CO7-1

Development of Albumin Nano Particles Loaded with Gd(III)-Thiacalixarene Complex for Gd-NCT

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INTRODUCTION: Owing to a large thermal neutron capture cross section, gadolinium attracts growing attention as an alternative to boron in NCT [1]. Because free gadolinium ($Gd(OH_2)_9^{3+}$) has toxicity, a safe carrier of Gd to tumor not to release free Gd is required. We recently found that thiacalix[4]arene-*p*-tetrasulfonate (TCAS) self-assembled three Gd ions to form a sandwich-type complex, Gd_3TCAS_2 (Fig. 1) [2], the characteristic features of which is high kinetic stability and ^1H relaxation arising from the Gd center [3]. Nano-sized particles are frequently used as a drug carrier toward tumor by enhanced permeability and retention effect. We have so far studied nano-carriers for Gd_3TCAS_2 such as silica nano-particle (NP) [4] and albumin NP (ANP) [5–7] aiming at Gd-NCT. This FY, we compared the ability of three types of ANP installed with Gd_3TCAS_2 at the surface (shell type), inside (core type), and both at the surface and core of ANP (core-shell type) to deliver Gd into cell and NCT effect.

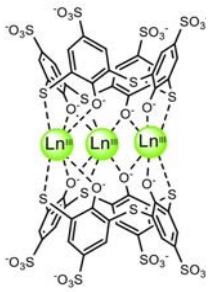

Fig. 1 Ln_3TCAS_2 complex.

EXPERIMENTS: *Preparation of ANPs.* We followed the procedure of previous reports [5–7]. *Cell experiment.* MCF-7 cells were seeded in a 6-well plate at a cell concentration of 1.0×10^5 cells/mL and incubated for 24 h. After supernatant was removed, RPMI medium and solution containing Gd in a form of shell, core, and core-shell ANPs, free Gd_3TCAS_2, 4) Gd-DTPA, 5) PBS (as control) were added to each well and incubated for 24 hr. The concentration of Gd in the medium to incubate MCF-7 was set to be 50 μM. After washing with PBS, the cells were detached from the well and transferred to tubes to be irradiated with thermal neutron for 20 min. The cell viability was assessed by the colony assay [7].

RESULTS: The largest amount of Gd delivered to MCF-7 cells was found with core-shell ANP (1.33 ± 0.06 nmol/10^6 cells), owing to the loading capacity of Gd_3TCAS_2 on ANP (8.13%) larger than that of shell (1.10%) and core (1.44%). The cytotoxicity of core-shell ANP was not observed up to 100 μM. Cell viability after neutron irradiation suggests that the largest NCT effect was obtained with free Gd_3TCAS_2 (Fig. 2). Among ANPs, core-shell type showed meaningful NCT effect, indicating that it can be a promising candidate for Gd agent in NCT.

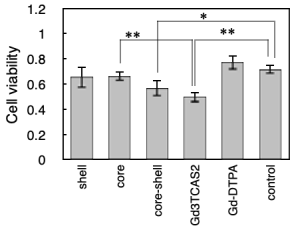

Fig. 2 Cell viability of MCF-7 cells incubated in the presence of Gd agents followed by washing out of free Gd agents and irradiation with thermal neutron. Incubation: 50 μM Gd for 24 h. Neutron fluence: 1.1×10^{12} thermal neutrons cm^{-2}, 1.9×10^{11} epithermal neutrons cm^{-2}.

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CO7-2

Development of Theranostic Agents for Neutron Capture Therapy (NCT) and Its Companion Diagnostics

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INTRODUCTION: Boron neutron capture therapy (BNCT) using ^{10}B-labeled agents with companion diagnostics is promising for cancer theranostics. If BNCT and companion positron emission tomography (PET) imaging can be performed with compounds with the same structure, it will be possible to predict the therapeutic and side effects of the compound for BNCT with higher accuracy. Recently, our research group developed a probe, *closo*-dodecaborate-(Ga-DOTA)- c(RGDfK) (**1**) (Fig. 1), containing *closo*-dodecaborate ($[B_{12}H_{12}]^{2-}$) as a boron cluster, [$^{67/68}$Ga]Ga-DOTA as a stable $^{67/68}$Ga complex for single photon emission computed tomography (SPECT) or PET imaging, and an arginine–glycine–aspartic acid (RGD) peptide targeting $\alpha_v\beta_3$ integrin expressed on the cancer cell membrane [1].

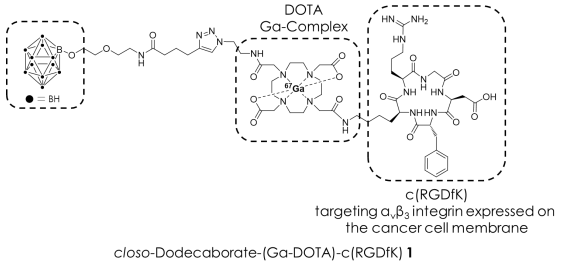

Fig 1. Chemical structure of *closo*-dodecaborate-[^{67}Ga](Ga-DOTA)-c(RGDfK) ([^{67}Ga]**1**).

[^{67}Ga]**1** showed high accumulation in tumors and low accumulation in non-target tissues. However, the synthetic method of **1** resulted in low yields and the radiolabeling time exceeding 6 h. Consequently, its application to ^{68}Ga ($T_{1/2}$: 68 min) was not feasible.
In this study, [^{10}B]**2** with a slightly modified synthetic method and structure was synthesized. Moreover, the precursor for **2** could also coordinate with gadolinium, which is expected to be useful as an element for neutron capture therapy, to from a heptacoordinate complex. Therefore, [^{10}B]**3** with two beneficial isotopes, ^{10}B and Gd, for NCT was also synthesized and evaluated.

EXPERIMENTS: [^{10}B]BPA (192 μM), [^{10}B]**3** (16 μM), and a mixture of [^{10}B]BPA (192 μM) and **3** (16 μM) were dissolved in FBS-free EMEM medium. In a microtube, U-87MG cells were suspended in the prepared medium and shaken for 3 h. After removing drug-containing medium, the amount of boron was measured using ICP-MS. [^{10}B]BPA (192 μM), [^{10}B]**2** (16 μM) and [^{10}B]**3** (16 μM) were dissolved in FBS-free EMEM medium. In a microtube, U-87MG cells were suspended in the prepared medium and shaken for 3 h. Neutron irradiation (1 MW) was performed for 30 min. The irradiated cells were incubated for 24 h. After adding WST-8 and incubating for 90 min, the absorbance at 450 nm was measured using a plate reader.

RESULTS: By combining a *closo*-dodecaborate derivative with an NHS ester structure and DOTA-Lys-RGD with an amino group, a precursor was obtained that is capable of metal coordination in the final step. Compound **2** was obtained with a yield of 6.6% (6 steps). Compound **3** was obtained with a yield of 4.8% (6 steps). In the cell uptake study, the amount of uptake of a mixture of [^{10}B]BPA and **3** was almost the same as the sum of uptake of [^{10}B]BPA and [^{10}B]**3**. In the neutron irradiation experiment in vitro, [^{10}B]BPA, [^{10}B]**2**, and [^{10}B]**3** showed significantly lower cell viability than the control group. These results suggest that [^{10}B]**2** and [^{10}B]**3** would be useful as theranostic drugs for NCT.

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CO7-3

Optimization of polymer-BPA conjugates for non-clinical studies

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INTRODUCTION: Boronophenylalanine (BPA) has been the most powerful drug in boron neutron capture therapy (BNCT). BPA can selectively accumulate within tumors through LAT1 [1]. Meanwhile, BPA is sometimes exchanged with extracellular amino acids including tyrosine due to the antiport mechanism of LAT1 and shows short-term retention in a target tumor, compromising therapeutic effect [2]. In this regard, we found that poly(vinyl alcohol) (PVA) can form complexes with BPA through boronate esters in aqueous solution and that the PVA-BPA complex can be internalized within tumor cells via LAT1-mediated endocytosis, prolonging the retention time of BPA [3].

In this study, we prepared various compositions of PVA-BPA and evaluated their BNCT effects.

EXPERIMENTS: PVA-BPA complexes with various compositions were intravenously administered to the mice bearing subcutaneous BxPC-3 tumors. When intratumoral boron concentrations reached their highest values, the tumor was irradiated with epi-/thermal neutrons at 1 MW for 50 min using the Kyoto University Research Reactor (KUR).

RESULTS: While all the samples exhibited the strong antitumor effects, PVA-BPA with a specific composition showed considerably high response rate 42 days after irradiation (Fig. 1). This result is consistent with boron concentration indicated in biodistribution study (data not shown).

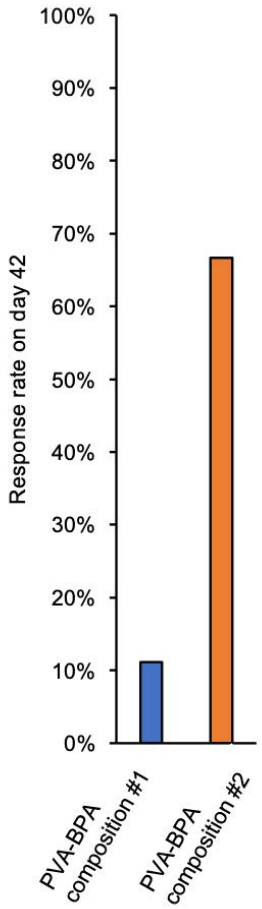

Fig. 1. BNCT effects of PVA-BPA.

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CO7-4

Development of novel boron delivery systems improving accumulation contrast

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a treatment that kills cancer cells by nuclear reaction between thermal neutrons and boron atoms (^{10}B). In a clinical condition, the boron concentration in the tumor must be 25 ppm or higher, and the boron concentration ratio between the tumor and blood (T/B ratio) and the surrounding normal organs (T/N ratio) must be 2.5 or higher to obtain an efficient therapeutic effect while keeping radiation exposure to normal tissue low. Although many studies have developed drug delivery systems (DDSs) such as polymeric micelles [1] and lip-osomes [2], these conventional DDSs should show appreciably prolonged retention in the blood-stream to increase the chance of leakage from tumor vessels into tumor tissue and the eventual tumor accumulation. Thus, simply converting conventional DDSs to BNCT does not necessarily result in a high T/B ratio, and even if high tumor accumulation is achieved, the dose of thermal neutron irradia-tion is limited and does not lead to improved therapeutic effects. Here, we developed a DDS that can actively increase the T/B ratio in a light-responsive manner.

EXPERIMENTS: The DDS was synthesized by conjugating low-molecular-weight boron drugs, which are immediately excreted by the kidney as a single agent, with the side chains of a derivative of the biocompatible poly(2-hydroxypropyl methacrylate) (PHPMA) via a photolabile linker. The accumulation of the boron drug in a tumor and various organs was investigated in a murine subcutaneous tumor model after intravenous injection. The similar experiment was conducted with photoirradiation and the result was compared with that without photoirradiation. Based on the results of the revealed pharmacokinetics, the conditions for neutron capture therapy were determined, and the BNCT effects were investigated.

RESULTS: The DDS could increase the T/B ratio in a light dose-dependent manner, thereby accomplishing significantly enhanced BNCT effects under the condition mimicking a practical situation (Fig. 1).

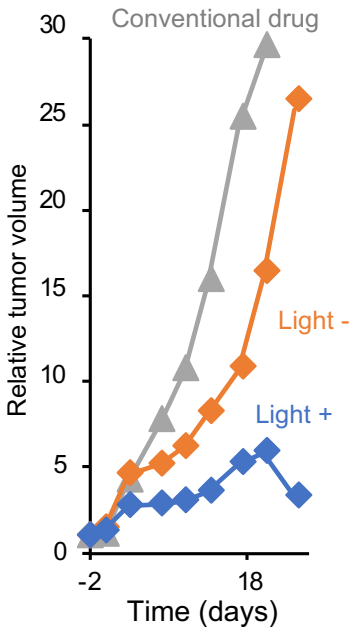

Fig. 1. BNCT effects.

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CO7-5

Elucidating the effects of boron neutron capture therapy on host immunity in mice tumor models

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a type of radiation therapy that utilizes a reaction in which boron atoms (^{10}B) capture neutrons and cause them to fission into alpha particles and lithium nuclei [1]. By selectively delivering boron atoms in the form of drugs to tumor cells, subsequent neutron irradiation can selectively induce nuclear reactions in the delivered cancer cells, resulting in the death of the cancer cells. In particular, immune cells are known to be more radiosensitive than normal cells and can be killed even by small doses of radiation [2]. On the other hand, it has also been shown that irradiation of tumor tissue releases the immune escape mechanism, which is designed to prevent tumor tissue from being attacked by the immune system, and that irradiation of tumors makes it easier for immune cells to attack tumor cells [3]. BNCT is also a type of radiation and may have some effect on the number and function of host immune cells after BNCT, but there have been only a few reports on the immune response after BNCT in detail [4]. The purpose of this study is to elucidate the effects of BNCT on host immune cells.
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EXPERIMENTS: A tumor model was created in which mouse-derived malignant melanoma cells B16 and mouse-derived squamous cell carcinoma SCCVII were transplanted subcutaneously into the lower leg skin of C57BL/6 and C3H mice, respectively. Mouse-derived malignant melanoma cells B16 and mouse-derived squamous cell carcinoma SCCVII were transplanted subcutaneously into the lower leg skin of C57BL/6 and C3H mice, respectively. Subcutaneous tumor size was assessed after treatment between BNCT or BNCT plus immunotherapy (anti-PD-1 antibody) group. Tumor tissue was removed after BNCT and BNCT + immunotherapy combination treatment, and the tumor tissue was enzymatically treated and separated into single cells. Then, flow cytometry was used to examine the percentage of T cells infiltrating the tumor and memory function.
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RESULTS: We found that the combination group of immunotherapy and BNCT showed better long-term tumor growth inhibition in the tumor curve. The BNCT group showed significantly increased expression of chemokines. Since the combination of BNCT and immunotherapy showed a long-term inhibition of tumor growth compared to the control group and the BNCT alone group, we focused on the memory function of lymphocytes, which is involved in immune memory and shows a long-term inhibition of tumor growth, and examined the percentage of CD3+ T cells with a memory function among the lymphocytes infiltrating the tumor. However, contrary to the hypothesis, no significant differences were found between the treatment groups or in comparison with the control group. Flow cytometric results showed that although lymphocytes in both the X-ray and BNCT groups decreased temporarily after irradiation, they recovered more quickly in the BNCT group than in the X-ray-irradiated control group.
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CO7-6

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

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INTRODUCTION: The purpose of this study is to explore the possibility of applying BNCT, which has been developed as a cancer therapy [1], to intractable diseases other than cancer (intractable non-tumor diseases) using mouse models, in order to further develop BNCT as a medical field and to discover potential indications of BNCT for diseases other than cancer [2]. Intraperitoneal administration of β-glucan to mice (Balb/c-derived offsprings) induces an immune response and symptoms similar to collagen disease in some mice models [3]. We aim to develop boron agents targeting the cause of inflammation based on boronated-antibodies.

