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

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

\textsuperscript{1}Institute of Engineering Innovation, School of Engineering, The University of Tokyo, Tokyo, Japan; \textsuperscript{2}Cooperative Unit of Medicine and Engineering, The University of Tokyo Hospital, Tokyo, Japan; \textsuperscript{3}Research Institute of Healthy Living, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan; \textsuperscript{4}Veterinary Medical Center, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; \textsuperscript{5}Department of Human and Molecular Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; \textsuperscript{6}Graduate School of Humanities, Seisen University, Tokyo, Japan; \textsuperscript{7}Laboratory of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan; \textsuperscript{8}Department of Surgery, Keisai-kgi Honyou Hospital, Hanamaki, Japan; \textsuperscript{9}Department of Surgery, Sodegaura Satukidai Hospital, Sodegaura, Japan; \textsuperscript{10}Department of Pharmacology, Kumamoto Institute Branch, LSI Medience Ltd. Co., Uto, Japan; \textsuperscript{11}Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan; \textsuperscript{12}Institute for Integrated Radiation and Nuclear Science, Kyoto University, Osaka, Japan; \textsuperscript{13}BNCT Joint Clinical Institute, Osaka Medical Pharmaceutical University, Osaka, Japan; \textsuperscript{14}Department of Cardiovascular Surgery, The University of Tokyo Hospital, Tokyo, Japan; \textsuperscript{15}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 $^{10}$B and thermal neutrons. The accumulation of $^{10}$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 $^{10}$B-borono-dodecaborate ($^{10}$BSH) by liposomal BDS in rabbit hepatic cancer models. Materials and Methods: We prepared $^{10}$BSH-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 $^{10}$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 $^{10}$BSH-containing liposomal BDS, without damage to normal cells. Conclusion: The present results demonstrate the applicability...
of $^{10}$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 ($\mu$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 $^{10}$B and thermal neutrons, which induces high-linear-energy transfer of $\alpha$ particles and lithium recoil. These particles ($\alpha$ and $^7$Li) destroy cells within an approximately 10-$\mu$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 $^{10}$B-boronododecaborate ($^{10}$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 $^{10}$B carrier (18, 19).

The accumulation of $^{10}$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 $^{10}$BSH-entrapped TF-PEG liposomes with $^{10}$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 $^{10}$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: distearylphosphatidylcholine (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 $^{10}$BSH-entrapped stealth (PEG) liposomes was as follows: DSPC:cholesterol:PEG-2000=1.0:1.0:0.11 (mol ratio) + $^{10}$BSH (125 mmol/l). The enclosed boron concentration entrapped in PEG liposomes was 4700 ppm.

$^{10}$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 μg streptomycin/penicillin under 5% 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, $^{10}$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: $2\times10^{12}$ 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°C. Harvested tumor samples were sliced into 6-$\mu$m-thick sections using a cryostat and deposited on glass slides before being stained with hematoxylin and eosin.
Pathological findings of tumors after intra-arterial injection of $^{10}\text{B}$-borono-dodecaborate-entrapped transferrin-conjugated polyethylene glycol-distearyloxyboron lipid-coated ($^{10}\text{BSH}$-TF-PEG-DSBL) liposomes. Histological and electron microscopic observations were performed. Rabbits were sacrificed 24 and 48 h after the injection of $^{10}\text{BSH}$-PEG-DSBL liposomes. Livers were resected and stored in Optimal Cutting Temperature compound frozen at −80°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.
Measurement of $^{10}\text{B}$ accumulations in each organ in vivo. We administered $^{10}\text{B}$-SH-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, and the hepatic artery injection of $^{10}\text{B}$ liposomes was performed. The administered volumes were 5.6 ml for $^{10}\text{B}$-PEG-DSBL liposomes and 3.3 ml for $^{10}\text{B}$-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 $^{10}\text{B}$ liposomes. The number of rabbits in each group was three.

We also measured $^{10}\text{B}$ 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 $^{10}\text{B}$-TF-PEG-DSBL liposomes. Tissues were weighed immediately after dissection and recorded as wet weights. Results were expressed as μg $^{10}\text{B}$ per g tissue. Mean values and standard deviations were calculated.

**Results**

*Characterization of liposomes.* Mean $^{10}\text{B}$ concentrations in liposomes were measured using ICP-AES. The concentration of $^{10}\text{B}$ entrapped in $^{10}\text{B}$-SH-PEG-DSBL liposomes was 2700 ppm, and that in $^{10}\text{B}$-SH-PEG liposomes was 4,700 ppm. The $^{10}\text{B}$ concentration entrapped in $^{10}\text{B}$-SH-TF-PEG-DSBL liposomes was 3,200 ppm.

