CO7-1 Synthesis and evaluation of a novel boron neutron capture therapy agent

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INTRODUCTION:

Only p-dihydroxyboronophenylalanine (BPA) is currently used in BNCT as a boron agent. However, BPA has issues such as low tumor accumulation and selectivity, large single-dose amounts, and water solubility, so there is a need to develop novel agents that show more favorable distribution in the body and higher tumor uptake. Many novel drugs are currently under development, including boron-containing porphyrins, amino acids, peptides, monoclonal antibodies, liposomes, various nanoparticles, boron cluster compounds, and copolymers.

Prostate cancer is the most commonly diagnosed form of cancer and the second leading cause of cancer-related deaths in men in the world. The prostate specific membrane antigen (PSMA) is an attractive target for diagnosis and therapy of prostate cancer because of its high expression in prostate cancer.[1,2]

PSMA is expressed in normal prostate tissue, primary prostate cancer, and lymph node metastases of prostate cancer,[2] although PSMA expression in prostate cancer is 10- to 100-fold higher than that in normal prostate tissue.[3]

PSMA exhibits GCP-II (glutamatecarboxy peptidase-II) activity. Previously, several asymmetric urea compounds (X-CO-Glu) were reported to act as GCP-II inhibitors. We also demonstrated the high affinity of asymmetric urea compounds (maleimido-Cys-CO-Glu) for PSMA *in vivo*.[4]

Given this background, in this study, we designed and synthesized a novel BNCT agent targeting PSMA (BP-1).

EXPERIMENTS:

Synthesis: W-Prep 2XY (Yamazen Corporation, Osaka, Japan) was used for silica gel column chromatography on a Hi Flash silica gel column (40 mm, 60 Å, Yamazen). Silica gel 60 F254, 0.5 mm (Merck KGaA, Darmstadt, Germany), was used for preparative thin layer chromatography (PTLC). A LC-20AD (Shimadzu Corporation, Kyoto, Japan) equipped with a SPD-20A UV detector (ρ; 220 and 254 nm) (Shimadzu) and a NDW-351 radioisotope detector (Hitachi Aloka Medical, Ltd., Tokyo, Japan) was used for high performance liquid chromatography (HPLC). The eluent consisted of a binary mixture of 0.1% trifluoroacetic acid (TFA) in H2O (solvent A) and 0.1% TFA in methanol (solvent B). Nanopure water was prepared by MQ Integra15 (Nihon Millipore, Tokyo, Japan). Mass spectra were recorded on a LCMS-2010 EV (Shimadzu). 1H NMR spectra were recorded on a LNM-AL500 (JEOL) using CDCl3 or D2O as the solvent and tetramethylsilane as an internal standard (Euriso-top, Saint-Aubin, France). Two human prostate carcinoma cell lines were purchased from DS Pharma Biomedical (Osaka, Japan):

Cell culture: LNCaP (PSMA-positive) and PC-3 (PSMA-negative). The cells were cultured as previously reported in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% fetal bovine serum, glutamine, and antibiotics (penicillin/ streptomycin) in a humidified CO2 incubator (37°C/5% CO2).

In vitro assay: BP-1 was administered to the LNCaP (PSMA-positive) and PC-3 (PSMA-negative). Then the cells were incubated for 1hr and irradiated (neutron, 1MW) for 30min at KUR. After irradiation, the colony formation assay was conducted.

RESULTS:

BP-1 containing ^{10}B could be synthesized in 30-40% total yield. In the absence of neutron irradiation, no cellular damage was observed within the range of drug concentrations tested in this study. However, when neutron irradiation was conducted, cell damage was observed in a drug concentration-dependent manner. The basic usefulness of BP-1 was demonstrated, but further validation, including reproducibility, is needed.

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CO7-2 The evaluation of boron neutron capture therapy (BNCT) to the novel mouse model of pelvic recurrence of colorectal cancer

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Introduction

Colorectal cancer is the most common cancer worldwide. Surgical resection is the mainstay treatment of colorectal cancer. However, local recurrence still occurs in 5% to 13% of patients after curative resection. When the tumor is unresectable, it needs alternative therapeutic strategy.

In this study, we investigated the effectiveness of boron neutron capture therapy to pelvic colorectal cancer using the mouse model of pelvic recurrence of colorectal cancer.

Material and methods

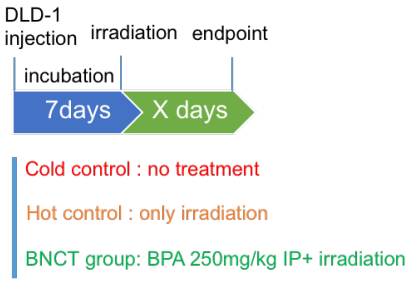
We used Boronophenylalanine (BPA) as a boron compound. DLD-1 cells were concentrated to 1.0×10^6/100μL in 0.1ml of PBS and injected into the pelvic cavity of each mouse. Animals were divided into three groups (nine animals per group); control, neutron irradiation only, BNCT with BPA (i.p.).

Result

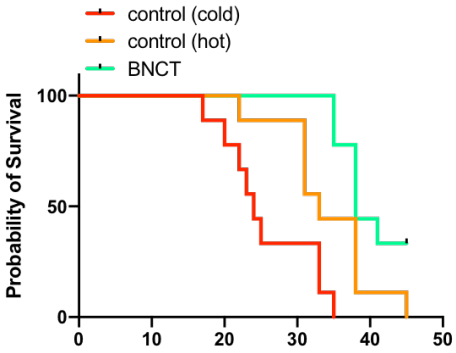
The nine mice were irradiated in the hot control and BNCT groups. BPA was administrated 4 h before irradiation in the group of BNCT. The experiment protocol was shown in **Figure. 1A**. The results showed that animal survival was significantly prolonged in the BNCT group compared with cold and hot control groups. (**Figure. 1B**) (VS. cold control, P<0.0001; VS. hot control, P<0.05) Kaplan-Meier plots also showed that animal survival was significantly prolonged in the hot control group compared with cold control. (P<0.05) The cold and hot control group showed a remarkable weight loss compared to the BNCT group. (**Figure. 1C**) These findings suggested that BNCT improved the survival CRC pelvic recurrence condition and no severe side effects of dehydration like diarrhea in the BNCT group.

Figure.1 Overall survival and change in body weight after BNCT.

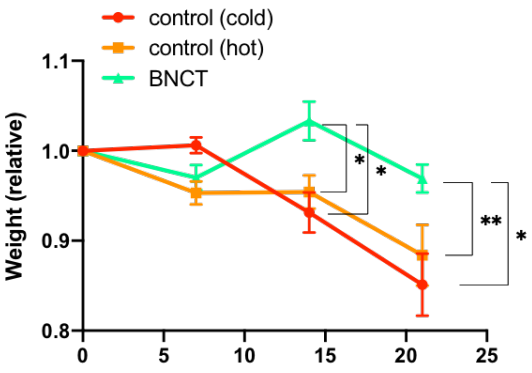
A Experimental protocol



B Overall survival



C Body weight



R3001

CO7-3 The abscopal effect as the late influence after the head neutron-irradiation of mice

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INTRODUCTION: It is reported that immune response is activated by partial radiation. The influence on immune organization of the mouse at the time of the head irradiation is not well known. The purpose of this study is to evaluate the relative biological effectiveness in the severe combined immunodeficiency (SCID), so-called SCID mice, those are having well-known high radiation sensitivity following thermal neutron irradiation for mice cranial.

Late effects of radiation are the influence to appear three months later after radiation. Here, I report the abscopal effect as the late influence after the head neutron-irradiation of mice.

EXPERIMENTS: CB17/Icr-*Prkdc*^{scid}/CrICrIj (SCID mice) were obtained from Charles River Inc. As a comparison experiment for the SCID mice, Balb/c and C3H/He mice were obtained from Japan Animal Inc.

Neutron irradiation was performed as follows. The Heavy Water Facility of the Kyoto University Research Reactor (KUR) was used. Mice were restrained in a plastic box on a radiation board. Neutron fluence was measured by radioactivation of gold foil and gamma-ray doses by TLD. I set the mouse so that the head part hit an irradiation port in the case of the escape pre-stick and irradiation 1Gy on the head in KUR 1MW. After neutron irradiation by KUR, mice were kept in animal breeding facilities and observed.

RESULTS: As shown in Table 1, the survival rate 12 months after the partial head-irradiation of the neutron by KUR. The partial head-radiation dose was about 1Gy that does not cause the bone-marrow death to a mouse. For example, by the experiment of the acute radiation damage of the SCID mouse, the dose of $LD_{50/30}$ (the dose that 50% die within 30 days after radiation exposure) is reported around 4Gy.

As for the SCID mouse, the death began four months after head irradiation, and the number of deaths became constant 12months from eight months. In case of Balb/c mouse, the death began seven months after head irradiation, and the number of deaths became constant 12months from eight months. With C3H mouse, the death began eleven months after head irradiation, and the number of deaths became the plateau from 12months.

As a result of dissecting a mouse over time, and having observed the internal organs, the involution of the spleen was accepted after irradiation with SCID mouse and Balb/c mouse four months after irradiation. Figure 1 shows that the splenic weight per body weight 4 months after head irradiation by 1MW KUR.

Table1. Survival rate 12months after head irradiation by KUR (1MW,1Gy) .

	C3H (n=20)	Balb/c (n=20)	SCID (n=21)
Survival rate	0.75±0.08	0.67±0.08	0.38±0.04

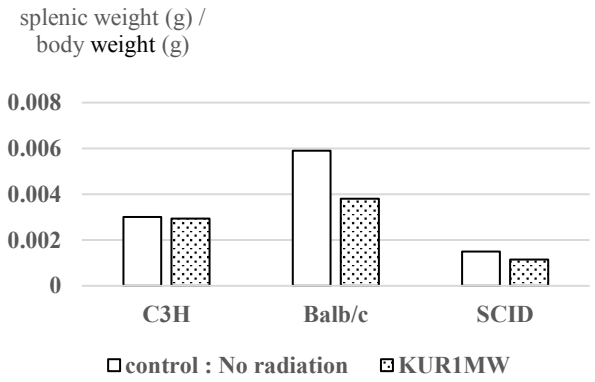

Fig.1 Involution of the spleen. The splenic weight per body weight was compared with no radiation mouse. SCID mice and Balb/c mice shows the involution of the spleen at 4 months after irradiation.

DISSCUSSION

In the previous study, I reported that the abscopal effect as the acute influence after the head neutron-irradiation of mice. In experiment of the apoptosis induced in the splenic cells 24 hours after head irradiation, the RBE values of SCID mice was 1.67, comparing the RBE values of C3H/He mice was 2.12. SCID mouse show extreme sensitivity to ionizing radiation, because cells lack functional DNA-dependent protein kinase.

In this study, the radiosensitive SCID mouse had a low survival rate and splenic regression damage, a so-called, the abscopal effect as the late influence after the head neutron-irradiation. It is predicted that the involution of the spleen causes immunodeficiency and death. It is my research theme that details mechanism to explain it in the future study.

CO7-4 A Fundamental Investigation on Correlation Between Important Elements for Activation and Major Elements in Concrete and Cements for Radiation Shielding

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INTRODUCTION: In several years, we performed neutron activation analyses to more than several hundreds of samples for radiation shield concrete and raw materials by KUR facilities. Based on the above NAA, a little database of important elements for activation for shield concrete and their raw materials were established to perform low activation shielding design for specific facilities [1]-[4]. On the other hand, we have also developed database of shielding materials with analytical major element data for them. The relationship between major elements and elements for activation is discussed in this paper.

DESCRIPTION of FIGURES: Figure 1 is described the correlation between two contents of Cobalt and Europium in concrete and cement. Figure 2 and Figure 3 show the correlation of contents of Cobalt to those of Silicon and Calcium for them. 24 kinds of shield concrete used in radiation facilities in Japan were prepared and irradiated in KUR, focusing to estimate Europium, Cobalt and Cesium (detail procedure was described in past report [4]), which are marked as blue circles in the figures. Nine kinds of cements are also symbolized as black triangles in the figures. Contents of Silicon and Calcium were estimated by x-ray fluorescence analysis (XRF), as well as other elements such as Aluminum, Iron, Sodium.

DISCUSSION: Figure 1 indicates the relatively positive correlation of Europium and Cobalt contents in concrete and cements, which were similar tendency as past works [4]. The relationships between such elements, which are important elements for activation, and major elements in concrete and cements are described in two following figures. Figure 2 indicates the relatively positive correlation between Silicon contents and Cobalt contents in concrete, while Silicon contents and Cobalt contents in cements are uncorrelated. Figure 3 also shows little correlation between Calcium contents and Cobalt contents.

CONCLUSION: Correlations of Silicon and Calcium contents to Cobalt contents are shown, which introduce the discussion of the relationship between major elements and important elements for activation in shield concrete based on material database for concrete and those raw materials.

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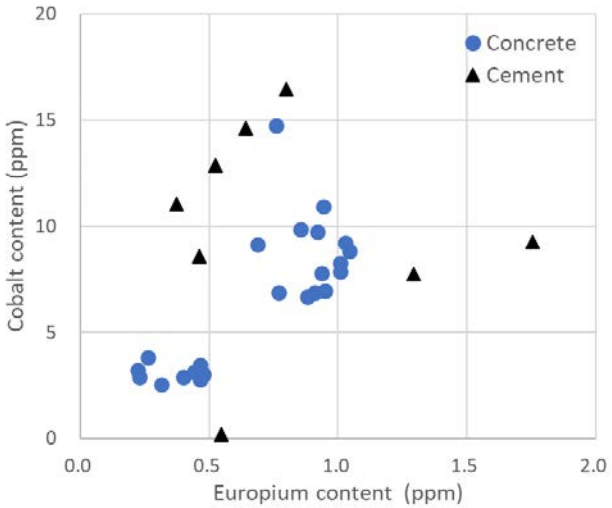

Figure 1 Correlation of Eu and Co in concrete and cement.

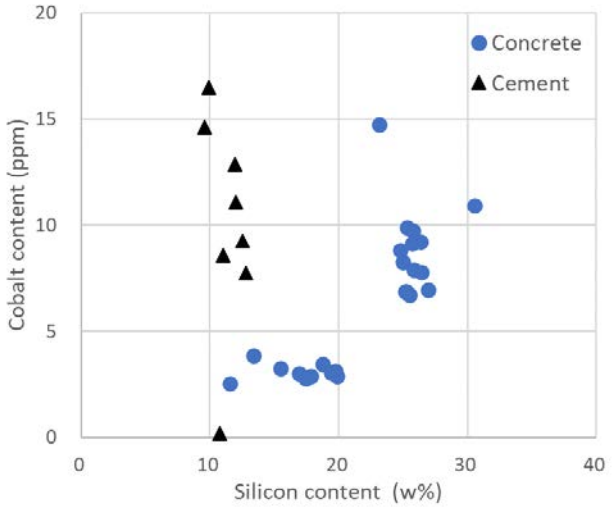

Figure 2 Correlation of Si and Co in concrete and cement.

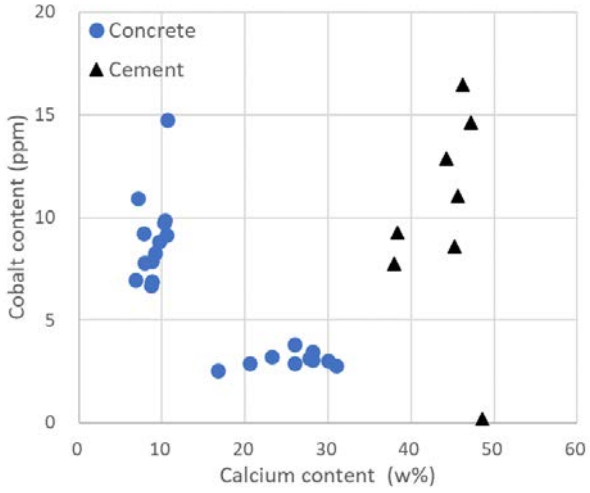

Figure 3 Correlation of Ca and Co in concrete and cement.

CO7-5 Development of Amino Acid Derivatives Containing ^{10}B-Clusters for BNCT

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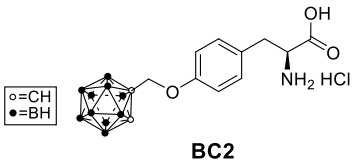
INTRODUCTION: Cancer cells alter amino acid and sugar metabolism in order to actively proliferate and metastasize. Proliferating cancer cells increase their uptake of glutamine for energy metabolism, causing glutamine addiction. Glutamine is transported into cells through ASCT2, and the imported glutamine can be used or exchanged through the L-type amino acid transporter (LAT1 or SLC7A5) for hydrophobic or aromatic amino acids such as isoleucine, valine, methionine, tryptophan, and phenylalanine. It is known that these transporters are overexpressed in a variety of tumor cells. Focusing on this characteristic of malignant tumor "glutamine addiction," we have designed, synthesized, and evaluated boron cluster-containing amino acid derivatives to develop boron carriers that can efficiently accumulate ^{10}B atoms in tumors via amino acid transporters that are highly expressed in tumors. As reported previously, **BC2** showed the best cellular uptake among the carborane-containing amino acid derivatives, and we further investigated its uptake process in the present study.



BC2

EXPERIMENTS AND RESULTS: Intracellular boron concentrations were examined over time when **BC-2** and L-boronophenylalanine (BPA) were administered to T98G cells at 10 μg ^{10}B/mL. As Fig. 2 shows, the boron concentration reached its maximum at 2 hours was maintained up to 20 hours. Next, the intracellular boron uptake was then measured at different **BC-2** concentrations over a 2-hour uptake time. The intracellular boron uptake was then measured at different **BC-2** concentrations over a 2-hour uptake time. The results showed that the boron concentration at 20 μgB/mL was 645 ng B/10^6 cells, approximately 2.5 times higher than at 10 μgB/mL (251 ng B/10^6 cells).

Next, to evaluate neutron sensitizing ability of the compounds, T98G cells were treated with 10 or 20 μg ^{10}B/mL boron carrier for 2 h. Then the cells were washed with PBS, suspended in serum containing medium and aliquoted into Teflon tubes for irradiation. Cells were irradiated using the neutron beam at the Heavy Water Facility of the Kyoto University Research Reactor (KUR) operated at 1 MW power output. The survival rates of the irradiated cells were determined using conventional colony assays. The D_{10} of BNCT was calculated from survival curve shown in Fig 2.

and treated for 20 hours, after which the collected cells were dissolved in nitric acid to be measured the boron concentration by ICP-AES. As a result, intracellular boron uptake was highest in BC-2, with a very high value of about 185 ng ^{10}B/10^6 cells, which is more than 10 times higher than BPA (Fig. 2).