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EXPERIMENTS: When 30 mg of laminarin, a β-glucan, was administered intraperitoneally to mice, swelling of bilateral wrist joints was observed from 7 days after administration, and 10-14 days after administration, redness of the wrist joints was observed, suggesting clear inflammation. No obvious diarrhea was observed in this mouse model or at the β-glucan dose.

In order to make specific boron compounds targeting the cause of inflammation, we attached boron compounds to the stationary portion (Fc portion) of a commercially available IgG antibody. The number of boron atoms was quantified by binding the FITC-bound boronated module to the isotype antibody and measuring the fluorescence intensity per antibody molecule.

A new boron drug targeting the IL17 receptor was prepared by the above method, and its therapeutic effect was examined by comparing it with that of the neutron alone irradiation group, the L-BPA administration group, and the boron drug targeting the immune response group.

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RESULTS: After binding a boronated module to a commercially available anti-mouse IL17 receptor and administering 200 μg to a mouse model of arthritis created by intraperitoneal administration of β-glucan, the therapeutic effect on arthritis was observed by irradiating bilateral wrist joints with neutrons. Mice with arthritis were prepared under the same conditions, and the same neutron irradiation was performed on the following two control groups; control group 1: Neutron irradiated without boronated antibody, control group 2: Neutron irradiated with boronated derivatives of amino acids, which are commonly used as boron drugs for BNCT. No obvious improvement in arthritis was observed when evaluated by scoring over time after neutron irradiation.

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CO7-7

Development of Fluorescent Dodecaborate Conjugated anti-EGFR Antibody as Theranostic Type Boron Carrier for BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) has been recognized as an essential treatment for refractory cancers such as glioma, head and neck cancer, and melanoma in recent years. 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) and mercaptododecaborate (BSH), 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. BSH, a class of water-soluble anionic boron cluster compounds with low toxicity, is clinically used as boron carrier for the treatment of brain tumors. However, tumor selectivity and cell membrane permeability of BSH is slightly low. In the course of our developing studies on new boron carrier for BNCT, we have designed and synthesized thiododecaborate ($[B_{12}H_{11}S]^{2-}$) unit-containing tumor seeking compounds such as amino acids, peptides and antibodies [1-3]. In BNCT, development of theranostic type boron carrier is highly noted, because visualization of the boron distribution and determination of tumor/normal ratio by non or minimally invasive examination are very important for the planning of BNCT. To develop the theranostic type boron carrier for BNCT, we have been developed bifunctional boron cluster containing compounds which linked Near-infrared (NIR) fluorescent dye (Cy5.5) and alkyl linker (Cy-BSH-OSu) to conjugate with tumor seeking compounds. In this paper, we report the conjugation of novel NIR fluorescent boron cluster with antibody, and the biological evaluation of boronated antibody as boron carrier for BNCT.

RESULTS and Discussion: In this study, anti-EGFR Mab Cetuximab was chosen as tumor target moiety. The synthetic route of bifunctional boron cluster containing compounds was illustrated in Fig. 1. The S-alkylation of cyanoethyl BSH (**1**) with 6-bromohexanoic acid was achieved by previously reported method in good yield. After deprotection of S-cyanoethyl group, the brominated Cy5.5 was treated to give Cy-BSH-OH followed by treatment with an ion-exchange resin. The succineimide ester type compound (Cy-BSH-OSu) was prepared by the reaction of Cy-BSH-OH with di(N-succinimidyl)carbonate (DSC). The conjugation of Cy-BSH-OSu boron compounds with Cetuximab were proceeded in 0.1 M Na_2HPO_4 to give Cy-BSH-Cetuximab (The number of Cy-BSH per antibody was about 3). In the next step, we evaluated the Cy-BSH-Cetuximab as boron carrier using EGFR high expressed tumor cell (A431 human carcinoma). Cy-BSH-Cetuximab was delivered boron atom to A431 cell, and cotreatment with EGF induce macropinocytosis enhanced the cellular uptake of Cy5.5-DB-Cetuximab. Further evaluation of boronated compounds as boron carrier for BNCT and is now under investigation.

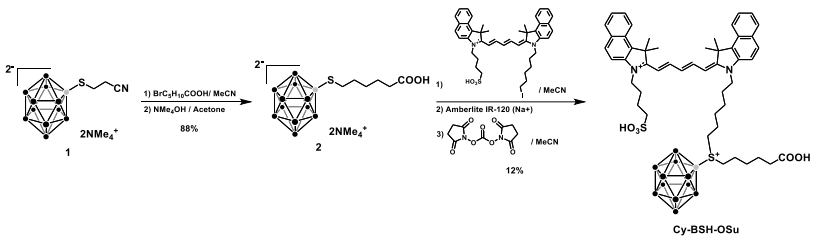

Fig 1. Synthesis of Cy-BSH-OSu.

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CO7-8

HER-2 targeted boron delivery system using the complex of β-1,3-glucan-boron nitride nanotube complex

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INTRODUCTION: With minimal invasiveness, boron neutron capture therapy has been considered as one of the promising modalities to treat cancer.[1] As cell destruction can be attained in cells uptaking boron agents actively, the deliverability of boron to tumor tissue is the key to enhance therapeutic benefits of BNCT. For these points of views, various types of delivery platforms have been studied such as liposomes and nanogels. Here, boron nitride nanotubes (BNNT) have been expected as a promising candidate as a boron agent for BNCT due to their large contents in each material and their one-dimensional morphology. Despite of their fascinating properties of BNNT, the biomedical application of BNNT was limited because of their poor dispersibility in aqueous media. In this work, we demonstrate the applicability of HER-2 targeting antibody conjugated β-1,3-glucanBNNT (HER-2-GL/BNNT) complex as a boron agent for BNCT.
EXPERIMENTS: Protein A mimicking moiety (PAM) was introduced to GL as antibody conjugate unit. The substitution degree of PAM was quantified by ^{1}H-NMR. Complexation of BNNT with PAM-GL was conducted high-speed vibration milling (HSVM) and the complex was extracted by water. The basic characterization of the complex was addressed by dynamic light scattering (DLS), transmission electron microscopy (TEM), and inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Conjugation of anti-HER-2 antibody with PAM-GL/BNNT was examined by fluorescence resonance energy transfer (FRET) using fluorescently labeled GL and antibody. BNCT activity *in vitro* was evaluated toward human ovarian cancer cells overexpressing HER-2 (SK-OV-3). Finally, tumor targeting delivery using HER-2-GL/BNNT in tumor xenograft model mice by *in vivo* imaging system and ICP-AES.
RESULTS: Synthesized PAM-GL could disperse BNNT at 540 ppm via HSVM and the hydrodynamic diameter of the complex was determined to be 80 nm (PDI, 0.16). TEM observation without staining revealed rod like structure. After complexation with antibody, FRET was observed, indicating the antibody was successfully conjugated with PAM-GL/BNNT complex. In addition, conjugation with antibody did not significantly change the hydrodynamic diameter of the complex and morphology. BNCT activity of HER-2-GL/BNNT complex toward SK-OV-3 was 29 times higher than that of L-BPA/fructose complex. To address these differences in BNCT activity, we compared cellular uptake amount of HER-2-GL/BNNT with L-BPA/fructose complex in SK-OV-3. After exposure to boron agents, we quantified the accumulated boron atom within cells using ICP-AES. As a result, HER-2-GL/BNNT enhanced cellular uptake of boron atom to SK-OV-3 cells by 12-fold. Finally, we demonstrated deliverability of the system using tumor xenograft model mice, which was established by transplantation of SK-OV-3 to nude mice, by *in vivo* imaging system. After administration of HER-2-GL/BNNT via intravenously, the complex was gradually accumulated in tumor tissue with time and the accumulation reached peaks at 6 h-post injection. Moreover, the HER-2-GL/BNNT could enhance the accumulated amount of BNNT in tumor tissue compared with GL/BNNT complex, indicating our system work as HER-2 targeting system.

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CO7-9

Evaluatiomnof in vitro tumor-killing effects of surface-modified gadolinium-loaded chitosan nanoparticles for gadolinium neutron capture therapy

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INTRODUCTION: Gadolinium neutron-capture therapy (Gd-NCT) is a cancer therapy that utilizes γ-rays and electrons emitted due to ^{157}Gd (n, γ) ^{158}Gd reactions. We have been developing gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs) to control Gd delivery in Gd-NCT. Accumulation of Gd in Gd-nanoCP treated tumors is based primarily on the bioadhesive (cationic), biocompatible (nontoxic), and biodegradable (bioerodible) properties of chitosan nanoparticles. Our previous studies demonstrated that neutron-capture reactions after intratumoral (i.t.) injection of Gd-nanoCPs in tumor-bearing mice could significantly suppress tumor growth; however, the inhomogeneous distribution of Gd-nanoCPs in tumor masses prevents complete cure. One can expect that increased dispersion stability of Gd-nanoCPs will improve the heterogeneous distribution of Gd in tumor tissues and increase the tumor-killing effect of electrons by shortening the adhesion length between Gd-nanoCPs and tumor cells. Thus, we aimed to investigate the impact of PEG modifica-tion on the *in vitro* tumor-killing effect of Gd-nanoCPs in Gd-NCT

EXPERIMENTS: Gd-nanoCP was prepared using chitosan and Gd-DTPA through the previous-ly developed w/o emulsion-droplet coalescence technique. The condensation reaction of the amino group present in intact Gd-nanoCP and NHS-activated ester-PEG prepared direct PEG-modified Gd-nanoCP (PEG-Gd-nanoCP). Mean particle size and zeta potential of the resultant Gd-nanoCPs were measured by Zetasizer® (Malvern). Gd concentration of tumor tissue was determined by in-ductively coupled plasma atomic emission spectrometry (ICP-AES, SPS3100) followed by incin-eration of each sample. Tumor-killing effect was evaluated by a cellular viability assay with SCC VII cells after thermal neutron irradiation.

RESULTS: Mean particle diameter and zeta potential of the Gd-nanoCP, and PEG-Gd-nanoCP, were 171 and 168 nm, 15 and 20 mV, respectively. No difference was observed in the average parti-cle size. Still, there was a difference in the settling tendency when left to stand, suggesting that PEG-Gd-nanoCP has higher dispersion stability. The tumor-killing effect of Gd-nanoCPs and PEG-Gd-nanoCP was increased in a concentration-dependent manner. Most notably, PEG-Gd-nanoCP exhibited a stronger tumor-killing effect than Gd-nanoCP at the same Gd dose. This tumor-killing effect could be ascribed to the higher association between Gd-nanoCPs and tu-mor cells, improved distribution of Gd in cells exposed to PEG-Gd-nanoCP, and increased influ-ences due to Auger and Coster-Kronig electrons, which have shorter path lengths and more vital tumor-killing ability than do γ-rays. Therefore, improving the dispersion stability of Gd prepara-tions within the tumor cells is crucial for achieving uniform Gd distribution. The improvement in dispersion stability with surface modification possibly led to the homogenization of Gd distribution because the particle sizes of the two Gd formulations did not differ significantly.

