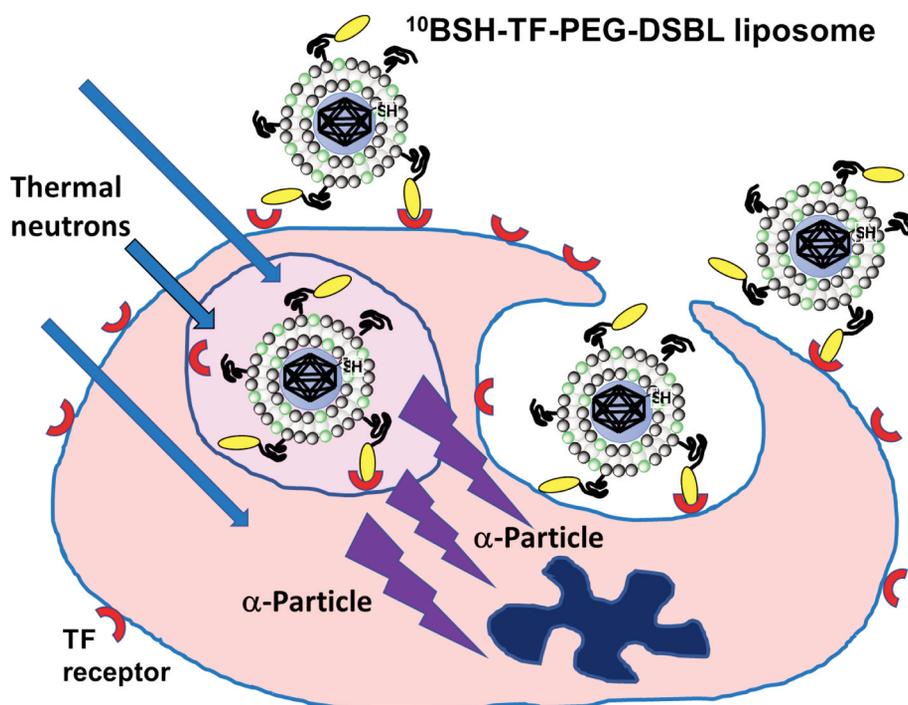


Figure 1. We propose the application of  $^{10}\text{B}$ -borono-dodecaborate-entrapped transferrin-conjugated polyethyleneglycol-distearoylboron lipid-coated ( $^{10}\text{BBSH-TF-PEG-DSBL}$ ) liposomes as a delivery carrier of boron compounds for intra-arterial injection in boron neutron capture therapy: As TF-PEG has an endocytic role, it can deliver  $^{10}\text{B}$  atoms into cancer cells. Use of DSBL increases the  $^{10}\text{B}$  concentration in cancer cells.

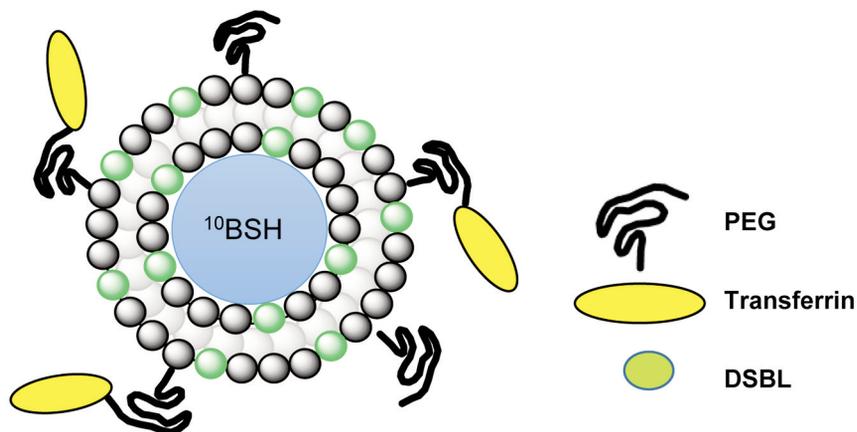


Figure 2. Schema of  $^{10}\text{B}$ -borono-dodecaborate ( $^{10}\text{BBSH}$ )-entrapped transferrin-conjugated polyethyleneglycol (PEG)-distearoylboron lipid (DSBL)-coated liposomes.

Pathological findings of tumors after intra-arterial injection of  $^{10}\text{BBSH-PEG-DSBL}$  liposomes. Histological and electron microscopic observations were performed. Rabbits were sacrificed 24 and 48 h after the injection of  $^{10}\text{BBSH-PEG-DSBL}$  liposomes. Livers were

resected and stored in Optimal Cutting Temperature compound frozen at  $-80^{\circ}\text{C}$ . Six-micrometer-thick sections of the same specimens were stained with hematoxylin and eosin for light microscopy to assess the induction of necrosis, hyalinization by BNCT.

