

I-1. PROJECT RESEARCHES

Project 5

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In this research project, twenty research projects were included. In this summary, three research projects (P5-1, P5-2, P5-3) could not be reported due to unexpected or uncontrolled events.

P5-4: Boronophenylalanine loaded mesoporous silica nanoparticles (BPA-MSN) as a new boron-delivery agent were investigated using chicken egg tumor model. Thermal neutron irradiation following BPA-MSN injection dramatically inhibited tumor growth. Growth inhibition was not observed in the tumors irradiated by thermal neutron with no injection, empty BSA or free BPA injection.

P5-5: As new boron-delivery agents, BPA or BSH bounded to 7-mer IFLLWQR peptide, designated IF7, were tested in this study. Pharmacokinetic studies revealed that IF7-BSH injection accumulate ^{10}B in the tumor more than twice compared with free BSH injection.

P5-6: Cancer-loaded human artificial three-dimensional tissue was investigated as the tool for evaluating accumulation ratio of BPA between cancer cells and normal tissues.

P5-7: Aggregation of boron carbide (B_4C) which is a candidate material for BNCT should be prevented in clinical use. Femosecond laser irradiation reported to enhance colloidal stability of various nanoparticles to aggregated B_4C nanoparticles failed to stable B_4C nanoparticle colloid due to a high hardness and chemical stability of B_4C .

P5-8: BPA nanosuspensions (median diameter: 144 nm) were made using stabilizers, macrogol 15 hydroxystearate and soybean lecithin. BNCT with subcutaneous-injected BPA nanosuspensions was equally efficacious in that with intravenous injected BPA-fructose.

P5-9: Therapeutic efficiency of pteroyl *closo*-dodecaborate conjugate (PBC) using F98 glioma-bearing rats *in vivo* boron neutron capture therapy (BNCT) was investigated. BNCT in combination with BPA (i.v) and PBC (convection enhanced delivery, CED) showed significantly longer survival time.

P5-10: Novel *p*-boronophenylalanine based boron carriers (BADB1-3), in which the medium-chain alkyl

sulfoniododecaborate is linked to C-terminal of L-BPA was evaluated about the cytotoxicity, water solubility, and cellular uptake. The BADB-1 is useful candidate as new boron carrier with viewpoint of low cytotoxicity and high cellular uptake.

P5-11: Novel boron-containing low molecular compound (Compound A) was investigated with tumor-growth delay assay. BNCT with Compound A (250 mg/kg) showed tumor growth inhibitory effect comparable to that with BPA (500 mg/kg).

P5-12: Two different types of phenylboronic acid-installed polymeric nanoparticles with pinacol protection (Pinacol-NP) and without any protection (PBA-NP) were investigated with tumor-growth delay assay. BNCT with Pinacol-NP exhibited comparable antitumor effect with BPA-fructose even at a 100-fold lower effective dose of ^{10}B .

P5-13: As novel boron delivery agents, 2-boryl-1,2-dideoxy-D-glucose derivatives uptaken into the tumor cell via glucose transporters (GLUT) and sodium dependent glucose cotransporters (SGLT) were investigated. The lead compound was found.

P5-14: Poly vinyl alcohol (PVA)-BPA exhibited strong BNCT effect using a hypovascular tumor model (BxPC3). PVA should offer great potential as the additive boosting therapeutic potential of BPA.

P5-15: Newly synthesized boronated polymer exhibited comparable antitumor effect to sorbitol-BPA. The boronated polymer may be useful in the clinical application.

P5-16: This study revealed that disulfide bond-containing maleimide-functionalized *closo*-dodecaborate (SSMID) are bound to form a covalent bond to the albumin through the Lys residues.

P5-17: Doxorubicin DXR-encapsulated boron liposome was tested in this study. All the mice treated with the DXR boron liposome died within 20 days. The boronated liposome significantly suppressed tumor growth.

P5-18: Artificial oligonucleotides bearing hydrophobic boron-containing fluorophore (BODIPY-ODN) was investigated as a new boron compound.

P5-19: BPA-BNCT exhibited suppression of tumor growth using two head and neck tumor models (SAS and HSG).

P5-20: This study revealed that RGD sequence binding GD-DTPA/CaP nanomicelle has the promising possibility as novel active targeting GdNCT agent.

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INTRODUCTION: Advance in Nanotechnology resulted in the generation of nanoparticles that have diameter in the range of 40-400 nm. We have been developing mesoporous silica nanoparticles (MSNs) that contain thousands of pores. An important feature of this type of nanoparticles is that they have a large surface area where a variety of reagents can be attached. MSNs are efficiently taken up into cancer cells. This involves the use of the endocytosis mechanism. MSNs are localized to the perinuclear localization, as lysosomes are located at these sites. MSNs can be designed to accumulate in tumor in animal models.

Our experimental plan is to prepare BPA-loaded MSN (BPA-MSN). We have designed experiments to prepare MSN that has BPA attached. We then examine tumor accumulation of BPA-loaded MSN. Then, the samples will be exposed to neutron beam at the nuclear reactor. BNCT efficacy will be compared to that of free BPA.

EXPERIMENTS: MSN were synthesized by the sol-gel process using TEOS as a building material and CTAB as a templating agent to produce pore structure. Diameter of MSN is 100 nm and the nanoparticle is surface modified by the addition of phosphonate. BPA was attached to MSN and the presence of Boron on MSN was confirmed by ICP-OES.

To carry out BNCT, we employed chicken egg tumor model established by transplanting human ovarian cancer cells. The experiments involved purchasing fertilized eggs and incubating till day 10 of development. A window was made on the egg shell and ovarian cancer cells were transplanted. Tumor was formed on the CAM (chorioallantoic membrane) three days later at which time BPA-loaded MSN were injected intravenously. After a day and half when MSN was accumulated in the tumor, eggs were irradiated with neutron beam at the reactor. The exposure time was set for 1 hour. After the exposure, the eggs were incubated for 3 days and the tumor size and weight were examined.

The setup developed for the exposure to neutron beam is shown in Figure 1. Two different setups have been developed, one accommodating 4 eggs while the other setup accommodating about 20 eggs.

RESULTS: We observed dramatic inhibition of tumor growth when BPA-MSN was injected. The tumor weight at the end of the experiment was 17 mg when BPA-MSN was injected, while control eggs with no injection had a tumor of 50 mg. Tumor weight of eggs with empty MSN injection was 45 mg. Eggs with free BPA injection had tumor weight of 40 mg. These results clearly show the effect of MSN formation of BPA on BNCT efficacy.

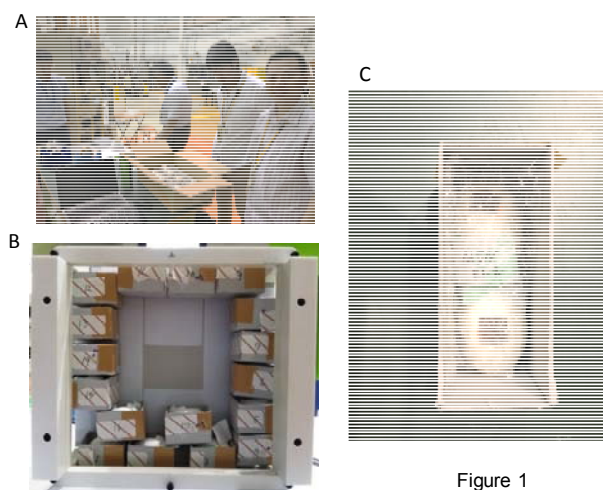


Fig. 1. Two different setups for carrying out BNCT experiments using chicken egg tumor model. A: BNCT experiments. B: Setup for 22 eggs. C: Setup for 4 eggs.

FUTURE PROSPECTS:

We have now demonstrated the power of our nanoparticles to improve BPA efficacy. Systematic experiments will be carried out to further investigate the use of nanoparticles in BNCT.

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- [4] K. Matsumoto *et al.*, Enzymes **44** (2018) 103-116.

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INTRODUCTION: In Aomori Prefecture, "Prefectural Government Refuge against the shortest life time with high death rate" is taken as the prefectural government, Hirosaki University (Faculty of Medicine / Graduate School of Science and Engineering) received this, "Next generation A (Aomori) - Research and development on BNCT treatment "started. The main core technologies related to next-generation A-BNCT therapy are (1) development of new boron medicines, (2) A-BNCT treatment technology and (3) POST / BNCT regeneration technology, and this joint research aims at technological development for practical application concerning (1). New boron drug development In Hirosaki University, IF7-B series (10B-IF7) in which boron nuclide ¹⁰B is bound to 7-mer peptide (IFLLWQR; IF7 peptide) 1) -2) which selectively binds to tumor vascular endothelial cells, ¹⁰B BSH-IF 7, ¹⁰B PA-IF 7). In this core research, demonstration experiments on the pharmacokinetics, toxicity test and BNCT treatment effect for practical application of the relevant IF7-B series are conducted by nuclear reactor irradiated animal experiments

EXPERIMENTS: In the fiscal year ending March 31, 2010, we will mainly focus on pharmacokinetic examinations by small animal experiments of IF7-B series drugs (3 species). An animal tumor model in which mouse bladder cancer cells (MBT 2) were seeded at the mouse thigh in Hirosaki University was prepared and injected with a boronic agent (IF 7 - BPA, IF 7 - BSH, BPA, BSH) from the mouse tail vein. Mice were sacrificed after drug administration (after 20 minutes, 40 minutes, 60 minutes, 90 minutes, 120 minutes, 240 minutes), and the tumor area and normal organs (liver, kidney, heart, bladder, lung, Brain, spleen, skin, blood) to Teflon containers (10 in total) and then transported to the Kyoto University Reactor. After transportation, measured boron concentration in prompt gamma ray assay(PGA) in E3 duct compartment.

RESULTS: Fig.1 shows the ¹⁰B concentration in the organ of 13 sites taken out from the mouse specimen after 0~40 minutes of BPA, IF7-BPA. From these results, the accumulation amount of BPA and IF7-BPA in tumor cells was up to about 10 ppm of ¹⁰B accumulation up to 40 minutes after injection, but no significant difference was observed between the two agents.