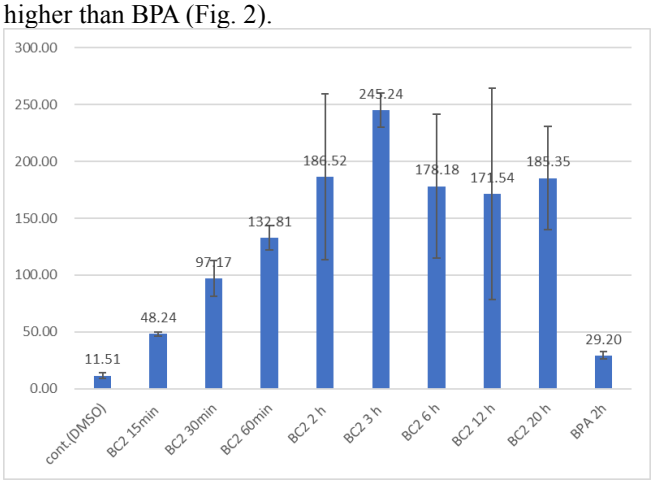
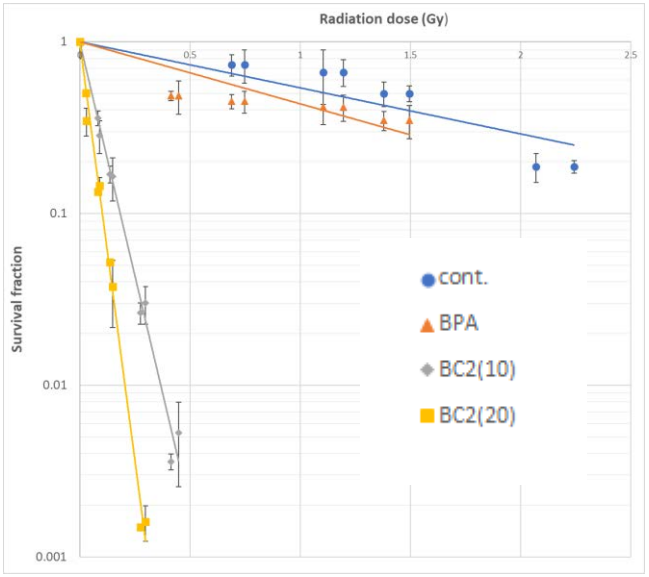


Fig. 1. Uptake of **BC2** in T98 cells.



	DMSO	BPA	BC2(10)	BC2(20)
D10 (Gy)	3.73	2.77	0.18	0.10
ER	0.96	1.29	19.46	34.78

Fig. 2. Survival curves after irradiation on T98G with **BC2**.

The cell-killing effect of neutron irradiation in the presence of BC-2 was enhanced in a dose-dependent manner. The Enhancement Ratio (ER) of **BC2** (20 μgB/mL) was 34.8, 30 times that of L-BPA (10 μgB/mL) (ER=1.29).

CO7-6 Enhancement of the cancer cell-killing effects of boron neutron capture therapy by overexpression of *LAT1* in cancer stem cell-like cells

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INTRODUCTION: Outcome from BNCT largely depends on amount of intracellular accumulation of boron compound. L-type amino-acid transporter 1 (LAT1) [1], through which boronophenylalanine (BPA) is transported into cells, is frequently expressed in various types of tumor cells including glioblastoma but not in normal cells [2]. We transfected *pCMV/LAT1-GFP* plasmids into a glioblastoma cell line, T98G, and selected several clones. The sensitivity of cancer cells to neutron and γ-ray fluences was well correlated with the expression level of LAT1 and the level of BPA uptake in the clones [3]. These results suggest that overexpression of LAT1 in cancer cells results in enhanced anticancer effects of BNCT and BNCT combined with gene therapy is beneficial for tumors with low LAT1 expression. In this study, we transfected *pCD133-TRE/LAT1-tdTomato/IRES/tTA* plasmids including a positive-feedback loop into glioblastoma cell line, T98G. The plasmids were designed to overexpress LAT1 tagged with tdTomato on cytoplasmic membranes of CD133 expressing cancer cells selectively. We obtained several transfectants which stably overexpress LAT1 in hypoxic microenvironment of spheroids. We used transfectant spheroids in which LAT1 is selectively overexpressed in CD133 positive cancer cells. We have already shown that the CD133 positive cancer cells in spheroids are model cells of cancer stem cells [4]. This study examined enhanced effects of LAT1 overexpression on BNCT in the transfectant spheroids.

EXPERIMENTS: Spheroids formed with T98G/K10 cells (LAT1-overexpressed in CD133 positive cell selectively) and T98G/KC6 cells (neo control vector-transfected clone) were treated with medium containing ^{10}BPA (0, 20, 40 ppm) or PBS for 2 hours. The spheroids in 1.5-ml cryo-tubes were irradiated with the fluences (boron dose 4.8 Gy + γ-rays 0.4 Gy for 20 min irradiation, boron dose 10.0 Gy + γ-rays 0.6 Gy for 40 min irradiation) from KUR. The irradiated spheroids were transferred from the cryo-tubes to non-adherent dishes and then cultured with DMEM for 3 or 7 days. The spheroids were fixed with 10% formalin after the culture of spheroids.

RESULTS: As shown in Fig. 1, volumes of T98G/K10 and T98G/KC6 spheroids treated with PBS (0 ppm) were relatively decreased (approximately 20% reduction) after the irradiation for 20 min or 40 min compared with non-irradiated spheroids. When T98G/K10 and T98G/KC6 spheroids were treated with 40 ppm ^{10}BPA, the significant difference in reduction percentage of the volume between T98G/K10 and T98G/KC6 were observed after the irradiation. The reduction percentages in T98G/KC6 spheroids were approximately 20% and 30% after 20 min and 40 min irradiation, respectively. On the other hand, the reduction percentages in T98G/K10 spheroids were approximately 50% and 60% after 20 min and 40 min irradiation, respectively. These results suggests that overexpression of LAT1 in CD133 positive cells (cancer stem cell-like cells) may result in enhanced sensitivity of spheroids to neutron and γ-ray fluences.

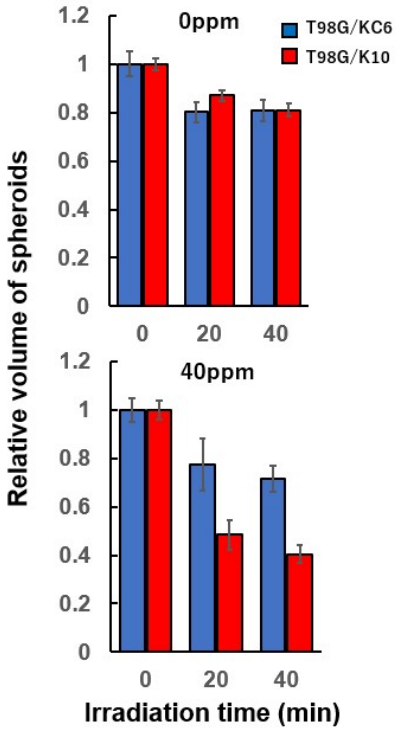

Fig. 1. Sensitivity of spheroids including LAT1 overexpressed CD133 positive cells to neutrons. Relative volumes of spheroids were measured in ^{10}BPA- or PBS-treated spheroids after neutron and γ-ray irradiation.

CONCLUSION: This study proposed that overexpression of LAT1 in cancer stem cells causes enhanced anticancer effects of BNCT. BNCT combined with gene therapy seems to be beneficial for tumors including cancer stem cells resistant to radiation cancer therapy.

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CO7-7 Effects of overexpression of *LAT1* in cancer stem cell-like cells on suppression of tumor growth by boron neutron capture therapy

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[4]Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Outcome from BNCT largely depends on amount of intracellular accumulation of boron compound. L-type amino-acid transporter 1 (LAT1) [1], through which boronophenylalanine (BPA) is transported into cells, is frequently expressed in various types of tumor cells including glioblastoma but not in normal cells [2]. We transfected *pCMV/LAT1-GFP* plasmids into a glioblastoma cell line, T98G, and selected several clones. The sensitivity of cancer cells to neutron and γ-ray fluences was well correlated with the expression level of LAT1 and the level of BPA uptake in the clones [3]. These results suggest that overexpression of LAT1 in cancer cells results in enhanced anticancer effects of BNCT and BNCT combined with gene therapy is beneficial for tumors with low LAT1 expression. In this study, we transfected *pCD133-TRE/LAT1-tdTomato/IRES/tTA* plasmids including a positive-feedback loop into glioblastoma cell line, T98G. The plasmids were designed to overexpress LAT1 tagged with tdTomato on cytoplasmic membranes of CD133 positive cancer cells selectively. We confirmed several clones which stably overexpress LAT1 in hypoxic microenvironment of spheroids. In this study, we examined enhanced effects of LAT1 overexpression on BNCT using the clones in which LAT1 is selectively overexpressed in CD133 positive cancer cells. We have already shown that the CD133 positive cancer cells in spheroids are model cells of cancer stem cells [4]. We transplanted the clone cells into nude mice and performed neutron irradiation on tumors.

EXPERIMENTS: We transplanted tumors formed with a clone (T98G/K10, *pCD133-TRE/LAT1-tdTomato/IRES/tTA*-transfected, LAT1-overexpressed cells in CD133 positive cell selectively), or a clone (T98G/K4, *pCMV/LAT1-GFP*-transfected, LAT1-overexpressed cells) into femoral region of nude mice. Accumulated amounts of ^{10}BPA in blood and tumor were measured using prompt gamma-ray assay (PGA) on 1 h after ^{10}BPA s.c. injection (100 mg/kg, 1 h before irradiation). The transplanted tumors into mice were irradiated with thermal neutron beam at the fluences of 3.3 x 10^{12} n/cm^{-2} on 1h after the ^{10}BPA injection.

RESULTS: Averages of the relative tumor volumes in ^{10}BPA-treated mice and PBS treated mice are shown in Fig. 1. Tumor growth in ^{10}BPA-treated T98G/K4 (n=6) was strongly suppressed after neutron irradiation compared with that in PBS-treated T98G/K4 (n=6). In contrast to this, tumor growth in ^{10}BPA-treated T98G/K10 (n=6) was weakly suppressed after neutron irradiation compared with that in PBS-treated T98G/K10 (n=6). The difference in tumor growth rate was observed between ^{10}BPA-treated T98G/K4 and T98G/K10, but not observed between PBS-treated T98G/K4 and T98G/K10. These results suggest that CD133 expressing cell-selective LAT1 overexpression is effective in ^{10}BPA-treated cells compared with PBS-treated cells. In further experiments, we plan to compare the effectiveness of CD133 expressing cell-selective LAT1 overexpression between LAT1 non-transfected tumors and T98G/K4 or T98G/K10 tumors.

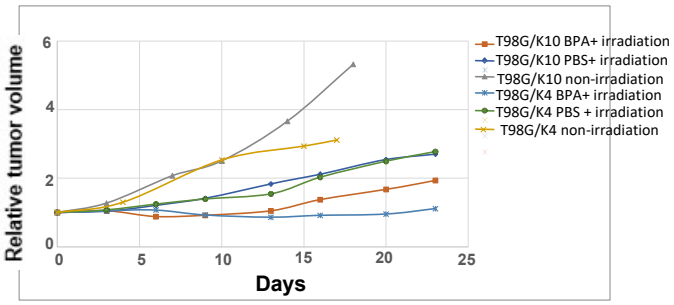

Fig. 1. Tumor growth curves of LAT1 overexpressing tumors (T98G/K4) and CD133 expressing cell selective LAT1 overexpressing tumors (T98G/K10).

CONCLUSION: We obtained preliminary data from this study. On the basis of the data, we plan to perform further detailed experiments in this study.

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CO7-8 Preliminary Experiment of Tumour Growth Suppression by Intra-Tumoral Injection of Boron-Polyplex/Polymer for Boron Neutron Capture Therapy to Pancreatic Cancer Model *in vivo*

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INTRODUCTION:

For effective Boron-neutron capture therapy(BNCT), it is necessary to accumulate Boron atoms into the tumor tissues selectively. We performed the experiments of boron delivery systems for BNCT using boronododecaborane($^{10}B_{12}H_{11}SH$; ^{10}BSH) [1, 2, 3]. The ^{10}BSH is difficult to be kept in the cytoplasm and nucleus in the cancer cells, so we need to develop some functional delivery systems. In this study, we evaluated ^{10}BSH / hyaluronic acid / protamine-mixed with cationic liposome (^{10}Boro-plex) or polymer (^{10}Boro-PEI) as neutron capture therapy agent by in vivo experiment on AsPC-1 human pancreatic tumor-bearing mice. We also use electroporation combined catinic liposome / polymer as an method for selective delivery of compound into the cells by open the membrane poles elcectorically in the field of gene therapy and chemotherapy [4, 5].

EXPERIMENTS:

^{10}Boro-plex were prepared mixed with 1.3mL of ^{10}BSH(6mg/mL), 0.15mL of a solution of 10mg/mL hyaluronic acid sodium, and 0.05mL of 10mg/mL of protamine incubating at room temperature for 30min, then, these mixing solutions were poured into cationic Liposome; Genetransfer. ^{10}Boro-PEI were prepared mixed with 1.3mL of ^{10}BSH(6mg/mL), 0.20mL of a solution of 10mg/mL hyaluronic acid sodium, and 0.10mL of 10mg/mL of protamine incubating at room temperature for 30min, then, these mixing solutions were poured into 0.40mL of Polyethyleneimine(PEI; Exgen). Human pancreatic cancer AsPC-1 cell was used for the *in vivo* anti-tumor effect evaluation. We prepared AsPC-1($5x10^5$) model by transplanting to right lower leg. Electroporation was performed after intra-tumoral injection of 0.1mL of ^{10}Boro-plex, then, we performed thermal neutron irradiation at Institute for Integrated Radiation and Nuclear Science, Kyoto University (average neutron fluence of 3.0×10^{12} n/cm^2). The change in tumor growth and survival rate of the mice reflected the anti-tumor effect of ^{10}Boro-plex. While measuring the size of tumor, the weight change was also recorded for evaluation of the toxicity of these samples.

RESULTS:

The experimental results showed that tumor growth suppression in ^{10}Boro-plex injected group by NCT was 4.5 times superior compared with non-irradiated group (only ^{10}Boro-plex injection), and tumor growth suppression in ^{10}Boro-PEI injected group was 2.3 times superior compared with non-irradiated group (only ^{10}Boro-PEI injection). No significant weight loss were observed after treatment suggesting low systemic toxicity of this system.

We attempted to enhance of retention of ^{10}Boron atoms by mixing ^{10}Boro-plex / ^{10}Boro-PEI. The mechanism of transfection in ^{10}Boro-plex and ^{10}Boro-PEI is thought as endocytosis and proton-sponge effect, respectively. Electroporation is very effective to increase the ratio of transfection of the genes and compounds into the cytoplasms of cancer cells.

It is easily heterogeneous of the concentration of ^{10}B in the tumor by intratumoral direct injection, so it is thought that combination with the methods of gene delivery system and electroporation was effective to accumulate the ^{10}B compound into the cancer cells. We hope to apply these techniques including gene therapy ansd electrochemotherapy acutually using in clinical to BNCT for local advanced cancers. We also consider and develop the more safety and stable ^{10}B delivery systems.

Table1. Tumor growth suppression of by intra-tumoral injection of ^{10}Boro-plex / ^{10}Boro-PEI and Electroporation with thermal neutron irradiation on AsPC-1 model *in vivo*

	Tumor growth rate		
	Day8	Day15	Day25
BNCT			
^{10}Boro-plex+EP	**3.02±0.44**	**2.71±1.69**	**3.84±3.68**
^{10}Boro-PEI+EP	**3.14±1.17**	**4.62±3.28**	**6.15±5.61**
Non NCT			
^{10}Boro-plex+EP	**5.86±3.35**	**9.02±5.14**	**17.35±13.54**
^{10}Boro-PEI+EP	**4.52±1.12**	**5.79±1.72**	**13.86±2.86**

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R3046

CO7-9 Enhancement of Tumour Growth Suppression by Electroporation with Intra-Tumoural Injection of Gadolinium-Polyplex for Gadolinium-Neutron Capture Therapy to Pancreatic Cancer Model *in vivo*

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INTRODUCTION:

Gadolinium reacts thermal neutron and offers cytotoxic effect by 1μm-range high LET Auger electron, and long-range gamma rays on Gadolinium-neutron capture therapy(GdNCT) [1, 2, 3]. It is necessary to accumulate high concentration of Gadolinium atoms into the tumor tissues selectively for effective GdNCT. Electroporation is an method for selective delivery of compound into the cells by open the membrane poles elcectorically in the field of gene therapy and chemotherapy [4, 5]. In this study, we evaluated the electroporation with intra-tumoral injection of ^{157}Gadolinium-Polyplex; ^{157}Gd-plex (gadolinium / hyaluronic acid / protamine-mixed with cationic liposome) by in vivo experiment on AsPC-1 human pancreatic tumor-bearing mice.

EXPERIMENTS:

^{157}Gd-plex were prepared mixed with 1.5mL of Gadolinium compound “Magnescope” (MW: 753.86), 0.2mL of a solution of 10mg/mL-hyaluronic acid sodium, and 0.1mL of 20mg/mL of protamine incubating at room temperature for 30min, then, these mixing solutions were poured into cationic Liposome; Genetransfer. Human pancreatic cancer AsPC-1 cell was used for the *in vivo* anti-tumor effect evaluation. We prepared AsPC-1($5x10^5$) model by transplanting to right lower leg. Electroporation was performed after intra-tumoral injection of 0.2mL of ^{157}Gd-plex, then, we performed thermal neutron irradiation at Institute for Integrated Radiation and Nuclear Science, Kyoto University (average neutron fluence of 2.0×10^{12} n/cm^2). The change in tumor growth and survival rate of the mice reflected the anti-tumor effect of ^{157}Gd-plex. While measuring the size of tumor, the weight change was also recorded for evaluation of the toxicity of these samples.

RESULTS:

Thirty percent of tumor growth suppression was achieved in the ^{157}Gd-plex+EP group in NCT groups compared with non-irradiated group. The tumor growth suppression of the ^{157}Gd-plex+EP group was superior than the only ^{157}Gd-plex injected group by NCT.