CO7-10

The effect of boron neutron capture therapy (BNCT) to gastrointestinal stromal tumor(GIST)

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INTRODUCTION: Gastrointestinal stromal tumors (GIST) are the most frequent soft-tissue sarcomas substantially arising from the gastrointestinal tract. Surgery is the first choice of treatment for primary GISTs. However, local recurrence or metastasis still occurs in 10% to 50% of patients after curative resection. Imatinib mesylate (imatinib) classified in a tyrosine kinase inhibitors is the primary agent of choice used to treat GISTs. On the other hand, drug resistance to imatinib poses a major obstacle to treatment efficacy.
In this study, we investigated the effectiveness of
boron neutron capture therapy (BNCT) to GIST-T1 and imatinib-resistant GIST-T1(GIST-T1/IM-R) using the mouse model.
****

EXPERIMENTS: We used Boronophenylalanine (BPA) as a boron compound. In vitro study, Cytotoxicity by BNCT with BPA was evaluated by colony forming assay. GIST-T1 and GIST-T1/IM-R cells were incubated with 20 μg B/mL BPA and boron-free medium for 1h at 37 ◦C in 5% atmospheric CO2. In vivo study, GIST-T1 cells were concentrated to 2.0×10^7/100μL in 0.1ml of PBS and injected into the right leg of each mouse. Animals were divided into three groups (5-7 animals per group); the cold control (no treatment, no neutron irradiation), hot control (neutron irradiation only), and BNCT (intraperitoneal BPA administration and neutron irradiation) groups.
****

RESULTS: In vitro study, **Fig.1** showed the results of the neutron irradiation for GIST-T1 and GIST-T1/IM-R cells. The Survival Fractions of GIST-T1 and GIST-T1 (GIST-T1/IM-R) cells decreased as the neutron fluence increased. In vivo study, **Fig.2** showed that tumor volume (mm^3) significantly reduced in the BNCT group compared with the cold and hot control groups. All mice did not have symptoms such as diarrhea and survived until the endpoint. Remarkable weight loss was not observed in three groups at the endpoint. (**Fig.3)**
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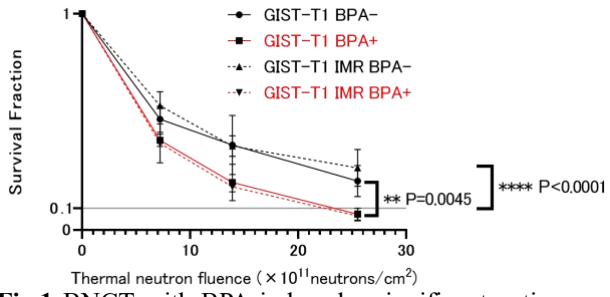

Fig.1 BNCT with BPA induced a significant anti-cancer effect in both GIST-T1 and GIST-T1 IMR compared to control.(****P<0.0001, **P=0.0045)

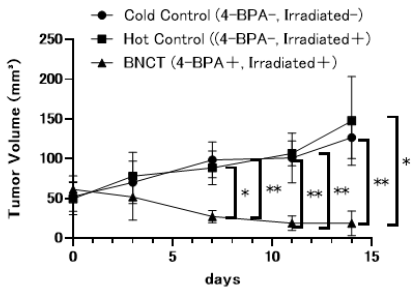

Fig.2 BNCT with BPA induced a significant anti-cancer effect compared to Cold and Hot control in GIST bearing mice. (*P<0.05, **P<0.01)

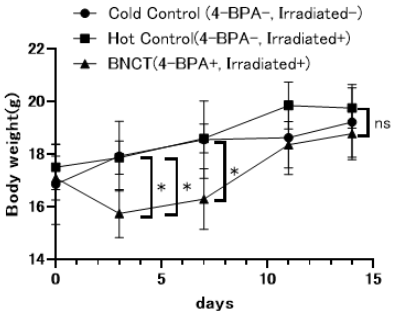

Fig.3 Body weight loss were observed a week after irradiation in BNCT group. There were not a significant difference in body weight two weeks after irradiation. (*P<0.05)

Ongoing study: We are making a mouse model of GIST-T1/IM-R, planning to investigate effect of BNCT. Also, we intend to clarify the mechanism of the antitumor effect using HE and TUNEL staining.
We will continue this study and the results will be published in the future.

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(doi) 10.1371/journal.pone.0249650

CO7-11

Evaluating the Efficacy and Safety of Boron Neutron Capture Therapy in Treating Metastatic Spinal Tumors

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INTRODUCTION: Metastatic spinal tumors have been treated with multidisciplinary interventions such as surgical resection, fixation, and radiation therapy. Despite the advancements in radiation therapy techniques, it remains challenging to achieve better quality of life and activity of daily living. This study aims to evaluate the efficacy and safety of boron neutron capture therapy (BNCT) for the treatment of metastatic spinal tumor using a mouse model. Through this investigation, we aim to contribute to the evolving landscape of therapeutic strategies, potentially offering an innovative alternative that could enhance clinical outcomes for patients afflicted with this condition.
****

EXPERIMENTS: ***In vitro:*** A549 human lung adenocarcinoma cells were exposed to neutron irradiation at 1 MW reactor power for 0-30 minutes and photon irradiation at doses of 0-8 Gy, aiming to assess their biological impact on the cell lines. For neutron irradiation, cells were exposed to 4-borono-L-phenylalanine (Boronophenylalanine; BPA) at a concentration of 10 μg Boron/mL for 24 hours before irradiation. The biological effects were evaluated using colony-forming assay to confirm the effect on cellular viability and proliferative capacity. ***In vivo:*** For the in vivo assessment, a mouse model was developed using metastatic spinal tumors derived from A549 cells. The mice bearing these tumors were randomly assigned into three groups: a control group receiving no treatment (untreated; n = 10), a group subjected to neutron irradiation alone (neutron only; n = 9), and a BNCT group (BNCT; n = 10. The BNCT group received an intravenous injection of BPA at a dose of 250mg/kg, followed by neutron irradiation 2.5 h later. Overall survival and neurological function of the hindlimb, and any adverse events were assessed post-irradiation.
****

RESULTS: ***In vitro:*** Neutron irradiation, particular with BPA, revealed a markedly enhanced cell-killing effect in comparison to photon irradiation. ***In vivo:*** In the in vivo assessment involving a metastatic spinal tumor mouse model, the BNCT group demonstrated significantly prolonged survival in comparison to both the untreated group, ($p < 0.01$) and the neutron only group ($p < 0.05$) as determined by the log-rank test. Furthermore, preservation of hindlimb neurological function was significantly more pronounced in the BNCT group relative to the untreated ($p = 0.0004$) and neutron only groups ($p = 0.0051$), based on multivariate analysis of variance. Notably, an assessment of adverse events across the three study groups showed no significant occurrences, indicating a favorable safety profile of BNCT treatment in this experimental setting.
****

These findings emphasize the potential of BNCT not only to prolong survival but also to preserve quality of life by preserving neurological function without inducing adverse effects. These results therefore advocate further exploration and development of BNCT as a feasible and promising treatment option for patients with metastatic spinal tumors.

CO7-12

ASCT2-Targeted Boron Agent Enhances BNCT Efficacy in Glioma Treatment

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a cutting-edge particle irradiation technique that utilizes the nuclear reaction triggered by the irradiation of non-radioactive boron-10 with thermal neutrons, selectively annihilating tumor cells that have incorporated boron. Although BNCT has demonstrated efficacy in the treatment of malignant gliomas, the reliance on Boronophenylalanine (BPA) for boron delivery has exposed certain limitations, including resistance observed in specific cell types, tissues, and varieties of cancer. This resistance highlights the necessity for innovative strategies to enhance BNCT's clinical efficacy and applicability. This study pivots towards the Alanine-serine-cysteine transporter 2 (ASCT2) - a transporter distinct from LAT1-investigating its potential as a gateway for the development of innovative boron carriers, with the aim of expanding the clinical applicability and effectiveness of BNCT.
****

EXPERIMENTS: This research embarked on in vitro investigations to evaluate boron accumulation in F98 and C6 rat glioma cells, and 9L rat gliosarcoma cells, following 24 hours of exposure to BPA and ASCT2-targetting new compound (GluB-2), each at a concentration of 10 μg B/mL. Complementary in vivo studies involved the intravenous administration of these compounds to an F98 rat brain tumor model, with the subsequent measurement of boron distribution within the tissues at intervals of 2.5, 6, and 24 hours post-administration. The therapeutic efficacy of these treatments was then evaluated through neutron irradiation experiments. The outcomes of these interventions were measured in terms of the survival periods of the treated subjects, offering valuable data on the viability and effectiveness of the investigated boron carriers in extending the life span of animals afflicted with glioma.
****

RESULTS: In vitro experiments revealed that GluB-2 achieved a significantly higher cellular boron concentration in F98 cells compared to BPA ($p=0.02$). In vivo, the peak of boron distribution in tumors occurred at 2.5 hours post-BPA treatment and at 6 hours post-GluB-2 treatment, with GluB-2 showing a superior maximum intratumor boron concentration. Survival analysis revealed mean survival periods of 28.0±2.5 days for the neutron-only group, 37.7±5.0 days for the BPA IV + BNCT group at 2.5 hours, 55.1±19.9 days for the GluB-2 IV + BNCT group at 6 hours, and 25.3±1.4 days for the untreated group. Remarkably, the GluB-2 IV + BNCT group demonstrated significantly prolonged survival compared to the BPA IV + BNCT group ($p<0.001$, log-rank test). These findings highlight the potential of GluB-2 as an effective boron carrier for enhancing BNCT efficacy in glioma treatment.
****

These results suggest that GluB-2 holds promise as a superior boron carrier for enhancing the efficacy of BNCT in treating glioma.

CO7-13

Basic research on new BNCT strategies for melanoma

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INTRODUCTION: The starting point for melanin synthesis in vivo is aromatic amino acids such as phenylalanine and tyrosine, and melanin synthesis is enhanced in cutaneous malignancies such as malignant melanoma. Boron-phenylalanine (BPA), a boron atom bonded to these amino acids, was created as a melanoma-targeted boron drug, and the success of BPA-BNCT was demonstrated in the 1989 Lancet article by Dr Mishima et al: "Treatment of malignant melanoma by single Malignant melanoma by single thermal neutron capture therapy with melanoma-seeking 10B-compound", published by Dr Mishima et al in the Lancet in 1989 [1]. This clinical study paved the way for the effectiveness of BNCT with boron drugs with cell-specific uptake and neutron irradiation of the cancer tissue area.

Melanoma is a cutaneous malignancy with an incidence of 1-2 per 100 000 people and is considered a rare cancer. In Australia, it is a disease with regional and racial variation, with an incidence of around 35 per 100 000 people. Surgery is the standard treatment of first choice for localized melanoma, and the prognosis is very good for Stage I melanomas, which have a low likelihood of spreading 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 usefulness of BNCT for localized melanoma is that there is no pain or functional impairment associated with surgery, which makes BNCT highly useful for melanoma patients, many of whom are elderly.

Clinical trials with BPA-BNCT for cutaneous malignancies (melanoma, angiosarcoma) are currently underway at the National Cancer Centre, and the results of the efficacy in these trials are expected.

While the efficacy of mainly surgical therapies in localized melanoma has been established, the development of treatments in inoperable advanced-stage melanoma is setting a new direction for malignancies as a whole. In particular, the discovery of immune checkpoint inhibitors targeting PD-1 by Professor Honjo Tusk and colleagues, who were awarded the 2018 Nobel Prize in Physiology or Medicine, has led to new breakthroughs in cancer treatment. Immune cells in our bodies are quick to attack foreign invaders such as viruses. On the other hand, they are unable to attack cancer cells that originate from our own cells because they are our own cells, even if they multiply in the body. Dr Honjo and colleagues have shown that the reason for the immune cells' inability to recognize antigens on cancer cells is due to the binding of an immune checkpoint molecule called PD-1 on the surface of cytotoxic T cells.

****

EXPERIMENTS: We purchased the B16-F10 mouse melanoma cell line to create a melanoma model, B16-F10 has high melanin synthesis capacity and has a large amount of black mel-anin pigment even in cultured cells. We used melanoma model mice transplanted with B16-F10 into C57BL/6 mice and Balb/c nu/nu mice. Animal experiments were performed after strict approval by the ethics committees for animal experiments at Okayama University and Kyoto University.

Experiments were conducted targeting CD8-positive T cells to verify the immune effects of cytotoxic T cells.**********

RESULTS: We used BPA as an effective boron agent for melanoma and confirmed the anti-tumor effect of neutron irradiation. The results suggest that anti-tumor immunity is influenced by the function of CD8-positive T cells. 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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CO7-14

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. 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 the present study, we planned to develop a boron drug for pancreatic cancer, a small molecule compound that does not utilize the EPR effect and targets glucose transporters. We focused on the tumor marker carbohydrate antigen CA19-9, called carbohydrate antigen 19-9, in the classification of pancreatic cancer. It has a high positive predictive value for cancer and is known to be elevated in uterine, ovarian and lung cancers, etc. Pancreatic cancers with high CA19-9 levels are known to have a poor prognosis and a CA19-9 high human pancreatic cancer cell line and CA19-9 low pancreatic cancer cells were used in the present experiment. Experiments were conducted with three different boron agents: glucose boron, BPA and BSH.

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RESULTS: The glucose boron drug was found to be efficiently introduced into cells via glucose transporters (GLUTs), which are highly expressed in pancreatic cancer. The anti-tumor effect of a novel drug agent in a mouse model of pancreatic cancer was confirmed in vitro and in vivo by neutron irradiation in a nuclear reactor, CA19-9, at the Institute for Complex Nuclear Science, Kyoto University, where a high therapeutic effect was obtained in a pre-experiment. The results are further developed and reported as a novel boron drug for targeting pancreatic cancer with high CA19-9 levels.

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CO7-15

Evaluation of A Retinoid X Receptor-binding BSH Derivative for Inhibition of Cell Proliferation under Neutron Irradiation

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INTRODUCTION: While boron compounds that target nuclear receptors have been reported, there is no information regarding the use of these compounds for neutron irradiation. The substances identified as these molecules contain carboranes, which are boron clusters (Ref. 1). The authors chose to create more hydrophilic boron compounds because they believed that although carboranes' high lipid solubility enhances intracellular storage, it may also cause cytotoxicity and reduce water solubility. Through synthesizing several compounds that bind to the retinoid X receptor (RXR), the authors discovered that CBTF-EE-BODIP (**1a**), in combination with the fluorescent cluster BODIPY that contains boron, had RXR-binding characteristics (Ref. 2). Thus, compound **1b** was made by substituting BSH for the BODIPY moiety of **1a**. Its intracellular concentration was then determined, and colony experiments using neutron irradiation and medication therapy were carried out on the RXR-expressing human breast cancer cell line MCF-7.

EXPERIMENTS: Chemical compounds were synthesized by the authors. MCF-7 cell line was obtained from RIKEN BioResource. A maximum exposure concentration of **1b** was set at 100 μM and a 24-hour exposure period. Following chemical exposure, cells were harvested, trypsin-EDTA treated, and washed with PBS. All of the resultant cells were lysed using RIPA buffer, and ICP-MS (Agilent 7900/MassHunter) was used to measure the content of ^{10}B in the resulting samples. For neutron irradiation experiment, the cells treated were then centrifuged in a 15 mL centrifuge tube, aspirated off, and 2 mL of media was added to count the number of cells. After transferring one milliliter of the cell suspension into 1.5 mL Eppendorf tubes, the samples were exposed to 1.2×10^{12} cm^{-2} (5 MW, 2 min) of thermal neutron fluence. After that, cells were sown at a density of 1×10^3 cells/well in 12-well plates and cultivated for 7 days at 37°C with 5% CO_2. Following the medium removal, cells were stained with crystal violet, fixed in 80% EtOH, and the number of colonies was counted. To calculate the colony formation rate, the total number of colonies was divided by the total number of implanted cells. Also, WST-8 experiment was performed according to supplier's protocol, after adding 10 μL of WST-8 reagent to each well, cells were incubated at 37°C for 2 hours and absorbance at 450 nm was measured with a plate reader.