Fig.2 shows the ¹⁰B concentration in the organ of 13 sites taken out from the mouse specimen after 0~40 minutes of BPA, IF7-BPA. From these results, the accumulation amount of BPA and IF7-BPA in tumor cells was up to about 10 ppm of ¹⁰B accumulation up to 40

minutes after injection.

Fig.2 shows BHS and IF7-BSH (Fig.1 (a)) and the new boron drug IF 7-BSH (Fig.1 (b)) by tail injection, respectively. However, in experiments with IF7-BSH, experiments were conducted using multiple specimens.

According to this result, the accumulation degree of ¹⁰B in the tumor site reaches up to about 20 ppm in 10 minutes after the introduction in BSH. On the other hand, it can be seen that IF7-BSH reaches 40 ppm more than twice that at the same elapsed time.

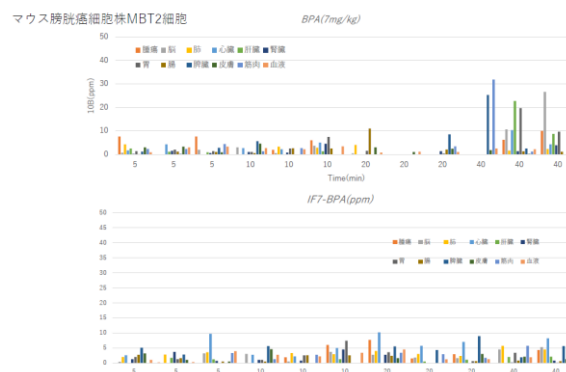


Fig.1 ¹⁰B condensation in various internal organs including tumor site. In the case of BPA and IF7-BPA.

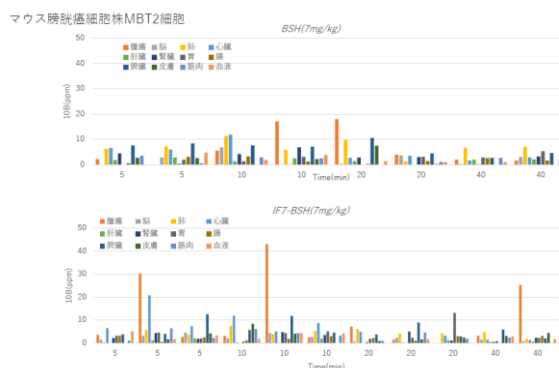


Fig.2 ¹⁰B condensation in various internal organs including tumor site. In the case of BSH and IF7-BSH.

From these results, while the accumulation performance of ¹⁰B to the tumor site of the newly developed IF7-BPA was not significantly improved as compared with the conventional agent BPA. However, in the case of IF7-BSH, it has been found that it has more than twice the integration performance comparing with the conventional agent BSH.

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INTRODUCTION: In Aomori Prefecture, "Prefectural Government Refuge against the shortest life time with high death rate" is taken as the prefectural government, Hirosaki University (Faculty of Medicine / Graduate School of Science and Engineering) received this, "Next generation A (Aomori) - Research and development on BNCT treatment "started [1-5]. The main core technologies related to next-generation A-BNCT therapy are (1) development of new boron medicines, (2) A-BNCT treatment technology and (3) POST / BNCT regeneration technology, and this joint research aims at technological development for practical application concerning (2). Pharmacokinetics experiments of ¹⁰BPA drugs using α -radiography were performed using cancer-loaded human artificial three-dimensional tissue NHDF / BxPC3 prepared by the LBLA (Layer By Layer Assembly) method, and the performance of the ¹⁰BPA for treatment of BNCT under the ¹⁰B(*p,n*)⁷Li reaction was evaluated.

EXPERIMENTS: Cancer-loaded human artificial three-dimensional tissue were prepared using human neonatal fibroblasts (NHDF (LONZA)) and red fluorescent protein (RFP) labeled (Anti- Cancer Japan) human pancreatic cancer cell line BxPC3 was used[7-13]. Organization of both cells was carried out using bovine serum-derived fibronectin (Wako) and porcine-derived gelatin (Wako) by the LBLA method. DMEM with 10% FBS, antibiotics was used as a medium for culturing after artificial tumor tissue production. Incorporation of ¹⁰B into the prepared artificial tumor cell tissue was carried out using boron drug ¹⁰BPA (boronophenylalanine C₉H₁₂BNO₄ molecular weight 209.01 (treatment concentration of ¹⁰B: 20 - 50 ppm) of 3% weight concentration (fructose complex). Irradiation experiments using these samples were conducted at the E-2 port of Kyoto University Reactor KUR, and irradiation was performed for 30 minutes under an irradiation flux of 1.4×10^9 n / cm².

RESULTS: Fig.1 shows the respective structures of the NHDF and NHDF/BxCP3 samples and the results of observation of the alpha ray track in each sample after neutron irradiation. The fluorescent tissue (NHDF (a), NHDF / BxCP3 (b) and (c)) is shown on the left side of the figure, and the alpha track images ((a) 'to (c)') are shown on the right side. The hole in the middle (black part to white part) is the above position confirmation hole, and the circular broken line (yellow) in both images shows the part with relatively high BxCP3 concentration on the NHDF / BxCP3 sample as a result of fluorescence observation. Comparing the α track concentrations of

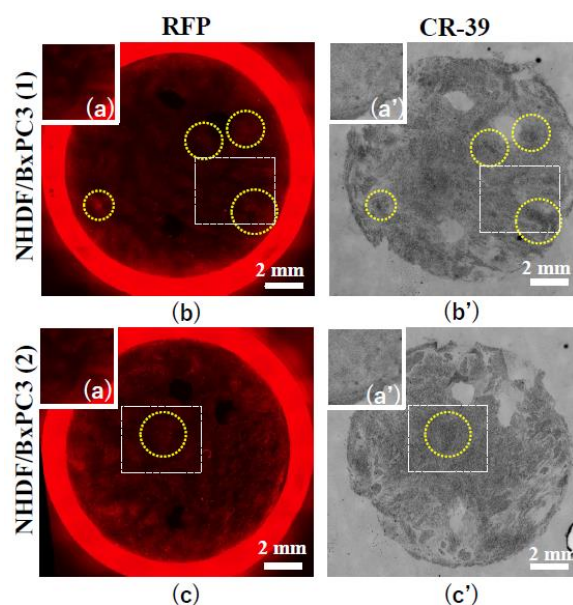


Fig.1 Fluorescence(left) and α radiography imaging(right) of NHDF and NHDF/BxPC3 3D cell structure($\times 4$): NHDF(a)(a') and NHDF/BxPC3(b)(b') and (c) (c')

NHDF and NHDF / BxCP3 samples, it can be seen that the concentration in BxCP3 is higher and the track height is higher in the high density BxCP3 site (circular broken line part). This means that it corresponds to the concentration of ¹⁰B taken up into each tissue of NHDF and NHDF / BxCP3 samples after BPA immersion and gives a concentration of ¹⁰B uptake proportional to each tissue concentration in both samples.

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PR5-4 An Attempt to Prepare Stable Colloid of Boron Carbide Using a Femtosecond Laser

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INTRODUCTION: Boron carbide (B_4C) is one of candidate materials for boron neutron capture therapy (BNCT) agent because of a chemical stability and high B atom content. Size reduction and aggregation prevention of B_4C particles are necessary for an application study for BNCT to avoid particle sedimentation. Seo, Y. et al. reported that an enhanced colloidal stability of various nanoparticle suspensions by a femtosecond laser irradiation of suspension [1]. In this study, we attempt to disperse aggregates and obtain stable suspension of B_4C nanoparticles by a Ti:sapphire femtosecond laser irradiation.

EXPERIMENTS: B_4C nanoparticles (EMJAPAN CO., LTD. NO-B4C-2-5) were dispersed in ethanol at 200 ppm concentration. The B_4C suspension of 6 ml in a glass vessel was irradiated over surface with a Ti:sapphire femtosecond laser (pulse width: 100 fs (FWHM), wavelength: 800 nm, and energy density: $8.5 \text{ mJ pulse}^{-1}$). The suspension was agitated using a magnetic stirrer during the irradiation for 1 h. B_4C nanoparticles were collected with a centrifugation and mounted on a Si wafer to observe a scanning electron microscope (SEM) image. B_4C particle size distribution and zeta potential were analyzed using a dynamic light scattering (DLS).

RESULTS: B_4C particles 100 to 200 nm in diameter were observed before laser irradiation (Fig. 1 (a)). Although primary particles were clearly observed in the particles before irradiation, these particles solidly aggregated and formed agglomerates larger than $1 \mu\text{m}$ in size as shown in particle size distribution (Fig. 2 black line). No noticeable shape change of particles were observed after laser irradiation (Fig. 1(b)). Particle size distribution after laser irradiation remained almost the same as that before irradiation (Fig. 2 red line). Zeta potentials of B_4C particles before and after irradiation were the same value -12.4 mV . These results are possibly due to a high hardness and chemical stability of B_4C . A fabrication of stable B_4C nanoparticle colloid is still a future challenge.

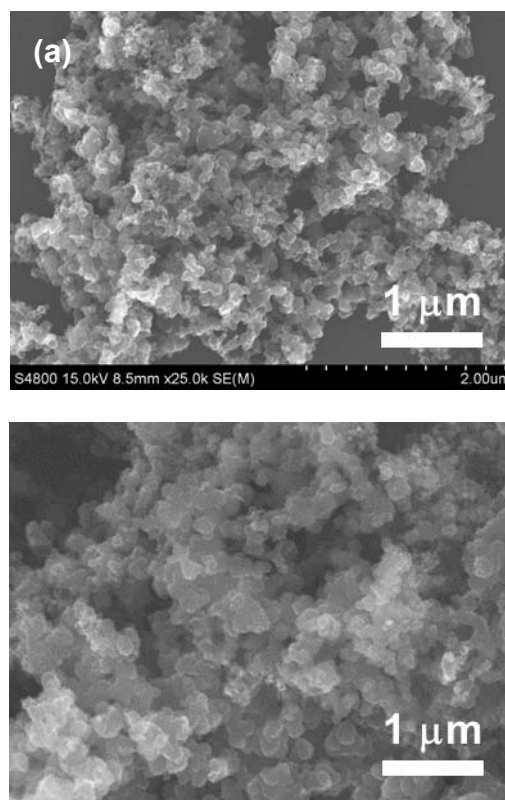


Fig. 1. SEM images of B_4C nanoparticles (a) before and (b) after laser irradiation.