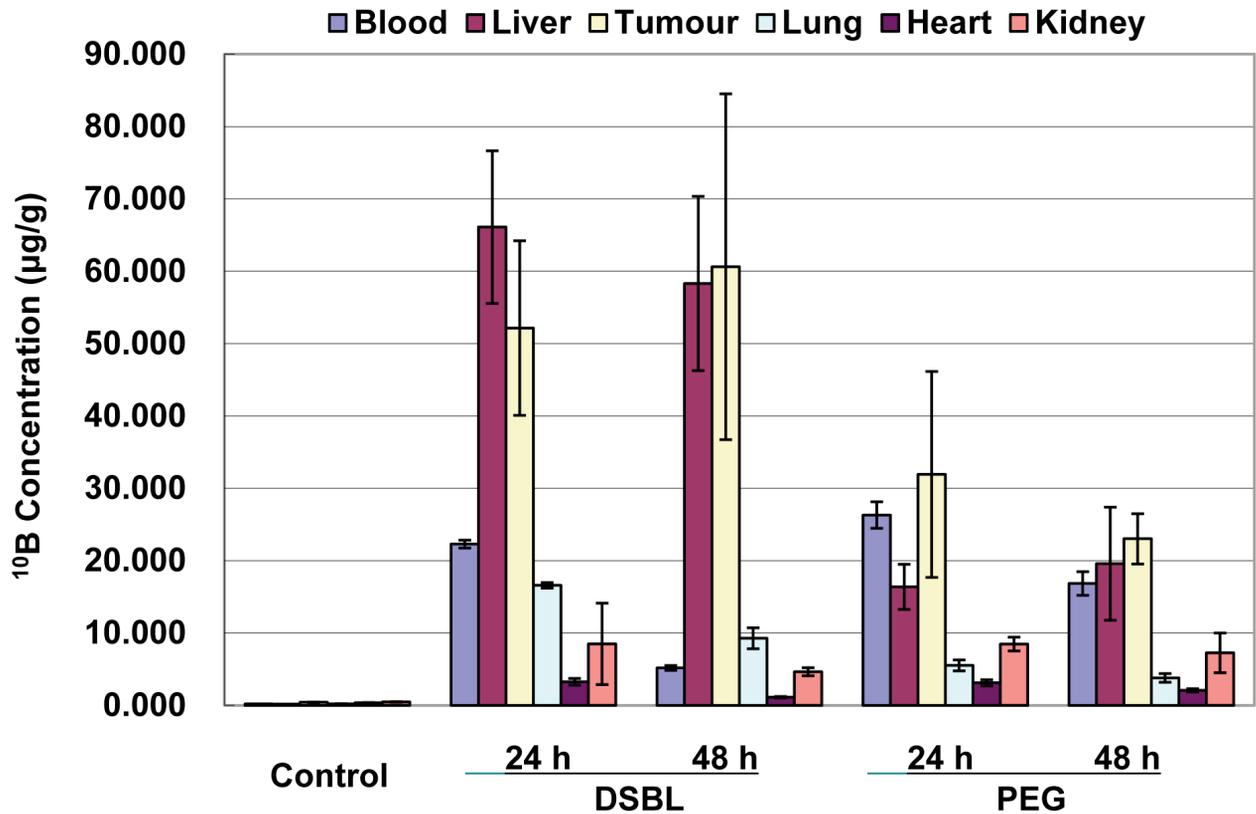


Figure 3. Measurement of boron concentrations in tumors and each organ after the intra-arterial injection of boron-entrapped polyethyleneglycol (PEG) liposomes in the VX-2 hepatic cancer-bearing rabbit model. Two types of <sup>10</sup>B-borono-dodecaborate (<sup>10</sup>BSH)-PEG liposomes were prepared: Distearoylboron lipid (DSBL) type and simple entrapping PEG type. Concentrations of <sup>10</sup>B were measured by inductively coupled plasma atomic emission spectroscopy at Juntendo University. Mean values and standard deviations were calculated (n=3).

Measurement of <sup>10</sup>B accumulated in each organ in vivo. We administered <sup>10</sup>BSH-containing liposomes (15 mg/kg) to tumor-bearing rabbits. Regarding the hepatic artery injection technique, the abdomen was opened under general anesthesia, the proper hepatic artery facing the liver was identified, the catheter or injection needle was inserted, and the hepatic artery injection of <sup>10</sup>BSH liposomes was performed. The administered volumes were 5.6 ml for <sup>10</sup>BSH-PEG-DSBL liposomes and 3.3 ml for <sup>10</sup>BSH-PEG liposomes. Boron concentrations in blood, VX-2 liver tumors, normal liver tissues, and other organs were measured using ICP-AES at 24 or 48 h after the intra-hepatic arterial injection of <sup>10</sup>BSH-PEG-DSBL or <sup>10</sup>BSH-PEG liposomes under general anesthesia in the VX-2 rabbit liver tumor model. Mean values and standard deviations were calculated. The number of rabbits in each group was three.