RESULTS: **1b** gave an intracellular boron content of *ca* 1 fmol/cell. This is equivalent to the macrocyclic polyamine compounds reported by Ueda et al. (Ref. 3), albeit being less than borofaran. It was therefore anticipated that this would prohibit cell proliferation when exposed to neutron radiation. WST-8 and colony assays were conducted on cells that had been exposed to compound **1b** for 24 hours at a final concentration of 100 μM. The cells were then exposed to neutron irradiation. The outcomes demonstrated that compound **1b** clearly inhibited cell proliferation. These results shed light on the potential utility of boron compounds that bind to nuclear receptors as boron delivery carriers for BNCT and indicate that **1b** may be employed as an intracellular 10B carrier.

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CO7-16

Intracellular Uptake of BSH in Combination with An Existing Drug and Effects of Neutron Irradiation

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INTRODUCTION: A cancer treatment called boron neutron capture therapy (BNCT) uses the nuclear reaction that occurs between neutrons and boron-10 (^{10}B). The amount of boron present in the cancer tissue is crucial since the treatment's efficacy is dependent on the collision of neutrons and ^{10}B. Two of the substances that have undergone clinical testing are BSH, a boron cluster, and borofaran, a phenylalanine derivative. Borofaran is authorized for use in medicine, and its absorption into cancer cells is facilitated by the amino acid transporter LAT-1. Tissues with low LAT-1 expression induce low borofaran delivery. BSH, on the other hand, is an anionic substance with incredibly limited cell membrane permeability. Thus, the goal of this study was to provide a novel approach.

BSH is an anionic material with extremely low cell membrane permeability. Michiue et al. reported employing the surfactant peptide A6K to deliver BSH intracellularly as a remedy (Ref. 1). The purpose of this study was to see whether any other medications, aside than A6K, might be utilized to enhance BSH's intracellular delivery. For intellectual property purposes, this study's result, which is an existing medication that increases BSH's intracellular delivery capacity, shall be referred to as drug A.

EXPERIMENTS: Using mouse B16BL6 cells, the intracellular delivery of BSH was investigated: After cultivating B16BL6 cells in six-well plates, the cells were exposed to the test compound-containing media for six hours. Following this, PBS rinsed, and cell lysates were made. The procedure used to identify CR-39 cells using BNCR was as follows: In 6 well plates (2 cm square), cells were cultivated, immersed in CR-39 medium, and then exposed to the test compound-containing medium for two hours. Following a PBS wash, the cells were processed to create cell lysates. Neutrons were used to irradiate the CR-39 cells. At KUR, a 20-minute exposure to 1 MW of neutron radiation resulted in a thermal neutron fluence of 2.22×10^{12} cm^{-2}. Samples were divided into four categories: BSH (500 μM) alone, BSH (500 μM) + drug A (0.002 mg/mL), BSH (500 μM) + drug A (0.02 mg/mL), and BSH (500 μM) + drug A (0.2 mg/mL).After neutron irradiation, the materials were treated with alkali according to Ref 1 and photographed using an optical microscope and processed in accordance with Ref. 2. Image J performed an analysis on the photographs.

RESULTS: Examining the intracellular translocation of BSH alone and in combination with already-approved medication A, it was shown that the latter significantly increased intracellular boron concentration as compared to a 2-hour exposure to 500 μM BSH alone. BSH alone or in conjunction with drug A was administered to B16BL6 cells seeded on CR-39 that had measurable BNCR, and the cells were exposed to neutron radiation. After irradiation, etch pits on CR-39 were measured. The findings showed that treatment with drug A raised the BNCR and, in a concentration-dependent manner, the intracellular absorption of BSH.

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CO7-17

Evaluation of Retinoid X Receptor Degradation by a RXR-Binding BSH Derivative under Neutron Irradiation

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INTRODUCTION: In order to achieve intracellular boron concentrations necessary to generate boron neutron capture reactions (BNCR), we have concentrated on developing boron agents that specifically target intracellular receptors. Among the several retinoid X receptor (RXR) binding compounds that we have so far produced, CBTF-EE-BODIP (**1a**, Fig. 1) with a fluorescent group that contains bo-ron, BODIPY, demonstrated RXR binding (Ref. 2). This time, we made **1b** (Fig. 1) by substituting BSH for the fluorescent group BODIPY moiety of **1a**, and we discovered that it inhibits cell proliferation when exposed to neutrons (R5047). Our goal in this work was to verify whether neutron irradiation cleaves RXR itself when it is attached to **1b**.

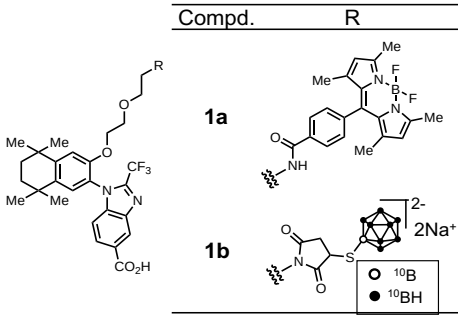

Figure 1. Chemical structures of **1a** and **1b**.

EXPERIMENTS: Chemical compounds were synthesized by us, and HPLC verified that they were at least 95% pure. RXR ligand binding domain (RXRα-LBD) was kindly provided by Prof. Nakano at the University of Shizuoka. Reporter gene tests toward RXR were conducted following Refs. 1 and 2. The radiation level of the neutron beams was 2.9×10^{13} cm^{-2}. RXRα-LBD (10 μM), **1b** (200, 100, 50 μM, converted to ^{10}B concentrations of 24, 12, 6 ppm), bexarotene (20 μM or no addition), and buffer (10 mM HEPES, 150 mM NaCl, 2 mM $MgCl_2$, 5 mM DTT, 5% DMSO) were the preparation methods for irradiated samples (100 μL). The aforesaid buffer was used to dilute the irradiated samples ten times, then they were combined with a 4:1 composition of BPB-containing buffer and electrophoresed for 70 minutes at 250 V and 20 mA. CBB staining was used to assess the RXRα-LBD concentration.

RESULTS: The binding affinity of **1b** to RXRα-LBD measured using the fluorescent RXR ligand, **1a**, was *K*i = 3 μM. When **1b** was present at the concentration ratios, neutron irradiation did not produce band splitting that would have indicated RXR cleavage; instead, a 24-hour incubation with 100 μM **1b** produced a concentration of 1 fmol/cell, and additional neutron irradiation at 1.2×10^{12} cm^{-2} inhibited cell growth. It has been discovered that there is inhibition of cell proliferation (R5047). The results of this experiment indicate that there is no cleavage of RXR in these circumstances, which may indicate that boron compounds that target nuclear receptors are effective boron delivery vehicles for BNCT.

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CO7-18

Mechanism of Glioma Resistance After BNCT via Small Extracellular Vesicles

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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 immuno-therapy. 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 locally or distantly is inevitable after BNCT. And distant recurrences, named cerebrospinal fluid (CSF) space dissemination, more frequently occur after BNCT compared with the cases after standard radiation-chemotherapy. Reasons for recurrence after BNCT have not been fully elucidated. Small extracellular vesicles (sEVs) are small membrane vesicles with sizes ranging from 50 to 150 nm. They serve as functional mediators and promote intercellular communication during physiological and pathological processes. including migration, treatment resistance, and metastasis in cancer. miRNAs are encapsulated in lipid membranes such as extracellular vesicles in blood and body fluids, exist stably, are taken up by the cells they reach, and act negatively on target genes, performing post-translational modification. In this study, we investigate miRNAs in sEVs secreted from glioblastoma cells after BNCT using microarray, which may explain the possible mechanism of local recurrence or CSF dissemination after BNCT.

EXPERIMENTS:

BPA Treatment and Neutron irradiation

Glioblastoma U87 MG cells were treated with 25 ppm of BPA in the culture media for 2 hours and irradiated with thermal neutrons in plastic tubes for 70 min.

After irradiation, they were plated into dishes and cultured for 3 days in the 5 % CO2 incubator. Then, sEVs released into the medium were collected by column chromatography, and miRNAs in sEVs were comprehensively investigated using microarrays.

RESULTS: An increase in 21 individual miRNAs (ratio>2) and a decrease in 2 individual miRNAs (ratio<0.5) were detected in BNCT cells compared to non-irradiated cells. Up-regulated miRNAs included miR-650, a prognostic marker in malignant glioma, miR-3147, which may serve an oncogenic role in vulvar squamous cell cancer, miR-4725-3p, which is known to be involved in xanthohumol, a prenylated flavonoid extracted from the hop plant Humulus lupulus L., inhibition of glioma invasion, and miR-4270, that is reported to modulate radiation sensitivity in nasopharyngeal squamous carcinoma.

CO7-19

Rational Design, Multistep Synthesis and in Vitro Evaluation of Poly (glycerol) Functionalized Nanodiamond Conjugated with Boron-10 Cluster and Active Targeting Moiety for Boron Neutron Capture Therapy

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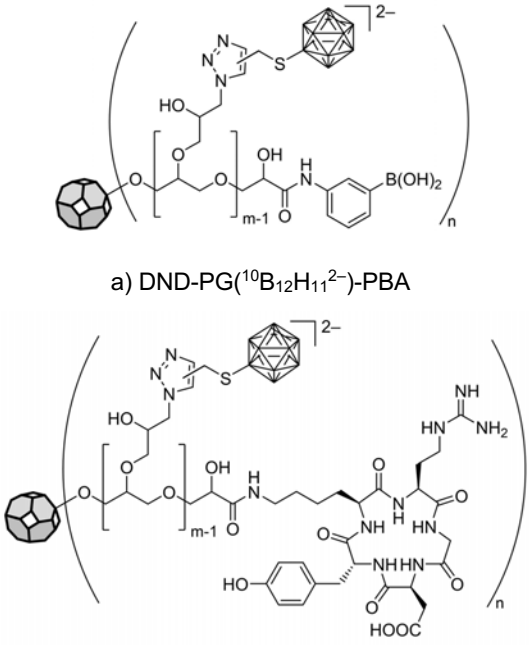
Boron neutron capture therapy (BNCT), advanced cancer treatment utilizing nuclear fission of ^{10}B atom in cancer cells, is attracting increasing attention. As ^{10}B delivery agent, sodium borocaptate (^{10}BSH, $^{10}B_{12}H_{11}SH{\cdot}2Na$), has been used in clinical studies along with L-boronophenylalanine (BPA). Recently, this boron cluster has been conjugated with lipids, polymers or nanoparticles to increase selectivity to and retentivity in tumor. In this work, we designed the anticancer nanoformulations for BNCT consisting of poly(glycerol) functionalized detonation nanodiamonds (DND-PG) as a hydrophilic nanocarrier [1], the boron cluster moiety ($^{10}B_{12}H_{11}^{2-}$) as a dense boron-10 source, and phenylboronic acid or RGD peptide as an active targeting moiety (Fig. 1) [2]. Some hydroxy groups in PG were oxidized to carboxy groups (DND-PG-COOH) to conjugate the active targeting moiety [3]. Some hydroxy groups in DND-PG-COOH were then transformed to azide to conjugate $^{10}B_{12}H_{11}^{2-}$ through click chemistry [4]. The nanodrugs were evaluated *in vitro* using B16 murine melanoma cells in terms of cell viability, BNCT efficacy and cellular uptake. As a result, $^{10}B_{12}H_{11}^{2-}$ moiety is found to facilitate cellular uptake probably due to its negative charge. Upon thermal neutron irradiation, the nanodrugs with $^{10}B_{12}H_{11}^{2-}$ moiety exhibited good anticancer efficacies with slight differences with and without targeting moiety [5].



a) DND-PG($^{10}B_{12}H_{11}^{2-}$)-PBA

b) DND-PG($^{10}B_{12}H_{11}^{2-}$)-c(RGDyK)

Fig. 1. Chemical structures of DND-PG conjugated with ^{10}B cluster ($^{10}B_{12}H_{11}^{2-}$) and phenylboronic acid (PBA) (a) or cyclic peptide (L-Arg-Gly-L-Asp-D-Tyr-L-Lys) (b).

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CO7-20

Development of Novel Small-molecule Boron Neutron Capture Therapy Drugs Targeting Tumor-specific Enzymatic Activity

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INTRODUCTION: In boron neutron capture therapy (BNCT), p-boronophenylalanine (BPA), the only approved BNCT agent, is selectively taken up by tumor cells through LAT1, which is a biomarker-transporter over-expressed in tumor cells. However, BPA faces the following challenges: 1) BPA accumulation is insufficient in some types of cancer because of poor LAT1 expression, and 2) BPA gradually leaks out of cells over time. Therefore, the development of new BNCT drugs that target another cancer biomarker and have a mechanism for prolonged intracellular retention is necessary to expand the indications for BNCT and improve therapeutic effect.
In this project, we focused on aminopeptidase activities which were proved to be higher in cancer cells than peripheral normal cells, and by utilizing these enzymatic activities we designed and synthesized EP-4OCB-FMA, a novel small-molecule carborane-containing drug candidate targeting DPP-4 activity which is known to be upregulated in esophageal cancers in human patients [1]. This drug is designed to stay inside cells for a long time by generating aza-quinone methide species by being hydrolyzed by DPP-4, which is enough nucleophilic to form a covalent bond with intracellular nucleophiles such as proteins and glutathione. Last year, we conducted BNCT experiments with tumor bearing mice by injecting EP-4OCB-FMA intratumorally, and found that tumor growth was suppressed in a drug-, enzyme-, and neutron-irradiation-dependent manner. These results indicate that EP-4OCB-FMA is a useful BNCT drug that targets tumor cell-selective enzymatic activity with an intracellular retention ability. So this year, we started to inject EP-4OCB-FMA systemically, and examined the blood half-life and accumulated boron atoms in tumors and surrounding normal tissues by LC-MS and E3.