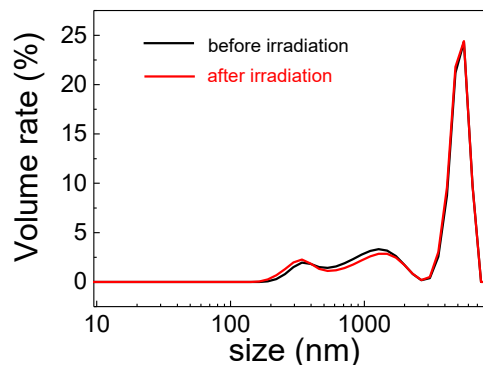


Fig. 2. B_4C particle size distribution analyzed with DLS measurement.

REFERENCE:

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PR5-5 Nanoparticulate *p*-borono-L-phenylalanine formulations for boron neutron capture therapy: Biodistribution after subcutaneous administration

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INTRODUCTION: The successful treatment of cancer by boron neutron capture therapy (BNCT) requires selective delivery of large amounts of ¹⁰B isotope to tumor cells. Although *p*-borono-L-phenylalanine (L-BPA) spontaneously accumulates in tumor cells, pharmaceutical drawbacks include poor water-solubility (1.6 mg/mL) and rapidly decreased tissue concentration after administration. In clinical BNCT, BPA-Fructose complex (BPA-Fr) is a continuously infused intravenously to maintain adequate ¹⁰B concentration in blood [1]. A more effective ¹⁰B carrier is required to increase the success of BNCT. In the present study, an attempt was made to formulate a BPA nanosuspension (NS) that would be expected to accumulate more efficiently than BPA-Fr after local administration. The biodistribution of the BPA-NS formulation after subcutaneous (s.c.) administration was evaluated. In a tumor bearing animal model was established to investigate the *in vivo* biodistribution of L-BPA and antitumor effects after BNCT.

EXPERIMENTS: Macrogol 15 hydroxystearate (Solutol® HS 15, SO) and soybean lecithin (SL) were used as stabilizers. BPA-NS using SO and SL was prepared by a wet-milling method with the use of a Pulverisette-7 planetary ball mill (Fritsch). The obtained BPA-NS was sonicated using a 2510J-DTH water-bath sonicator (Branson Ultrasonics Co.) for 5 min at room temperature. Biodistribution was assessed using male B16F10 melanoma bearing C57BL/6J mice. BPA-Fr and BPA-NS (500 mg BPA/kg) were administered via s.c. injection to the mice. At a predetermined time after administration, the mice were sacrificed and blood and tissue samples were immediately collected. The concentration of ¹⁰B in the blood was measured by inductively coupled plasma atomic emission spectroscopy. In the BNCT trial, tumor-bearing mice were divided into BNCT group and control group. The tumors in the left hind legs were exposed to thermal neutron irradiation at the Insti-

tute for Integrated Radiation and Nuclear Science, Kyoto University.

RESULTS: BPA-NS displayed a mass median diameter of 144 nm with 52.1 mg BPA/mL. The BPA concentration of BPA-NS was equivalent to BPA-Fr. After s.c. administration, the maximum ¹⁰B concentration in tumors was 43 ppm (1 h) for BPA-Fr and 36 ppm (6 h) for BPA-NS. In addition, concentrations of ¹⁰B in tumors were 5 ppm at 12 h after administration of BPA-Fr and 22 ppm 12 h after administration of BPA-NS. The previously described prolonged retention of ¹⁰B in blood following s.c. route of administration [2, 3] may account for a longer retention of over 20 ppm in tumors for 12 h. The area under the ¹⁰B tumor concentration-time curve (AUC_{tumor}) were calculated to be 313.5 µg·h/mg for BPA-Fr and 599.6 µg·h/mg for BPA-NS. BPA-NS displayed significantly higher AUC_{tumor} values than BPA-Fr (P<0.0006), which could reflect the dissimilar transport rate of the BPA formulations into the bloodstream after s.c. administration. In the BNCT trial, growth of tumor masses was observed in the control group, while the BNCT group showed an equivalent suppression of tumor growth. These results suggested that boron accumulates specifically in the tumor after s.c. administration of BPA formulations and that BNCT after s.c. dosing of BPA-NS or BPA-Fr is equally efficacious in the treatment of BNCT after intravenous administration of BPA-Fr.

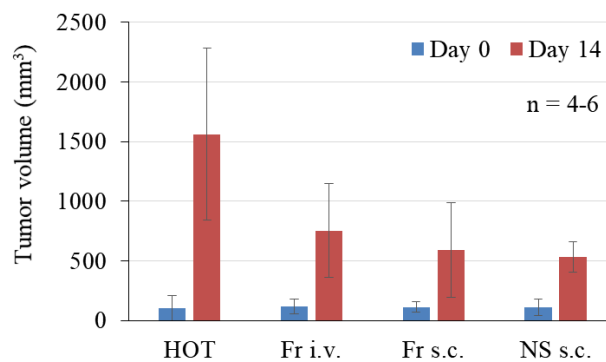


Fig. 1. Tumor volumes after thermal neutron beam irradiation of BNCT and HOT control groups.

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Evaluation of boron neutron capture therapy (BNCT) using brain tumor bearing rats or mice model

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Main study

Introduction

Folic acid (FA) has high affinity for the folate receptor (FR), which is limited-expressed in normal human tissues and over-expressed in many cancer cells, including glioblastoma.

We evaluated a newly developed pteroyl closo-dodecaborate conjugate (PBC) as boron carrier in which the pteroyl group is known to interact with FR. The purpose of this study was to evaluate the therapeutic efficiency of PBC using F98 glioma-bearing rats *in vivo* boron neutron capture therapy (BNCT).

Materials and Methods

We used two boron compounds; Boronophenylalanine (BPA) and PBC. For *in vivo* study, F98 glioma bearing rats were divided to five groups: untreated controls, neutron irradiation only, BNCT with BPA (i.v.), BNCT with PBC (convection enhanced delivery; CED), and BNCT in combination with BPA (i.v.) and PBC (CED).

Results

The survival data following BNCT are summarized in Table. 1, and the Kaplan-Meier survival plots are shown in Fig. 1. Median survival time (MST) in all BNCT groups were significantly longer than that in the untreated control group ($p = 0.0013$, $p = 0.0029$, and $p = 0.0013$ by log-rank test, respectively).

Only the combined group showed significantly longer survival compared with the neutron irradiation control ($p = 0.0042$ by log-rank test). In addition, the combined group showed the longest MST and the highest percent increase in life span value among all treated groups (65.2%; Table. 1), as well as a significantly longer survival compared with the other BNCT groups (vs. BPA [i.v.]: $p = 0.0152$, vs. PBC [CED]: $p = 0.0058$ by log-rank test).

Group	n	Survival time
		Median
Untreated controls	6	23.0
Irradiated controls	6	26.0
BPA (i.v.)	6	30.0
PBC (CED)	5	31.0
PBC (CED) + BPA (i.v.)	6	38.0

Table. 1 Survival times of F98 glioma-bearing rats following tumor implantation.

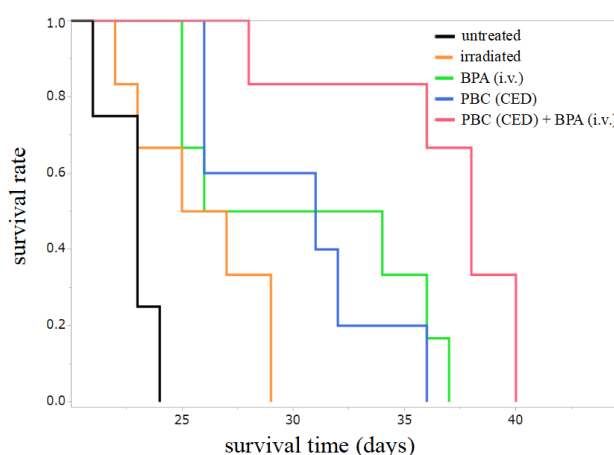


Fig. 1 Kaplan-Meier survival curves for F98 glioma-bearing rats of untreated controls, neutron irradiation only, BNCT with BPA (i.v.), BNCT with PBC (convection enhanced delivery; CED), and BNCT in combination with with BPA (i.v.) and PBC (CED).

Ongoing study

We developed another novel boron drug (AAL) that combines the characteristics of BPA and BSH, which has a boron cluster in its structure and targets an amino acid transporter. As in the main study, BPA was administered i.v. and AAL was administered by CED, neutron irradiation was performed on F98 glioma bearing rats. As a result, in combination of AAL (CED) and BPA (i.v.), a significant prolongation in survival time was obtained compared with the single agent groups (data not shown). We will continue this study; the study results will be published in the future.