We attempted enhancement of retention of gadolinium atoms by mixing ^{157}Gd-plex. The experimental results showed that the tumor growth suppression of ^{157}Gd-plex+EP group was revealed superiority compared to the group with ^{157}Gd-plex group after NCT, and no significant weight loss were observed after treatment suggesting low systemic toxicity of this system. We would like to consider the techniques to perform EP in the body. The ^{157}Gd-plex will become one of the candidates for Gd delivery system on NCT. Moreover, the body weight of the mice did not decrease after the treatments, which indicate the safety of ^{157}Gd-plex / ^{157}Gd-plex+EP and the GdNCT.

Table1. Augmentation by electroporation of tumor growth suppression by GdNCT with intra-tumoral injection of ^{157}Gd-plex on AsPC-1 model *in vivo*

	Tumor growth rate		
	Day8	**Day15**	**Day25**
GdNCT			
^{157}Gd-plex+EP	**1.604±0.517**	**2.657±1.024**	**4.017±1.615**
^{157}Gd-plex	**2.217±0.569**	**4.034±1.254**	**7.522±1.499**
Non NCT			
^{157}Gd-plex+EP	**3.221±0.621**	**7.259±1.563**	**12.655±2.750**

Tumor growth suppression in ^{157}Gd-plex+EP group by NCT was 3times superior compared with non-irradiated group. Tumor growth suppression in ^{157}Gd-plex+EP group was 1.8times superior compared with ^{157}Gd-plex group in NCT groups.

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CO7-10 Identification of host immunostimulatory effects induced by boron neutron capture therapy

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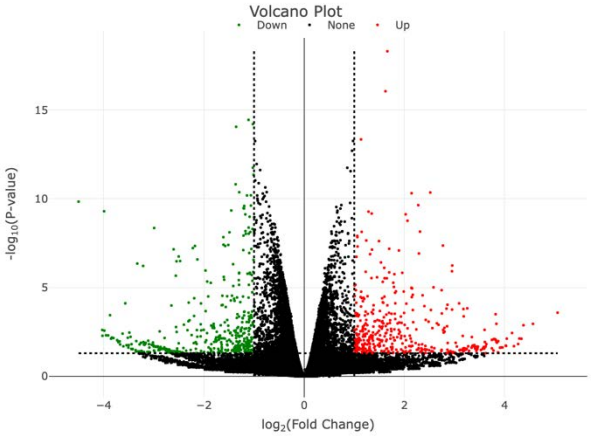
INTRODUCTION: Various tumor immunostimulatory effects have recently been demonstrated following X-ray or gamma irradiation, including an increase in major histocompatibility complex (MHC) molecules on the tumor surface [1], activation of antigen-presenting capacity by dendritic cells [2], and induction of diverse T-cell receptor clones [3]. It has not been clarified whether BNCT has the same immunostimulatory effects as X- or gamma-irradiation. Wet has shown in previous experiments using the reactor until last year that the anti-tumor effect of BNCT is weakened when the immune function of mice is weakened immediately before BNCT. In this fiscal year, we aim to elucidate the specific mechanism of immune activation by BNCT and to clarify the effect of the combination of BNCT and immunotherapy by mouse experiments.

EXPERIMENTS: The spleen was removed from the mice and the splenocytes were collected. The splenocytes were activated and the activated spleen cells were treated with L-BPA followed by neutron irradiation. The subsequent degree of apoptosis of each immune cell was measured using a flow cytometer. Blood was withdrawn from C3H mice in which SCC7 tumor cells were implanted subcutaneously in the lower limbs, or C3H mice without tumor cells, and the blood was dried completely, and the dried blood was irradiated with neutrons. After irradiation, the specimens were subjected to radio-activation analysis. B16 mouse melanoma cells were implanted subcutaneously in C57BL/6 mice and conducted neutron irradiation, BNCT with L-BPA, BNCT with L-BPA combined with anti-PD-1 antibody (immunotherapy). Each tumor tissue was subjected to RNA-seq analysis.

RESULTS: Each immune cell in the splenocytes showed a lower rate of apoptosis after BNCT than the tumor cells such as murine melanoma cell line, B16, and murine squamouse cell carcinoma cell line, SCC7. Among the T cells, CD8+ T cells and CD4+ T cells showed a similar rate of apoptosis as the immuno-suppressive regulatory T cells. There was no significant difference in the effect of BNCT on activated T cells compared to the non-activated state. Trace metals, which were expected to change by radio-activation analysis, were not detected in the blood of the carcinoma-bearing mice. RNA-seq results showed that the BNCT group expressed significantly more factors that induce and attract immune cells than the X-rays group.

Fig1. Volcano plot of RNA-seq analysis



The right side of the figure shows the group of genes more expressed in the BNCT group and the left side shows the group of genes more expressed in the X-ray group.

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CO7-11 Basic research to expand the indication of neutron capture therapy to non-neoplastic diseases

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INTRODUCTION: In 2020, boron neutron capture therapy (BNCT) using L- BPA (borophalan, trade name: stevoronin) as a boron-based μm-scale cancer treatment with neutron irradiation is covered by insurance to the treatment of head and neck cancer patients in Japan. Clinical trials are currently underway to expand the indications of BNCT other than head and neck cancer including malignant brain tumors and malignant melanoma. The challenges of BNCT are attenuation of thermal neutrons in the body, accumulation of the boron drug in normal tissues, and difficulty in uniformly accumulating the boron drug in the tumor tissue [1]. The first challenge, attenuation of thermal neutrons in the body, can be solved by multi-port irradiation from several directions The second issue, the accumulation of boron drugs in normal tissues, could be improved by modifying the pharmacokinetics of boron drugs with simultaneous use of pharmacokinetics modifiers. The third problem, the uniform accumulation of boron-based drugs in tumor tissues, is originated to the feature of tumor cells: tumor cells are a population of cells with heterogeneous characteristics. Multiple BNCT irradiations and the use of multiple boron drugs are possible solutions to this problem [2].

The potential of BNCT greatly depends on boron agents. While many new boron agents have been developed and antibody is also tried to be used as a new boron drugs for BNCT, the treatment effect of the antibody-based BNCT had not been enough [3]. One of the big reasons for that is the tumor heterogeneity, one of the challenges of BNCT mentioned above. If we change BNCT target different pathologic cells instead of tumor cells, we may be able to improve morbid conditions using the BNCT principle. This study aims to establish antibody-based BNCT for a new therapeutic stragety for non-tumor diseases, thereby expanding the range of therapeutic indications for BNCT.

EXPERIMENTS: We have created a boronated module using maleimide to bind boron clusters to antibodies. Mice were injected intraperitoneally with β-glucan to induce immunoreactive inflammation (inflammation-induced mouse model). With the boronated module, we developped a boronated antibody targeting immune cells that cause inflammation, administered the boronated antibody to the inflammation-induced model mice, and examined the therapeutic effect of neutron irradiation on the inflammatory site compared to the neutron-alone group.

RESULTS:
Approximately 40 boron per antibody molecule was found to be bound by the maleimide-based method. To investigate the possibility that boronation of antibodies may inhibit the binding of antibodies to their target protein binding, the fluorescent boronated antibody to the CD8a molecule were prepared and co-stained with fruorescent anti-CD8b antibody with CD8+ T cells. Both CD8a and CD8b were well-stained with the antibodies, which shows the target specificity of the boronated antibody was maintained. Inflammation-induced mouse models were treated with the boronated antibodiy targeting inflammatory cells and irradiated with 5 MW 12-minute neutron locally to the site of inflammation. The intensity of local inflammation was evaluated over time using the inflammation evaluation score compared to the neutron-alone irradiation group. However, no improvement in inflammation was observed. With these results, the boronation module of the antibody was modified to increase the amount of boron that can be bound per antibody molecule.

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R3050

CO7-12 Mechanism of Glioma Resistance After BNCT Conferred by Glioma Niche

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) have been applied to recurrent malignant glioma and even after standard therapy (surgery, chemo-radiation therapy) because of the selective damage to the tumor. Especially, glioblastoma (GBM) is the most miserable cancer, whose patient survival is 14.6 months and remarkably resistant to chemo-radiation and immunotherapy. With BNCT, we achieved better local control and survival benefit in malignant glioma using thermal neutrons produced by the reactor in Kyoto University. However, the recurrence is inevitable after BNCT. Reasons for recurrence after BNCT have not been fully elucidated.

We reported glioma stem cells which are known to be resistant to chemo-radiation therapy, take up a boron compound, *p*-boronophenylalanine (BPA) and can be targeted by BPA-BNCT [1]. In this study, we investigated whether the glioma niche influences the survival of glioma cells after BNCT.

EXPERIMENTS:

Cell culture: We used human GBM cell line, U87MG *delta EGFR* and human microglia cell line, HMC3 as a component of glioma niche. Both cells were cultured in Dulbecco's Modified Eagle Medium（DMEM） containing 10% fetal bovine serum at 37 °C in CO_2 incubator.

Boronophenylalanine (BPA) Treatment and Thermal Neutron Irradiation: We treated U87MG *delta EGFR* cells with medium containing BPA at the concentration of 20 ppm for 30 minutes. The BPA was formulated and its concentration was measured as previously described [2]. After we trypsinized and rinsed the cells, cells were collected in Eppen tubes with DMEM containing BPA at the concentration of 20 ppm and irradiated with thermal neutron for 20 minutes.

Co-culture system: After thermal neutron irradiation, 10E5 U87MG *delta EGFR* cells were disseminated into the bottom dish of 6 well plates. And the same number of HMC3 cells were disseminated into the insert (pore size, 0.4 μm), and co-cultured with U87MG *delta EGFR* cells in 6 well plates. Five days after co-culture, U87MG *delta EGFR* cell numbers were counted and compared with mono-culture of U87MG *delta EGFR* cells.

RESULTS: The cell numbers of non-irradiated mono-culture and co-culture groups were 7.2E5 cells and 4.2E5 cells. While, the cell numbers of BNCT mono-culture and co-culture groups were 2.1E5 cells and 1.0E5 cells (Figure 1). Compared to the mono-culture, cell growth of U87MG *delta EGFR* cells in co-culture system was reduced to 25.3 % in non-irradiated control group and to 27.4 % in BNCT group. In this co-culture system, microglia did not affect glioma cell growth after BNCT. We will further examine the various ways of co-culture system using 3D co-culture, co-culture before and after BNCT etc.

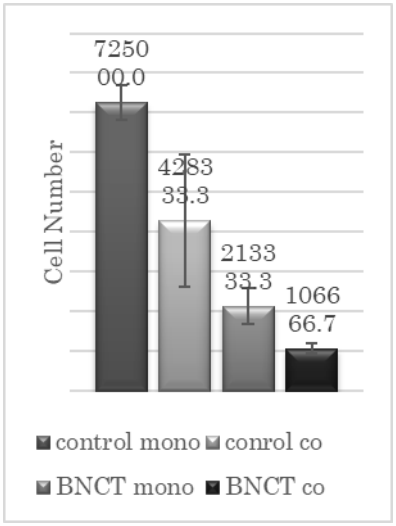

Figure 1. Cell number of U87MG *delta EGFR* cells of non-irradiated control and BNCT groups in mono-culture or co-culture with HMC3 cells 5 days after BNCT.

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CO7-13 Solbilization of All-*Trans*-Retinoic Acid as Macrophage Polarizer by β-1,3-Glucan

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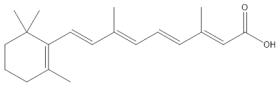
INTRODUCTION: Boron neutron capture therapy (BNCT) is a potent cancer therapy that exhibits cancer selectivity at the cellular level and has few side effects. However, in many cases, it will not be completely cured due to recurrence/metastasis (distant dissemination). One of the reasons is the involvement of tumor-associated macrophages (TAM) present in the stroma of tumor tissue. It has become a major problem that TAM not only promotes neovascularization and tumor regrowth/metastasis, but also enhances antitumor immunity [1]. Since TAM exhibit an M2 phenotype that promotes tumor progression, conversion of M2 TAM toward a tumoricidal M1 phenotype is a promising anti-cancer therapy. Recently Takeya *et al.* reported that natural compounds possessing an inhibitory effect for signal transducer and activator of transcription-3 (STAT3) suppressed M2 polarization [2]. Although all-*trans*-retinoic acid (ATRA) is one of well-known and strong STAT3 inhibitor [3], handling of ATRA is not easy owing to its poor water-solubility. Herein, water-solubilization of ATAR by using β-1,3-glucan is attempt in order to efficiently and selectively deliver the M2→M1 polarizer to M2 macrophages by targeting dectin-1 expressed in M2 macrophages.



Scheme. Chemical structure of ATRA

EXPERIMENTS: β-1,3-glucan was used after purification from black yeast (*Aureobasidium pullulans*). For the preparation of nanogels with excellent blood stability and high efficiency of dectin-1 mediated endocytosis, the molecular weight of β-1,3-glucan was reduced by sonication. The complex nanogel of ATRA with β-1,3-glucan was prepared *via* a dialysis technique based on a supuramolecular strategy. The hydrodynamic diameter of the complex was measured using a Zetasizer Nano ZS (Malvern, UK). After lyophilization of aqueous ATRA/β-1,3-glucan complex solution, the residue was re-dissolved in DMSO. The concentration of ATRA was estimated by molar extinction coefficient of ATRA in DMSO (ε, 41500). In rectal cancer transplantation model, pharmacokinetics of ATRA/β-1,3-glucan complex nanogel was evaluated with co-complexation of Indocyanine Green using *in vivo* imaging system VISQUE® InVivo Smart-LF (Vieworks, Korea).

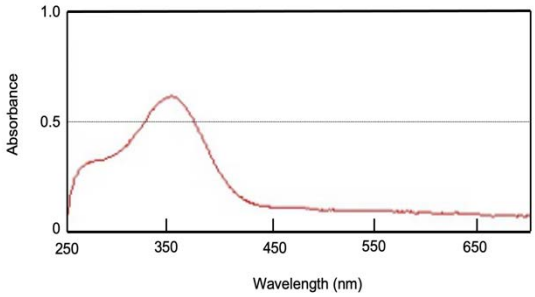


Fig.1. UV-vis absorption spectrum of ATRA/β-1,3-glucan nanogel in DMSO.

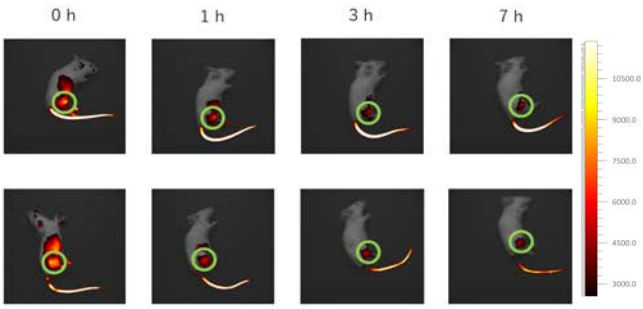


Fig. 2. Pharmacokinetics of ATRA/β-1,3-glucan nanogel in tumor-bearing mouse. Tumor tissue is marked with circle.

RESULTS: According with Fig. 1, water-solubilization of hydrophobic ATRA by the complexation with β-1,3-glucan was revealed. The concentration of ATRA in aqueous solution was estimated as 1.10 mM. Dynamic scattering measurement revealed ATRA/β-1,3-glucan complex diameter of 80 nm and a size corresponding to the tumor targeting EPR effect [4].

Tumor tissue accumulation of ATRA/β-1,3-glucan complex nanogel was immediately confirmed using a mouse rectal cancer transplantation model in a few hours (Fig. 2).

In conclusion, we succeed to prepare the water-soluble ATRA/β-1,3-glucan nanogel. We will evaluate the effect of the administrating of the macrophage polarizer on the tumor growth inhibitory effect and examine the BNCT sensitizing effect by controlling the polarity of TAM in near future.

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CO7-14 Carborane bearing pullulan nanogel/boron oxide nanoparticle hybrid for BNCT

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INTRODUCTION: With noninvasiveness and efficient damage inducibility for genomic DNA, boron neutron capture therapy (BNCT) is one of the most promising modalities for cancer treatment. Cell destruction in BNCT is achieved by energy from the particles which was generated by the nuclear reaction between ^{10}B and low-energy thermal and/or epithermal neutron, that is boron neutron capture reaction, and effective ranges of the energy from these particles are corresponding to the size of a cell (<10 μm), suggesting that accumulation of boron agents toward cancer cells with high specificity is significant to maximize benefits from BNCT. Currently, L-boronophenylalanine (L-BPA) and disodium mercaptoundecahydro-*closo*-dodecaborate (BSH) are clinically available as boron agents for BNCT, however these two types of boron agents have several issues in delivery including short circulation time in blood stream, tumor selectivity, and the leaky feature from cells. For these points of views, delivery platforms for boron agents are urgent requests to expand applicability of BNCT.

In this study, we demonstrated efficient tumor growth suppression using organic-inorganic hybrid nanogels (HBNGs) comprising of carborane bearing pullulan nanogels and hydrophobized boron oxide nanoparticles (Figure 1). Our HBNGs technology is based on a hybrid combination of boron oxide nanoparticles for boron contents with a boronated nanogel carrier. Previously, we showed facile tumor-targeted boron agent delivery based on EPR effect using carborane bearing pullulan nanogel with high boron contents in each nanoparticle. In addition, the nanogels uptaken by cancer cells are accumulated in nuclear membrane, which is advantageous to induce efficient DNA double strand break toward cancer cells.[1,2] These advantages of carborane bearing pullulan nanogel in BNCT encouraged us to develop the HBNGs systems by coupling carborane bearing pullulan nanogel with hydrophobized boron oxide *via* supramolecular chemistry approach.[3,4] In the present study, we demonstrated applicability of HBNGs as a boron agent for BNCT *in vitro* and *in vivo*. We then evaluated therapeutic efficacy of BNCT against tumor xenograft mice.

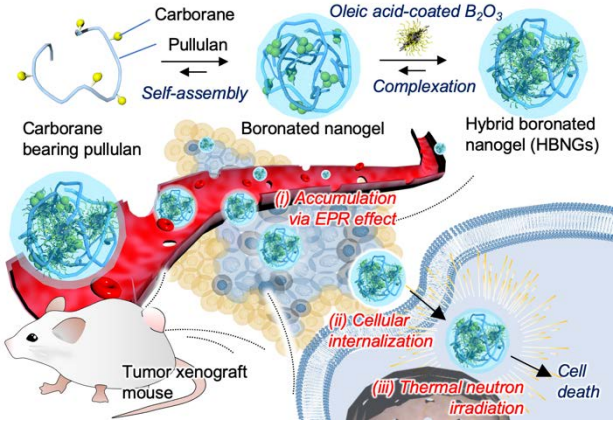

Fig. 1. Schematic illustration of this study. BNCT with hybrid nanogel for cancer therapy.