We also measured <sup>10</sup>B concentrations in blood, VX-2 liver tumors, and normal liver tissues using ICP-AES 24, 48, 72, or 120 h after the intra-hepatic arterial injection of <sup>10</sup>BSH-TF-PEG-DSBL liposomes. Tissues were weighed immediately after dissection and recorded as wet weights. Results were expressed as µg <sup>10</sup>B per g tissue. Mean values and standard deviations were calculated.

## Results

**Characterization of liposomes.** Mean <sup>10</sup>B concentrations in liposomes were measured using ICP-AES. The concentration of <sup>10</sup>B entrapped in <sup>10</sup>BSH-PEG-DSBL liposomes was 2700 ppm, and that in <sup>10</sup>BSH-PEG liposomes was 4,700 ppm. The <sup>10</sup>B concentration entrapped in <sup>10</sup>BSH-TF-PEG-DSBL liposomes was 3,200 ppm.

**Comparison of <sup>10</sup>B accumulation in each organ and biodistribution of <sup>10</sup>BSH-PEG and <sup>10</sup>BSH-PEG-DSBL liposomes after intra-hepatic arterial injection.** The <sup>10</sup>B concentrations in the VX-2 tumor using <sup>10</sup>BSH-PEG-DSBL and <sup>10</sup>BSH-PEG liposomes were 52.14±12.07 and 31.92±14.23 µg/g, respectively, 24 h after the intra-arterial injection, and were 60.62±23.89 and 23.02±3.47 µg/g, respectively, 48 h after (Figure 3). The <sup>10</sup>B concentration in the tumor using DSBL liposomes was two-fold higher than that of <sup>10</sup>BSH-PEG

liposomes. The amount of boron that accumulated was also two-fold higher in hepatic tumors than in normal hepatic tissue (31 ppm on average) with  $^{10}\text{B}$ BSH-PEG liposomes at 24 h after the intra-arterial injection (Figure 3).

*Histological and electron microscopic findings of tumors after intra-arterial injection of  $^{10}\text{B}$ BSH-PEG-DSBL liposomes.* Pathological findings revealed no damage to normal hepatic tissue with this dosage injection of  $^{10}\text{B}$ BSH-PEG-DSBL liposomes (Figure 4).

*Hepatic biodistribution of  $^{10}\text{B}$ BSH-TF-PEG-DSBL liposomes after intra-hepatic arterial injection.* Modifications to the surface of PEG liposomes by conjugation with TF increased the  $^{10}\text{B}$  concentration in tumors to around 25  $\mu\text{g/g}$  at 72 h after the intra-hepatic arterial injection of  $^{10}\text{B}$ BSH-TF-PEG-DSBL liposomes into the VX-2 rabbit hepatic tumor model. The concentration of boron in normal hepatic tissue was 10-15  $\mu\text{g/g}$  72 h after the intra-hepatic arterial injection (Figure 5). The tumor/normal liver  $^{10}\text{B}$  concentration ratio was at least 1.5-fold higher until 120 h after intra-hepatic arterial injection (Figure 5). When  $^{10}\text{B}$ BSH-TF-PEG-DSBL liposomes were administered intra-arterially at a dose of 6.4 mg  $^{10}\text{B}$ /kg, we observed the selective uptake of  $^{10}\text{B}$  by cancer tissues compared with that by normal hepatocytes in the VX-2 rabbit hepatic cancer model. TF-PEG-liposomes maintained a high  $^{10}\text{B}$  concentration in tumors, with concentrations higher than 25  $\mu\text{g/g}$  for at least 72 h after intra-arterial injection. This high retention of  $^{10}\text{B}$  in tumor tissue indicates that the binding and concomitant cellular uptake of extravasated TF-PEG liposomes occurred by TF receptor and receptor-mediated endocytosis, with the concentration of  $^{10}\text{B}$  in hepatocytes eventually decreasing, resulting in a tumor/normal tissue ratio of 2.0 at 48 h after liposomal injection.