*Comparison of $^{10}\text{B}$ accumulation in each organ and biodistribution of $^{10}\text{B}$-PEG and $^{10}\text{B}$-SH-PEG-DSBL liposomes after intra-hepatic arterial injection.* The $^{10}\text{B}$ concentrations in the VX-2 tumors using $^{10}\text{B}$-PEG-DSBL and $^{10}\text{B}$-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 injection (Figure 3). The $^{10}\text{B}$ concentration in the tumor using DSBL liposomes was two-fold higher than that of $^{10}\text{B}$-PEG.

Figure 3. Measurement of boron concentrations in tumors and each organ after the intra-arterial injection of boron-entrapped polyethylene glycol (PEG) liposomes in the VX-2 hepatic cancer-bearing rabbit model. Two types of $^{10}\text{B}$-borono-dodecaborate ($^{10}\text{B}$SH)-PEG liposomes were prepared: Distearoylboron lipid (DSBL) type and simple entrapping PEG type. Concentrations of $^{10}\text{B}$ were measured by inductively coupled plasma atomic emission spectroscopy at Juntendo University. Mean values and standard deviations were calculated (n=3).
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 $^{10}$BSH-PEG liposomes at 24 h after
the intra-arterial injection (Figure 3).

**Hepatic biodistribution of $^{10}$BSH-TF-PEG-DSBL liposomes
after intra-hepatic arterial injection.** Modifications to the
surface of PEG liposomes by conjugation with TF increased
the $^{10}$B concentration in tumors to around 25 μg/g at 72 h after
the intra-hepatic arterial injection of $^{10}$BSH-TF-PEG-DSBL
liposomes into the VX-2 rabbit hepatic cancer model. The
concentration of boron in normal hepatic tissue was 10-15
μg/g 72 h after the intra-hepatic arterial injection (Figure 5).

The tumor:normal liver $^{10}$B concentration ratio was at least
1.5-fold higher until 120 h after intra-hepatic arterial injection
(Figure 5). When $^{10}$BSH-TF-PEG-DSBL liposomes were administed intra-arterially at a dose of 6.4 mg $^{10}$B/kg, we
observed the selective uptake of $^{10}$B by cancer tissues
compared with that by normal hepatocytes in the VX-2 rabbit
hepatic cancer model. TF-PEG-liposomes maintained a high
$^{10}$B concentration in tumors, with concentrations higher than
25 μg/g for at least 72 h after intra-arterial injection. This high
retention of $^{10}$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 $^{10}$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 $^{10}$BSH-TF-PEG-DSBL liposomes at Kyoto
University Institute for Integrated Radiation and Nuclear Science. The fluence of thermal neutrons was 2×10$^{12}$ n/cm$^{-2}$
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 $^{10}$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
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, ×200) and electron microscopy (original magnification, ×600) after intra-hepatic arterial injection of $^{10}$B-borono-dodecaborate-entrapped polyethylene glycol distearoyl boron lipid-coated ($^{10}$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 $^{10}$BSH-PEG-DSBL liposomes than in non-treated control groups by electron microscopy.
as an intracellular drug delivery system by receptor-mediated endocytosis (25).

The presence of a ligand facilitates the entry of $^{10}$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 $^{10}$BSH-entrapped PEG liposomes (18). Furthermore, $^{10}$BSH-TF-PEG liposomes achieved superior tumor growth suppression by BNCT (19), and using neutron capture autoradiography, we demonstrated that $^{10}$B atoms more selectively accumulated in tumors using $^{10}$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). $^{10}$BSH-DSBL-PEG liposomes have been developed, and their significant antitumor effects were observed in mice injected with these liposomes (15 mg $^{10}$B/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 ($Na_4B_{24}H_{22}S_2$) 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 $[B_{20}H_{17}NH_3]_3$-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 $^{10}$B into cancer cells after the
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 $^{10}$B-borono-dodecaborate-entrapped transferrin-conjugated polyethylene glycol-distearoylboron lipid-coated liposomes.