RESULTS AND DISCUSSION: Carborane bearing pullulan nanogels were prepared as previously reported.[1] To obtain hybrid nanoparticle (HBNGs), the hydrophobized boron oxide nanoparticles were injected to dispersion of nanogels and the boron oxide nanoparticles were encapsulated mainly *via* hydrophobic interaction. Dynamic light scattering measurement revealed that the hydrodynamic diameter of HBNGs is to be 180 nm, which is corresponding to the size for enhanced permeation and retention effect (EPR effect) that is known as passive tumor targeting effect. Transmission electron microscopy revealed that the structures derived from boron oxide nanoparticles agglomerated within nanogels. This feature is ideal to achieve efficient boron neutron capture reaction.

We demonstrated therapeutic efficacy of BNCT *in vitro* using current system. In this study, we employed L-BPA/fructose complex, which is used in clinical, as a comparison. As a result, both systems could induce cell death toward murine colon carcinoma cell line (Colon26) boron concentration dependently. Moreover, IC_{50} value using HBNGs were 6 times lower than that using L-BPA/fructose complex, suggesting our hybrid system are potentially available as a boron agent for BNCT.

In conclusion, our system based on hybrid nanoparticles enabled to induce cell death toward cancer cell lines by irradiating thermal neutron with high efficiency. We will address therapeutic efficacy *in vivo* in the near future.

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R3058

CO7-15 Optimization of polymeric BPA for non-clinical studies and basic study on its analogues

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INTRODUCTION: Boronophenylalanine (BPA) has been considered as the most powerful drug in boron neutron capture therapy (BNCT). Since BPA can be internalized into cells through LAT1 amino acid transporters expressed on many tumor cells, BPA can accumulate selectively within malignant tumors [1]. Although BPA has proven its promise in clinical studies, it was also reported that intracellular BPA is sometimes exchanged with extracellular amino acids including tyrosine due to the antiport mechanism of the amino acid transporter, leading to short retention time in a target tumor [2]. This unfavorable efflux of intracellular BPA is likely to compromise the ultimate therapeutic effect; thus, it is important to prolong the intracellular retention of BPA.

In this regard, we recently found that polymers possessing multiple hydroxy groups or sugar moieties can form complexes with BPA molecules through boronate esters in aqueous solution and that the polymer-BPA complexes are internalized into cultured tumor cells via LAT1-mediated endocytosis and entrapped mainly in endo-/lysosomes, resulting in prolonged retention in the intracellular compartment by preventing the unfavorable efflux [3, 4]. The polymer-BPA complexes can exhibit the prolonged tumor retention even in *in vivo* condition and significantly enhance the BNCT effects. In particular, poly(vinyl alcohol)-BPA (PVA-BPA) complexes showed the considerably strong BNCT effects in subcutaneous tumor models [3]. Considering the ease of manufacturability PVA-BPA, its clinical translation appears to be promising. Thus, in this study, we prepared PVA-BPA complexes with various compositions and compared their therapeutic effect to optimize the drug formulation for non-clinical studies.

EXPERIMENTS: BALB/c mice bearing subcutaneous CT26 tumors were prepared by subcutaneous injection of the cell suspension. PVA-BPA complexes with various compositions were prepared in aqueous solutions, and they were intravenously injected to the mice at a dose of 10 mg BPA/mouse. As a control, conventional sorbitol-BPA complexes were subcutaneously injected to the mice at a dose of 10 mg BPA/mouse. The thermal neutrons were irradiated to the tumor using KUR 3 h after injection. The 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, all the PVA-BPA complexes exhibited the enhanced BNCT effects even compared with the conventional sorbitol-BPA that was subcutaneously injected. We are now investigating possible side effects of these complexes and will find out the optimal composition for non-clinical studies.

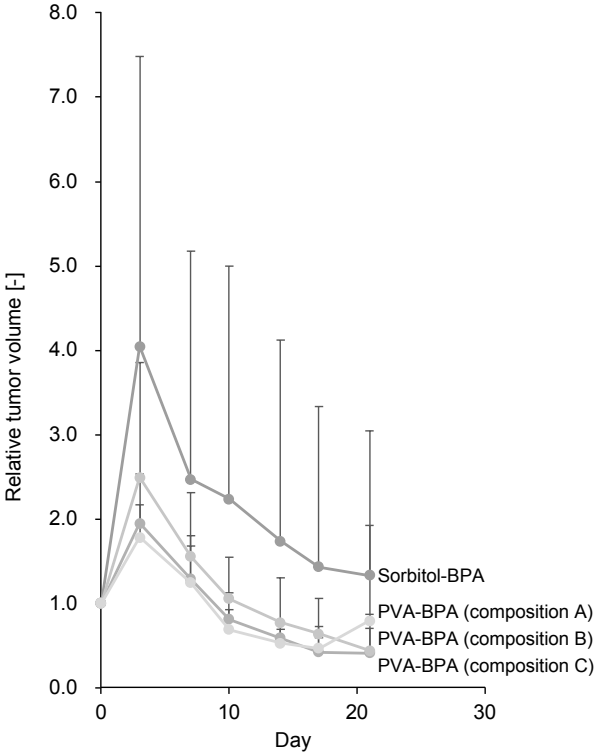

Fig. 1. Antitumor efficacy of sorbitol-BPA and PVA-BPA complexes at various compositions to subcutaneous CT26 tumor models.

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CO7-16 Development of Boron-Folic Acid Complex and BNCT Antitumor Effect

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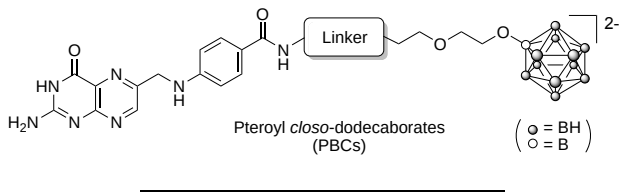
INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is an expecting cancer therapy for the treatment of harsh and un-operatable malignant tumors. The efficiency of boron agent depends highly on tumor selectivity, sufficient amount of boron agent in tumor site, non-toxicity, tumour/normal tissues ratio (>3) and absorption of thermal neutrons by boron. In 2020, accelerator-based BNCT for head and neck cancer using L-BPA was approved by the Pharmaceuticals and Medical Devices Agency in Japan, making BNCT more accessible treatment [1]. L-BPA is known to actively accumulate into tumor cells thorough L-type amino acid transporter 1 (LAT-1). However, there are still many patients for whom L-BPA is not applicable, thus the development of novel boron carriers applicable to various cancers including L-BPA-negative tumors is required for further development for BNCT.

We focused on folate receptor (FRα). Folate is known to be taken into cells via the folate receptors, that are overexpressed on the surface of many cancer cells [2]. We have developed water-soluble pteroyl-*closo*-dodecaborate conjugates (PBCs) and examined their cell uptake using folate receptor (FRα) positive and negative cells. We found that PBCs showed significant cell uptake by FRα positive cells, especially U87MG glioblastoma cells. In addition, PBCs were found to be adequately low cytotoxic with IC_{50} values in the range of 1~3 mM toward selected human cancer cells, low enough to use as BNCT boron agents [3].



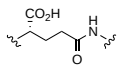
Compound	Linker	
PBC1	—	
PBC2		(glutamine)
PBC3		(glycine)
PBC4		(alanine)

Fig. 1. Chemical structures of PBCs

In this study, we examined *in vivo* biodistribution of PBCs in colon 26 tumor-bearing mice.

EXPERIMENTS: Tumor-bearing BALB/c mice (female, 5-6 weeks old) were prepared by injecting subcutaneously (s.c.) a suspension (1.0×10^6 cells/ 50 µl PBS/ mice) of CT26 cells. The mice were kept on a regular chow diet and water for a week. The tumor-bearing mice were injected *i.v.* with 200 µl of PBC1, PBC3 or L-BPA-fructose complex solution dissolved in ultrapure (Milli-Q) water at the 1500 ppm [B]. At intervals of 1 hours for 6 h, the mice were lightly anesthetized and blood samples were collected from heart. The mice were then sacrificed by cervical dislocation and dissected. Liver, spleen, kidney, brain, and tumor were excised, washed with saline, and weighted. Each tissue was digested with 1 mL of HNO_3 at 90 ℃ for 3 h, and then the digested samples were diluted with distilled water. After filtering through a membrane filter (0.5 µmφ, 13JP050AN, ADVANTEC, Japan), boron concentrations were measured by ICP-OES.

RESULTS: As shown in Fig. 2, boron concentrations of PBC 1 and PBC 3 in blood were lower than that of L-BPA. In contrast, accumulations of PBC 3 in liver and kidney were significantly higher compared with those of PBC-1 and L-BPA. A similar accumulation of PBC 1 to L-BPA was observed in tumor at 1 h after injection, however PBC 1 was rapidly cleared from blood after 3 h.

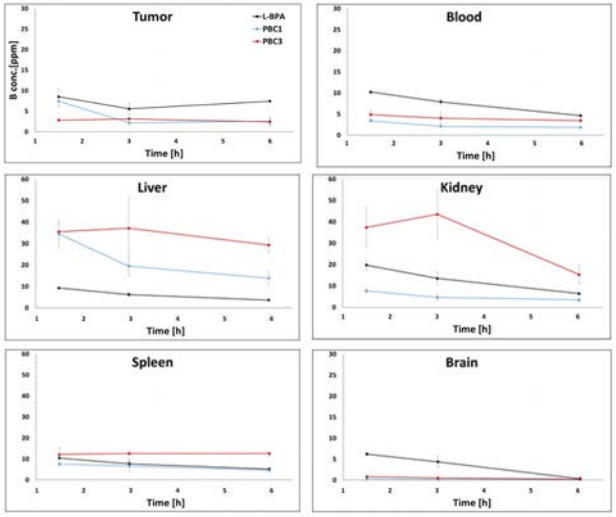


Fig. 2. Time-dependent boron distribution of PBC 1, PBC 2, and L-BPA in CT26 mouse colon tumor-bearing mice. Each boron agent was injected into the mice via the tail vein at a dose of 15 mg[B]/kg. Data are expressed as means ± SD (n = 3).

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CO7-17 Development of Gadolinium-Boron Complexes for MRI-Guided BNCT

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a promising cancer treatment of harsh and un-operatable malignant tumors. An important condition for effective planning of BNCT treatment of patients is the control of the distribution of the boron-containing drug in the body and its accumulation in tumors. Since the ^{10}B isotope is non-radioactive, non-invasive measurements in the body are a fairly difficult task and usually require either the introduction of radioactive labels for positron emission tomography (PET) [1].

We focused on the Gd-based magnetic resonance imaging (MRI), one of the most widely used methods of medical diagnostics. The ^{157}Gd isotope has the highest thermal neutron capture cross section of all stable nuclides in the periodic table, which exceeds the thermal neutron capture cross section of the ^{10}B isotope by more than 60 times. The reaction of neutron capture by ^{157}Gd induces complex inner shell transitions that generate prompt γ-emission displacing an inner-core electron, which in turn results in internal-conversion electron emission, and finally in the Auger electron emission, together with soft X-ray and photon emission. Therefore, the synthesis of compounds containing both boron and gadolinium is of particular interest for the further development of neutron capture cancer therapy. We have developed maleimide-functionalized *closo*-dodecaborate (MID) albumin conjugates that demonstrated high and selective accumulation in tumor tissue with no toxicity in the absence of thermal neutrons, hence a promising boron delivery system [2].

In this study, we designed Gd-functionalized MID albumin conjugates aiming MRI-guided BNCT.

EXPERIMENTS: To a solution of bovine serum albumin (BSA) in 10 mM HEPES buffer (pH 7.4) was added Gd-complex ligand. The reaction solution was shaken at 800 rpm for 12 h at room temperature. The reaction solution was subjected to six cycles of ultracentrifugation with a 30 kDa filter to remove excess ligand before the addition of MID. The reaction solution was shaken for another 12 h at 37°C. The final BSA-Gd-MID conjugate was obtained after filtration of excess MID via ultracentrifugation. The concentration of ^{10}B and Gd was estimated via ICP-OES. The conjugate solution was diluted with PBS for the biodistribution study.

CT26 tumor bearing mice (Balb/cCrSlc nu/nu female, 5−6 weeks old, 16−20 g) were injected via the tail vein with 200 μ L of MID-BSA or Gd-MID-BSA (25 mg [^{10}B]/kg). The whole bodies of mice were placed in an acrylic mouse holder and fixed on a 5-mm-thick thermo-plastic plate. At 12 h after administration, the right thighs of mice were irradiated with neutrons in the KUR nuclear reactor. BNCT effects were evaluated on the basis of the changes in tumor volume of the mice.

RESULTS: Previously, we demonstrated that thermal neutron irradiation was performed using the colon 26 tumor-bearing mice injected with MID-BSA in a dose range of 7.5-30 mg [^{10}B]/kg and observed efficient tumor growth suppression 2 weeks after irradiation [3,4]. Therefore, we compared antitumor effects of Gd-MID-BSA with MID- BSA at a dose of 25 mg [^{10}B]/kg. The results are shown in Fig. 1. Tumor of cold-control mice grew up very slowly, whereas the tumor growth of mice injected with MID-BSA and Gd-MID-BSA was suppressed. Notably, hot-control mice also suppressed tumor growth similar to the mice injected with MID-BSA and Gd-MID-BSA. These results indicate that complete tumor growth suppressions were observed even in the mice without ^{10}B injection probably due to the significantly high neutron fluence. Therefore, it is considered necessary to revise the irradiation plan for the next irradiation experiment.

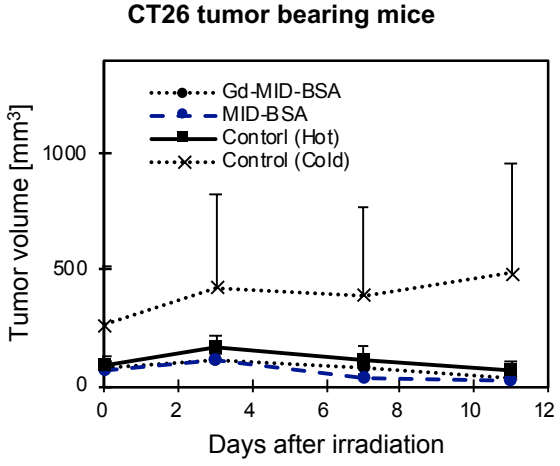

Fig. 1. Antitumor effect in colon 26 (CT26) tumor bearing mice. Gd-MID-BSA and MID-BSA were intravenously injected at a dose of 25 mg [^{10}B]/kg via the tail vein, and the tumors were irradiated with thermal neutron (hot) for 12 min ($3.0\text{-}4.2\times10^{12}$ neutrons/cm^2) 3 or 6 hours after injection or without irradiation (cold).

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CO7-18 Water-soluble dodecaborate-containing pyrazolopyrimidine for BNCT

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INTRODUCTION: Recently, boron neutron capture therapy (BNCT) has been recognized as an essential treatment for refractory cancers such as glioma, head and neck cancer, and melanoma. Although many types of boron compounds, including amino acids, peptides, nucleic acids, anticancer drugs, and liposomes have been reported as boron delivery agents for BNCT, only two compounds, *p*-borono-L-phenylalanine (L-BPA, Boropharan-10B, Fig.1-**1**) and disodium mercapto-*closo*- undecahydro-dodecaborate (**2**) ($[B_{12}H_{11}SH]^{2-}2Na^+$, BSH, Fig.1-**2**), are clinically used in the treatment of cancer with BNCT. In light of these factors, novel useful boron-pharmaceuticals for BNCT are in high demand.

The translocator protein (TSPO) is an 18 kDa protein composed of at least three subunits. TSPO is localized in the mitochondria and plays important roles in several biological processes, such as cholesterol metabolism, proliferation, and apoptosis. Because TSPO is highly expressed in several disease states, including Alzheimer's disease, Huntington's disease, and cancer, it is a potentially useful drug target. TSPO overexpression has been reported in several cancers, including breast, colorectal, prostate, and ovarian cancer and glioma, and appears to be an indicator of poor prognosis in patients with lymph-node negative breast cancer Therefore, TSPO ligands labeled with positron emission atoms, such as DPA-713 (Fig. 1-**3a**), and DPA-714 (Fig. 1-**3b**), have been reported as potential cancer imaging agents. In paticular, pyrazolopyrimidine derivatives DPA-713 and DPA-714 show the high specific affinity for TSPO. Arylpyrazolo[1,5-α]pyrimidine acetamide moiety of DPA-714 seem to be important role on TSPO binding affinity, alkyl ether derivatives at the 4'-position of the phenyl ring are repoterd.

In this study, three novel dodecaborate-containing pyrazolopyrimidine TSPO ligands were developed (Fig. 1): DPA-BSH (**4a**), DPA-BSCN (**4b**), DPA-BSTPG (**4c**). Here, we present the biological evaluation of novel boron compounds **4a-c** as boron carrier for BNCT.

RESULTS and Discussion: To evaluate the potential of the dodecaborate-containing DPA derivatives as BNCT agents, we used two breast cancer cell lines, MCF-7 and MDA-MB-231 which exhibit low and high TSPO expression, respectively. We applied equal dosages of boron to the tumor cells *via* treatment with the novel compounds and L-BPA (6.0 Bpm, 0.6mM) as well as a high dosage of L-BPA (20 Bppm, 2.0 mM), and then measured the intracellular boron concentrations by ICP-OES.

As shown in Fig. 2, all three novel dodecaborate-containing DPA derivatives successfully delivered boron to tumor cells, and MDA-MB-231 cells with overexpressed TSPO consistently took up more novel boron compounds than MCF-7 cells. Although DPA-BSH (**4a**) and DPA-BSCN (**4b**) delivered moderate levels of boron, DPA-BSTPG (**4c**) delivered a notably higher level than L-BPA in both MDA-MB-231 and MC F-7 cells. Notably, the MDA-MB-231 intracellular boron concentration achieved by using DPA-BSTPG (**4c**) was 5–6 times greater than that achieved by using a more highly concentrated dose of L-BPA (DPA-BSTPG: 0.05 mM, BPA: 2.0 mM).

In conclusion, we synthesized novel dodecaborate-containing pyrazolopyrimidine derivatives (Fig. 1-**4a-c**). The result of the *in vitro* evaluation suggest that these compounds are useful boron carriers targeting TSPO over-expressing cells. DPA-BSTPG (**4c**), in particular, delivers a large amount of boron to breast cancer cells. The *in vivo* evaluation of DPA-BSTPG is ongoing, and the results will be reported in the near future.