*Morphological and pathological analyses after BNCT.* In order to examine the tumor growth-suppressing effects of BNCT, the VX-2 rabbit hepatic cancer model was subjected under general anesthesia to thermal neutron irradiation 48 h after the intra-arterial injection of  $^{10}\text{B}$ BSH-TF-PEG-DSBL liposomes at Kyoto University Institute for Integrated Radiation and Nuclear Science. The fluence of thermal neutrons was  $2 \times 10^{12}$  n/cm<sup>2</sup> on the surface of the beam port, and details of the physical dose from each neutron energy range, including measurement results on the rear side, are shown in Table I. Rabbits were observed for 2 weeks, sacrificed, and liver tumors and the intra-abdominal state were then examined. After BNCT, the suppression of tumor growth was noted with thermal neutron irradiation after the intra-arterial injection of  $^{10}\text{B}$ BSH-TF-PEG-DSBL liposomes, with smaller tumor sizes in the group treated with these liposomes than in the untreated group and the group treated only with thermal neutron irradiation (Figure 6; Table II). Many tumor nodules were detected in the liver, abdominal cavity, and peritoneum in the control group. Hematoxylin and

Table I. Irradiation mode at the Kyoto University Institute for Integrated Radiation and Nuclear Science for boron neutron capture therapy for the hepatic cancer model.

Position	Thermal neutron fluence (cm <sup>-2</sup> )	Epithermal neutron fluence (cm <sup>-2</sup> )
Surface	$2.0 \times 10^{12}$	$8.9 \times 10^{11}$
Rear side	$1.6 \times 10^{12}$	$2.8 \times 10^{11}$

Irradiation mode: Thermal neutron mode: OO-0000-F. 1 MW: 45 min. Cd ratio: ~9.4.

Table II. Tumor growth suppression by boron neutron capture therapy (BNCT) after an intra-arterial injection of  $^{10}\text{B}$ -borono-dodecaborate-entrapped transferrin-conjugated distearoyl-boron lipid-coated ( $^{10}\text{B}$ BSH-TF-PEG-DSBL) liposomes into the VX-2 hepatic tumor model. VX-2 cells were inoculated into the left lobe of the liver of female New Zealand White rabbits. Two weeks after the inoculation, tumor-bearing rabbits were irradiated with thermal neutrons (fluence:  $2 \times 10^{12}$  n/cm<sup>2</sup>) 48 h after intra-arterial injection of  $^{10}\text{B}$ BSH-TF-PEG-DSBL liposomes. After irradiation, the effects of BNCT were evaluated based on the tumor volume after 14 days (calculated as  $1/2 \times \text{length} \times \text{width}^2$ ). Values are the means  $\pm$  standard deviation.

Treatment	Number of rabbits	Tumor size (mm <sup>3</sup> )
$^{10}\text{B}$ BSH TF-PEG-DSBL liposomes	4	$452.0 \pm 280.5$
Thermal neutrons only	2	$1,474.3 \pm 37.8$
Untreated	1	2,232.6

eosin staining results shown in Figure 5 indicate the effectiveness of cancer cell killing by BNCT. Electron microscopic findings revealed apoptotic bodies and nuclear degradation in cancer cells (Figure 7).

## Discussion

PEG liposomes have a long circulation time and biodistribution patterns and pharmacokinetics that enhance their systemic therapeutic effects. The PEG liposome formulation prevents rapid uptake by the reticuloendothelial system and, thus, liposomes remain in the circulation for long periods. 'Stealth' liposomes in tumors combine slower plasma clearance and higher vascular permeability compared with conventional liposomes, and form depots of drugs in the perivascular space after extravasation which are available to neighboring cells within tumors for a few days (19, 20). Ishida *et al.* previously reported that PEG liposomes with an average diameter of 100-200 nm had the longest circulation time and the greatest accumulation in solid tumors *in vivo* (20). Maruyama *et al.* developed TF-binding PEG liposomes