Development of S-Alkylthiododecaborate Containing *p*-Boronophenylalanine for BNCT

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INTRODUCTION: BNCT is based on the nuclear fission reaction of a ¹⁰B-atom with thermal and/or epithermal neutrons to yield high linear energy transfer α particles (⁴He) and recoiling ⁷Li nuclei in tumor cells, and has attracted attention in term of its potential therapeutic effects on malignant tumors.^[1] A boron delivery agent (boron carrier) with high therapeutic efficiency and low adverse effects is crucial for successful BNCT. Although many kinds of boron compounds such as amino acids, nucleic acids and sugars have been reported as boron carriers for BNCT, only two compounds are used clinically for the treatment of cancer with BNCT: *p*-borono-L-phenylalanine (BPA, **1**) and mercaptocloso-undeca-hydrododecaborate (BSH, **2**) (Fig. 1).^[2,3] Unusual boron amino acids represented by BPA have long being recognized as tumor seeking compounds due to structural analogy to usual L-amino acid, because L-amino acid transport system (LAT1) is enhanced compared with normal tissues to sustain the proliferation of tumor cells.^[4]

In the course of our developing studies on new boron carrier for BNCT, we have designed and synthesized thiododecaborate ([B₁₂H₁₁S]²⁻) unit-containing L-amino acids, a new class of tumor seeking and water soluble amino acid. Recently, medium-chain alkyl sulfoniododecaborate ([B₁₂H₁₁S⁺octyl]¹⁻) unit containing amino acids (AS-DBA, **3**) showed high cell membrane permeability, low cytotoxicity and high water-solubility, and these compounds could deliver large amount of boron to several kinds of tumor cells.^[5]

To develop a new boron carrier for BNCT, we designed and synthesized novel *p*-boronophenylalanine based boron carrier (BADB1-3, **2a-c**), in which the medium-chain alkyl sulfoniododecaborate is linked to C terminal of L-BPA. These novel boron compounds have

enhanced hydrophobicity and cell membrane permeability owing to a reduced negative charge (-1) and the introduced alkyl chain. Here, we present the biological evaluation of novel boron compounds **2a-c** as boron carriers for BNCT.

RESULTS and Discussion: To evaluate the BADBs, we examined the cytotoxicity, water-solubility, and cellular uptake of AS-DBAs, and compared them with that of BPA, BSH, and DBA.

The water solubility of BADB-1 (S-Octyl, **2a**) was higher than that of BPA and **2b**, **2c** (BSH, **1a-c**, **2a**: >40 g/L, BPA: 1.6 g/L, **2b,c**: <10g/L). The cytotoxicity of BADB **1a** was marginally low (IC₅₀ of **1a** is 0.1 mM in F98 glioma and B16 melanoma cells). However, the cytotoxicity of **2b,c** was higher than 0.1mM.

In the next step, we measured the boron concentrations in tumor cells by ICP-OES (Fig. 2). The intracellular boron concentration of BADB-1 in C6 and B16 cells was 2-3 times greater than that of L-BPA, with fewer doses of the compound.

Our results show that BADB-1 is useful candidate as ¹⁰B carriers. The *in vivo* evaluation of BADB-1 is ongoing and the results will be reported soon.

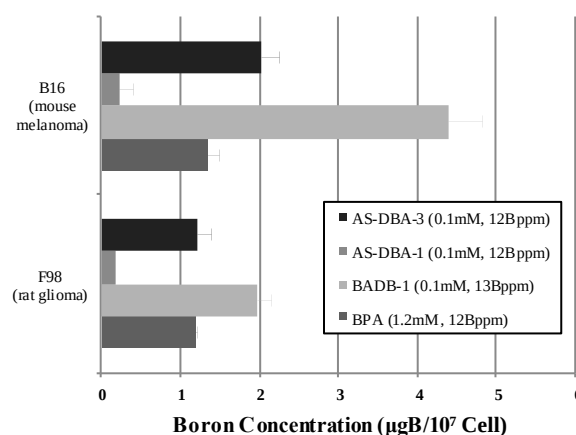


Fig. 2. Boron-uptake test against cancer cells.

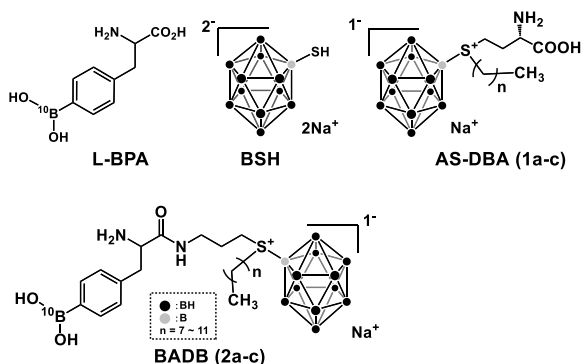


Fig. 1. Boron Carrier.

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INTRODUCTION: Boron neutron capture therapy (BNCT) is gaining attention as a state-of-the-art minimally invasive cancer treatment [1]. Up to now, clinical studies using boronophenylalanine (BPA) and sodium borocaptate (BSH) as ¹⁰B delivery agents for neutron capture reaction have been conducted [2,3]. However, continuous administration of their high concentrations is needed to keep sufficient ¹⁰B tumor concentration. Therefore, we have developed novel boron-containing low molecular compounds efficient in accumulation and retention in tumor.

EXPERIMENTS: 3x10⁶ of mouse colon carcinoma (CT26) cells were injected in the right thigh of 5-week-old female Balb/c mice. Two weeks after injection tumor-bearing (avg. 360 mm³) mice were used in this study. Two dosage of compound A (88.8 mg/kg with 24.1 mg [¹⁰B]/kg, n=5 and 250 mg/kg with 68.3 mg [¹⁰B]/kg, n=6) were administrated by tail vein injection 24 hours before irradiation. Fructose-BPA (500 mg/kg with 24.1 mg [¹⁰B]/kg, n=5) was administrated by tail vein injection 2 hours before irradiation as a positive control. Groups with irradiation only (n=4) and untreated mice (n=6) were used as controls.

The irradiation was performed with thermal neutrons with a flux of 1.8-4.0 x 10¹² neutrons/cm² over 1 hour at Kyoto University Research Reactor (KUR).

The tumor size and body weight were measured in the period starting prior the treatment till 26 days after irradiation. And the tumor volume (mm³) was calculated using the following formula.

$$\text{Tumor volume} = (\text{long diameter}) \times (\text{short diameter})^2 / 2$$

RESULTS: The results of thermal neutron irradiation is shown in Fig. 1. The effect on body weight in all individuals was not found (a). Compound A administrated group and BPA administrated group significantly suppressed the tumor growth as compared with other control groups (b). In particular, Compound A (250 mg/kg) administrated group showed tumor growth inhibitory effect comparable to BPA administrated group until 18th day.

We assume that Compound A is a candidate boron compound for further investigation that showed high accumulation in tumor even 24 hours after injection.

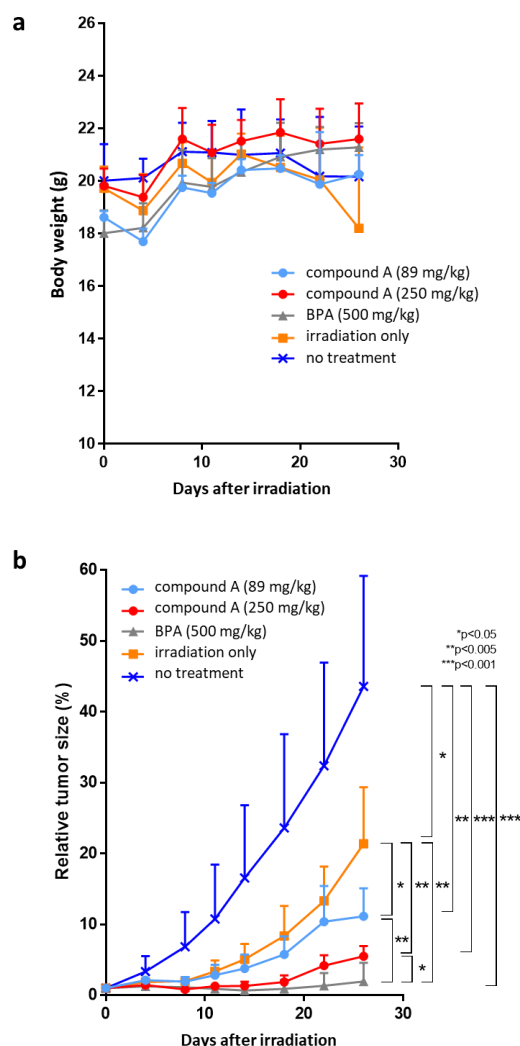


Fig. 1. BNCT of tumor-bearing mice with ¹⁰B-enriched compounds. (a) Mice body weight after thermal neutron irradiation. (b) Tumor growth ratio after 1 hour - thermal neutron irradiation (1.8-4.0 x 10¹² neutrons/cm²) with the injection of compound A 24 hours, and BPA 2 hours before irradiation, and irradiation only and untreated groups as controls. Comparison using the t-test was performed using mice 26 days after irradiation.

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INTRODUCTION

The boron neutron capture therapy (BNCT) is based on a binary approach that combines non-invasive thermal neutron irradiation and administration of boron-10 (¹⁰B) compounds, which can result in small-range nuclear fission, followed by tumoricidal effects. For the last decade, much efforts have been made on the establishment of safe and stable neutron sources such as linear accelerators, so that in the near future the BNCT could propagate much rapidly and widely, through the local hospitals. Nevertheless, most of the BNCT agents, whether in pre-clinical development or clinical trials, have shown significant obstacles, preventing further clinical translations. For instance, one of only clinically approved BNCT agent, boronophenylalanine (BPA), is a low-molecular weight compound with extremely rapid renal clearance, thus requires continuous intravenous infusion, usually begins few hours before the irradiation and even continues during the irradiation. Thus, the boron concentration in the blood circulation at the moment of neutron irradiation should be considerably high, not only narrowing down therapeutic window of the BNCT agent, but also inducing critical adverse effects to the non-target tissues.

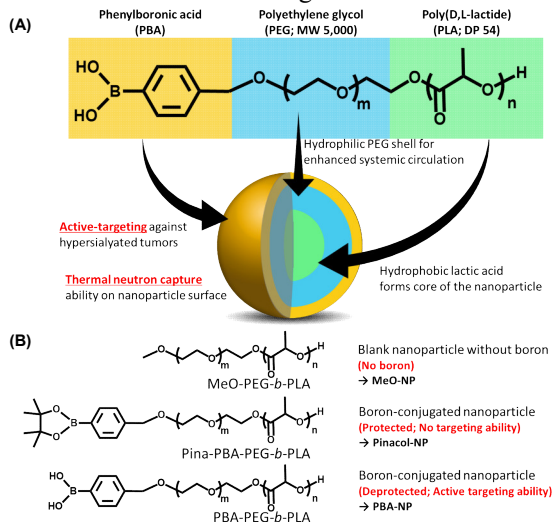


Figure 1. (A) Schematic design of the PBA-installed nanoparticle (PBA-NP). (B) Chemical structure of the MeO-PEG-*b*-PLA, Pina-PBA-PEG-*b*-PLA, and PBA-PEG-*b*-PLA.