EXPERIMENTS: EP-4OCB-FMA was then administered intravenously to obtain the blood half-life and various kinetic parameters. Specifically, EP-4OCB-FMA was injected intravenously into mice at a dose of 40 mg/kg intravenously by tail, and after intraperitoneal administration of a triad of anesthetics at a predetermined time, blood was collected from the heart. Then, protein was removed from the collected whole blood and the concentration of EP-4OCB-FMA was analyzed by LC-MS. For comparison, EP-FMA without carborane skeleton was also evaluated.

RESULTS: The blood half-life of EP-4OCB-FMA was estimated to be 0.40 h. Given that the concentration of EP-FMA without carborane was so low that the blood half-life could not be calculated, it is suggested that the blood half-life is considerably prolonged by the carborane structure. This may be due to the interaction of EP-4OCB-FMA with blood albumin via the carborane backbone, as described above. On the other hand, however, the blood half-life of 0.40 h was not sufficient, suggesting that it was lost from the blood very quickly. The poor blood retention is likely due to excretion of the drug from the liver and kidneys, and indeed high boron concentrations have been detected in urine. From these results, we started to re-design the probe molecule targeting another peptidases whose structure is based on the Protide chemistry, and completed its synthesis. Evaluation of the accumulated boron atoms with living cells are now on going, and BNCT experiments will be conducted next year.

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CO7-21

Synthesis of PEPT1-targeted boron containing dipeptides for pancreatic cancer therapy

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INTRODUCTION: Peptide transporter 1 (PEPT1) is of interest because it is expressed in various types of cancer cells. It has been reported that PEPT1 is highly expressed in pancreatic cancer cells. Earlier studies showed that boron containing dipepetides are taken up by PEPT1 transporter. However, BNCT efficacy of these dipeptides has not been investigated. We have synthesized ^{10}B containing dipeptides that have higher solubility and longer retention time in the tumor than BPA. We investigated whether these ^{10}B-dipeptides have better BNCT efficacy than BPA using KUR.

EXPERIMENTS: We synthesized three types of dipeptides. They were intravenously injected to CT26-tansplanted BALB/c mice or FaDu-transplanted nude mice. Boron contents in the tumor were investigated by ICP. We then investigated whether these reagents exhibit improved BNCT efficacy compared with BPA by neutron irradiation at KUR. They were intravenously injected into mice 2 hours before neutron irradiation (12 minutes at 5MW). After neutron irradiation, tumor volume and body weight were measured for 6 weeks (up to 42 days after irradiation).

RESULTS: The dipeptides synthesized showed higher solubility than BPA; they can be dissolved in solvents that include water. When injected into tumor-bearing mice, a significantly higher concentration of boron was detected in tumor samples compared to BPA. We also observed a longer retention time (>4 hours) in the tumor.
We then investigated BNCT efficacy of these boron compounds. They were intravenously injected into CT26-transplanted mice 2 hours before neutron irradiation. These mice were held to 12 mouse holder and placed in front of KUR, and neutron irradiation was carried out. Tumor was almost disappeared with dipeptides-injected mice and tumor regrowth was not observed up to 27 days after the irradiation. In contrast, tumor regrowth was observed in BPA-injected mice. Similar results were obtained using FaDu-transplanted mice. FaDu tumor was almost eliminated with these boron reagents. These results show that newly synthesized dipeptides provide a promising reagent for BNCT. We are in the process of preparing a paper to be submitted for publication.

CO7-22

Construction of novel boron-containing silica nanoparticles and BNCT experiments

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INTRODUCTION: We continue to develop novel boron containing nanoparticles for BNCT. We have previously developed BPA-BPMO and BSH-BPMO and have used tumor spheroids and mouse models to demonstrate their BNCT efficacy. However, it is necessary to improve their tumor accumulation capability. Tumor accumulation is influenced by various features of nanoparticles. To do this, we are systematically changing features of the nanoparticles including size and surface charge. We are also including PEG surface modification. To examine tumor accumulation, we are using mouse model systems and ICP measurements.

EXPERIMENTS: Various nanoparticles were examined for their tumor accumulation in mouse models. We are particularly interested in small size nanoparticles that have weakly positive surface charge as well as PEG. These nanoparticles will be injected intravenously into mice and tumor accumulation of the nanoparticles will be investigated by Rhodamine-B fluorescence as well as by ICP measurement of boron and silicon. CT26 as well as FaDu transplanted mouse models will be used. In addition, we will use the CAM model prior to the mouse experiments to gain insight into the tumor accumulation of the nanoparticles. For the mouse experiments, different routes of nanoparticle administration including iv, ip and sc will be tested. To achieve successful tumor accumulation by the EPR mechanism, it is necessary for the nanoparticles to have prolonged circulation time. We will test 3, 12, 24 and 36 hours after injection to test tumor accumulation. Nanoparticles will be intravenously injected to mice at 5 mg/mouse or 2 mg/mouse. We will dissect tumor as well as various organs which include liver, lung, spleen, intestine and kidney. Nanoparticle fluorescence will be detected with an inverted fluorescence microscope. Moreover, tumor and organs will be ashed with the mixture that includes perchrolic acid and hydrogen peroxide and then silica and boron contents in tumor and each organs will be examined by ICP analysis.

RESULTS: We tested nanoparticles that have less than 50 nm of diameter. SEM and TEM microscopy were used to analyze the nanoparticles. We found that these nanoparticlea are homogeneous and well dispersed.
As a preliminary test, we used the CAM model, a simple and convenient model that uses fertilized eggs. After incubation for eight days, a window was made on the egg shell and human cancer cells are placed on the CAM membrane. Tumor growth was observed in four to five days. At four days, the nanoparticles were injected intravenously and tumor accumulation of the nanoparticles was confirmed.
We then tested tumor accumulation of the nanoparticles in the CT26 transplanted mouse models. Examination of the tumor and various organs including liver, lung and kidney by fluorescent microscope showed excellent tumor accumulation. Fluorescence in the liver was observed but this was less than that found with the tumor. Maximum tumor accumulation was observed 24 hours after the injection.

CO7-23

Antitumor effect of boron neurton capture therapy in cervical cancer mouse model

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INTRODUCTION: In Japan, approximately 10,000 women develop cer-vical cancer and 2,900 women die from the disease annually. The incidence and mortality rates of cervical cancer in Japan are on the rise. Squamous cell carci-noma (SCC) is the most common histological type at 80%, and adenocarcinoma (Adeno) accounts for about 20%. Main treatment options for cervical cancer are surgery or radiation therapy. However, sometimes se-rious adverse events occur, the new treatment modali-ties for cervical cancer are needed. In the present study, we investigated the efficacy and safety of boron neutron capture therapy (BNCT) for cervical cancer in a mouse model.

EXPERIMENTS: BPA (L-isomer) was supplied by Stella pharma (Osaka, Japan) and converted to fructose complex. 4-6 week-old female nude mice (BALB/c Slc-nu/nu) were purchased from SLC, Japan. Patient-derived xenograft (PDX) was created using cervical cancer tissue (SCC/Adeno). PDX is a procedure in which a patient's tumor tissue is transplanted into immunocompromised mice, and the response to treatment has been reported to be highly consistent with the effect on the pa-tients themselves. [1] Treatment was initiated 4-6 weeks af-ter cell injection. Mice were divided into cold control (no treatment), hot control (neutron irradiation only) and BNCT (peritoneal BPA fol-lowed by neutron irra-diation) groups. 2.5 hours before neutron irradiation, BPA (250 mg/kg) was injected intraperitoneally into mice in the BNCT group. After irradiation, tumor size and mouse weight were measured, and tumor volume was calculated as follows.
V=ab2/2

RESULTS: Fig.1 shows the tumor volume of squamous cell car-cinoma PDX in the cold control, hot control and BNCT groups. The tumor was suppressed in the BNCT group than in the hot control group ($P<0.05$). The body weight was no remarkable change in the both groups. Fig.2 shows the tumor volume of adenocarcinoma PDX in the hot control and BNCT groups. The tumor volume did not differ between the both groups.

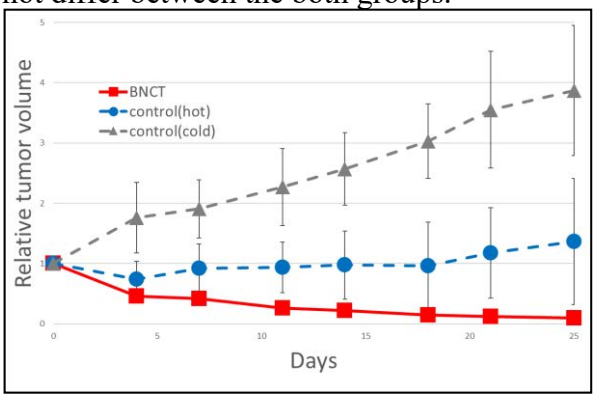

Fig.1 Antitumor effect on subcutaneous PDX (squamous cell carcinoma) tumor model.
Tumor grows curves in the cold control (no treat-ment) and the hot control (irradiation only) and BNCT (irradiation after BPA administration) groups (n=12).

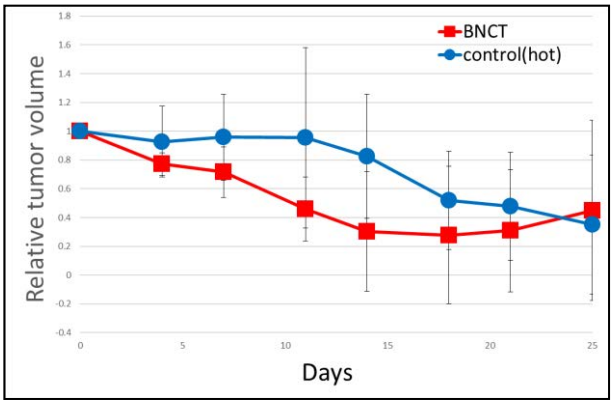

Fig.2 Antitumor effect on subcutaneous PDX (adeno carcinoma) tumor model.
Tumor grows curves in the hot control (irradiation only) and BNCT (irradiation after BPA administra-tion) groups (n=9). (n=12).

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CO7-24

Quantitative Analysis of Elements Causing Activation in Radiation Shielding Concrete Using Internal Standards

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INTRODUCTION: Concrete is widely used as radiation shield in nuclear reactors and accelerator facilities because of its flexibility, sufficient supply and low cost. On the other hand, in the cases of the reactors or high energy accelerators, once these facilities start operating, the shielding concrete becomes radioactive by nuclear reaction with neutrons generated. Under these circumstances, low-activation concrete is one of the ways to mitigate the problems such as human exposure and decontamination of the facilities. The application of low-activation concrete is particularly effective in accelerator facilities for BNCT, which uses high intensity neutrons. To estimate the level of activation, we have performed neutron activation analyses (NAA) on more than several hundred samples of shielding concrete and raw materials using KUR facilities [1]. In this report, we describe the internal standards utilized to reduce the uncertainties regarding to the experimental procedure of NAA, such as fluctuation of neutron flux and detection efficiency of a gamma-ray detector.

EXPERIMENTS: Three nuclides produced by reactions of ^{151}Eu(n,γ)^{152}Eu, ^{59}Co(n,γ)^{60}Co and ^{133}Cs(n,γ)^{134}Cs in concrete and raw materials were subject to quantitative NAA. An in-house reference powder samples (std_0) and commercially available rock reference materials (std_1) with known concentrations of Eu, Co and Cs were prepared as the internal standards. These standards and the samples subjected were encapsuled and irradiated simultaneously for 1 hour at Pn-2 under 1 MW operation of KUR. In addition to the capsules with the regular samples, the capsule with 12 standard samples (std_0) was prepared. The gamma-ray spectrum of each sample was measured one by one with an HP-Ge detector with automatic measurement system, where the photo-electric peaks at 1408 keV for ^{152}Eu, 1333 keV for ^{60}Co, and 795 keV for ^{134}Cs were analyzed.

RESULTS: Fig. 1(a) shows the inter-capsule variation of peak count rates with decay correction between 8 capsules. A similar trend was observed for all nuclides by the capsules, reflecting the flux fluctuation. Fig. 1(b) shows the intra-capsule variation of the count rates of 12 standards. A similar trend was observed for all nuclides by the samples, suggesting the influence of flux distribution in the capsule as well as insufficient uniformity of the elements between the standard samples.

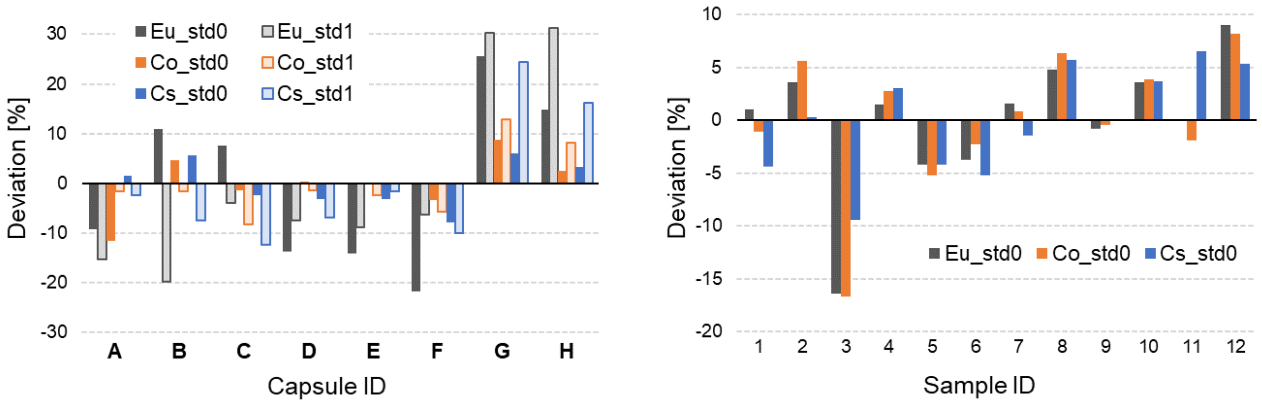

Fig. 1 (a) Inter- and (b) intra-capsule variation of peak count rates of the standard samples.