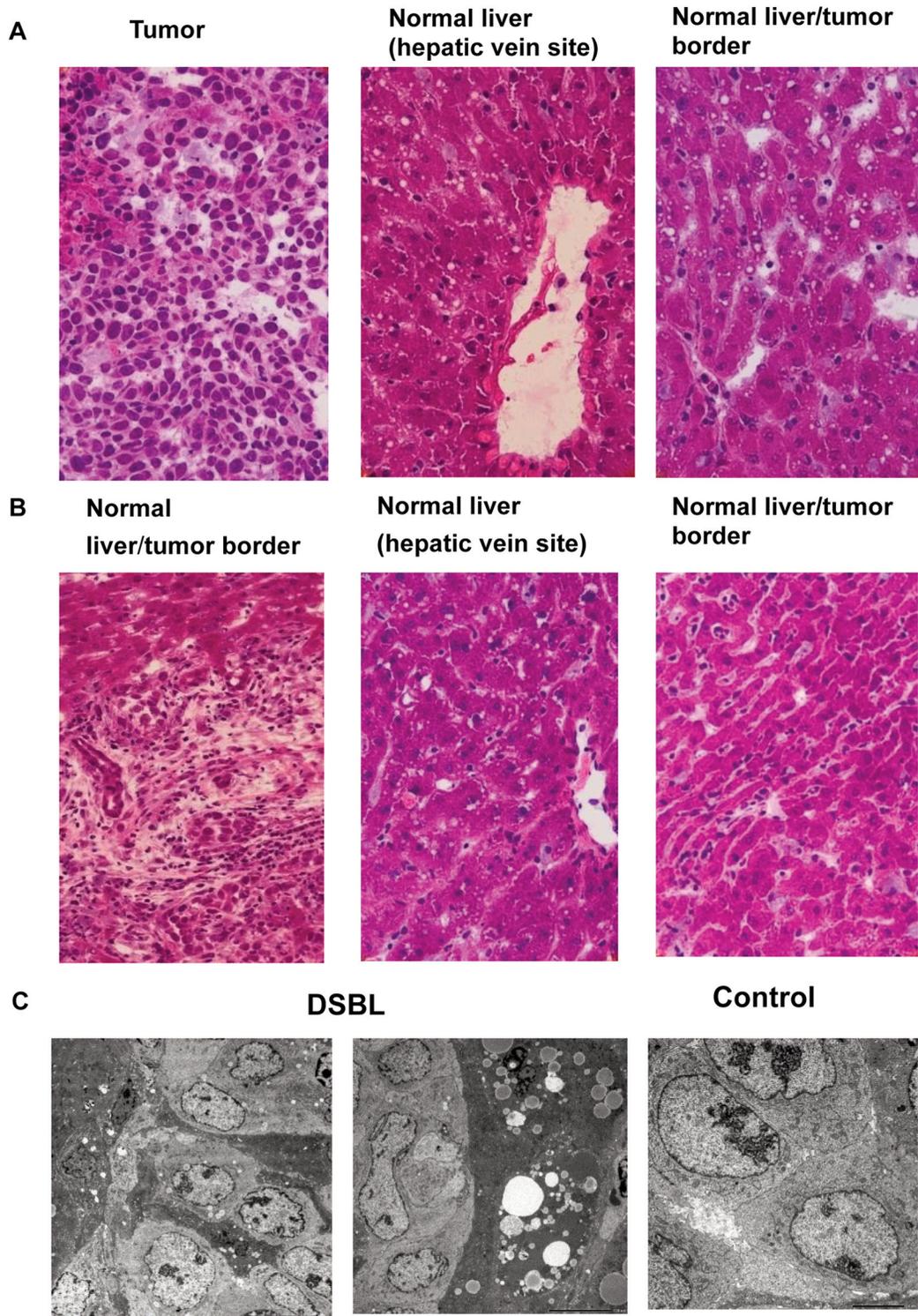


Figure 4. Pathological and histological examinations. No damage was observed in normal liver tissue under light (original magnification,  $\times 200$ ) and electron microscopy (original magnification,  $\times 600$ ) after intra-hepatic arterial injection of  $^{10}\text{B}$ -borono-dodecaborate-entrapped polyethyleneglycol distearoyl boron lipid-coated ( $^{10}\text{B}$ BSH-PEG-DSBL) liposomes. A: Fat droplets were detected at the boundary of the tumor and normal hepatic tissue 24 h after administration. Degeneration and hyalinization were not observed in hepatocytes. B: Fat droplets were only noted in the liver vein surrounding the hepatic lobulus after 48 h. This phenomenon may have been due to the drainage of lipids constructed of liposomes from the liver. C: More fatty vesicles were detected in VX-2 tumor tissues 24 h after the intra-arterial injection of  $^{10}\text{B}$ BSH-PEG-DSBL liposomes than in non-treated control groups by electron microscopy.