Figure 7. Electron microscopy findings (x600) of tumors after boron neutron capture therapy using intra-hepatic arterial injection of $^{10}$B-borono-dodecaborate-entrapped transferrin-conjugated polyethylene glycol-distearoylboron lipid-coated liposomes. Nuclear deformation and apoptotic bodies were detected in tumor cells.
intravenous injection of $^{10}$B-borono-phenylalanine (42). BNCT with an intravenous injection of TF-PEG liposomes with a high content of $^{10}$BSH has the ability to destroy malignant cells at the edge of the tumor mass, which is a hypervascular area. Experiments with these new $^{10}$B delivery systems that combine $^{10}$BSH-TF-PEG liposomes using the electric pulse technique to enhance the uptake of $^{10}$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 $^{10}$B$^\mathrm{SH}$-TF-PEG-DSBL liposomes increased tumor retention of $^{10}$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 $^{10}$B$^\mathrm{SH}$ 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 $^{10}$BSH-entrapped WOW emulsion, and performed a clinical study on BNCT using $^{10}$B$^\mathrm{SH}$-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 injectional 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 $^{10}$B$^\mathrm{SH}$-entrapped TF-PEG liposomes consisting of boron lipids (DSBL) as boron delivery systems in BNCT. The results demonstrated that $^{10}$B$^\mathrm{SH}$-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 $^{10}$B$^\mathrm{SH}$-TF-PEG-DSBL liposomes without damage to normal cells. These results demonstrate the potential of $^{10}$B$^\mathrm{SH}$-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 $^{10}$B$^\mathrm{SH}$-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, HS, MS, YS, ND, YN, YF, and HT. Contributed reagents, materials, analysis tools or data; HY, YM, AS, HS, YS, TS, MN, HY, MO, JN, and HT. Wrote the article; HY, ND, HN, MS, and HT.

Acknowledgements

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan (No. 11691202 and No. 11557092 to Hironobu Yanagie), and a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan (No. 2008-Nano-004 to Hiroyuki Nakamura). We express our appreciation to Mrs. Yuriko Sakurai and Mrs. Kikue Mouri for their preparation of pathological samples.

References

1. Chen Z, Xie H, Hu M, Huang T, Hu Y, Sang N and Zhao Y: Recent progress in treatment of hepatocellular carcinoma. Am J Cancer Res 10(9): 2993-3036, 2020. PMID: 33042631.
2. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A and Roberts LR: A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 16(10): 589-604, 2019. PMID: 31439937. DOI: 10.1038/s41575-019-0186-y
3. Kanematsu T, Inokuchi K, Sugimachi K, Furuta T, Sonoda T, Tamura S and Hasuo K: Selective effects of Lipiodolized antitumor agents. J Surg Oncol 25(3): 218-226, 1984. PMID: 6199624. DOI: 10.1002/jso.2930250317
4. Hirash S, Shimizu M, Nakashima T, Iwata K, Uchiyama F, Tateno S, Tamura S and Setoguchi T: Arterial-injection chemotherapy for hepatocellular carcinoma using monodisperse poppy-seed oil microdroplets containing fine aqueous vesicles of epirubicin. Initial medical application of a membrane-emulsification technique. Cancer 75(6): 1245-1254, 1995. PMID: 7882276. DOI: 10.1002/1097-0142(19950315)75:6<1245::aid-cncr2820750606>3.0.co;2-u
5. Hirash S, Tabata N, Kondo KH, Maeda Y, Shimizu M, Nakashima T and Setoguchi T: Size of lipid microdroplets effects results of hepatic arterial chemotherapy with an anticancer agent in water-in-oil-water emulsion to hepatocellular carcinoma. J Pharmaco Exp Ther 289(2): 816-819, 1999. PMID: 10215657.
6. Ikushima I, Higashi S, Ishii A, Seguchi K, Iryo Y and Yamashita Y: Ultrasoundselect transcatheter infusion of epirubicin in water-in-oil-in-water emulsion for small hepatocellular carcinoma. Br...
Biomed Pharmacother 60(1): 43-50, 2006. PMID: 16260113. DOI: 10.1016/j.biopharma.2005.05.011
19 Maruyama K, Ishida O, Kasaoka S, Takizawa T, Utoguchi N, Shinohara A, Chiba M, Kobayashi H, Eriguchi M and Yanagie H: Intracellular targeting of sodium mercaptoundecahydro-dodecaborate (BSH) to solid tumors by transferrin-PEG liposomes, for boron neutron-capture therapy (BNCT). J Control Release 98(2): 195-207, 2004. PMID: 15262412. DOI: 10.1016/j.jconrel.2004.04.018

10 Yanagie H, Ogata A, Sugiyama H, Eriguchi M, Takamoto S and Barth RF, Mi P and Yang W: Boron delivery agents for neutron capture therapy. Sci Technol Adv Mater 2(8-6): 3059-3065, 2010. PMID: 20371186. DOI: 10.1016/j.scitam.2010.03.050

12 Mi P: Stimuli-responsive nanocarriers for drug delivery, tumor imaging, therapy and theranostics. Theranostics 10(10): 4557-4588, 2020. PMID: 32292515. DOI: 10.7150/thno.38069

16 Yanagie H, Tomita T, Kobayashi H, Fujii Y, Takahashi T and Hasumi K, Nariuchi H and Sekiguchi M: Application of boronated anti-CEA immunoliposomes to tumor cell growth inhibition in <i>in vivo</i> boron neutron capture therapy model. Br J Cancer 63(4): 522-526, 1991. PMID: 2021537. DOI: 10.1038/bjc.1991.124