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CO7-25

Examination of improvement of BNCT treatment efficiency by L-phenylalanine deficiency in mice tumor models

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INTRODUCTION: L-Boronophenylalanine (L-BPA), a boronated isotope of L-phenylalanine (Phe), is used as a boron drug and is taken up by the tumor through its enhanced metabolism of amino acids. LAT1 is an exchange transporter, releasing one amino acid molecule into the extracellular space for the uptake of one amino acid molecule into the cell [1]. However, L-BPA is also taken up by normal cells via LAT2 and other pathways [2], and the improvement of the boron concentration ratio (T/N ratio) between tumor and normal tissues has become an issue. In this study, we attempted to improve the therapeutic effect of BNCT by restricting Phe and improving L-BPA uptake.

EXPERIMENTS: 5.0 x 10^5 human tongue carcinoma-derived cell lines SAS were transplanted into the thighs of 6-week-old female nude mice. Two weeks after transplantation, the carcinoma-bearing mice were fed Phe-free feed, and 500 mg/kg of L-BPA was administered after 24 hours. At 0.5, 1, 2, and 3 hours after administration, each organ (blood, brain, lung, liver, tongue, intestine, muscle, skin, and kidney) containing tumor was sampled and boron concentration was measured by ICP-AES. Carcinoma-bearing mice were fed Phe-free feed for 24 hours, and 2 hours after administration of 500 mg/kg L-BPA, they were neutron irradiated. Neutron-irradiated carcinoma-bearing mice were kept until approximately one month after irradiation, during which time their body weight and tumor size were regularly measured.

RESULTS: Although Phe restriction did not significantly increase L-BPA uptake in non-tumor organs, Phe restriction significantly increased L-BPA uptake in tumor tissue.
Furthermore, the change in tumor size after neutron irradiation was strongly suppressed in the L-BPA neutron-irradiated group compared to the control and neutron-irradiated-only groups. In particular, tumor size in the Phe-restricted L-BPA and neutron irradiated group was significantly reduced compared to the L-BPA and neutron irradiated group without Phe restriction. There was no change in body weight in all groups after neutron irradiation.

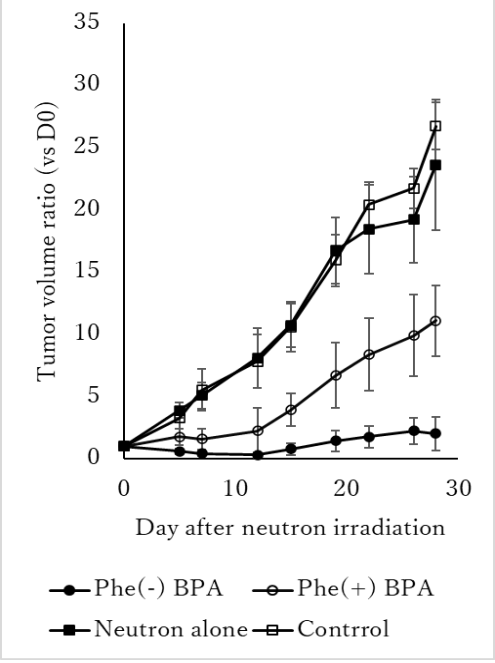

Fig. 1. Change over time in tumor size ratio after Phe-restricted BNCT.

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CO7-26

Establishment of BNCT equivalence evaluation methods with different neutron sources (iBNCT accelerator and KUR reactor)

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INTRODUCTION: In recent years, clinical trials and regulatory approvals using accelerator based BNCT devices have been underway in Japan. However, currently, one device is being applied for approval for one case, and bioequivalence evaluation between the devices is essential to make effective use of the few valuable accelerator-based neutron sources and to further expand the range of indications. Recently, papers on optimal methods for BNCT experiments have been reported [1,2], but there are no reports specifically evaluating equivalence among multiple facilities. The purpose of this study was to evaluate the bioequivalence between an accelerator-based neutron source (iBNCT001) developed by the University of Tsukuba and a reactor-based neutron source (KUR) at the Institute for Integrated Radiation and Nuclear Science, Kyoto University which has long been used for BNCT research, using cells and mice.

EXPERIMENTS: For *in vitro* experiments, cells were treated with p-boronophenylalanine (L-BPA) for 1 h in prepared medium at ^{10}B concentrations of 0, 10, 25, and 40 ppm, and neutron irradiation was performed by iBNCT001 and KUR. For *in vivo* experiments, 1x10^6 cells/10 μL were transplanted into the right lower limb thigh of 7–8-week-old nude mice and irradiated with neutrons when the tumor grew to 8-10 mm in diameter after 2 weeks. Irradiation of cells and mice was performed using the same system in iBNCT001 and KUR. The dose corresponding to the proton charge (mC) or irradiation time (min) was calculated by Monte Carlo calculations using the PHITS code.

RESULTS: The cell survival curves were plotted on the horizontal axis of the biological isoeffective dose (GyEq) calculated from the BPA treatment and neutron fluence at each boron concentration. The difference between the two facilities (iBNCT and KUR) was ±3~7% when comparing the isoeffective dose, D_{10} (the dose required to achieve 10% survival) (Table 1). In the analysis of anti-tumor effects in the carcinoma-bearing mice, tumor growth curves were obtained by measuring tumor size at least once a week after exposure to isoeffective doses (4.02 and 4.04 GyEq or 8.04 and 8.08 GyEq) in the iBNCT and KUR. Although the trend of enhanced antitumor effect with increasing dose was similar at both centers, a 25-45% difference between iBNCT and KUR was observed when the effect ratios were compared at 7, 14, 21, and 28 days after irradiation, respectively. The reason for the large differences in the in vivo experiments could be due to differences in feeding facilities, food, temperature and humidity during the experiment, and the person who measured tumor size.

^{10}B conc. (ppm)	0		10		25		40	
Facility	iBNCT	KUR	iBNCT	KUR	iBNCT	KUR	iBNCT	KUR
D_{10}	8.99	9.71	2.43	2.31	2.92	2.84	2.91	2.77
iBNCT/KUR	0.93		1.05		1.03		1.05	

Table 1. Isoeffective doses at iBNCT and KUR and differences between the two facilities.

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CO7-27

Development of Boron Agents for MRI-Guided BNCT

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a promising treatment for tough and inoperable malignant tumors. To achieve the highest BNCT effect, thermal neutrons should be irradiated at the point when the boron concentration in tumors reaches its peak. Magnetic Resonance Imaging (MRI) is a widely-used, non-invasive diagnostic method. Consequently, gadolinium (Gd) contrast agents with boron sources have been developed for MRI-guided BNCT. Furthermore, the ^{157}Gd isotope possesses the highest thermal neutron capture cross-section. Therefore, synthesizing compounds containing both boron and gadolinium can not only help estimate the biodistribution of boron drugs under MRI guidance but also aid in the development of efficient neutron capture cancer therapy [1]. We have developed maleimide-functionalized *closo*-dodecaborate (MID) albumin conjugates that demonstrate high and selective accumulation in tumor tissue with no toxicity in the absence of thermal neutrons, thus representing a promising boron delivery system [2]. In this study, we synthesized Gd complexes to functionalize MID albumin conjugates for MRI-guided BNCT.

EXPERIMENTS: Bovine serum albumin (BSA) was conjugated with Gd-DO3A-Mal and then purified using an ultrafiltration filter. The resulting Gd-BSA conjugate was functionalized with MID and purified by ultrafiltration to obtain Gd-MID-BSA. We monitored the accumulation of Gd-MID-BSA in tumors using a 9.4 T MR scanner. T1-weighted images of the tumor were taken at predose and 3, 6, 12, and 24 h postdose. Subsequently, the therapeutic effect was examined by irradiating thermal neutrons on tumor mouse models injected with Gd-MID-BSA. Gd-MID-BSA and MID-BSA were intravenously injected into CT26 tumor-bearing mice at a dose of 5 mg[^{10}B]/kg, followed by thermal neutron irradiation of the tumor with 3.1–3.4 × 10^{12} neutrons/cm^2 at 24 h after injection.

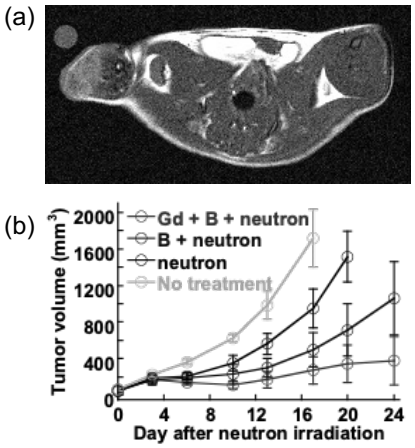

Fig. 1. (a) Representative T_1-weighted images of the tumor at 3h postdose. (b) Tumor growth curves after irradiation of the thermal neutron.

RESULTS: As shown in Fig. 1a, MRI signals in the tumors significantly increased at 3 h after injection and plateaued by 24 h, indicating prolonged blood retention and efficient accumulation of Gd-MID-BSA in the tumor. Tumor growth curves after thermal neutron irradiation are plotted in Fig. 1b. Tumor growth in the group injected with Gd-MID-BSA was significantly suppressed compared to the other groups, including MID-BSA, demonstrating that the neutron capture reaction of ^{157}Gd assisted in the BNCT effect. These results contribute to the advancement of MRI-guided neutron capture therapy as a potential treatment for malignant tumors [3].

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CO7-28

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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INTRODUCTION: L-type amino-acid transporter 1 (LAT1) [1], through which boronophenylalanine (BPA) is transported into cells, is 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 overexpressing LAT1. The sensitivity of clones 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 showed 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 our later study [4], we transfected pCD133-TRE/LAT1-tdTomato/IRES/tTA plasmids into T98G cells. The plasmids were designed to overexpress LAT1 tagged with tdTomato on cytoplasmic membranes of CD133 positive cancer cells (cancer stem cell-like cells) selectively. We established several clones which stably overexpress LAT1 in hypoxic microenvironment of spheroids including CD133 positive cells and showed that overexpression of LAT1 in CD133 positive cells results in enhanced sensitivity of spheroids to neutron and γ-ray fluences. In KUR experiments performed in 2022, we obtained preliminary data indicating that tumor regrowth after BNCT is relatively delayed in mice with tumors formed with the pCD133-TRE/LAT1-tdTomato/IRES/tTA plasmid-transfected cells compared with mice with control cells. The aim of this study is to confirm the preliminary experiment.

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/KC2, control plasmid-transfected, LAT1-nonoverexpressed 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 thermal and epithermal neutron fluence for 60 min irradiation was 5.1×10^{12} cm^{-2}. The total dose was 1.40 Gy for neutrons (1.077 Gy) and γ-rays (0.323 Gy), and the estimated boron dose was 0.325 Gy/ppm.

RESULTS: Tumor growth in ^{10}BPA-treated T98G/K10 and T98G/KC2 mice was strongly suppressed for approximately 40 days after neutron irradiation. However, such suppression was not observed in PBS-treated T98G/K10 and T98G/KC2 mice. We are now continuously measuring tumor size after BNCT in ^{10}BPA-treated T98G/K10 and T98G/KC2 mice. Significant differences in the tumor regrowth between two groups are not observed at present stage (data not shown). We plan to do additional experiments to confirm the effect of BNCT on tumor regrowth using CD133 expressing cell-selective LAT1 overexpression cells and control cells.

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CO7-29

Enhancement of Tumour Growth Suppression by Electroporation with Intra-Tumoural Injection of ^{10}Boron-Polyplex for Boron Neutron Capture Therapy to Pancreatic Cancer Model *in vivo*

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INTRODUCTION: It is necessary to accumulate high concentration of Boron atoms into the tumor tissues selectively for effective Boron neutron capture therapy(BNCT) [1, 2]. Electroporation is an method for selec-tive delivery of compound into the cells by open the membrane poles electorically in the field of gene therapy and chemotherapy [3, 4]. In this study, we evaluated the electroporation with intra-tumoral injection of ^{10}Boron-Polyplex; ^{10}Boro-plex (^{10}Boron / hyaluronic acid / protamine-mixed with cationic liposome) by in vivo experiment on AsPC-1 human pancreatic tumor bearing mice.

EXPERIMENTS: ^{10}Boro-plex were prepared mixed with 1.5mL of ^{10}BSH(^{10}B:23809 ppm) or ^{10}BPA(^{10}B:1290 ppm), 0.3mL of a solution of 10mg/mL hyaluronic acid sodium, and 0.15mL of 20mg/mL of protamine incubating at room temperature for 30min, then, these mixing solutions were poured into cationic Liposome; Lipofectamine 3000(0.15mL+reaction solution 0.15mL). We prepared human pancreatic cancer AsPC-1($5x10^5$) model by transplanting to right lower leg. Electroporation was performed after intra-tumoral injection of 0.2mL 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/cm2). The change in tumor growth and survival rate of the mice reflected the anti-tumor effect of ^{10}Boro-plex.

RESULTS: Tumor growth suppression was achieved in the ^{10}Boro-plex+EP group in NCT groups compared with non-irradiated group. The experimental results showed that 10Boro-plex+EP group was revealed the tu-mor growth suppression, and no significant weight loss were observed after treatment suggesting low systemic toxicity of this system.