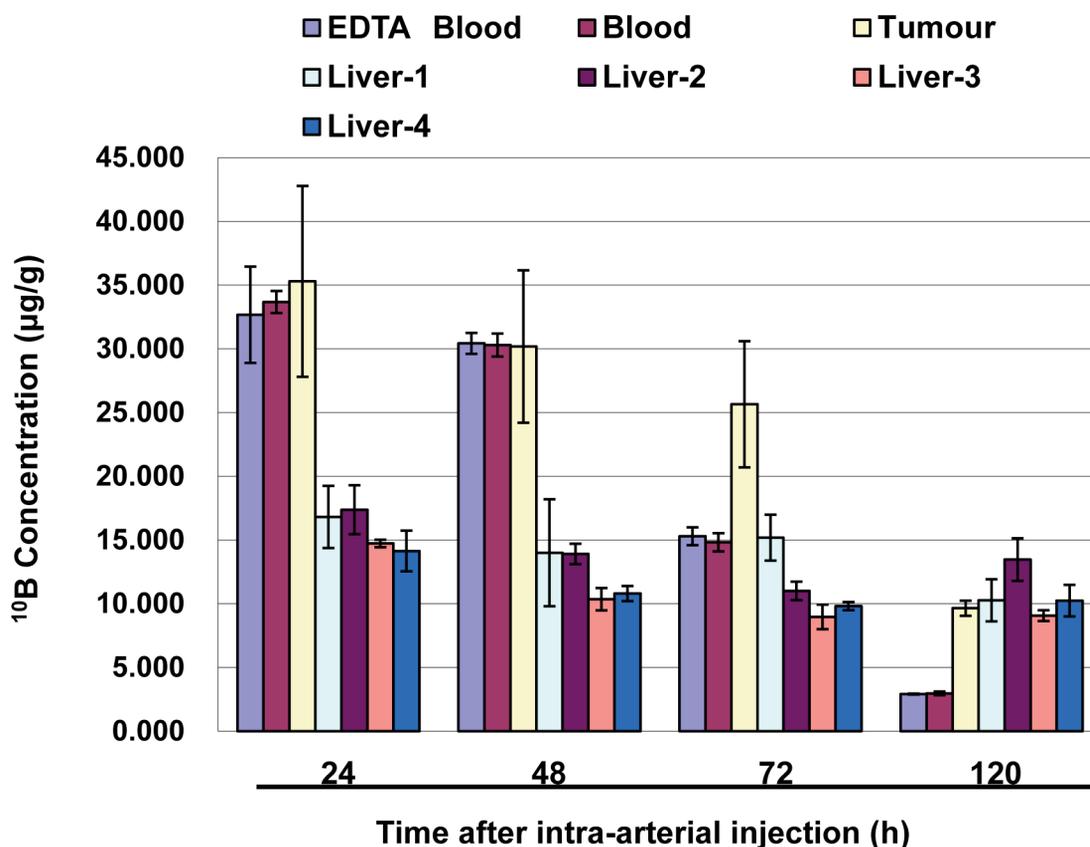


Figure 5. Boron concentrations in tumors and normal hepatic tissue in the VX-2 rabbit hepatic cancer model after intra-hepatic arterial injection of  $^{10}\text{B}$ -borono-dodecaborate -entrapped transferrin-conjugated polyethyleneglycol distearoyl-boron lipid liposomes. Liver-1: Normal liver near tumor in left lobe of liver; Liver-2: normal liver edge in left lobe of liver; Liver-3: normal liver central area in right lobe of liver; Liver-4: normal liver edge in right lobe of liver.

as an intracellular drug delivery system by receptor-mediated endocytosis (25).

The presence of a ligand facilitates the entry of  $^{10}\text{B}$ -containing compounds into cells through receptor-mediated endocytosis (11). We showed the suppression of tumor growth in a human pancreatic cancer model by BNCT after repeated intravenous injections of  $^{10}\text{B}$ BSH-entrapped PEG liposomes (18). Furthermore,  $^{10}\text{B}$ BSH-TF-PEG liposomes achieved superior tumor growth suppression by BNCT (19), and using neutron capture autoradiography, we demonstrated that  $^{10}\text{B}$  atoms more selectively accumulated in tumors using  $^{10}\text{B}$ BSH-entrapped TF-PEG liposomes than conventional liposomes (26).

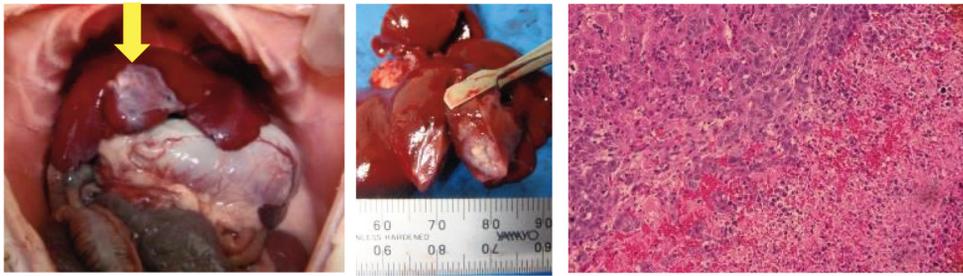
Nakamura *et al.* reported several boron lipids constructed of liposomes, which increased the uptake of boron-10 atoms in cancer cells, and termed these liposomal Boron Delivery Systems (27).  $^{10}\text{B}$ BSH-DSBL-PEG liposomes have been developed, and their significant antitumor effects were observed in mice injected with these liposomes (15 mg  $^{10}\text{B}/\text{kg}$ ) after

thermal neutron irradiation (22). Furthermore, liposomes composed of the closo-dodecaborate lipids DSBL and dipalmitoyl boron lipid exhibited strong cytotoxicity with thermal neutron irradiation, and these lipid liposomes were taken up into the cytoplasm by endocytosis without degradation (21). TF-loaded nido-carborane liposomes also achieved a higher survival rate with BNCT in tumor-bearing mice (28).