Herein, to address aforementioned limitations, we designed and synthesized phenylboronic acid (PBA)-installed polymeric nanoparticles, as illustrated in Fig. 1. The PBA groups on the nanoparticles not only grant thermal neutron capturability but also provide strong affinity to the sialic acid, supposed to facilitate effective accumulation of the nanoparticles to hypersialated metastatic tumor cells.

EXPERIMENTS

Alkoxyated 4-(hydroxymethyl)phenylboronic acid pinacol ester was used as an initiator for anionic ring-opening, sequential polymerization of ethylene oxide and D, L-lactic acid. Accordingly, ¹H NMR analysis confirmed that prepared polymer is comprised of pinacol ester-protected PBA (Pina-PBA), hydrophilic polyethylene glycol (PEG) chain (*c.a.* 110 units), and hydrophobic poly(lactic acid) (PLA) chain (*c.a.* 40 units), hence abbreviated as Pina-PBA-PEG-*b*-PLA (Fig. 1B). Furthermore, deprotection of the pinacol ester was conducted by transesterification with 1,4-phenylenediboric acid, resulted in PBA-PEG-*b*-PLA. Those polymers were separately dissolved in dimethylformamide and subsequently dialyzed against pure water, to remove organic solvent and to generate the nanoparticles through hydrophilic-hydrophobic interactions. Two different PBA-installed nanoparticles, either with pinacol protection (Pinacol-NP) and without any protection (PBA-NP), were prepared and analyzed by static and dynamic light scattering measurements. In vivo feasibility of those nanoparticles was evaluated on a C57BL/6j mice model, subcutaneously inoculated with B16-F10 mouse melanoma cells on the right thigh. When average tumor volume was reached around 50 mm³, the mice were subcutaneously injected either with saline, BPA, Pinacol-NP, or PBA-NP (*n* = 6). It is noteworthy that applied timeframe between drug injection and thermal neutron irradiation was different between low molecular weight compounds groups (2 h for saline and BPA) and nanoparticles groups (48 h for Pinacol-NP and PBA-NP), as their pharmacokinetics were found to be significantly altered. All the irradiation experiments were carried out in the Institute for Integrated Radiation and Nuclear Science, Kyoto University.

RESULTS

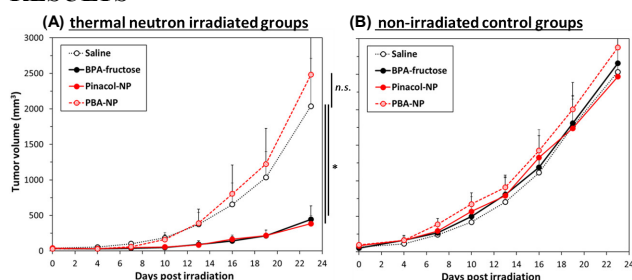


Figure 2. Tumor growth profile of the B16-F10 melanoma tumor bearing mice, subcutaneously injected with saline, BPA-fructose (24 mg ¹⁰B/kg), Pinacol-NP and PBA-NP (0.24 mg ¹⁰B/kg). (A) thermal neutron irradiated groups and (B) non-irradiated control groups. (*n* = 6, **p* < 0.05)

Aforesaid nanoparticles, either with or without pinacol ester protection, were highly stable in physiological condition, as their average hydrodynamic diameters were sustained between 70–80 nm for at least 24 h (data not shown). This suggests that the nanoparticles might demonstrate long-term circulation and attenuated renal clearance profile, once they enter the bloodstream. Notably, in vivo irradiation experiments ensured feasibility of the Pinacol-NP, as it exhibited comparable antitumor effects with the BPA-fructose complex (Fig. 2), even at a 100-folds lower effective dose (0.24 mg ¹⁰B/kg for Pinacol-NP and PBA-NP, and 24 mg ¹⁰B/kg for BPA). On the contrary, the PBA-NP exhibited almost no antitumor effect as its tumor growth profile is similar to the saline, presumably because of non-specific association with sialic acids on circulating cells and enzymes, which might accelerate the clearance through reticuloendothelial system. This speculation could be supported by a significantly lower tumor accumulation of the PBA-NP, compared to the Pinacol-NP (data not shown).

PR5-10 Design, Synthesis, and Evaluation of Glucose-type Boron Carriers for BNCT

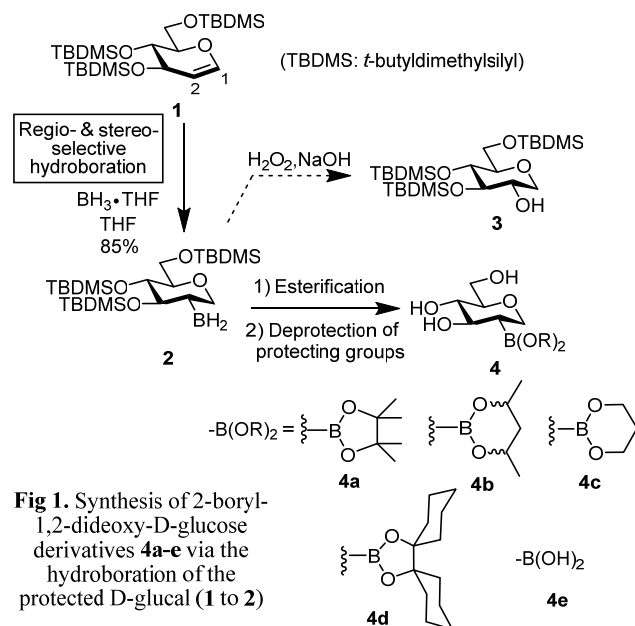
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INTRODUCTION: Boron neutron capture therapy (BNCT) is one of powerful therapies for local tumor control in the treatment of brain tumor, melanoma, and so on [1]. To date, only two boron-containing drugs, L-4-boronophenylalanine (BPA) and BSH (sodium mercaptoundecahydrododecaborate, $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$), have been approved as clinically test compounds, and development of better BNCT agents is highly required.

It is well described that tumor cells metabolize D-glucose by anaerobic glycolysis, which provides 2 moles of ATP per mole of D-glucose. Therefore, the rapid growth and proliferation of tumor cells demand a drastic increase in D-glucose uptake and metabolite flux using glucose transporters (GLUT) and sodium dependent glucose cotransporters (SGLT, known as the Warburg effect). An enhanced uptake of D-glucose and glucose transporter expression are common in cancer cells and provide clinically valid targets for cancer diagnosis and treatment using derivatives such as 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) and 2-deoxy-D-glucose (2-DG). In this paper, we report new BNCT agents **4a-d** based on the D-glucose scaffold having borane at the C2 position of D-glucose by the hydroboration of D-glucal derivatives **1** that contain a double bond between the C1 and C2 positions (Fig. 1) [2].



EXPERIMENTS and RESULTS: Synthesis of 2-boryl-1,2-dideoxy-D-glucose derivatives **4** was carried out via the regio- and stereoselective hydroboration of the protected D-glucal **1**, as shown in Figure 1. Hydroboration is typically used to convert alkenes to alcohols. A boryl intermediate such as **2** is obtained by the treatment of an alkene with a borane reagent, and are then generally converted to alcohol such as **3** by treatment with hydrogen peroxide (H_2O_2) with the retention of stereochemistry. However, we use the boryl intermediate **2** for the synthesis of BNCT agents **4a-e** after esterification of **2** with diols and deprotection of the hydroxyl groups, without the reaction with H_2O_2 . Cytotoxicity and cellular uptake activity of **4a-d** in cancer cells was evaluated by MTT assay and ICP-MS (inductivity coupled plasma-mass spectrometer) (Fig. 2). These data and the results of enzymatic phosphorylation indicate that **4e** could be a lead compound of tumor-accumulating BNCT agents.

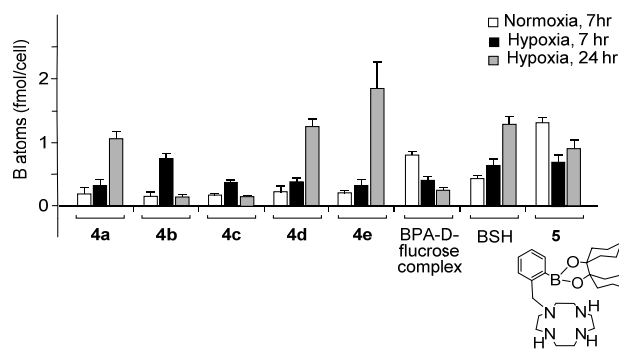


Fig. 2. Cellular uptake assays of **4a-e**, BPA-D-fructose complex, BSH, and the reference compound **5** [3] in HeLa S3 cells, as determined on ICP-MS after incubation with 30 μM of the drugs under normoxic and hypoxic conditions at 37 $^{\circ}\text{C}$ for 7–24 hr. Data represent the mean $\pm$ SD of at least three replicates.

The advantages of **4a-e** are: i) **4a-e** have high solubility in water and low toxicity; ii) **4a-e** are translocated to the cancer cells through GLUT1 and iii) the uptake pathway of 2-borylsugar is different from those of BSH and BPA, which allows us to use the combination of 2-borylsugar, BSH, and/or BPA. These findings suggest that 2-boryl-1,2-dideoxy-D-glucose derivatives, especially **4e** could function as a lead compound of tumor-accumulating BNCT agents. The improvement of the design of these B-carriers are now in progress.

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PR5-11 Investigation of therapeutic potential of poly(vinyl alcohol)-boronophenylalanine complexes in subcutaneous hypovascular tumor models

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INTRODUCTION: Boronophenylalanine (BPA) is the most powerful drug in clinical boron neutron capture therapy (BNCT). BPA can accumulate selectively within target tumors through the large neutral amino acid transporter 1 (LAT1), which is overexpressed on many cancer cells [1]. Although BPA has exhibited strong BNCT effect, its therapeutic effect has been sometimes compromised by quick clearance from tumors. The clearance from the tumor may be due to antiport mechanism of LAT1. Intracellular BPA may be exchanged with extracellular amino acids when extracellular BPA concentration decreases [2].