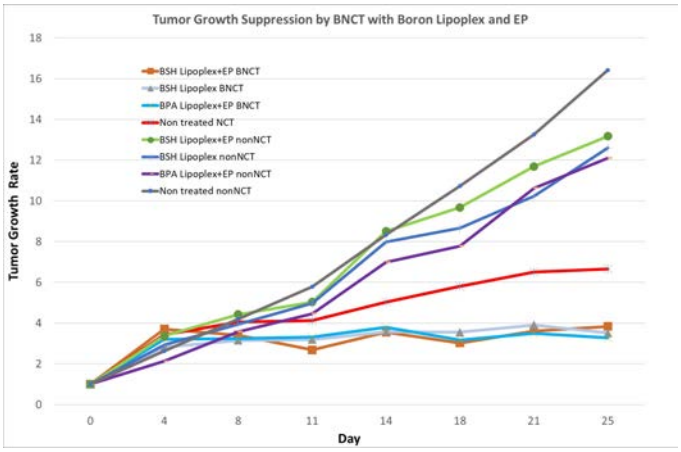

Figure1. Tumor growth suppression in ^{10}Boro-plex+EP group by NCT was superior compared with non-irradiated group.

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R5089

CO7-30

Tumor Growth Suppression of Gadolinium Neutron Capture Therapy with Gd_2O_3 polymeric nanocarries

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INTRODUCTION: On the basis of Gadolinium-neutron capture therapy(GdNCT), Gadolinium atoms react thermal neutron and offers cytotoxic effect by 1μm-range high LET Auger electron, and long-range gamma rays [1, 2, 3]. Recently, nanoscale drug becomes more and more popular because it can promote the accumulation of Gd agents in tumor through the enhanced effect of per-meability and retention (EPR). In this study, we built a series of Gd entrappednanomicelle(Gd_2O_3) to improve the accumulation in tumor and evaluated its antitumor effect through the comparison of tumor size.

EXPERIMENTS: Preparation of polymeric nanocarriers; A hydrophobic part, Poly-γ-Benzyl-L-Glutamate (PBLG) was combined to hydrophilic polymer. PEG was used for modify molecular weight and water solubility, and P(Asp) was used for combining to gadolinium chelator. Gd3+ was oxi-dated to Gd_2O_3 for stability improvement. Due to the certain size, this nanomicelle tends to accumulate near tumor tissue and keep a long retention time. After 24h injection into colon 26 tumor-bearing mice with Gd_2O_3 nanomicelle, the tumor-bearing mice received thermal neutron irradiation at Nuclear Reactor Facility of Kyoto Univ Institute for Integrated Radiation & Nuclear Science (3.0×10^{12} n/cm^2).

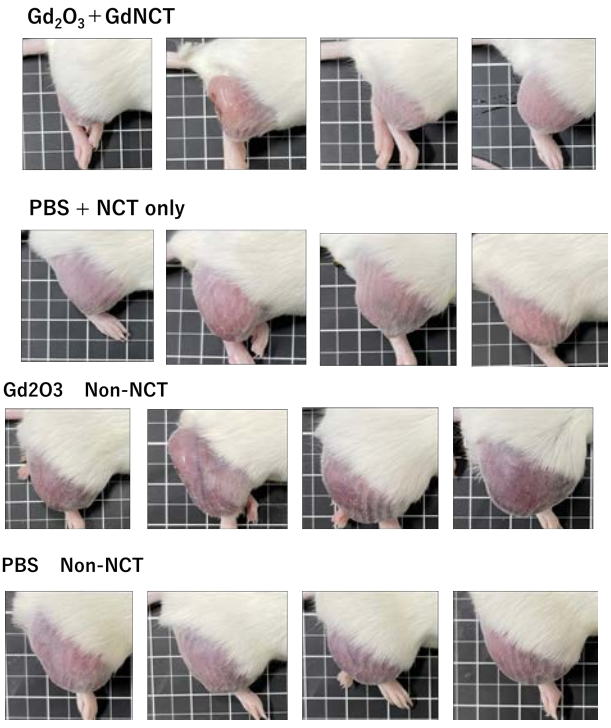

Figure 1. Tumor growth suppression by GdNCT using Gd_2O_3 nanomicelle.

RESULTS: Tumor growth was suppressed with the injection of Gd_2O_3 nanomicelle and according to the comparison in the control group, the suppression worked not only through the irradiation, but the influence of Gd_2O_3 nanocarriers. We hope to increase the concentrations of Gd atoms entrapped into the nanomicelle, and more selective targeting to cancer cells using ligands.

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CO7-31

Research and Development of New Technology for Boron Neutron Capture Therapy

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) relies on accumulating boron-containing compounds within cancer cells, followed by irradiation with a neutron beam. Ensuring a high accumulation of boron-containing compounds within the cells is crucial to optimize the nuclear reaction between ^{10}B and neutrons in cancer cells. One of the most widely used boron carriers for BNCT is L-*p*-boronophenylalanine (L-BPA), which is taken up by cancer cells due to their elevated amino acid transport mechanisms [1]. However, a challenge in using L-BPA for BNCT is the rapid decrease in intracellular L-BPA levels caused by the efflux of L-BPA from cancer cells through specific transporters. This study aims to experimentally validate an approach to enhance the effects of neutron irradiation on cancer cells by inhibiting the transporters responsible for L-BPA efflux. Employing cultured cells and tumor-bearing mice as model systems, we investigated the potential of this approach to improve the efficacy of BNCT using L-BPA.

EXPERIMENTS: For the cell irradiation experiment, the mouse-derived tumor cell line 4T1 was treated with L-BPA by adding it to the culture medium. After a washing step, the cells were incubated for 60 min in a medium either with or without inhibitors that target the transporters responsible for L-BPA release. The cells were then harvested using Hanks' Balanced Salt Solution (HBSS) and transferred to 1.5 mL tubes to serve as samples for neutron irradiation. A control sample was also prepared by irradiating cells that had not been loaded with L-BPA. After neutron irradiation, cell numbers were adjusted, and the cells were seeded into 10 cm dishes. Viable cells were evaluated for colony formation to assess cell survival.

For the in vivo mouse irradiation experiment, human-derived tumor cell line MCF7 and mouse-derived 4T1 were subcutaneously implanted into nude mice and Balb/c mice, respectively, to establish tumor models. Mice were administered 125 mg/kg and 400 mg/kg of L-BPA for MCF7 and 4T1, respectively, via tail vein injection. This was followed by administering a transporter inhibitor (25mg/kg as active stereoisomer), responsible for preventing L-BPA release, at 1 h and 1.5 h post-L-BPA injection. As a control, mice were prepared for neutron irradiation without L-BPA loading but received the inhibitor treatment alone. Neutron irradiation was conducted at 5 MW for 20 and 30 min for MCF7 and 4T1, respectively. The therapeutic effects on tumor regression were subsequently compared between the experimental and control groups after neutron irradiation.

RESULTS: In cell irradiation experiments, 4T1 cells were treated with L-BPA and washed. The cells were then divided into two groups: Group 1 was incubated for 60 min in RPMI 1640 cell culture medium containing an inhibitor of the transporter responsible for L-BPA release. Group 2 was incubated for 60 min in a culture medium without the inhibitor. Group 3 was prepared as an additional control, consisting of cells not treated with L-BPA. All groups were then subjected to neutron irradiation. After irradiation, cell viability was assessed by measuring colony formation in low-density cultures. The results indicated no significant difference in cell survival between Group 2 and Group 3 (non-BNCT), suggesting that the intracellular L-BPA content had decreased to levels less effective for BNCT within 60 min after L-BPA treatment. In contrast, the survival rate in Group 1 was significantly lower than in Group 2, demonstrating an enhancement of BNCT efficacy by inhibiting the transporter responsible for L-BPA release.

In in vivo mouse irradiation experiments, MCF7 cells and 4T1 cells were implanted into the hind limbs of mice to form tumors. Mice were then administered L-BPA intravenously and divided into two groups. Group 1 received an intravenous injection of a transporter inhibitor responsible for L-BPA release at 1 h and 1.5 h after L-BPA administration. Group 2 did not receive inhibitor treatment following L-BPA administration. Additionally, Group 3 was prepared by administering saline instead of L-BPA, followed by injections of the transporter inhibitor at 1 h and 1.5 h post-saline administration. For each group, mice were anesthetized 2.5 h after administering L-BPA or saline and subjected to neutron irradiation. Tumors were excised two weeks after irradiation, and their sizes were measured. The results indicated that, for both MCF7 and 4T1 tumors, tumor sizes in Group 1 were significantly smaller than in Group 2, demonstrating that inhibiting the transporter responsible for L-BPA release significantly enhanced BNCT efficacy.

The results of this study have confirmed the proposed concept for optimizing L-BPA-based BNCT.

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CO7-32

Early responses of tumor cells to BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a cutting-edge cancer treatment approach that employs high-energy alpha particles and lithium nuclei generated by nuclear reactions. This method offers precision, brevity, and minimal side effects. To enhance the effectiveness of BNCT, it is essential to conduct comprehensive analysis of its response to cancer cells and therapeutic efficacy. We conducted extensive research on the consequences of BNCT on tumor cells, such as cell death and various biological responses. Using cancer cell lines and xenograft mouse models can significantly bolster this endeavor.

EXPERIMENTS: Neutron irradiation experiments at the KUR reactor were conducted at a constant power level of 1 MW in all cases. Gold foil activation analysis and thermoluminescence dosimeters (TLDs) were employed. The irradiation mode used at KUR was OO-0000F, with a Dr ratio of approximately 9.4. The total physical dose was calculated using the flux-to-dose conversion factor [1]. The relevant data is presented in Tables 1-3.

The human squamous cell line SAS and malignant melanoma A375 cells were incubated with ^{10}B-boronophenylalanine fructose complex (BPA) (Catchem) for 2.0 hrs in suspension. The cell survival was analyzed by colony formation assay and culture supernatants were harvested at 6 and 24 hrs for RNA and proteins isolation and molecular analysis.

Mouse melanoma cell lines B16F10 and the variant were grafted to the hind legs and were locally irradiated using ^{6}LiF containing thermal neutron shield. Mice were injected with BPA at 500 mg/kg bodyweight approximately 30 min before irradiation. Mice were euthanized on days 7 and 13 after irradiation, and blood, tumors, and other organs were analyzed.

RESULTS: The measurement of thermal neutron fluence and doses for mice (Table 1) and cells were shown in Table 1 & 3. A potential role of HMGB1 as an early biomarker was indicated by cellular and *in vivo* responses to BNCT and proteome analysis of exosomes derived from SAS cells showed basic data for identifying biomarkers for the response to BNCT [2, 3].

Table 1. Irradiated doses for local irradiation of mice on Dec. 5, 2023 (Cart, irradiation room).

Irradiation time	Irradiation (min)	Position	Fluence (/cm²)		(Gy)					
			Thermal neutron (/cm²)	Epi-thermal neutron	Thermal neutron (GY)	Epi-thermal neutron (GY)	Fast neutron (GY)	Gamma ray (GY)	Physical dose (GY)	B-10* (1 ppm)
12:27-13:27	60	Center	4.10E+12	7.30E+11	5.40E-01	5.80E-02	4.00E-01	3.10E-01	1.30E+00	3.00E-01
13:38-14:38	60	Center	4.20E+12	7.60E+11	5.70E-01	6.00E-02	4.20E-01	2.30E-01	1.30E+00	3.20E-01

Table 2. Irradiated doses at cells on Dec.12, 2023 (Single layer, E-4 rail port)

Irradiation time	Irradiation (min)	Position	Fluence (/cm²)		(Gy)					
			Thermal neutron (/cm²)	Epi-thermal neutron	Thermal neutron (GY)	Epi-thermal neutron (GY)	Fast neutron (GY)	Gamma ray (GY)	Physical dose (GY)	B-10* (1 ppm)
10:41-10:51	10	Center	1.10E+12	1.90E+11	1.40E-01	1.50E-02	1.10E-01	7.80E-02	3.40E-01	8.00E-02
11:02-12:02	60	Center	6.10E+12	1.10E+12	8.10E-01	8.70E-02	6.00E-01	4.30E-01	1.90E+00	4.50E-01
12:21-12:29	8	Center	8.90E+11	1.60E+11	1.20E-01	1.30E-02	8.80E-02	7.40E-02	2.90E-01	6.60E-02
12:30-12:36	6	Center	7.00E+11	1.20E+11	9.30E-02	9.90E-03	6.90E-02	4.80E-02	2.20E-01	5.20E-02
12:38-12:42	4	Center	4.50E+11	8.10E+10	6.10E-02	6.50E-03	4.50E-02	3.80E-02	1.50E-01	3.40E-02
12:44-12:46	2	Center	2.40E+11	4.20E+10	3.20E-02	3.40E-03	2.30E-02	1.90E-02	7.70E-02	1.80E-02

Table 3. Irradiated doses at cells on January 17, 2024 (Single layer, E-4 rail port)

Irradiation time	Irradiation (min)	Position	Fluence (/cm²)		(Gy)					
			Thermal neutron (/cm²)	Epi-thermal neutron	Thermal neutron (GY)	Epi-thermal neutron (GY)	Fast neutron (GY)	Gamma ray (GY)	Physical dose (GY)	B-10* (1 ppm)
8:32-8:34	2	Center	2.50E+11	4.40E+10	3.30E-02	3.50E-03	2.40E-02	1.20E-02	7.20E-02	1.80E-02
8:37-8:41	4	Center	5.10E+11	9.00E+10	6.80E-02	7.20E-03	5.00E-02	2.80E-02	1.50E-01	3.80E-02
8:43-8:49	6	Center	7.80E+11	1.40E+11	1.00E-01	1.10E-02	7.70E-02	1.40E-02	2.10E-01	5.80E-02
8:51-8:59	8	Center	9.90E+11	1.80E+11	1.30E-01	1.40E-02	9.80E-02	1.20E-02	2.60E-01	7.40E-02
9:08-9:10	2	Center	2.50E+11	4.40E+10	3.30E-02	3.50E-03	2.50E-02	9.90E-03	7.10E-02	1.90E-02
9:12-9:16	4	Center	4.40E+11	7.90E+10	5.90E-02	6.30E-03	4.40E-02	2.10E-02	1.30E-01	3.30E-02
9:18-9:24	6	Center	6.60E+11	1.20E+11	8.70E-02	9.30E-03	6.50E-02	4.60E-02	2.10E-01	4.90E-02
9:25-9:33	8	Center	9.60E+11	1.70E+11	1.30E-01	1.40E-02	9.40E-02	3.40E-02	2.70E-01	7.10E-02
9:36-10:36	60	Center	6.90E+12	1.20E+12	9.20E-01	9.80E-02	6.80E-01	4.60E-01	2.20E+00	5.10E-01

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CO7-33

Development of boron carriers based on the characteristics of energy metabolism of cancer

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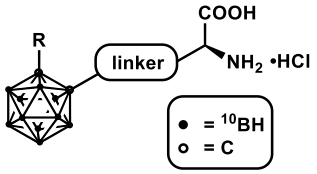
INTRODUCTION: Glutamine functions as a carbon and nitrogen source for biosynthesis, energy metabolism, and redox homeostasis in cancer cells. Dysregulated glutamine metabolism is a prominent metabolic hallmark of cancer cells. Thus, the glutamine metabolic pathway is a breakpoint in cancer therapy, as many cancer cells exhibit a marked dependence on glutamine, also known as 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 over-expressed in a variety of tumor cells. Therefore, we are developing boron carriers that efficiently accumulate 10B atoms in tumors by exploiting amino acid transporters that are highly expressed in cancers. We have synthesized various amino acid derivatives containing boron clusters as hydrophobic pharmacophores and screened them by boron uptake into cells, resulting in several promising candidate compounds.