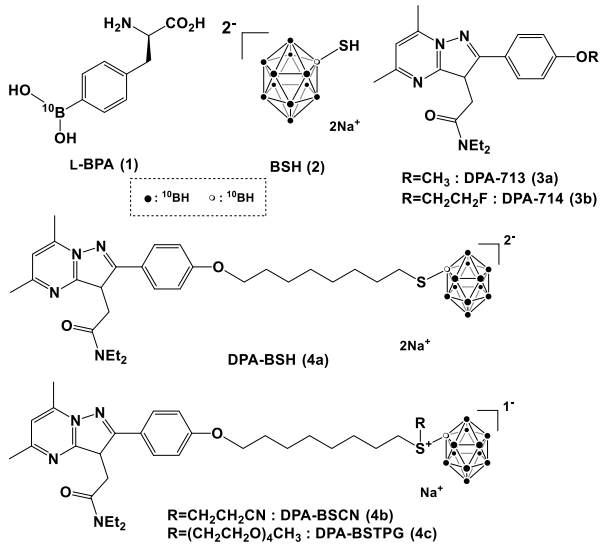

Fig. 1. Boroncompounds and TSPO targeted compounds.

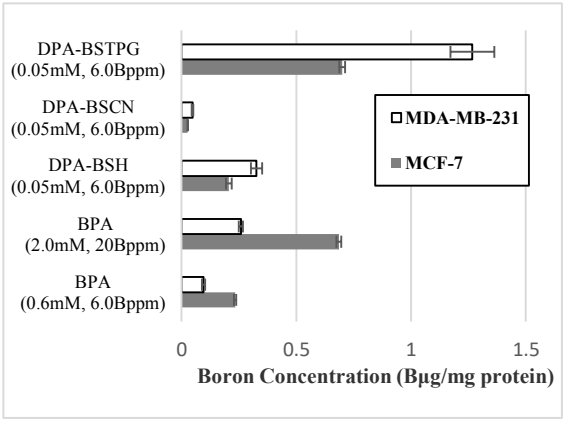

Fig. 2 Cellular uptake of boron in breast cancer cells.

R3082

CO7-19 Attempts to sensitize tumor cells by exploiting the tumor microenvironment

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INTRODUCTION: Hypoxia and glucose deprivation have been suggested to play important roles in resistance to radiation [1]. Attempts to sensitize tumor cells by exploiting the tumor microenvironment have been studied. A major mediator of the cellular hypoxic response, hypoxia inducible factor 1 (HIF-1), is a potential target for cancer therapy, because it transcriptionally regulates a number of genes, including those involved in glucose metabolism, angiogenesis and resistance to chemotherapy and radiation therapy [2]. We previously reported that the disruption of Hif-1α enhanced the sensitivity of murine squamous cell carcinoma (SCC VII) cells to gamma-ray [3]. We have investigated whether the disruption of Hif-1α affects the sensitivity of SCC VII cells to the boron neutron capture reaction (BNCR) with analysis of early DNA damage response, analysis of the intracellular ^{10}B levels, analysis of SLC7A5 expression profiles, and clonogenic assays. Moreover, we examined whether the disruption of Hif-1α affects the tumor growth after BNCT.

EXPERIMENTS: Seven-week-old female C3H/He mice were used in the present study. SCC VII and SCC VII Hif-1α-knockout (ΔHif-1α) cells were subcutaneously inoculated into the right hind legs of mice. BPA was subcutaneously administered into nuchal sites in tumor bearing-mice. After 60 min, mice received neutron irradiation for 12 min at 5 MW in the Kyoto University Research Reactor (KUR). Tumor volume and body weight was measured every three days.

RESULTS: In experiments using culture cells and tumor-bearing mice, we found that the survival of SCC VII Hif-1α-knockout (ΔHif-1α) cells was lower than that of SCC VII cells. Hypoxia-treated SCC VII cells exhibited the decreased intracellular concentrations of BPA and the down-regulation of the SLC7A5 protein levels. BPA uptake and the SLC7A5 protein were not decreased in hypoxia-treated ΔHif-1α cells, the survival of which was lower than that of SCC VII cells. More DNA damage was induced in SCC VII ΔHif-1α cells than in SCC VII cells. It was found that the disruption of the Hif-1α gene enhanced the direct cell-killing effects of BNCT with BPA [4].
Next, tumor growth in the right leg was measured in BNCT-treated and untreated (control) mice. In control groups, tumors continued to grow, regardless of the presence of Hif-1α gene. Tumor volume was smaller in SCC VII tumor-bearing mice treated with BPA-BNCT in than in control animals. Furthermore, in SCC VII ΔHif-1α tumor-bearing mice, the tumor volume was decreased and became undetectable at day 6 after BPA-BNCT.
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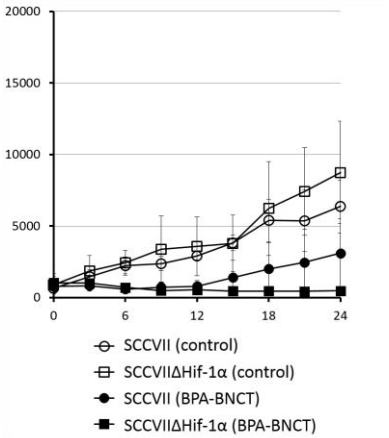

Fig. 1. Mean tumor volume as a function of time after BPA-BNCT. Tumor-bearing animals were administered BPA, followed by neutron irradiation (at day 0). Four to six tumors in each group were used.

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CO7-20 Establishment of a method for determining position in alpha autoradiography using CR39

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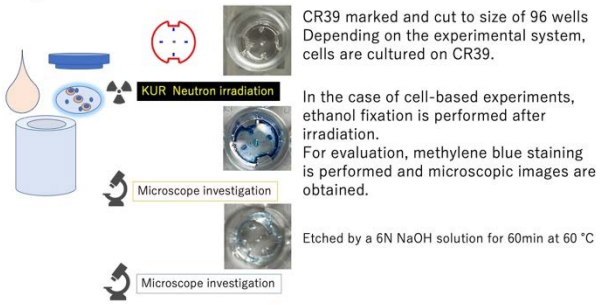
INTRODUCTION: The biodistribution of boron compounds in the microenvironment is an important factor in the success of boron neutron capture therapy, but an appropriate and simple method for visualizing it has not yet been established. In general, intracellular concentrations are measured by wet ashing of cell samples collected from suspended cell suspensions by plasma luminescence analysis, and the same is true for tissue and blood samples. Therefore, we conducted experiments to establish a measurement system using alpha autoradiography, which requires neutron irradiation but for which materials are relatively easy to obtain.

EXPERIMENTS: CR39 was marked and cut as shown in Fig. 1. A silicon cap was made to fit the 96 wells and sealed to prevent scattering of the radioactive materials during irradiation. Poly-lysine treatment was performed when cells were attached.

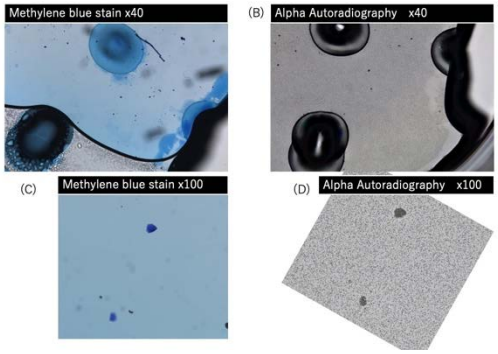
Figure 1



Marking was done from the back side of the detection surface and adjusted for laser power that did not penetrate the CR39. The cut CR39 was designed to fit the bottom of the 96-well well and to be easily removed.
Silicon caps were made by pouring DOWSIL™ 184 into appropriate molds and solidifying at 80°C for 2 hours.
Irradiation was performed at KUR, and this sample was irradiated for 15 minutes using rails.
After irradiation, samples with cellular components were washed, ethanol-fixed, and stored at room temperature. For analysis, as shown in the center of Figure 1, methylene blue staining was performed to obtain microscopic images, which were then etched, and the alpha-ray pits were reapplied to the microscopic images. Etching was performed in a 6N sodium hydroxide solution at 60°C for 60min.

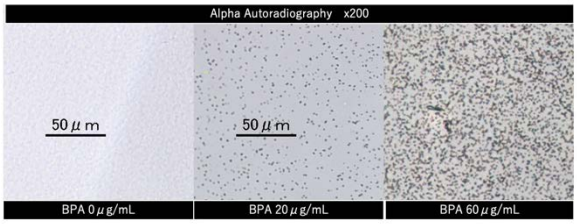
RESULTS: In this case, we analyzed the determination of their location and the acquisition of calibration images As shown in Fig. 2., the laser-marked points did not change much in size before and after etching, and their relative positions were maintained. Using a sample with cells on it, it was possible to match the positional information before and after etching based on the markers and the shape of the particles adhering to the surface. It was considered possible to determine the position of the marker alone if the enlarged images were fused together.

Figure 2



(A) and (C) are methylene blue stain image.
(B) and (D) are etched pit image by light microscope.
laser markers were useful for position matching.
alpha particle pathway made pits on image (C) and (D).

Figure 3



alpha pit on the CR39 with Boronophenylalanine solution. boron concentration were 0μg/mL (left), 20μg/mL (middle), 60μg/mL(Right).

Fig. 3. visualizes the alpha-ray range of the boron 10-thermal neutron reaction between the liquid surface (BPA solution) and the solid (CR39) when a solution of boronophenylalanine is placed on CR39. The density of alpha rays increases with concentration.

Disucussion: If the staining target is a cell, it is expected that by washing this area thoroughly before etching, more alpha rays will be seen in the cell-attached area on CR39 than in the surrounding liquid phase, if enough boron is incorporated into the area. No other experimental system is capable of irradiating cells in a living state, and it would be possible to evaluate this system for radiobiological studies in terms of visualization of alpha radiation rather than boron. Also, perhaps staining the pits could have applications in absorbance spectrometry and other applications. Although neutron irradiation is required, it may help in a rapid measurement system for boron concentration.

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CO7-21 Screening of Boron / Gadolinium Compounds for BNCT of Malignant Brain Tumors, 2021

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Malignant brain tumor cells infiltrate very quickly, and the tumor cells have already spread widely already at the time of diagnosis, and treatment needs to be performed promptly. However, from our clinical experiences so far, the microdistribution of tumor cells is not visualized at the time of treatment, another words the brain is full of tumor cells. Although it has been excluded from the indication for BNCT in many cases where macroselectivity cannot be clearly expected on MRI images, it still shows recurrence in many indications. Perhaps malignant brain tumor cells might be free to move back and forth in the brain in a variety of ways and forms. In other words, it is no exaggeration to say that the target of malignant brain tumors is not being visible.

The Rocher's theory that cell-selective treatment is possible in BNCT of malignant brain tumors may be achievable microscopically, mainly by devising biological carriers. However, since the physical depth of thermal neutrons is only within 6 cm or less from the brain surface even with intra-operative BNCT and about 4 cm with non-invasive BNCT, the Rocher's theory does not hold in macroselectivity [1]. Therefore, we have conducted therapeutic experiments while improving the micro/macro distribution of thermal neutrons by inserting voids into the brain parenchyma with craniotomy and replacing body fluids with heavy water, but distant recurrence in the brain after BNCT has not been controlled.

Our therapeutic strategies of BNCT for malignant brain tumors are (1) to suppress the infiltration of malignant brain tumor cells, and (2) to investigate the possibility of concomitant use of gadolinium capture reaction. The following is a report on the progress of the experiment last year.

(1) Becker, Flieger and Hosman have already synthesized boron compounds, B1 and B2, and their derivatives using an infiltration inhibitor as a ligand, and confirmed a high BNCT effect in in-vitro experiments in our screening tests. The absorbed does yielding the D_{37} (dose used to inhibit 63% colony formation) values were 0.27 Gy for B-1, 0.32 Gy for B-2, 0.82 Gy for BPA and 1.55 Gy for boron-free control. The relative killing effect of R-I to BPA is 0.82/0.27=3.0, and that of R-2 is 0.82/0.32=2.6. The survival fraction of B-1 and B-2 in a pre-incubation boron concentration at 0.143ppm ^{10}B and 0.101ppm ^{10}B respectively were similar, and these result suggest that B-1 and B-2 are actively accumulated and/or attached to the SCCVII cells. In the next, it is necessary to evaluate infiltration suppression and BNCT effect by in-vivo alpha track experiments. Suppression of infiltration of malignant brain tumor cell should be innovative in itself. If suppress of the infiltration of malignant brain tumor cells can be attained by this strategy, it may also improve the clinical malignancy that correlates with invasiveness, so that in that future the indications of another adjuvant treatment options might be able to be reconsidered.

(2) The possibility of gadolinium neutron capture therapy is controversial, but the total range of heavy-charged particles generated by the ^{10}B (n, α) ^{7}Li reaction is local close to the cell diameter, and the total dose distribution is probably too selective. In GdNCT, it is expected that the dose distribution will be improved by higher total kinetic energy of gamma rays, electron beams, Auger electrons, etc., by a single neutron capture reaction of ^{167}Gd (n, γ) ^{168}Gd. Zairov and Neikter have prepared gadolinium inclusion nanoparticles and repeated GdNCT experiments in vitro, and confirmed their low toxicity, tumor retention and concentration-dependent GdNCT effect. The figure shows survival fraction of their Gd nano compounds, R1 an R2 The absorbed does yielding the D_{37} (dose used to inhibit 63% colony formation) values were 0.55 Gy for B1, 0.56 Gy for B2, and 1.50 Gy for control. We will continue to investigate the possibility of GdNCT.

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R3107

CO7-22 The effects of radioactivation of animals on the environment for the BNCT to companion animals.

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Introduction

In the veterinary medicine field, a few studies have reported the use of BNCT for treatment[1)]. Because small animals such as dogs and cats have smaller bodies, neutrons can be delivered to deeper portion into organs than that in humans. This is why, many tumors developed in companion animals can be candidate of BNCT. BNCT is expected to expand its application in veterinary medicine in the future.

However, radioactivation of several atoms in the body are generated in the body following thermal or epitheramal neutron beams[2,3)]. Therefore, animals and their excretions after BNCT include radioactive materials, and there are concerns about the effects on owners and the environment around them. Although there are some reports on radio activation of experimental animals after thermal or epithermal neutron beams, no reports on the radioactivation of urine and feces have been reported. In this study, we evaluated the changes in surface doses from rats and their urine and feces activated by neutron irradiation over time.

Experiments

After the neutron irradiation, NaI (TI) scintillation detector (TCS-1172 , Hitachi, Tokyo) was used for dose rate measurements at the surface of the abdomen and at ambient 30cm away from rat within 8 days.

Radioactivation of the body was analyzed using an HP-Ge detector. In order to determine the radionuclide, it was necessary to obtain the detection efficiencies of the HP-Ge detector.
Radioactivity in the urine (0.5ml) and stool (0.1g) of irradiated rat was measured with Geiger Mueller counter.

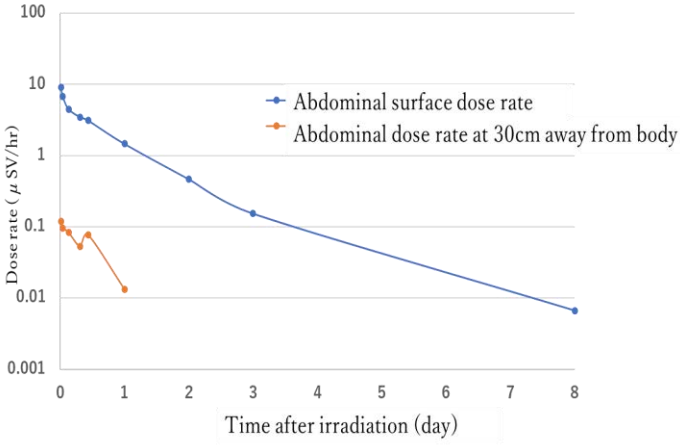
Result

The dose rate on the abdominal surface and the that at 30 cm away from the abdominal surface are shown in Fig1. The surface dose rate decreased to the same level as the background within about 8 days after irradiation, and the 30 cm dose rate decreased to the same level as the background at 1 day after irradiation.

The induced radionuclides were ^{24}Na, ^{38}Cl, ^{56}Mn, and ^{42}K from rat after the neutron irradiation.

The half-life of radioactivity in the urine and feces of radioactivated rats was about 14 days. The results suggested that the radionuclide in the urine and stools from the irradiated rats were ^{32}P.

Fig1. Abdominal surface dose rate and abdominal dose rate at 30cm away from body after irradiation.



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CO7-23 In Vivo Efficacy of BPA-Ionic Liquid as a Novel Compound for BNCT

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INTRODUCTION: In 2020, Steboronine® received manufacturing and marketing approval as the world's first BNCT drug. Its medicinal ingredient, p-boronophenylalanine (BPA), shows excellent antitumor effects after thermal neutron irradiation, but is known to require large doses to achieve clinical efficacy because of its low solubility. Therefore, we have been investigating the use of ionic liquids (ILs) as new solvents for BPA. We have already reported that ionic liquids, consisting of meglumine and serine, exhibit high BPA solubility and anti-tumor effects comparable to those of the BPA-fructose complex (BPA-Fru) [1]. Here we advance further and present *in vivo* antitumor effects of BPA-based IL (BPA-IL) after thermal neutron irradiation.

EXPERIMENTS:

1. Synthesis of BPA-IL

BPA-IL used in this study was synthesized using meglumine as a cation and BPA as an anion. Equimolar meglumine and BPA solutions were stirred at room temperature for 24 hours. After that, the water content of the IL was reduced to about 20 wt% by rotary evaporation at 80°C.

2. Anti-tumor effect using BPA-IL by BNCT

Female 3-week-old BALB/cA mice were purchased from CLEA Japan Inc. (Tokyo, Japan). The tumor model was prepared by grafting 2×10^6 of murine colon carcinoma cells (CT26) to the right thigh of mice (4 weeks old, weighing 16-20 g) to develop a tumor of 6-8 mm in diameter.

Ten days later, 40μL of BPA-IL was administrated via intravenous injection before irradiation was delivered at a dose of 24mg^{10}B/kg. Similarly, 200μL of BPA-Fru was administered. Two hours after injection, thermal neutron irradiation was performed with a flux of $1.2\text{-}1.3 \times 10^9$ neutrons/cm^2/s over 30 min. The tumor size was measured over time after the irradiation until day 30 and the volume was calculated using the previously applied formula [2].