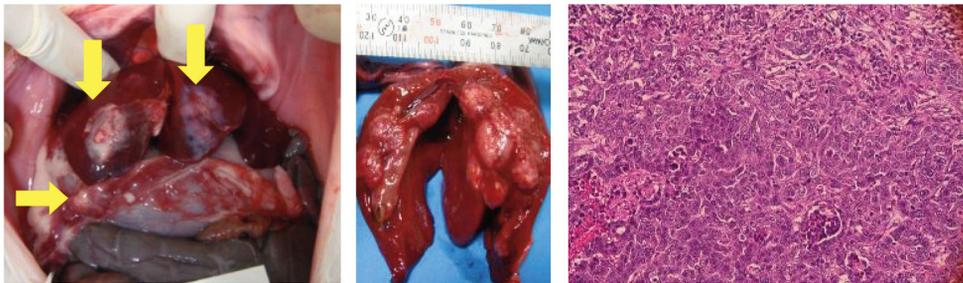
Boron compounds are currently being developed (29-35). Previous studies showed that tumor uptake after the administration of a sulfhydryl borane dimer ( $\text{Na}_4\text{B}_{24}\text{H}_{22}\text{S}_2$ ) was approximately two-fold that after the administration of equal amounts of boron as a monomer (36, 37). Feakes *et al.* encapsulated the apical-equatorial isomer of polyhedral borane [ $\text{B}_{20}\text{H}_{17}\text{NH}_3$ ] $^{3-}$  ion in liposomes prepared with 5% PEG-2000-distearoyl phosphatidyl-ethanolamine and found that the circulation time of these liposomes was prolonged, resulting in the continued accumulation of boron in tumors (38-41).

Cemazar *et al.* performed an electric pulse technique to enhance the accumulation of  $^{10}\text{B}$  into cancer cells after the

BNCT



Thermal neutron therapy only



Untreated control

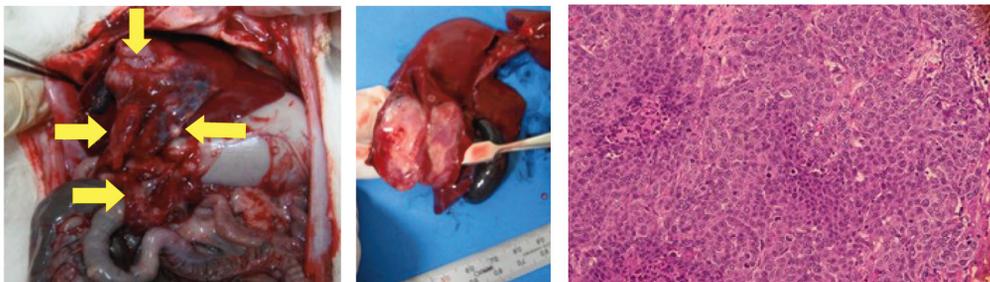


Figure 6. Tumor growth suppression by boron neutron capture therapy (BNCT). Morphological and pathological findings of VX-2 tumors after BNCT with intra-arterial injection of  $^{10}\text{B}$ -borono-dodecaborate-entrapped transferrin-conjugated polyethyleneglycol-distearoylboron lipid-coated liposomes.