We recently found that poly(vinyl alcohol) can form the complex with multiple BPA molecules through boronate esters in aqueous solution, and the complex, termed PVA-BPA, can be internalized into cells through LAT1-mediated endocytosis, thereby avoiding the untoward efflux and improving retention in tumors. Importantly, in subcutaneous hypervascular CT26 tumor models, PVA-BPA showed augmented therapeutic effect compared with conventional fructose-BPA complexes. In this study, to examine whether PVA-BPA can exhibit such therapeutic effect even in a hypovascular tumor model, we evaluated the antitumor activity of PVA-BPA in a subcutaneous BxPC3 tumor model.

EXPERIMENTS: BALB/c nude mice bearing subcutaneous BxPC3 tumors were used in this study. PVA-BPA or fructose-BPA complex was intravenously injected to the mouse (10 mg BPA/mouse), and the thermal and epithermal neutrons were irradiated to the tumor using KUR 3 h after the injection. Size of the tumor was measured using a caliper, and tumor volume (V) was calculated using the following equation:

$$V = 1/2 \times a \times b^2$$

where a and b denote major and minor axes of a tumor, respectively.

RESULTS: The pancreatic cancer BxPC3 cells are reported to form a subcutaneous hypovascular tumor having rich stroma inhibiting penetration of drugs, which characteristics can be generally found in intractable cancers. The key to treating such stroma-rich tumors is efficient penetration of drugs in the tumor, and macromolecules sometimes suffer from limited penetration.

As shown in Fig. 1, both fructose-BPA and PVA-BPA exhibited strong BNCT effect, and PVA-BPA

more efficiently suppressed the tumor growth. The possible explanation for this enhancement should be prolonged tumor retention of PVA-BPA. The quick clearance of fructose-BPA from the tumor might compromise the therapeutic efficacy. It is noteworthy that the use of PVA did not deteriorate the therapeutic potential, which may be explained by the molecular weight of PVA. We used PVA with the molecular weight of roughly 10,000. This smaller molecular weight compared with the other macromolecules or nanoparticles (including liposomes and polymeric micelles) might facilitate the intratumoral penetration. PVA should offer great potential as the additive boosting therapeutic potential of BPA.

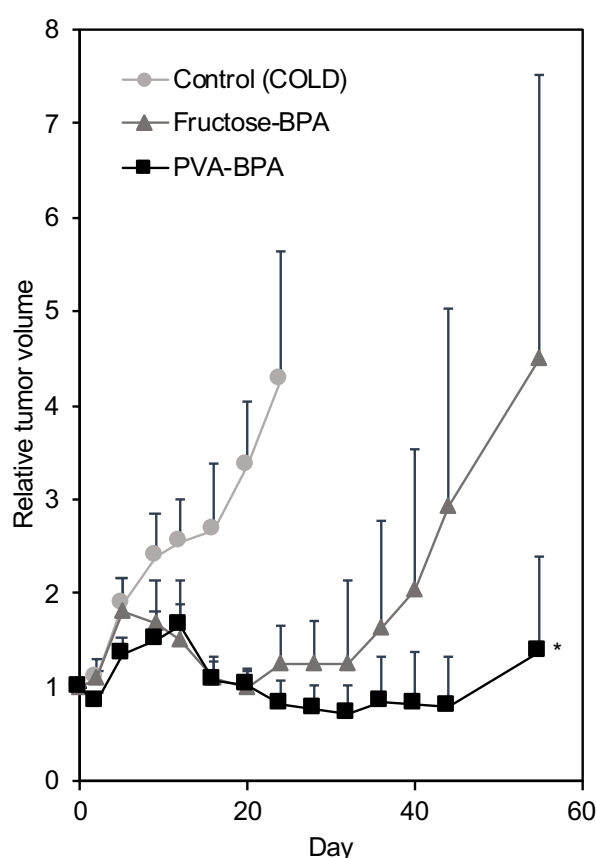


Fig. 1 Antitumor effect of fructose-BPA and PVA-BPA. At day 0, the samples were injected, and neutrons were irradiated to the tumor.

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INTRODUCTION: In boron neutron capture therapy (BNCT), successful treatment requires boron drugs that can selectively target tumors. Although boronophenylalanine (BPA) has demonstrated its therapeutic potential in many clinical studies, BPA can treat only the tumors overexpressing LAT1. Thus, novel boronated compounds that can accumulate within tumors through the other mechanism should be developed to extend the application of BNCT.

In this regard, polymeric carriers including polymer conjugates and polymeric micelles have attracted recent attention, because these polymeric carriers offer efficient tumor accumulation through enhanced permeability and retention effect [1]. In particular, polymer conjugates have the advantages of efficient penetration in tumors as well as quick clearance from normal tissues owing to their smaller size compared with polymeric micelles [2]. We recently synthesized biocompatible boronated polymers whose molecular weight can be easily controlled. We found that these polymers exhibited molecular weight-dependent tumor accumulation and retention in the blood. In this study, we compared the therapeutic effect of the optimized polymer with that of sorbitol-BPA complexes.

EXPERIMENTS: BALB/c nude mice bearing subcutaneous BxPC3 tumors were used in this study. Boronated polymers or sorbitol-BPA complexes were intravenously injected to the mouse (10 mg BPA/mouse), and the thermal and epithermal neutrons were irradiated to the tumor using KUR 2.5 h after the injection. Size of the tumor was measured using a caliper, and tumor volume (V) was calculated using the following equation:

$$V = 1/2 \times a \times b^2$$

where a and b denote major and minor axes of a tumor, respectively.

RESULTS: As shown in Fig. 1, our synthesized polymer exhibited comparable antitumor effect to sorbitol-BPA. Considering that sorbitol-BPA is the most powerful boron drug in clinical situations, our polymer may also have great potential for clinical BNCT. It is also important that the polymer did not significantly decrease the body weight of the mice, indicating that the polymer should not have acute toxicity. These results suggest that our polymer may be useful in the clinical application.

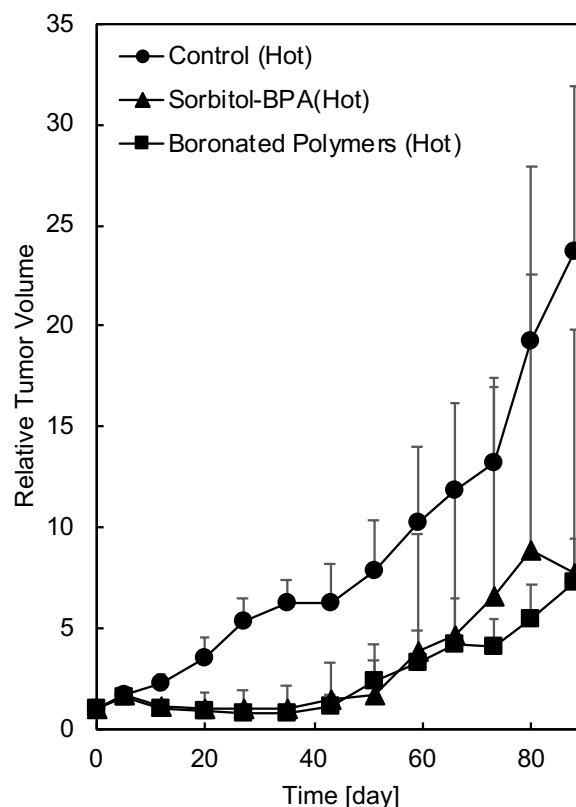


Fig. 1 Antitumor effect of boronated polymers and sorbitol-BPA. At day 0, the samples were injected, and neutrons were irradiated to the tumor.

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INTRODUCTION: Boron neutron capture therapy (BNCT) has been attracting growing interest as one of the minimally invasive cancer therapies. The phase II clinical study of accelerator-based BNCT for the treatment of brain tumor and head and neck cancer patients has been completed in Japan. We focused on a serum albumin as a nano biocarrier. Albumin is known to accumulate in malignant and inflamed tissues due to enhanced permeability and retention (EPR) effect. We developed maleimide-functionalized *closo*-dodecaborate (MID) for conjugation to bovine serum albumin (BSA).[1] In this paper, we designed disulfide bond containing MID (SSMID) for identification of the binding sites on albumin by MS/MS analysis.

EXPERIMENTS: For the modification with SSMID, a solution of BSA (final concentration 100 μ M) in PBS buffer was added SSMID (final concentration 10 mM). The solution was briefly vortexed and incubated at room temperature for 12 h. The mixture was added 5 $\times$ SDS-PAGE sample buffer, boiled at 95 $^{\circ}$ C for 5 min and subjected to SDS-polyacrylamide gel (10% acrylamide) electrophoresis (PAGE). The gel was stained by CBB and obtained image with a Molecular Imager ChemiDoc XRS (Bio Rad). The entire acrylamide gel was rinsed with ultrapure water for a few hours, and bands (spots) of interest were excised with a clean scalpel. The gel pieces cut into a cube size was transferred to a tube and they were washed with ultrapure water for 10 minutes three times. The gels pieces were treated with 100 μ L of 100 mM ammonium bicarbonate/acetonitrile (1:1, vol/vol) solution and incubate tubes for 10 min at 37 $^{\circ}$ C. The supernatant was removed and the gels were treated with 100 μ L of 100 mM ammonium bicarbonate solution and incubate tubes for 10 min. The supernatant was removed and the gels were treated with 100 μ L of 100 mM ammonium bicarbonate/acetonitrile (1:1, vol/vol) solution and incubate tubes for 10 min at 37 $^{\circ}$ C. The supernatant was removed and the gels were treated with 50 μ L of acetonitrile and incubate tubes at 37 $^{\circ}$ C until gel pieces become white and shrink. The shrunk gels pieces were treated with 50 μ L of 100 mM DTT / 100 mM ammonium bicarbonate solution, vortex briefly and incubate tubes for 30 min at 37 $^{\circ}$ C. The tubes were cooled down to room temperature and the supernatant was removed completely. For capping free SH

groups, 50 μ L of 100 mM iodoacetamide / 100 mM ammonium bicarbonate solution was added and incubated for 30 min at room temperature in the dark. The gels pieces were washed with 100 μ L of 100 mM ammonium bicarbonate solution and 100 μ L of 100 mM ammonium bicarbonate/acetonitrile (1:1, vol/vol) solution. The gel pieces were shrunk with acetonitrile. A solution of trypsin in Tris buffer (pH 8.0) were added to the dry gel pieces. The samples were incubated overnight at 37 $^{\circ}$ C. The digestion was quenched with adding TFA (final 0.1 %), and the samples were desalinated through Cleanup C18 Pipette Tips (Agilent). The solution (2 μ L) were mixed on MALDI plate mix with 1 μ L of CHCA.