On the last measurement day, a significant difference between tumor size in each group was calculated using independent t-test. The *p*-values representing significant differences are marked with the following number of asterisks: *: $p<0.05$, **: $p<0.01$, ***: $p<0.005$, ****: $p<0.001$, and ns: no significant difference.

RESULTS:

As shown in Figure 1, BPA-IL significantly inhibited tumor growth compared to the control group despite the lower dose volume compared to BPA-Fru. As shown in Figure 2, no significant side effects (e.g., weight loss) were observed after using BPA-IL similar to BPA-Fru.

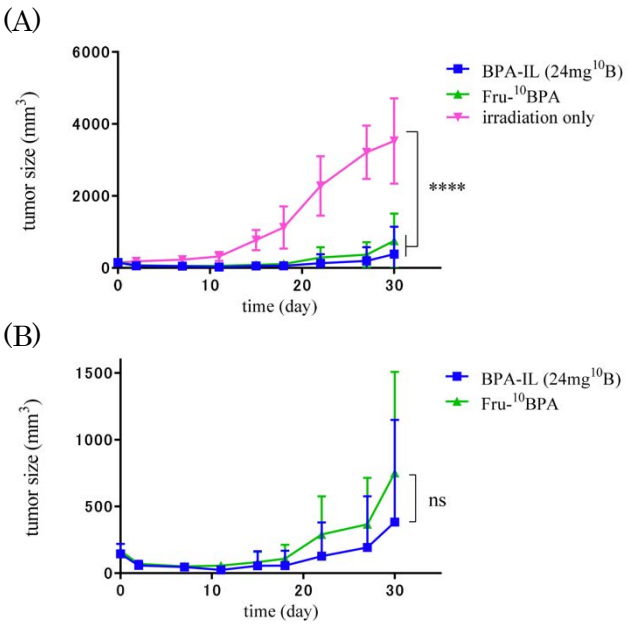

Fig.1) BPA-IL anti-tumor effect after BNCT (n=4).
(A) BPA-IL and BPA-Fru vs. irradiation only: ****
(B) BPA-IL vs. BPA-Fru: n.s.

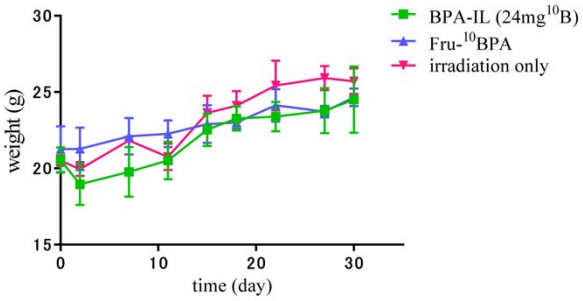

Fig.2) Mice body weight after BPA-IL injection with further neutron irradiation.

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ACKNOWLEDGMENTS:

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CO7-24 Preliminary study of the anti-tumor effect of BNCT using bubble liposome on the canine hemangiosarcoma model

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INTRODUCTION: Canine hemangiosarcoma is a common malignant tumor derived from vascular endothelial cells. Hemangiosarcoma often occurs in the spleen and liver and has a poor prognosis despite surgery and chemotherapy in veterinary medicine [1.2].

Boron neutron capture therapy (BNCT) is a new treatment that utilizes the nuclear reaction between boron and neutrons. BNCT is an effective treatment for malignant tumors in humans and is expected to be applied to canine malignant tumors. In order to enhance the therapeutic effect of BNCT, it is important to increase the concentration of boron in the tumor.

Recently, drug delivery using a combination of ultrasound and microbubbles has been reported to be effective in increasing drug concentrations [3]. The interaction of microbubbles and US increases the local permeability of blood vessels, allowing the injected drug to move easily out of the vessel and increasing the drug concentration in the tissue. The combination of liposomal doxorubicin, microbubbles, and ultrasound has been reported to inhibit tumors in veterinary medicine. [4].

In this study, we prepared the BSH-encapsulated liposomes and perfluorocarbon microbubbles for selective cancer targeting, and applied the BNCT to the canine hemangiosarcoma model by intravenous injection.

EXPERIMENTS: Canine hemangiosarcoma cell line (Ju-A1) was cultured in RPMI1640 supplemented with 10% FBS, L-glutamate, penicillin and streptomycin. 8×10^5 cells subcutaneously injected into the right hindlimb of nude mice.

BSH-encapsulated liposomes and bubble liposomes were injected via the tail vein, and ultrasound was applied to the tumors.

In vivo evaluation was performed on Ju-A1 tumor-bearing mice irradiated at nuclear reactor facility of Kyoto University Institute for integrated Radiation & Nuclear Science. Antitumor effect was evaluated on the basis of the change in tumor growth.

RESULTS: Tumor growth was suppressed in the group with BSH-encapsulated liposomes, microbubbles, and ultrasound. Tumor growth was significantly suppressed in the Lipo-Bubble-US group than in the non-irradiated control group.

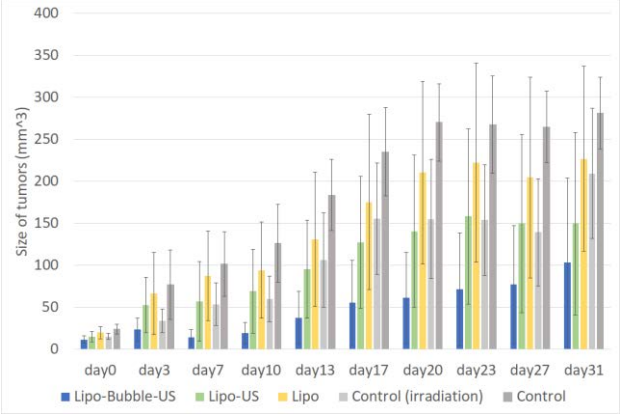

Figure 1. Tumor growth in the canine hemangiosarcoma model.

This preliminary result indicates that drug delivery systems using microbubbles and ultrasound have the potential to be applied to BNCT in canine hemangiosarcoma. In the future, we hope to apply this delivery system to clinical studies of BNCT in veterinary medicine.

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CO7-25 Development of boron-nutritional diagnostics in the plant using a neutron capture reaction

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INTRODUCTION: We are working on elucidating the physiological function of boron in plants using a boron-10 neutron capture (BNC) reaction. This is one of the effective applications of nuclear power in addition to generating electricity. Although boron is an essential nutritional element for all plants, a deficiency or an excess of boron causes various growth disorders. For the numerous and frequent crop disorders arising from boron-toxicity world-wide, noteworthy solutions have yet to be proposed, as a boron analysis method with high resolution has not been well-developed. We still have a poor understanding of the physiological function of boron in plants. In order to collect multidimensional information on how much boron is localized in specific tissue/cell at various stages of plant-growth, *in situ* high-resolution visualization technique capable of detecting the localization of boron is being developed by applying α-autoradiography with solid-state nuclear tracking detector, CR-39[1,2]. We expect that this technique will contribute to the establishment of the nutritional diagnosis method for boron in plants.

Here we report the result from BNC analysis of the boron-distribution in a seed of special tomato cultivar, 'Micro-Tom'.

EXPERIMENTS: Plant materials and growth conditions> Seeds used in analysis were harvested from a dwarf tomato cultivar, 'Micro-Tom' (*Solanum lycopersicum* L). Micro-Tom was originally derived for home gardens as the dwarf cultivar, but is now used as a major material for various plant researches[3]. The original seeds, which were obtained from Inplanta Innovations Inc., seeded on the moderately moisturized vermiculite and cultivated at 23°C under a 16-h light/8-h dark cycle in a 60%-humidified growth chamber. A week later, their seedlings were transferred to the hydroponic media containing major nutrients (1 mM $Ca(NO_3)_2$, 0.5 mM KH_2PO_4, 0.5 mM K_2SO_4, 1 mM $MgSO_4$, and 1.5 mM NH_4NO_3) and micronutrients (75 μM EDTA-Fe, 46 μM $H_3{}^{10}BO_3$, 9 μM $MnSO_4$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.8 μM Na_2MoO_4) under the same condition.

In situ visualization of boron in a Micro-Tom seed using neutron capture radiography> Mounted slice (10-μm thickness) of Micro-Tom seeds onto CR-39 (20 mm×30 mm) was irradiated with epithermal neutron for 20 min by applying to the pneumatic tube in the graphite thermal column (Tc-Pn) of Kyoto University Research Reactor (KUR). The irradiated CR-39 plate was etched in 6 M NaOH solution, and the resulting etch-pits were observed under an optical microscope.

RESULTS: Both Fig. 1(A) and 1(B) show cross sections prepared from the same Micro-Tom seed. Arrowed contents in Fig. 1(A) are the granular starch and embryo, respectively. On the other hand, Fig. 1(B) is a radiograph, which was generated by BNC reaction, and reveals distribution of boron in the cross section. A large number of etch-pits derived from boron-10 were imaged throughout the section as small black spots. Interestingly, the distribution amount of boron in the embryo was almost same as in the granular starch.

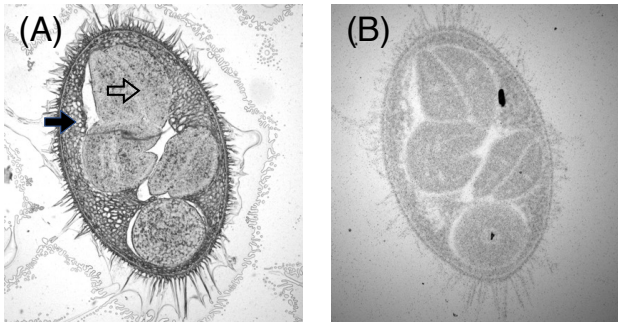

Fig.1 Detection of boron in the Micro-Tom seed using BNC reaction. (A): Optical microscopic images of the cross section of the seed. Black and white arrows indicate granular starch and embryo, respectively. (B): Radiograph.

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CO7-26 An Evaluation of the Response of Tumor Cells to BNCT

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INTRODUCTION: Nuclear reactions between thermal neutron and boron-10 generate alpha particle and lithium nuclei of high LET. The analysis of tumor cell response including cell death and various biological reactions as well as the effect on immune regulatory environment is important to consider clinical optimization of boron neutron capture therapy (BNCT). We used cancer cells and xenograft mouse models to analyze the responses to BNCT.

EXPERIMENTS:
Neutron irradiations at KUR reactor was operated at 1 MW in all experiments. We used gold foil activation analysis for the measurement of thermal neutron fluences and thermoluminescence dosimeter (TLD) for the measurement of the γ-ray doses including secondary γ-ray. Total physical dose calculation was carried out using the flux-to-dose conversion factor by the sum of the absorbed doses resulting from $^{1}H(n, \gamma)^{2}D$, $^{14}N(n, p)^{14}C$, and $^{10}B(n, \alpha)^{7}Li$ reactions, as previously described.
The cells were pretreated with ^{110}B-boronophenylalanine fructose complex (BPA) for 1.5-2.0 hrs. The cell survival of human squamous cell line SAS and murine melanoma cell line B16 cells was analyzed by colony formation assay, and cells were harvested at 6 hours and 24 hours with ISOGEN after BNCT. RNA and proteins were also isolated and RNA expression levels were examined using real-time PCR and protein levels were analyzed by ELISA.
For mouse experiement, we used mouse melanoma cell line B16 and B16F10mGM.The C57BL/6J male mice of 5 weeks old were transplanted with either B16 or B16F10mGM cells at bilateral legs 8 days before irradiation. The mice were placed on a board with the head facing out and the feet facing inward, with a circular cutout in the middle as the radiation port, and the left leg of the mouse was irradiated at the same time. Local irradiations to mouse hind legs were operated using ^{6}LiF containing thermal neutron shield. Mice were injected with BPA at 500 mg/kg bodyweight approximately 30 min or 60 min before irradiation.

RESULTS:
Table 1. Irradiated doses to cells (E-4 rail port) on August 24, 2021. (Cl: Closest, F: Farthest)

Irradiation time [min]	Position	Fluence [/cm2]		[Gy]					
		Thermal neutron [/cm2]	Epi-thermal neutron [/cm2]	Thermal neutron [Gy]	Epi-thermal neutron [Gy]	Fast neutron [Gy]	Gamma-ray [Gy]	Physical Dose [Gy]	B-10** (1ppm)
10	Cl	1.04E+12	1.84E+11	0.14	0.02	0.10	0.12	0.38	0.08
	F	7.76E+11	1.38E+11	0.10	0.01	0.08	0.12	0.31	0.06
60	Cl	6.40E+12	1.10E+12	0.85	0.09	0.63	0.62	2.20	0.47
	F	5.10E+12	9.10E+11	0.68	0.07	0.51	0.62	1.90	0.38
2	Cl	2.30E+11	4.00E+10	0.03	0.00	0.02	0.02	0.08	0.02
4	Cl	4.40E+11	7.80E+10	0.06	0.01	0.04	0.04	0.15	0.03
6	Cl	6.00E+11	1.10E+11	0.08	0.01	0.06	0.09	0.24	0.05
8	Cl	8.60E+11	1.50E+11	0.11	0.01	0.09	0.09	0.30	0.06

Table 2. Irradiated doses for local irradiation of mouse legs (cart, irradiation room) on October 28, 2021. (P: Position, Ce: Center)

Mouse No.	P	Fluence [/cm^2]		[Gy]					
		Thermal neutron	Epi-thermal neutron	Thermal neutron	Epi-thermal neutron	First neutron	γ-ray	Physical dose	B-10** (1ppm)
60	Ce	3.3E+12	5.8E+11	0.44	0.046	0.32	0.23	1.0	0.24
60	Ce	3.2E+12	5.8E+11	0.43	0.046	0.32	0.36	1.2	0.24

Table 3. Irradiated doses for local irradiation of mice (cart, irradiation room) on November 30, 2021. (Cl: Closest, F: Farthest, Ce: Center, V: vial, M: mouse)

Irradiation time [min]	Position	Fluence [/cm^2]		[Gy]					
		Thermal neutron	Epi-thermal neutron	Thermal neutron	Epi-thermal neutron	First neutron	γ-ray	Physical Dose	B-10** (1ppm)
60	Cl V	3.0E+12	5.4E+11	0.4	0.043	0.3	0.37	1.1	0.22
	F V	1.5E+12	2.7E+11	0.2	0.022	0.15	0.37	0.75	0.11
	Ce M	3.8E+12	6.8E+11	0.51	0.054	0.38	0.25	1.2	0.28
60	Ce M	3.9E+12	6.9E+11	0.52	0.055	0.38	0.31	1.3	0.29

The measurement of thermal neutron fluence and doses are shown in Tables 1-3.
The results from dynamic profiles of RNA and proteins suggested that presence of early responding genes were confirmed after BNCT. A potential role of HMGB1 as a biomarker for evaluation of early *in vivo* response to BNCT was also indicated [1].

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CO7-27 Development of ^{10}BPA-loaded mesoporous silica-based nanoparticles and evaluation in BNCT mouse experiments

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INTRODUCTION: Boron phenylalanine (BPA) has been developed as useful boron compound which is available for Boron neutron capture therapy (BNCT) therapy. We have recently developed a mesoporous silica-based nanoparticle which has biodegradable bond in the framework that are loaded with BPA, named biodegradable periodic mesoporous organosiliga (BPMO). These nanoparticles have a large surface area where BPA can be attached for BNCT application. In this study, we intratumor injected BPA-BPMO to mice transplanted with CT26 mouse colon cancer and evaluated the BNCT efficacy.

EXPERIMENTS: BPMO was synthesized by sol-gel synthesis of two precursors, bis[3-(triethoxysilyl) propyl] tetrasulfide and 1, 2-bis(triethoxysilyl) ethane. This resulted in the incorporation of tetrasulfide bonds into the framework of the nanoparticles. BPMO was then processed to modify with GOPTS (3-glycidyloxypropyl trimethoxysilane) that contain epoxy groups. After converting epoxy groups to diols, BPA was mixed with diol-BPMO to graft BPA using a method to chelate boron with diol groups. The synthesized nanoparticles were characterized by using SEM, TEM, FT-IR, nitrogen adsorption-desorption analysis and zeta potential. The amount of boron attached on the nanoparticles was examined by ICP, and boron content was determined.

Mouse model was established by transplanting mouse colon cancer cells CT26 to BALB/c mouse. After injection of BPA-loaded nanoparticles, amount of boron in the tumor was examined by ICP.

Irradiation of mice with neutron beams was carried out by placing mice in specially prepared holders. Irradiation was carried out with thermal neutron for 12 min at an operating power of 5MW. After the irradiation, mice were returned to cage, and tumor size was longitudinally and transversely measured every 2 days for up to 2 weeks.

RESULTS: Nanoparticles synthesized had approximately 80-100 nm of diameter and homogenous shapes examined by SEM and TEM microscopy. FT-IR analysis of BPMO showed diagnostic peaks of typical Si-O-Si, $-(CH_2)_2-$ and $-CH_2-$ vibrations. After loading of BPA to BPMO, we analyzed surface charge of BPA-BPMO which was negative due to modification with phosphonate. The zeta potential of BPA-BPMO was -41.38 mV.

Boron accumulation in the CT26 tumor was investigated with ICP. We were able to achieve delivery of boron in the tumor but the route of delivery needs to be further investigated. We also observed accumulation of BPA-BPMO in the tumor by confocal microscopy. As seen in Fig.1, the red fluorescence of BPA-BPMO could be observed in the part of the tumor when 6.25 mg/tumor of BPA-BPMO (equivalent to 125 µg/tumor of ^{10}B) was injected to tumor.

Investigation of the BNCT efficacy of BPA-BPMO was carried out with Kyoto University Nuclear Reactor and evaluated by measuring tumor size every 2 days for 2 weeks. After mice which was intratumor injected high concentration (6.25 mg/tumor of BPA-BPMO) or low concentration (0.625 mg/mouse of BPA-BPMO) were irradiated with thermal neutron, as seen in Fig.2, BNCT efficacy of BPA-BPMO was resulted in that was lower than that of free BPA, but constant tumor growth inhibition was seen.

We are currently preparing BSH-BPMO instead of BPA-BPMO to transport more boron, and carrying out mouse experiment.

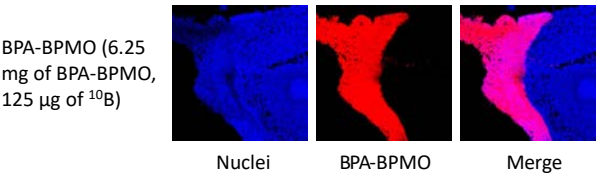

Fig.1 Tumor accumulation of BPA-BPMO by confocal microscopy.