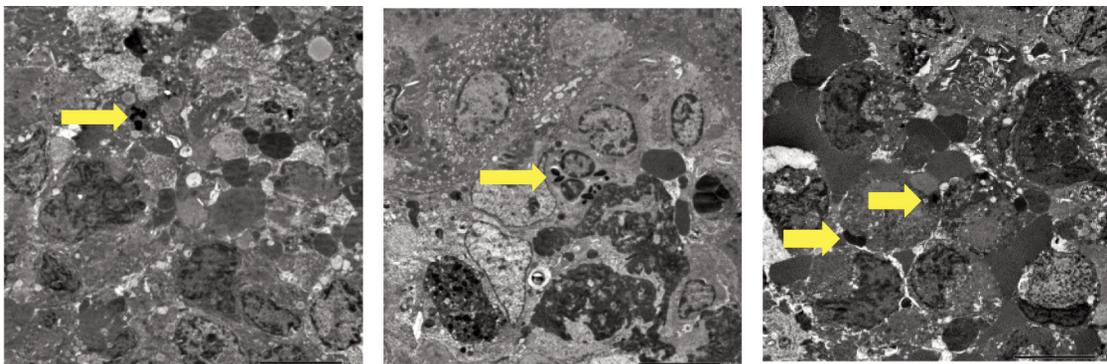


Figure 7. Electron microscopy findings ( $\times 600$ ) of tumors after boron neutron capture therapy using intra-hepatic arterial injection of  $^{10}\text{B}$ -borono-dodecaborate-entrapped transferrin-conjugated polyethyleneglycol-distearoylboron lipid-coated liposomes. Nuclear deformation and apoptotic bodies were detected in tumor cells.

intravenous injection of  $^{10}\text{B}$ -borono-phenylalanine (42). BNCT with an intravenous injection of TF-PEG liposomes with a high content of  $^{10}\text{B}$ SH has the ability to destroy malignant cells at the edge of the tumor mass, which is a hypervascular area. Experiments with these new  $^{10}\text{B}$  delivery systems that combine  $^{10}\text{B}$ SH-TF-PEG liposomes using the electric pulse technique to enhance the uptake of  $^{10}\text{B}$  will hopefully soon proceed to clinical BNCT trials.

The development of boron lipids for the construction of liposomes is very important because liposomes with boron atoms will increase the concentration of boron in cancer cells by liposomal endocytosis (21, 22, 28). The density of boron was previously shown to be higher in tumors than in normal hepatic tissue following modifications to the surface of PEG liposomes with TF, and tumor growth-suppressing effects were confirmed by thermal neutron irradiation (19, 21, 22, 28, 31). Intelligent targeting prevents accumulation in normal hepatic tissue and tumor selectivity in targeting is expected. In our study, the intra-arterial injection of  $^{10}\text{B}$ SH-TF-PEG-DSBL liposomes increased tumor retention of  $^{10}\text{B}$  atoms and also suppressed tumor growth *in vivo* upon thermal neutron irradiation. Pathological damage was not observed in normal hepatocytes after BNCT. The present results demonstrated the potential of  $^{10}\text{B}$ SH TF PEG-DSBL Lip as a novel intra-arterial boron carrier in BNCT for cancer.

Suzuki *et al.* reported preclinical studies and the treatment of patients with HCC by BNCT (43, 44). We also demonstrated that VX-2 tumor growth was suppressed by BNCT after an intra-hepatic arterial injection of  $^{10}\text{B}$ SH-entrapped WOW emulsion, and performed a clinical study on BNCT using  $^{10}\text{B}$ SH-entrapped WOW emulsion for patients with HCC (45, 46).

There were limitations to intra-arterial injection of drugs in these experiments as the proper hepatic artery of the rabbit is very thin, and it is very difficult to apply an injectable catheter to branches of hepatic arteries super-selectively. We hope to develop the boron-entrapped delivery systems for selective accumulation in tumors using this rabbit model, then proceed to a pilot clinical study of BNCT in the near future.

## Conclusion

We prepared  $^{10}\text{B}$ SH-entrapped TF-PEG liposomes consisting of boron lipids (DSBL) as boron delivery systems in BNCT. The results demonstrated that  $^{10}\text{B}$ SH-TF-PEG-DSBL liposomes deliver and facilitate the retention of boron atoms in cancer cells of tumor tissues in a rabbit hepatic tumor model. Tumor growth was suppressed by thermal neutron irradiation after an intra-arterial injection of  $^{10}\text{B}$ SH-TF-PEG-DSBL liposomes without damage to normal cells. These results demonstrate the potential of  $^{10}\text{B}$ SH-TF-PEG-DSBL liposomes as a novel boron delivery carrier in BNCT for

cancer using intra-arterial techniques. We intend to proceed to preclinical safety and clinical studies on patients with HCC using  $^{10}\text{B}$ SH-TF-PEG-DSBL liposomes in the near future.

## Conflicts of Interest

None declared.

## Authors' Contributions

Conceived and designed the experiments; HY, HN, MS, YS, HT, SM, KO, and HT. Performed the experiments; HY, MY, YM, AS, RM, YM, MS, YS, HT, SM, and KO. Analyzed and interpreted the data; HY, YM, AS, HN, MS, YS, ND, YN, YF, and HT. Contributed reagents, materials, analysis tools or data; HY, YM, AS, HN, MS, YS, TS, MN, HY, MO, JN, and HT. Wrote the article; HY, ND, HN, MS, and HT.

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