17 Yanagie H, Tomita T, Kobayashi H, Fujii Y, Nonaka Y, Saegusa Y, Hasumi K, Eriguchi M, Kobayashi T and Ono K: Inhibition of human pancreatic cancer growth in nude mice by boron neutron capture therapy. Br J Cancer 75(5): 660-665, 1997. PMID: 9043021. DOI: 10.1038/bjc.1997.118

18 Yanagie H, Maruyama K, Takizawa T, Ishida O, Oguard B, Matsumoto T, Sakurai Y, Kobayashi T, Shinohara A, Rant J, Skvare J, Ilic R, Kuhne G, Chiba M, Furuya Y, Sugiyama H, Hisa T, Ono K, Kobayashi H and Eriguchi M: Application of boron-entrapped stealth liposomes to inhibition of growth of tumor cells in the <i>in vivo</i> boron neutron-capture therapy model.
30 Michiue H, Sakurai Y, Kondo N, Kitamura M, Bin F, Nakajima K, Hirota Y, Kawabata S, Nishiki T, Ohmori I, Tomizawa K, Miyatake S, Ono K and Matsu H: The acceleration of boron neutron capture therapy using multi-linked mercaptoundeca-hydrododecaborate (BSH) fused cell-penetrating peptide. Biomaterials 35(10): 3396-3405, 2014. PMID: 24452095. DOI: 10.1016/j.biomaterials.2013.12.055

31 Masunaga S, Kasaka S, Maruyama K, Nigg D, Sakurai Y, Nagata K, Suzuki M, Kinashi Y, Maruhashi A and Ono K: The potential of transferrin-pendant-type polyethyleneeglycol liposomes encapsulating decahydrododecaborate-(10)B (GB-10) as (10)B-carriers for boron neutron capture therapy. Int J Radiat Oncol Biol Phys 66(5): 1515-1522, 2006. PMID: 17126210. DOI: 10.1016/j.ijrobp.2006.08.028

32 Takeuchi K, Hattori Y, Kawabata S, Futamura G, Hiramatsu R, Waniibuchi M, Tanaka H, Masunaga SI, Ono K, Miyatake S and Kirihata M: Synthesis and evaluation of dodecaboranethiol containing kojic acid (KA-BSH) as a novel agent for boron neutron capture therapy. Cells 9(6): 1551, 2020. PMID: 32630612. DOI: 10.3390/cells9061551

33 Maitz CA, Khan AA, Kueffer PJ, Brockman JD, Dixon J, Jalisatgi SS, Nigg DW, Everett TA and Hawthorne MF: Validation and comparison of the therapeutic efficacy of boron neutron capture therapy mediated by boron-rich liposomes in multiple murine tumor models. Transl Oncol 10(4): 686-692, 2017. PMID: 28683435. DOI: 10.1016/tran.2017.05.003

34 Nakase I, Aoki A, Sakai Y, Hirase S, Ishimura M, Takatani-Nakase T, Hattori Y and Kirihata M: Antibody-based receptor targeting using an Fc-binding peptide-dodecaborate conjugate and macropinocytosis induction for boron neutron capture therapy. ACS Omega 5(36): 22731-22738, 2020. PMID: 32954120. DOI: 10.1021/acsomega.0c01377

35 Nakagawa F, Kawashima H, Morita T and Nakamura H: Water-soluble closo-dodecaborate-containing pteroyl derivatives targeting folate receptor-positive tumors for boron neutron capture therapy. Cells 9(7): 1615, 2020. PMID: 32635272. DOI: 10.3390/cells9071615

36 Slatkin D, Micca P, Forman A, Gabel D, Wielopolski L and Fairclird R: Boron uptake in melanoma, cerebrum and blood from Na2B12H11SH and Na4B24H22S2 administered to mice. Biochem Pharmacol 35(10): 1771-1776, 1986. PMID: 3707608. DOI: 10.1016/0006-2952(86)90342-4

37 Elhanati G, Salomon Y and Bendel P: Significant differences in the retention of the borocaptate monomer (BSH) and dimer (BSSB) in malignant cells. Cancer Lett 172(2): 127-132, 2001. PMID: 11566486. DOI: 10.1016/s0304-3835(01)00649-8

38 Feakes DA, Shelly K, Knobler CB and Hawthorne MF: Na3[B20H17NH3]: synthesis and liposomal delivery to murine tumors. Proc Natl Acad Sci USA 91(8): 3029-3033, 1994. PMID: 8159700. DOI: 10.1073/pnas.91.8.3029

39 Feakes DA, Shelly K and Hawthorne MF: Selective boron delivery to murine tumors by lipophlic species incorporated in the membranes of unilamellar liposomes. Proc Natl Acad Sci USA 92(5): 1367-1370, 1995. PMID: 7877984. DOI: 10.1073/pnas.92.5.1367