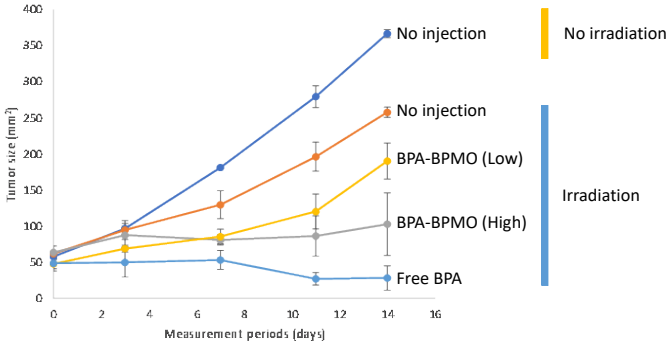

Fig.2 BNCT efficacy of BPA-BPMO.

R3150

CO7-28 Conjugation of Phenylboronic Acid Moiety through Multistep Organic Transformations on Nanodiamond Surface for an Anticancer Nanodrug of Boron Neutron Capture Therapy

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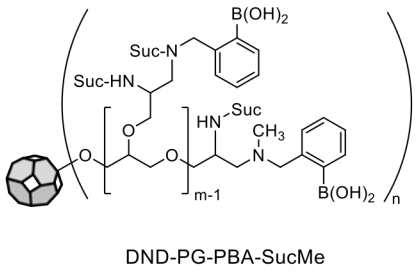
Detonation nanodiamonds (DNDs) have attracted considerable attention, in particular, in the field of nanomedicine due to its biocompatibility as well as various functionalities imparted by surface modification. Meanwhile, boron neutron capture therapy (BNCT) is an advanced cancer treatment utilizing nuclear fission reaction of ^{10}B upon neutron irradiation. Recently, quite a few boron-containing nanoparticles have been investigated to deliver ^{10}B atoms into cancer tissue selectively and retentively. In this study, we explored boronic acid functionalized DNDs as an anticancer agent for BNCT. Phenylboronic acid (PBA) moiety was introduced to polyglycerol (PG) modified DNDs (DND-PG) through multistep organic transformation (Figure 1 and 2), giving percent order of boron atoms. The process is scalable and reliable by simple covalent chemistry and the resulting product is well dispersed, and stable chemically and physically under physiological conditions. In the *in vivo* experiments, the resulting material was accumulated into the tumor to exert the BNCT efficacy upon neutron irradiation (Figure 3 and 4). These results demonstrate that the PBA functionalized DNDs are a promising candidate as an anticancer nanodrug for BNCT.

Reference: M. Nishikawa, H. G. Kang, Y. Zou, H. Takeuchi, N. Matsuno, M. Suzuki, N. Komatsu* *Bull. Chem. Soc. Jpn.*, 94(9), 2302-2312 (2021).

Figure 1. Schematic illustration for design of the three-layered structure of DND-based nanodrug.



DND-PG-PBA-SucMe

Figure 2. Chemical structure DND-based nanodrug including phenylboronic acid moiety for BNCT.

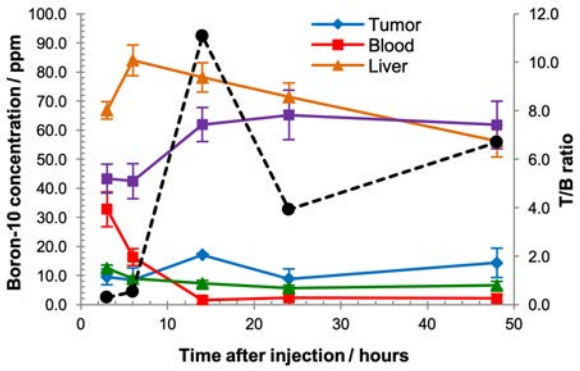


Figure 3. Pharmacokinetic study of DND-PG-PBA-SucMe-^{10}B; ^{10}B concentrations in tumor, blood and major organs (solid lines) and the concentration ratio of tumor (T) to blood (B) (T/B ratio, dotted line).

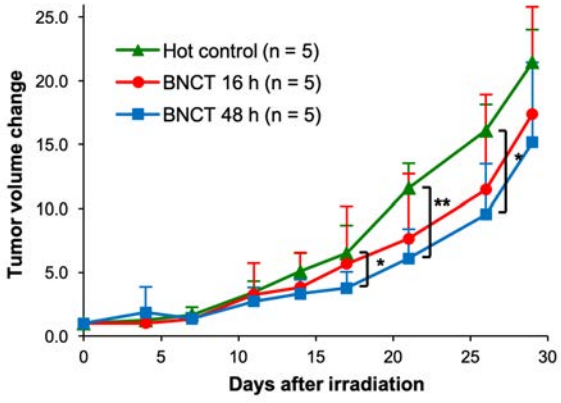


Figure 4. BNCT results of DND-PG-PBA-SucMe-^{10}B. Relative tumor volume was monitored in BNCT and control groups for 29 days after neutron irradiation. (n = 5, the Student's t-test, *p < 0.05, **p < 0.01).

CO7-29 The option of BNCT for oral squamous cell carcinomas

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INTRODUCTION:
The number of patients with oral and oropharyngeal cancer exceeds 20,000, and both mortality and morbidity rates continue to increase, with approximately 7,000 people currently suffering from oral cancer each year and more than 3,000 people dying in Japan [1]. The most common oral cancer is oral squamous cell carcinoma (OSCC)(90 %), and OSCC has a high potential for nodal metastasis and locoregional invasion, from which over 50% of patients die. As the most common histopathological grading of OSCC, the World Health Organization differentiation criteria, based on the Broders criteria and merely recognizes well-, moderately-, and poorly-differentiated variants of conventional OSCCs, is used for an indicator of prognosis. In consideration of the preservation of organ function, as well as appearance, Boron Neutron Capture Therapy (BNCT) for head and neck cancer is one of the effective treatments instead of surgical procedures, radiotherapy, chemotherapy, and combined therapy [2]. Therefore, the correlation between the tumor differentiation in OSCC and prognosis after BNCT is investigated in this study.
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EXPERIMENTS: The HSC-4 (JCRB cell Bank) as well-differentiated OSCC and HSC-3 (JCRB Cell Bank) as poorly-differentiated OSCC were cultured in MEM medium (Gibco, Thermo Fisher Scientific, USA) supplemented with 10 % fetal bovine serum (Sigma -Aldrich, USA) and 100 unit/ml penicillin and 100 mg/ml streptomycin (1% p/s) (Thermo Fisher Scientific, USA) for OSCC mouse model. Each cell lines ($1x10^6$ cells) were subcutaneously injected into the left hind legs of 6-week-old female Balb/c nude mice (Clea Japan Inc., Japan). After the injection of L-boronophenylalanine (BPA, Steboronine®, Stella pharma, Japan) to the OSCC mouse, the tumor was resected, centrifuged for 5 mins at 300×g then treated with 60% HNO3 and heated for 1 hour for measuring the boron concentration by the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, Agilent technologies 7500cx)[3]. After the administration of BPA, OSCC mice were irradiated by neutron for 40 minutes and the follow-up body weight and the diameter of tumor were measured. The tumor size was calculated according to the following formula.
Tumor volume [mm3] = (Long diameter [mm]) x (Short diameter [mm])2 /2
****

RESULTS and DISCUSSION:
The results of ICP-MS analysis showed the boron uptake is observed in both OSCC models. However, the boron uptake in poorly-differentiated OSCC is slightly higher than the well differentiated OSCC. After BNCT**,** the weight loss and the adverse effect were not observed in both OSCC. The tumor had significant growth in non-treatment group, compared to significant shrinkage in BNCT group. The tumor response rate is 84% in poorly differentiated OSCC and 96% in well differentiated OSCC.
These results indicate that BNCT is effective to both OSCC, although there are some slight differences. It was found that it is important to validate not only the boron concentration but also the boron distribution in this study. In 2022, the boron distribution and the boron neutron reaction in the tissue of well- and poorly -differentiated OSCC model would be investigated utilizing neutron induced autoradiography technique with a CR-39[4].
In near future, the multidisciplinary approach including BNCT will proposed for OSCC.
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[4]R Ogawara *et al.,* Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, Volume 467, 15, Pages 9-12 (2020).

CO7-30 New boron drug development research targeting pancreatic cancer

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INTRODUCTION:

Pancreatic cancer refers to malignant tumors arising from the pancreas, but generally refers to pancreatic ductal carcinoma. Ductal carcinoma originates from the pancreatic duct epithelium and accounts for 80-90% of all neoplastic lesions in the pancreas. According to national statistics, it was the fifth leading cause of death after lung cancer, stomach cancer, colorectal cancer, and liver cancer. Pancreatic cancer in our country has been on the rise in recent years, with more than 30,000 people dying from pancreatic cancer each year.

The number of pancreatic cancer deaths has increased more than eightfold in the past 30 years, and the disease is more common in people in their 60s and slightly more common in men. It has been associated with smoking, family history of pancreatic cancer, diabetes, and chronic pancreatitis.

Pancreatic cancer is difficult to detect in its early stages because there are few subjective symptoms. It is usually noticed after the disease has progressed to a more advanced stage by abdominal pain, weight loss, jaundice, and other symptoms. Therefore, when pancreatic cancer is diagnosed, it is often found in an advanced stage. In addition, although some people worry about pancreatic cancer when they experience back pain, it is not necessarily a characteristic symptom of pancreatic cancer. It is important to note that pancreatic cancer may be present in diabetics when their blood glucose control suddenly deteriorates.Ultrasonography, CT, MRI, endoscopic pancreatography, and angiography are used to diagnose pancreatic cancer. If pancreatic cancer is suspected, the pancreas cannot be seen from the surface of the body, so an ultrasound or CT scan is first performed to check for the presence of a mass in the pancreas. CT scan can also be used to check for metastasis of pancreatic cancer to other organs such as the lungs and liver.

One of the characteristic imaging findings of pancreatic cancer is that the normal pancreas is contrasted without contrasting the pancreatic cancerous area when contrast enhanced CT scan is performed.

Usually, malignant tumors have more pronounced tumor vascular growth than normal tissues due to the rapid development of tumor blood vessels to nourish the tumor. In addition, these tumor vessels maintain a very leaky structure to provide a high degree of oxygen and nutrition to the tumor and are easily detectable using contrast media. However, pancreatic cancer, despite being a malignant tumor, is characterized by the fact that tumor blood vessels are somewhat scarce compared to normal, and the stroma between tumor cells is hyperplastic, making it difficult to receive the contrast effect of contrast media.

DDS (Drug Delivery System) is a research field that delivers drugs such as anticancer agents to such malignant tumors. It has been reported that when a liposomal formulation containing a drug is administered to a tumor-bearing model, such a macromolecular drug accumulates specifically in the tumor by leaking from the tumor blood vessels. This effect is called the EPR effect (Enhanced Permeability and Retention effect) and has been proposed as a theory that minimizes drug damage to normal tissue and maximizes the effect on tumor tissue.

For pancreatic cancer that does not undergo contrast effect, we believe that it is difficult to use polymeric DDS formulations, which mainly have EPR effect, for future clinical applications. Therefore, we focused on PET (Positron Emission Tomography) using 18F-FDG, which is used in the diagnosis of pancreatic cancer. FDG is a test reagent of a glucose derivative called fluorodeoxyglucose F18. Glucose is labeled with 18F, a radionuclide, and is used as a test reagent for various types of cancer. In this study, we focused on glucose metabolism in cancer, and decided to develop a glucose-based boron drug [1].

EXPERIMENTS:

In this study, we decided to develop a boron drug for pancreatic cancer by targeting glucose transporters. In classifying pancreatic cancer, we focused on CA19-9, a tumor marker for pancreatic cancer, which is a type of carbohydrate antigen called carbohydrate antigen 19-9. Pancreatic cancers with high CA19-9 levels are known to have a poor prognosis, and we investigated the relationship between CA19-9 high human pancreatic cancer cell lines and CA19-9 high human pancreatic cancer To evaluate CA19-9, CA19-9 in the supernatant of cultured cells was measured by ELISA and observed by confocal laser microscopy with cell immunostaining using CA19-9 antibody.

RESULTS:

In this study, we have succeeded in synthesizing a glucose-binding boron drug and have filed a patent application. It was confirmed that the glucose boron drug was efficiently introduced into the cells via glucose transporters highly expressed in pancreatic cancer. The anti-tumor effect of the new drug in pancreatic cancer model mice was demonstrated at Institute for Integrated Radiation and Nuclear Science, Kyoto University with neutron irradiation. The results were further developed and reported as a novel boron drug. We thank many collaborators for their cooperation.

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CO7-31 Basic research on new BNCT strategies for melanoma

H. Michiue[1], T. Fujimoto[1,2], N. Kanehira[1,2], K. Igawa[1], Y. Sakurai[3], N. Kondo[4], T. Takata[3], and M. Suzuki[3]

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INTRODUCTION:

Melanoma has long been a target of BNCT. In particular, the effectiveness of BNCT has been recognized worldwide as evidenced by the publication of "Treatment of malignant melanoma by single thermal neutron capture therapy with melanoma-seeking 10B-compound" in the Lancet in 1989. In the article "Treatment of malignant melanoma by single thermal neutron capture therapy with melanoma-seeking 10B-compound" published in the Lancet in 1989, Dr. Mishima et al.[1]

In this report, the world's first successful case of BPA-BNCT for melanoma was reported. It is regarded as the cornerstone of the development of BNCT in Japan. Melanoma is a cutaneous malignancy with an incidence of 1-2 per 100,000 people and is considered a rare cancer. In Australia, the incidence is about 35 per 100,000 people, with regional and racial variation. Surgery is the standard treatment of first choice for localized melanoma, and the prognosis is very good for Stage I melanoma that is unlikely to spread to the regional lymph nodes.

The prognosis, mainly surgery, for localized melanoma is very good, with a 5-year survival rate of 95-100%, and the disease is reported to be curable by surgery. The utility of BNCT for local melanoma is reported to be high for melanoma patients, many of whom are elderly, because there is no pain or functional disability associated with surgery.

The boron drug BAP (p-boronophenylalanine), which was developed to target melanoma, is a derivative of L-phenylalanine, the starting material for melanin synthesis, and has been developed to target malignant melanoma from the beginning, and its concentration in melanoma is It is highly concentrated in melanoma. In recent years, its use in malignancies other than melanoma has been progressing, and its incorporation into various malignant tumors via LAT1, an amino acid transporter that is highly expressed in cancers, has been reported.

Immunotherapy for advanced-stage melanoma has been in the spotlight in recent years. For many years, the therapeutic efficacy of immunotherapy was considered limited, but the development of immune checkpons and inhibitors by Professor Honjo Tasuku and his colleagues, who received the 2018 Nobel Prize in Physiology or Medicine, has established immunotherapy as a fourth therapeutic approach alongside surgery, chemotherapy and radiation therapy, immunotherapy has been established as a fourth therapeutic modality alongside surgery, chemotherapy, and radiation therapy.

Immune cells in our body are quick to attack invading foreign substances such as viruses. On the other hand, they cannot easily attack cancer cells generated from our own cells because they are our own cells, even if they proliferate in our body. They discovered that the reason why immune cells are unable to attack cancer cells is due to the binding between an immune checkpoint molecule called PD-1, which exists on the surface of cytotoxic T cells, and a molecule called PD-L1 on the surface of cancer cells.

Furthermore, by developing a drug that inhibits the mechanism by which cancer cells escape from the immune mechanism, the foundation of immunotherapy that enables immune cells to smoothly kill cancer cells has been completed. This drug is called an immune checkpoint inhibitor. The efficacy of this immune checkpoint inhibitor was confirmed in patients with advanced-stage melanoma, marking the start of immunotherapy.

We believe that it would be very useful for the future development of BNCT to investigate the direction of combined immunotherapy using anti-PD-1 antibody, which has good results in melanoma, and BPA-BNCT, which is compatible with melanoma, and to verify the effectiveness of this combination through basic research using animal models.

EXPERIMENTS:

We purchased the B16-F10 mouse melanoma cell line to create a melanoma model, as B16-F10 has high melanin synthesis capacity and has a large amount of black melanin pigment even in cultured cells. Since B16-F10 originated from C57BL/6 mice, we used these mice as a melanoma model. Animal experiments were conducted after strict approval by the ethics committees of Okayama University and Kyoto University.

Melanoma model mice were created at Okayama University and then transported to t Institute for Integrated Radiation and Nuclear Science, Kyoto University, where BNCT treatment experiments were conducted and subsequent experiments were performed.

RESULTS:

We used BPA as an effective boron agent for melanoma and confirmed the anti-tumor effect of neutron irradiation. The results were favorable, and are useful for the development of BPA-BNCT for melanoma in the future

We would like to express our deepest gratitude to the many collaborators who assisted in this project..

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CO7-32 Quantitative analysis of the contribution of tumor vascular damage to the antitumor effect of X-ray using BNCR

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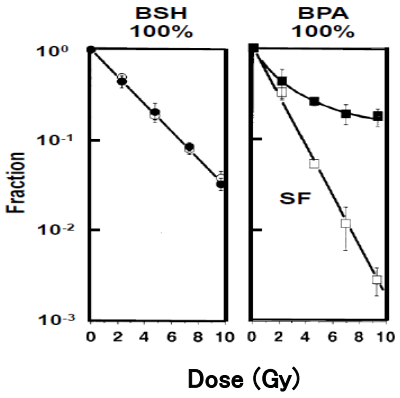
INTRODUCTION: Tumor tissue consists of tumor cells and stroma, especially the vasculature that supplies the cells with oxygen and nutrients. The antitumor effect in BNCT is almost specific to cells accumulating boron compounds. In this study, we compare the effect of this tumor cell-specific BNCT with the effect of X-rays that has no tumor cell specificity and quantitatively elucidate the effect of damage caused in the vascular system on the overall antitumor effect. This is the purpose of this research.

In FY2021, data from a previous paper using SCCVII tumors were re-analyzed to determine whether the effect of BNCT on solid tumors can be explained solely by its cellular-level killing effect.