RESULTS: Since the *closo*-dodecaborate of SSMID could be cleaved by DTT after conjugation with BSA, SSMID conjugation sites on BSA were identified by MS/MS analysis. After in-gel digestion was performed with trypsin, the resulting peptide fragments were analysed by MALDI TOF-MS. The mascot analysis identified at least three lysine residues, Lys221, Lys413 and Lys431, as conjugated sites in addition to Cys34 (Fig. 1).[2]

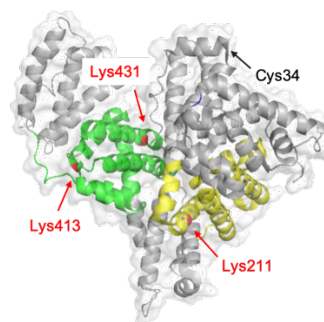


Fig. 1. MID-binding sites of albumin (PDB: 1E7F). Four SSMID modification residues (Cys34, Lys211, Lys413 and Lys 431) were identified by MS/MS analysis.

Because these Lys residues are located in the drug binding sites 1 and 2 in albumin, it is plausible that MID and SSMID are bound to these drug binding sites to form a covalent bond to the protein through these Lys residues. The fact that MID-BSA conjugation was prevented in the presence of warfarin and ibuprofen [3] also supports our hypothesis. We are now in a position to develop protein-based boron carriers with multiple functions, such as for in vivo imaging and active targeting in BNCT.

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PR5-14 Experiment on the Therapeutic Effect of Doxorubicin-Encapsulated Boron Liposome by Thermal Neutron Irradiation

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INTRODUCTION: There has been a growing interest in Boron Neutron Capture Therapy (BNCT) because they are expected to be next generation in minimally invasive cancer treatment. And the clinical trial was started using boronophenylalanine (BPA) as boron drugs in 2017. However it is difficult that all patients fully remit from cancer by BNCT only. The purpose of this study was to examine whether the combination therapy (BNCT and chemotherapy) using boron liposome get higher anti-tumor effect. Therefore we investigated the therapeutic effect of doxorubicin-encapsulated boron liposome prepared using PBL^[1].

EXPERIMENTS:

1. Preparation of PBL modified liposome

PBL modified liposome were prepared using bare liposome by post insertion method^[2]. The bare liposome were prepared from DSPC and cholesterol (1:1, molar ratio) by conventional lipid-film method^[3]. The resulting liposome were extruded with an extruder through a polycarbonate membrane with a 100-nm pore size, yielding each liposomes.

2. Encapsulation of doxorubicin (DXR) into PBL modified liposome

The liposome encapsulating doxorubicin were prepared by the pH-loading method^[4] and measured inclusion amount by fluorescence spectrometry. DXR concentration of the resulting liposome suspension was 3.3 mM.

3. Combination therapy using doxorubicin-encapsulated PBL-liposome for tumor-bearing mice

The tumor-bearing mice were prepared by grafting 5×10^6 of mouse colon carcinoma cells (CT26) to the right thigh of female BALB/cA mice (4 weeks old, weighing 16-20 g) to have a tumor diameter of 6-8 mm.

Table.1) Characterization of administration samples.

	PBL-liposome	DXR-encapsulated PBL-liposome	BPA
¹⁰ B conc. [mgB/kg]	10	10	10
Lipid conc. [mg/mL]	650	500	—
Interval time before irradiation [hour]	12	24	2

About 12 days after, each sample (Table.1) was administered by tail vein injection before irradiation. And the irradiation was performed with thermal neutrons with a flux of $1.8\text{--}4.0 \times 10^{12}$ neutrons/cm² over 1 hour.

The tumor size was measured over time after the irradiation until Day 26 and calculated using the following formula.

$$\text{Tumor size [mm}^3\text{]} = \frac{(\text{Long diameter [mm]}) \times (\text{Short diameter [mm]})^2}{2}$$

RESULTS: As shown in Fig. 1, significant body weight loss was observed in mice administration of DXR-encapsulated PBL-liposome. And all irradiated mice in this group died within 20 days. This suggests that side effect of DXR in the liposome occur by irradiation.

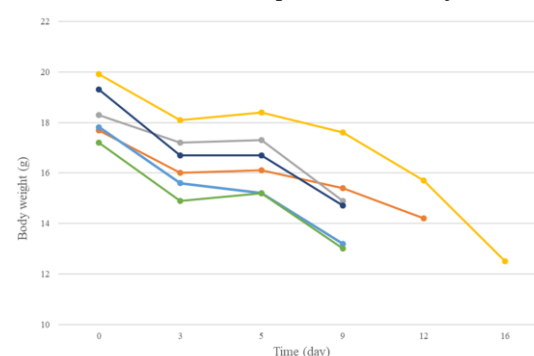


Fig.1) Body weight of mice after irradiation with the injection of DXR-encapsulated PBL-liposome. Data represent each result for six mice.

As shown in Fig. 2, PBL-liposome significantly suppressed the tumor growth as compared to other control groups, indicating its excellent candidate drug potential for BNCT.

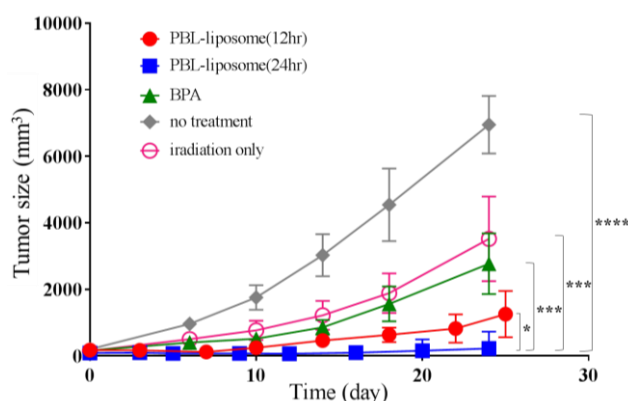


Fig.2) Antitumor effect of BNCT by PBL-liposome.

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INTRODUCTION: The functionalized oligonucleotides have been widely used for various scientific fields including gene therapy or diagnosis as well as DNA-based drugs. However, artificial oligonucleotides required toxic gene transfer agent such as cationic lipid for cellular uptake, and therefore there are increasing demands for easy methods to introduce them into cells. Recently, we reported that amphiphilic oligonucleotides with hydrophobic units formed aggregates in aqueous solution, and these aggregates showed unique properties.¹ The aggregate consisted of ODNs smoothly penetrated cell membrane and showed high stability in living cells. Thus, the aggregate act as favorable functional oligonucleotides in living cells. In this study, we attempted to apply artificial oligonucleotides bearing hydrophobic fluorophore BODIPY at strand end (BODIPY-ODN) to drugs for BNCT. We prepared these artificial oligonucleotides and characterized their properties in living cells and tissues.

EXPERIMENTS:

Measurement of critical aggregate concentration (CAC). To form the aggregate, indicated concentrations of BODIPY-ODN in phosphate buffer (5 mM, pH 7.0) were added to 10 μ M indocyanine green in acetonitrile. After the removal of solvent in vacuo, the resulting mixture was dissolved in water to form the aggregate and the fluorescence spectra of the resulting samples were measured using excitation at 774 nm.

In vivo experiments using BODIPY-ODN. BODIPY-ODN (100 μ M) in 50 μ L of saline were intratumorally injected into the A549 tumor-bearing mice. After 1 h, the mice were irradiated (neutron, 1 MW) for 45 min at KUR. Then, the change of the size of the tumors were measured.

RESULTS: Initially, we evaluated an aggregation property of 10 mer BODIPY-ODN. We identified the aggregate formation by measurement of fluorescence of indocyanine green (ICG). ICG showed bright emission in aqueous solution, while encapsulation of ICG into the aggregate consisting of amphiphilic molecules led to a suppression of fluorescence emission due to the concentration quenching even in the aqueous solution. Thus, we evaluated the aggregate formation of BODIPY-ODN by monitoring of ICG emission. As a result, we observed robust fluorescence of ICG in the absence of BODIPY-ODN, while the addition of BODIPY-ODN led to decrease of its emission intensity. Thus, BODIPY-ODN formed aggregate that encapsulated ICG in their hydro-

phobic core. The CAC values, which indicate the concentration of amphiphiles above which aggregate formation is observed, were estimated to be 430 nM for BODIPY-ODN.

Next, we demonstrated the cellular experiments of BODIPY-ODN using a human cell line of lung carcinoma A549 to characterize their behavior in living cells. We evaluated the cellular uptake of BODIPY-DNA aggregate. A549 cells were incubated with 2 μ M BODIPY-ODN for 24 h and imaged the fluorescence emission of BODIPY from the cells by microscopy. We observed high levels of emission from the cells, indicating that BODIPY-ODN penetrated cell membrane smoothly at this concentration. These results strongly indicate that the aggregate formation of BODIPY-ODN was key for its cellular uptake. Finally, we evaluated the function of BODIPY-ODN aggregate in vivo. For the experiments in vivo, we transplanted A549 tumor cells into the lower thigh of nude mice. To the tumor tissue, the aggregate consisted of BODIPY-ODN were intratumorally injected. After 1 h, the thermal neutrons were irradiated to mice, and then the changes of tumor size were monitored. We expected the shrinking of the tumor size, however, the negligible effects of BODIPY-ODN and irradiation were observed probably due to the low concentration of drugs in the tumor tissue (Fig. 1). The improvements of the method of administration and increase of drug amount were under investigation.

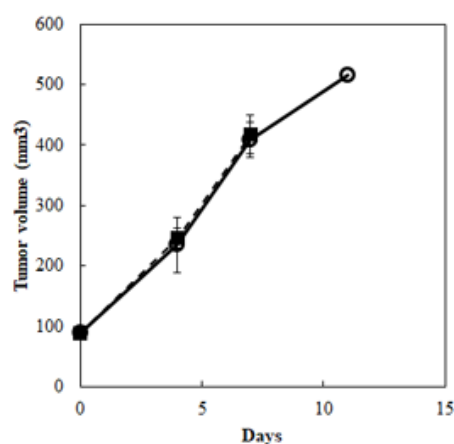


Fig. 1. Monitoring of the tumor volume after administration of BODIPY-ODN and irradiation. Open circle and solid line: administration of BODIPY-ODN. Filled square and dashed line: administration of saline.

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PR5-16 *In vivo* evaluation of boron neutron capture therapy for head and neck cancer

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INTRODUCTION: Head and neck cancer (HNC) is one of main target for Boron neutron capture therapy (BNCT) with consideration for functional and aesthetic outcomes. [1]. However, there are few facilities to evaluate *in vivo* evaluation of BNCT. We optimize HNC model mice to evaluate the novel boron compound in this study.

EXPERIMENTS: In pervious *in vitro* study, we used SAS (JCRB0260, Japan) and HSG-c5 cell line (JCRB1070, Japan) as human head and neck cancer cell line. The intracellular ¹⁰B concentration as sodium mercaptoundecahydrododecaborate (BSH, Katchem, Czech) and boronophenylalanine (BPA, Katchem, Czech) in SAS and HSG cell lines were measured by inductively coupled plasma-atomic emission spectroscopy. The result showed that the intracellular uptake and accumulation of BPA was higher than that of BSH in both SAS and HSG cell lines. Also, in boron neutron capture reaction for SAS and HSG cell lines, more suppressive effects on colony formation and cell viability in BPA compared with BSH were observed in each cell lines. Therefore, we used BPA as ¹⁰B to optimize in this *in vivo* study.

In this *in vivo* study, SAS or HSG cell lines were subcutaneously injected into the left hind legs of 6-week-old female Balb/c nude mice (Clea Japan Inc., Japan). Using L-boronophenylalanine (BPA, Katchem, Czech), fructose-BPA complex (200 mg/kg) was injected to the HNC tumor bearing mouse 2hrs before irradiation. The average fluence of the thermal neutron (0, 3, 4 X10¹² n/cm²) were irradiated. After neutron irradiation, the tumor size was calculated according to the following formula.

Tumor volume [mm³] = (Long diameter [mm]) x (Short diameter [mm])² /2

RESULTS: As shown in Fig. 1, irradiation groups showed significantly decrease in tumor size compared to non-irradiation group at 2 weeks after BNCT for HNC mice. In irradiation of SAS group, gradually tumor regrowth is shown at 3 weeks after irradiation. However, in irradiation of HSG group, the tumor growth is shown as non-irradiation group at 1 weeks after irradiation, at 3 weeks after irradiation, there are no regrowth of tumor. In non-irradiation group, tumor is increased and the growth speed of HSG group is significantly higher than that of SAS group. The optimization of *in vivo* BNCT protocol for head and neck cancer needs more consideration in another factor such as tumor size.

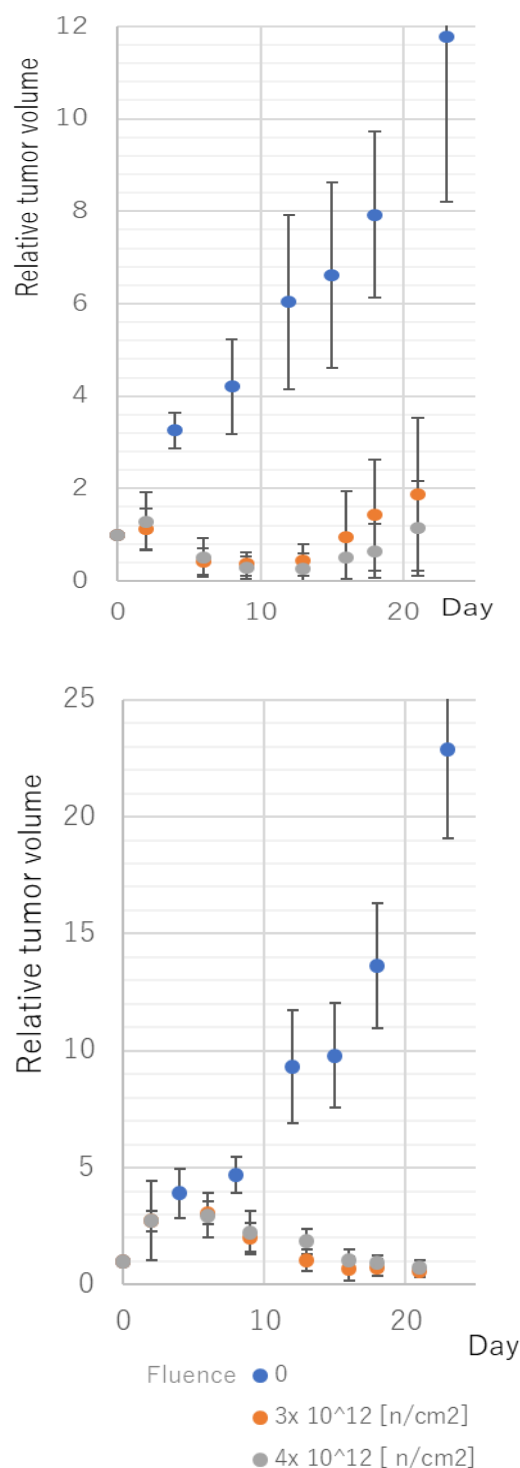


Fig. 1. Tumor growth ratio after thermal neutron irradiation (0, 3, 4 X 10¹² n/cm², each group n = 5) with BPA 2hrs before irradiation. (a) BNCT of

SAS-bearing mice, (b) BNCT of HSG-bearing mice

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Antitumor effectivity by Gd-neutron capture therapy using Arg-Gly-Asp(RGD) sequence binding Gd-DTPA-incorporated calcium phosphate nanoparticles

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INTRODUCTION: We had reported that the gadolinium neutron capture reaction (Gd-NCR) showed the tumour growth suppression, and could be applied to the intensive cancer treatments in near future [1,2,3].

Gadolinium-157 has been thought one of the attractive candidate atom for neutron capture therapy (NCT) agent because of its high thermal neutron cross section (255 000 barns). The range of induced high LET Auger electrons is few micron, so it is necessary to accumulate the ¹⁵⁷Gd atoms in the cancer cells for effective GdNCT [1,2,3].

In the recent targeting fields of pharmaceutical sciences, Arg-Gly-Asp(RGD) sequence is very useful, because RGD sequence can bind to the Integrin receptor of cancer cell surface. So, it can be used for cancer targeting by endocytosis mechanism of RGD sequence [4].

In this work, we prepared the RGD motif binded Gd-DTPA/CaP nanoparticles for selective cancer targeting, and augmentation of ¹⁵⁷Gd uptakes to cancer cells, then examined the GdNCR by intravenous injection.

EXPERIMENTS: *In vivo* evaluation was performed on colon-26 tumor-bearing mice irradiated for 60 minutes at nuclear reactor facility of Kyoto Univ Institute for Integrated Radiation & Nuclear Science with average neutron fluence of 2.0×10^{12} n/cm². Antitumor effect was evaluated on the basis of the change in tumor growth and survival rate of the mice.

RESULTS:

Tumor growth was suppressed in the groups of RGD sequence binding Gd-DTPA/CaP nanomicelle and bare Gd-DTPA/CaP nanomicelle compared with the non-irradiated groups with the injection of same DDS (data not shown). No acute toxicities were recognized in

the treated mice after GdNCT using intravenous injection of RGD sequence binding Gd-DTPA/CaP nanomicelle and bare Gd-DTPA/CaP nanomicelle.

The tumour volume was decreased after GdNCT. The abnormal change in the liver, the kidney, the heart, and the lung were not found in the histologic examination one month after Gd NCT in the treated groups of RGD sequence binding Gd-DTPA/CaP nanomicelle and bare Gd-DTPA/CaP nanomicelle.

In this experiment, the tumour decrease by bare Gd-DTPA/CaP nanomicelle was superior than the group of RGD sequence binding Gd-DTPA/CaP nanomicelle. We will confirm the size of nanomicelle and the Gd concentration in the nanomicelle, and the administrated volume of nanomicelle.

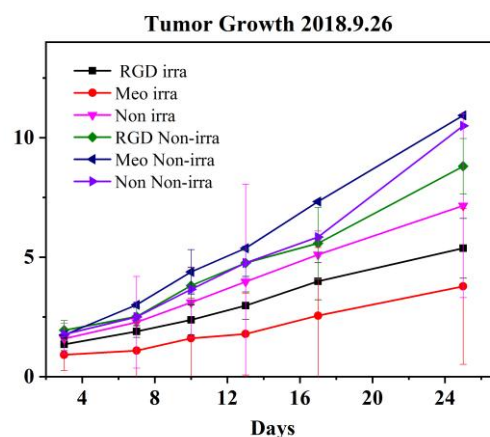


Figure 1. Tumor growth suppression in the group of RGD sequence binding Gd-DTPA/CaP nanomicelle and bare Gd-DTPA/CaP nanomicelle.

In the next experiments, we will check the expression of integrin receptors in many cancer cell lines. We hope to check the uptake of Gd atoms in the cancer cells by endocytosis. We will also evaluate of the mechanism of cytotoxicity on GdNCT, for examples, apoptosis, autophagy, senescence, etc. We hope to refer these results of toxicity examinations to the clinical studies of GdNCT for selection of target cancers in the future.

These results indicate that RGD sequence binding Gd-DTPA /CaP nanomicelle has the promising possibility as novel active targeting GdNCT agent.

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