EXPERIMENTS: The main mechanism of radiation-induced cell death is double-strand breaks in DNA. Unrepaired breaks therefore appear as micronuclei in the cytoplasm after nuclear division. Addition of cytochalasin B to the culture medium of irradiated cells does not inhibit nuclear division but does inhibit cell division, so that cells which have undergone post-irradiation division are identified as binuclear cells. The decrease in the proportion of cells without micronuclei is equal to the α value of the cell survival curve analyzed by the LQ model; the particles released from the BNCR are high-LET particles and cell survival can only be expressed in terms of α values. Therefore, if the distribution of the boron compound is non-uniform, the lines obtained from both the micronucleus and colony formation assays should bend in the middle. Conversely, if the boron drug is not taken up by the cells and is distributed almost uniformly in the extracellular space, the two curves after BNCT should overlap perfectly. The relationship between neutron fluence and cell survival at the level determining cure can be estimated from the neutron fluence and cure rate curves in tumor cure experiments. Combining these two neutron fluence and cell survival curves, and assuming a reasonable tumor cell count based on tumor size, the relationship between cell survival and neutron fluence can be estimated for the range from pre-neutron irradiation to the neutron fluence at which cure is achieved.

RESULTS and DISCUSSION: As shown in Figure-1, for BSH, which is not taken up by cells and is mostly present in the intercellular space, the cell survival curve showed an exponential decrease concerning neutron fluence, and the micronucleus test curve completely overlapped on cell survival curve. On the other hand, for BPA-BNCT, cell survival showed an exponential decrease to the 10^{-3} level, but the micronucleus test curve bent in the middle. This was thought to be due to heterogeneous accumulation of BPA in the cells. This discrepancy also suggests that the colony formation rate of cells which did not take up BPA was significantly lower than that of cells. Figure-2 shows the estimated slope of the cell survival curve at the level at which tumor cure is achieved in BNCT cure experiments with BSH and BPA. Combining the results of figures -1 and -2, the estimated cell survival curve in the full fluence range is shown in figure-3. From this, the tumor-forming potential of SCCVII tumor cells in mice was estimated to be between 10^{-3} and 10^{-4}. TD50 has not been determined but is considered to be an approximately reasonable figure based on transplantation experience. As can be seen from the present analysis, most of the anti-tumor effects of BPA-BNCT but also BSH-BNCT with higher blood boron concentrations, can be explained by the direct effect of BNCT on tumor cells and the contribution of vascular damage to tumor cure is estimated very small if present.

Figure-1.



Open symbol: Survival Fraction,

Closed symbol: Fraction of the binuclear cells without micronuclei

Figure-2

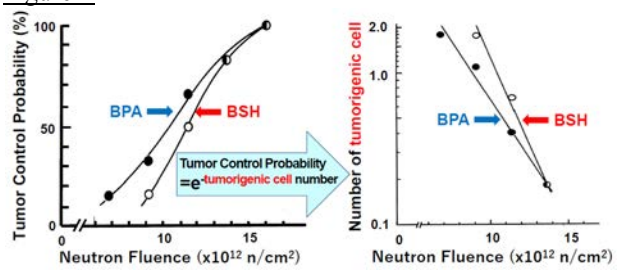
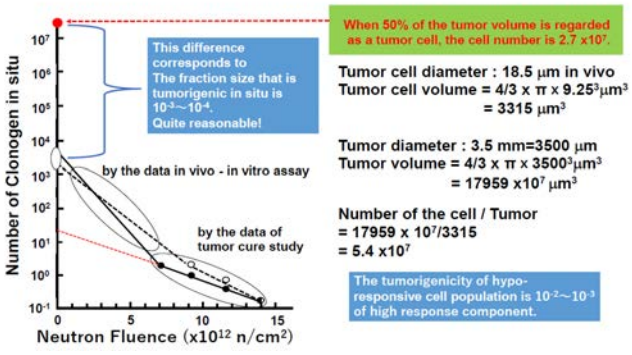


Figure-3



CO7-33 Study on Intracellular Protein Destruction by Boron Neutron Capture Reaction

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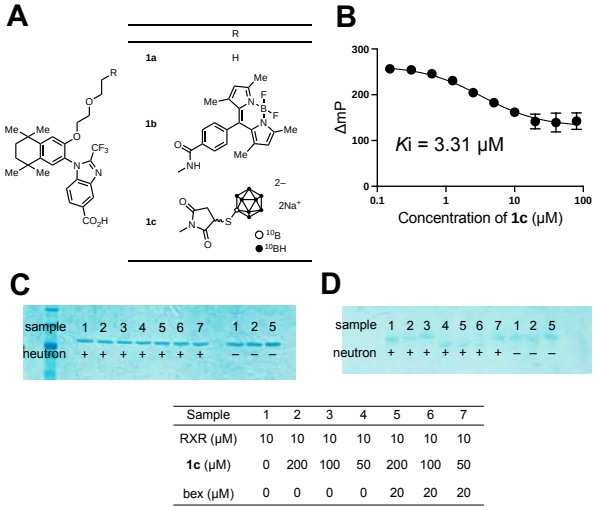
INTRODUCTION: Boron neutron capture therapy (BNCT) is a cancer treatment based on the nuclear reaction between boron-10 (^{10}B) and neutron. The high-energy particle beam after neutron capture by ^{10}B is reported to break the double-stranded DNA of cancer cells and induce apoptosis. However, the effect of the particle beam on intracellular organelles has not been investigated in detail. In this study, we investigate whether protein cleavage based on neutron irradiation occurs using the retinoid X receptor (RXR), one of nuclear receptors, and a boron-containing RXR ligand.

EXPERIMENTS: The compounds shown in Figure 1A and bexarotene were synthesized by the authors. The RXR ligand binding domain (RXR-LBD) was kindly gifted by Prof. Nakano, University of Shizuoka. RXR-LBD binding assay and reporter gene assay were performed according to references 1 and 2. Neutron beam was irradiated at 5 MW for 30–60 minutes. The irradiated sample (100 μL) was prepared as below; RXR-LBD (10 μM), CBTF-EE-BSH (200, 100, 50 μM, converted to ^{10}B concentration 24, 12, 6 ppm), bexarotene (20 μM or not), buffer (10 mM HEPES, 150 mM NaCl, 2 mM $MgCl_2$, 5 mM DTT, 5% DMSO). The irradiated sample was diluted 10 times with above buffer and mixed with BPB-containing buffer in a 4:1 composition, and electrophoresed at 250 V, 20 mA for 70 minutes. The concentration of RXR-LBD was estimated using CBB staining.

RESULTS: We have reported CBTF-EE (**1a**) as an RXR antagonist.[1] Also, it has been confirmed that CBTF-EE-BODIPY (**1b**), which was designed by inducing a fluorescent group at the end of the alkoxy chain of **1a**, functions as an RXR antagonist.[2] Thus, we designed and synthesized CBTF-EE-BSH (**1c**), which has ^{10}B cluster molecule BSH, and evaluated whether **1c** will function as an RXR ligand (Figure 1A). As a result of RXR-LBD binding assessment, it has been revealed that a *K*i value of **1c** was 3.31 μM (Figure 1B). Then, neutron irradiation was performed on each solution in which **1c** and RXR-LBD coexisted, and in which bexarotene, which is an RXR agonist and inhibits the binding of **1c** to RXR-LBD, was further added. After irradiation, the amount of RXR-LBD was estimated by SDS-PAGE, which can evaluate the migration distance according to the molecular weight of the protein, and CBB staining. Irradiation with 2.98×10^{13} /cm^{-2} as thermal neutrons and 5.53×10^{12} /cm^{-2} as extrathermal neutrons did not show (Figure 1C). Interestingly, NATIVE-PAGE, which can evaluate the three-dimensional structure of the protein, indicated that the binding of bexarotene becomes dominant by neutron irradiation (Figure 1D).



Sample	1	2	3	4	5	6	7
RXR (μM)	10	10	10	10	10	10	10
1c (μM)	0	200	100	50	200	100	50
bex (μM)	0	0	0	0	20	20	20

Figure 1. (A) Chemical structures of **1a**–**1c**. (B) Binding ability of **1c** toward RXR-LBD. Evaluation of RXR-LBD concentration by SDS-PAGE (C), and the 3D structure of RXR-LBD by NATIVE-PAGE (D).

DISCUSSIONS AND FUTURE PLAN: The neutron irradiation to the combination of **1c** and RXR-LBD could not induce RXR-LBD degradation. On the other hand, NATIVE-PAGE revealed that neutron irradiation induced the structural change of **1c** and the dominant binding of bexarotene to RXR-LBD, when **1c** and bexarotene coexisted. It remains whether this phenomenon is caused by BNCR. Irradiation of gamma ray or electron beams is a subject for future study.

In recent years, various fields have been challenging to produce boron delivery agents which could replace L-boronophenylalanine (BPA). Given that the BNCT targets tumor DNA cleavage, it is of interest to use nuclear receptor ligands as boron delivery agents. The reporter gene assay revealed that **1c** functions as an RXR antagonist. In the future, we will investigate the intracellular translocation ability of **1c** in detail and aim to provide insights into the availability of nuclear receptor ligands as intracellular ^{10}B delivery agents.

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CO7-34 Observation of Intracellular Boron Neutron Capture Reaction with a Novel Boron Compound

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a cancer treatment based on the nuclear reaction between boron-10 (^{10}B) and neutron. Since this therapeutic effect depends on the collision of ^{10}B with neutron, the concentration of boron in the cancer tissue is an important factor. However, for borofalan (4-boronophenylalanine), currently the only drug approved for BNCT, the boron concentration in cancer tissue is estimated from the blood concentration. Thus, we aimed to create a new BNCT agent whose boron concentration in cancer tissue is measurable.

Since BNCT agents are used in large doses, we focused on iodine contrast agents for X-ray CT, which are also used in similar large doses, and enable quantify iodine concentration noninvasively by X-ray CT. We hypothesized that this principle could be used to measure boron concentration in tissues. To test the above hypothesis, we designed and synthesized **1** (Figure 1A), and investigated the intercellular transferability, X-ray CT imaging ability, and boron neutron capture reaction (BNCR) in cancer cell B16BL6.

EXPERIMENTS: After obtaining iodine-containing boron compound **1**, the intracellular transferability was determined by following the steps as below. Cells were cultured in 35 mm petri dishes submerged 2 cm squares of CR-39 and exposed to medium containing the **1** for 2 h. The cells were then washed with PBS and the cell lysates were prepared. Then, the concentration of **1** was measured using LC-MS/MS. And the CR-39 was irradiated with neutrons at KUR. Neutron irradiation conditions were at E-3 for 2 hours, 1 MW for 10, 20 minutes, or 5 MW for 10, 20 minutes, respectively. Sample conditions; were as follows: blank, BSH (500 μM), **1** (500 μM), **1** (500 μM) + A6K (100 μM). After neutron irradiation, the samples were treated according to the literature [1] and imaged using an optical microscope (BZ-X700). X-ray CT scan was performed using Eminence STARGATE in aqueous solutions with iodine concentrations of 4000, 2000, 1000, and 500 ppm.

RESULTS: Chemical structure of **1** contains a boron cluster BSH, and two iodines for X-ray CT. The intracellular translocation of each condition was measured in B16BL6 cells, and 2-hour exposure at 500 μM of **1** gave an intracellular approximately 20-fold higher boron concentration than that of BSH, and a combination with A6K, which promotes intracellular delivery of BSH [2], approximately 100-fold higher boron concentration than that of BSH.

X-ray CT imaging of aqueous solutions of **1** was performed, and the correlation between the iodine concentration of **1** and the CT values was obtained. It was found that the iodine concentration which enables to quantify was more than 800 ppm.

B16BL6 cells seeded on CR-39, which can quantify BNCR, were irradiated with neutrons at KUR, and the trajectory of alpha particle on CR-39 was analyzed. As a result, while no etch pits were observed even after 2 hours of irradiation in E-3, a boron concentration-dependent number of etch pits was detected after 20 minutes of irradiation at 1 MW (thermal neutron fluence 2.22×10^{12} cm^{-2}). The BNCR on CR-39 after irradiation was quantified as etch pits (Table 1). The results showed a high correlation with the intracellular boron concentration (Table 1) calculated by LC-MS/MS (R^2 = 0.968, Figure 1B).

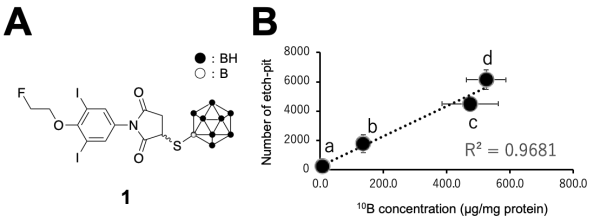

Figure 1. (A) The Chemical structure of **1**. (B) Correlation between BNCR generated on CR-39 and intracellular boron concentration.

Table 1. Comparison of conditions a–d, number of etch pits on CR-39 and intracellular boron concentration

	compounds	concentration (μM)	^{10}B concentration (μg/mg protein)	etch-pit (quantity/field)
a	blank	-	6.8	248.2
b	BSH	500	135.4	1785.6
c	**1**	500	473.7	4498.0
d	**1**/A6K	500/100	524.1	6148.2

DISCUSSIONS AND FUTURE PLAN: In this study, **1** was created and found to permeate B16BL6 cells in combination with A6K peptide, whose concentration is expected to be detectable by X-ray CT imaging. We are planning to perform CT imaging in tumor-bearing mouse. In addition, **1** was found to exhibit intracellular BNCR using CR-39. In accordance with the previous report [3], colony assays will be planned.

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CO7-35 Development of immune function targeted boron neutron capture therapy using novel nanoparticles

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INTRODUCTION: Recent studies have shown that irradiation of tumors with X-rays has various tumor immunostimulatory effects, and this is a new aspect of the antitumor effects of radiation therapy. Boron neutron capture therapy (BNCT) is a type of radiation therapy that uses the interaction of neutrons and boron nuclei (^{10}B) to emit heavy particles with high cell-killing effects at a micro-level range [1]. In this study, we aim to develop boron neutron capture therapy targeting immune functions by creating boron-containing nanoparticles that target immune functions and cell populations that have negative effects on anti-tumor immunity.

EXPERIMENTS: We attempted to develop BNCT using novel nanoparticles that target immunosuppressive cells by encapsulating the boron drug BSH in liposomes and binding proteins targeting immunosuppressive cells to their outer surface. Ligands were bound to BSH-encapsulated liposomes targeting CD25 and CD11b. The prepared boron drug was cultured with suppressor T cells and myeloid-derived suppressor cells (MDSCs), and the uptake capacity of the boron drug was evaluated using ICP-AES.

RESULTS: Contrary to expectations, the boron drug created did not accumulate on suppressive T cells and MDSCs.

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CO7-36 Basic Experiment of Boron Neutron Capture Therapy(BNCT) for a Rat Model of Malignant Spinal Cord Glioma

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INTRODUCTION

Boron neutron capture therapy (BNCT) is a particle irradiation which can destroy tumor cells with selectivity. Owing to this merit, BNCT has been expected to be adjuvant therapy for invasive or unresectable cancers. Actually, BNCT using BPA has been covered by insurance for head and neck cancer since 2020. At our institution, we have clinically applied BNCT since 2002 as an experimental adjuvant therapy for intracranial malignant gliomas using nuclear reactor as a neutron source.

Intramedullary spinal cord tumors (IMSCT) are rare neoplasms, accounting for 4% of central nervous system tumors, and for 15% of all primary spinal cord tumors. High-grade spinal cord gliomas make up 10% of them [1]. Due to such rarity, optimal management strategy remains controversial despite its poor prognosis. Imitating management of intracranial gliomas, intervention for IMSCT actually include surgical resection, radiation therapy, and chemotherapy. However, it is often difficult to obtain gross total removal for high-grade spinal cord glioma, and sometimes surgical compilations are inevitable. Furthermore, in previous reports, extent of removal makes no statistical difference in prognosis [2]. Similarly, adjuvant therapy is not estimated to prolong survival [3,4].

The objective is exploring the possibility that BNCT have antitumor effect against malignant spinal cord glioma, which is often unresectable and resistant to conventional adjuvant therapy.

MATERIAL AND METHOD

Experimental design

Twelve Fisher 344 rats received intramedullary injection containing 100,000 F98 rat glioma cells in 5µl Dulbecco's Modified Eagle Medium (DMEM). They were randomized into following 3 groups.

<Group1> untreated control group (n=4)
<Group2> neutron irradiation only (n=2)
<Group3> neutron irradiation 2 hours after BPA administration by intravenous injection (n=6)

Animals in group 3 received intravenous injection of BPA with dose of 250mg/kg b.w. (12mg B/kg) 2 hours before irradiation. A week after inoculation of F98 cells into a rat spinal cord, rats of group2 and group3 were irradiated at reactor power of 5 MW for twenty minutes. After irradiation, the animals were observed in KURNS. Euthanasia was performed when Basso, Bresnahan and Beattie (BBB) score (functional testing scale of hindlimb) was less than or equal to 5, or severe bladder and rectal obstruction was observed. The survival time since tumor cell implantation was calculated

Production of spinal cord tumor model

Animals were anesthetized with intraperitoneal injection of following anesthetics: medetomidine (ZENOAQ, Fukushima, Japan) (0.4mg/kg), midazolam (SANDOZ, Yamagata, Japan) (2.0mg/kg), and butorphanol (Meiji Seika, Tokyo, Japan) (5.0mg/kg). Placed prone on sterile field, their backs were sterilized and shaved. Longitudinal skin incision was made on the level of Th8 to Th10. The underlying paravertebral muscles were retracted, spinous process and lamina of Th9 was removed, and finally spinal cord was revealed. Cell implantation was performed at the median of bare spinal cord with a 28gauge Hamilton syringe. The needle was proceeded to a depth of 3mm from the dorsal surface of the spinal cord. Wounds were sutured with 3-0 silk braided.

RESULTS

Median survival of each group (untreated, irradiation only, irradiation after BPA i.v) was 15.8 ± 0.5 days, 18.5 ± 0.5 days, and 22.5 ± 2.0 days. There was significant difference between survival time of group 1 and group 3 ($p = 0.0071$, log-rank test). But, comparison between group 1 and group 2, group 2 vs group 3 shows no statistical difference ($p = 0.0439$, $p = 0.1489$ respectively).

DISCUSSION

This experiment resulted in a significant difference in survival time between the untreated group and BNCT group. None of the irradiated rats improved their BBB scores and eventually died, suggesting that therapeutic effect of BNCT was limited to retard the progression of tumor.

Although the irradiation and boron compound contribution followed previous experimental protocol for the rat brain tumor model, we consider it is necessary to optimize the experimental method for the spinal cord tumor model. Future assignment is unrevealing biodistribution of boron especially in normal spinal cord and spinal cord tumors to determine optimal conditions for boron compound administration. Besides, evaluation of the histopathology is indispensable to assess the antitumor effect and the presence of radiation necrosis.

CONCLUSION

It was suggested that BNCT is effective treatment for malignant spinal cord glioma.

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