In this study, we synthesized a new derivative, BCY2, and compared its cellular uptake and the tumor cell-killing effect under neutron irradiation with BC2.

EXPERIMENTS: Boron compounds are synthesized by a microwave reaction in which the corresponding alkyne reacts with decaborane in the presence of a Lewis base to construct car-borane. T98G was treated with 10 ε gB/mL boron carrier for 30 minutes or 1 hour, washed with cold PBS, dis-solved in nitric acid directly, and intracellular boron up-take was measured by ICP-AES. To evaluate the enhancement of neutron irradiation by the compound, T98G cells were treated with 10 or 20 ε g ^{10}B/mL boron carrier for 2 hours, then the cells were washed with PBS, suspended in serum-containing medium, dispensed into Teflon tubes, and irradiated. The cells were irradiated with a neutron beam at a power of 1 MW at the Heavy Water Facility of the Kyoto University Research Reactor (KUR). Survival of irradiated cells was measured using a conventional colony assay; D10 of BNCT was calculated from the survival curve shown in Fig. 2.

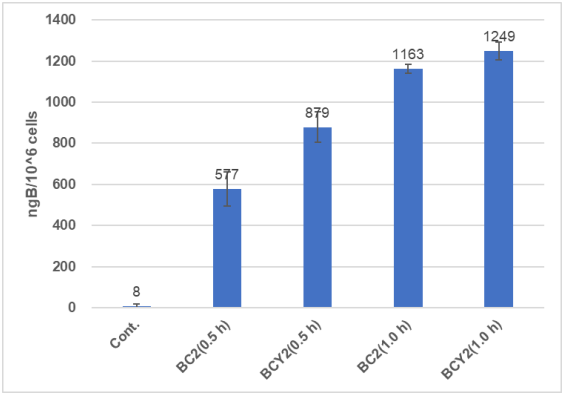
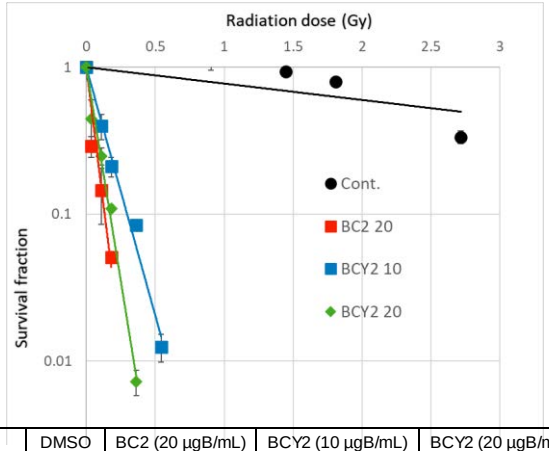


Fig. 1. Incorporation of boron carriers into T98G.

RESULTS: As previously reported, BC2, which showed the highest cellular uptake, increased intracellular boron content up to 2 hours treatment time and remained the same with further treatment time. Therefore, we compared the treatment times up to 1 hour and found that BCY2 tended to be taken up more than BC2, as shown in Fig. 1. When cells suspended in fresh medium after drug treatment were irradiated with neutrons, BCY2 also significantly enhanced the cell-killing effect of neutron beams to the same degree as BC2. This result suggests that these car-borane-containing amino acids remain in the cell without being excreted by the cell.

From the above, it was predicted that these compounds are taken up by cells by a different mechanism than BPA, which is currently under investigation. Due to the low water solubility of these compounds, structural modifications are being performed for pharmacokinetic analysis in a tumor-bearing mouse model.



	DMSO	BC2 (20 μgB/mL)	BCY2 (10 μgB/mL)	BCY2 (20 μgB/mL)
D10 (Gy)	8.959	0.133	0.296	0.172
ER	1.0	67.2	30.2	52.0

Fig. 2. Survival fraction of T98G cells treated with BC2 and BCY2 with mixed-neutron irradiation.

ACKNOWLEDGEMENTS: This research was supported by JSPS KAKENHI (JP19H03352 and JP26670059).

CO7-34

Detection of Boron-10 Compound in a Plant Seed Using a Neutron Capture Reaction

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INTRODUCTION: One of the optimal solutions to ensure a sustainable food supply is to increase and stabilize crop yields through plant breeding. However, because of the extremely low rate of naturally occurring mutant varieties, breeding methods that efficiently produce new plant varieties by artificially inducing mutations have been sought. Therefore, breeding using radiation or chemicals as mutagens continues to be practiced around the world. Fast neutrons can efficiently introduce random mutations into the target genomes, but they also have the disadvantage of causing cell death because of their high energy. Against this background, we have developed a boron neutron capture reaction breeding method (BNC breeding method), which can maintain a high survival rate while introducing random mutations. The BNC breeding method uses the nuclear reaction of $^{10}B(n,\alpha)^{7}Li$. The basic principle of this method is that thermal neutrons are irradiated toward the seeds of plants that have absorbed a ^{10}B compound, and the α-rays generated thereafter introduce mutations. The thermal neutrons irradiated are very low energy, equal to one to four millionth of fast neutrons, and are used merely as a trigger for this reaction. Therefore, the side reactions are expected to be much lower than those in the conventional method using fast neutrons. The objective of this study was to visualize the distribution of ^{10}B compounds absorbed in the tissues of rice seeds for the practical application of the BNC breeding method.

EXPERIMENTS: Materials> Rice seeds (*Oryza sativa* L. cv. Nipponbare) were kindly gifted from Dr. Segami, Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture. ^{10}B-boric acid aqueous solution was kindly gifted from Dr. Hattori, Research Center for BNCT, Osaka Metropolitan University.

In situ visualization of ^{10}B compounds in seeds> rice seeds were immersed in ^{10}B-boric acid sol. (10 mM) for 24 hrs. Slices (10-μm thickness) of the seeds were mounted onto a solid-state nuclear tracking detector, CR-39 (20 mm×30 mm) and irradiated with epithermal neutrons for 20 min by applying them 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 for 60 min at 70°C, and the resulting etch-pits were observed under an optical microscope.

RESULTS: Both Fig. 1(A) and 1(B) show close-up images of cross-sections prepared from the same rice seed, which was immersed in ^{10}B-boric acid sol. (10 mM). Fig. 1(A) is a bright-field image. Fig. 1(B) is an α-tracking autoradiograph, which was generated by the BNC reaction, and reveals the distribution of ^{10}B compounds in the cross-section. The areas enclosed by the circle indicate the embryo. A large number of etch-pits derived from ^{10}B were imaged throughout the section as small black spots. It was observed that a large amount of ^{10}B accumulated in the embryo compared to the area not circled (endosperm).

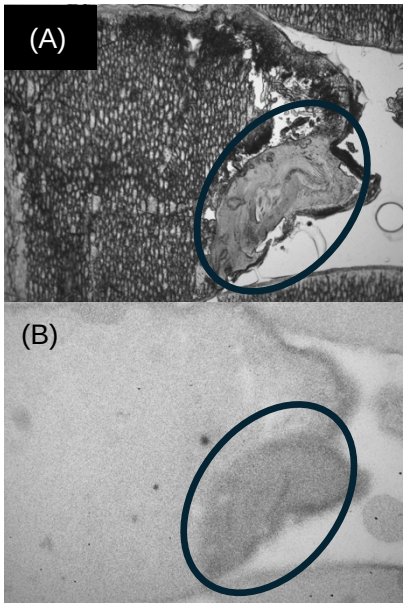

Fig. 1. Detection of ^{10}B compounds absorbed into the rice seed.

Tumor eradication by GdNCT-immunotherapy with gadolinium-containing nanoparticles

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Neutron capture therapy (NCT) as a promising radiotherapy of cancer has attracted great attention in recent years. The NCT using boron- and gadolinium-based sensitizers are called BNCT and GdNCT, respectively. GdNCT is capable of killing cancer cells with Auger electrons of high linear energy transfer (LET) as well as low-LET γ photons, which are generated by the capture reaction of Gd and thermal neutrons. ^{157}Gd with a natural abundance of 15.7% has the largest thermal neutron cross section of all the stable isotopes at 254,000 barns, which is much higher than that of ^{10}B (3,840 barns).[1] Despite its potential, GdNCT research is limited compared to BNCT, partly due to the scarcity of effective Gd-based sensitizers that can accumulate selectively in tumors.

Immune checkpoint blockade (ICB) is a groundbreaking immunotherapy that involves the use of immune checkpoint inhibitors to combat various types of cancer. These inhibitors work by blocking checkpoint proteins from binding with their partner proteins, essentially preventing the immune system from being suppressed and allowing it to attack cancer cells. This approach helps enhance the body's immune response against cancer. Recent findings suggest that combining GdNCT with ICB immunotherapy not only substantially inhibits primary tumor growth but also induces shrinkage in distant tumors receiving no GdNCT treatment.[2]

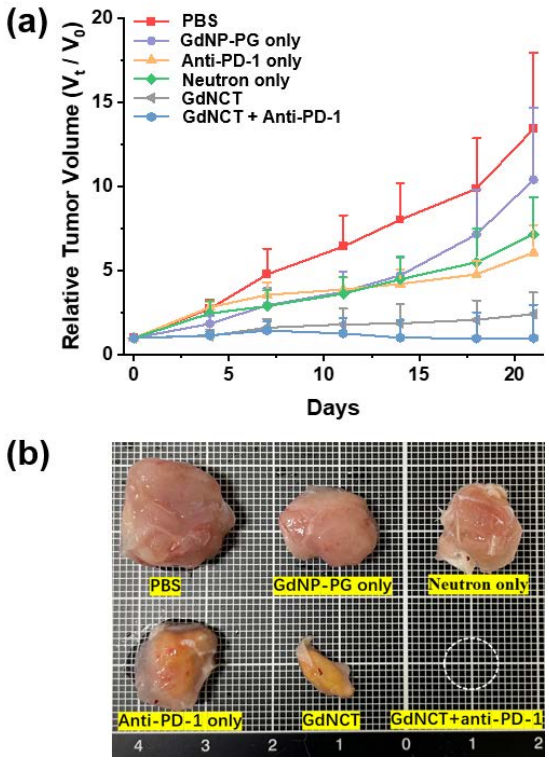

Figure 1. GdNCT-immunotherapy mediated with GdNP-PG nanoparticles. (a) Tumor growth curve (n=4); (b) Representative photographs of the CT26 tumors in different groups after treatment for 21 days.

We have recently developed GdNP-PG nanoparticles as an efficient sensitizer for GdNCT. In this work, we evaluated the efficacy of GdNCT-immunotherapy mediated with GdNP-PG nanoparticles. The mice bearing CT26 tumor were intravenously injected with the PBS dispersion of GdNP-PG. After the injection for 24 h, the mice were irradiated with thermal neutrons at a reactor power of 5 MW for 12 min. Subsequently, anti-PD-1, an inhibitor of immune checkpoint, was administrated through intraperitoneal injection on days 0, 4, 7, and 11. The preliminary results show that GdNCT significantly suppressed the growth of CT26 tumor as compared to control groups. More importantly, three out of four mice in GdNCT + anti-PD-1 group exhibited nearly complete tumor regression, and tumor recurrence was not observed after the treatment for more than 2 months. Further study to elucidate the mechanism of GdNCT-immunotherapy is in progess.

Reference:

[1] S. L. Ho *et al.,* *ACS Omega*, **7(3)** (2022) 2533-2553.
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CO7-36

MnO_2 modified albumin nanoboron drug for MRI image-guided BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) has shown significant efficacy for malignant glioma[1]. We constructed BPA-F&DOTA-Gd@LIPO using boron phenylalanine-fructose (BPA-F) complex as the boron capture agent and DOTA-Gd as the part that provides the MRI image, which were co-loaded in lipid nanoparticles using microfluidic method. The therapeutic effect of BPA-F&DOTA-Gd@LIPO after neutron irradiation was evaluated in this experiment.

EXPERIMENTS: The glioma cell line U87MG was seeded onto a 6-well plate, and after 24 hours of incubation, PBS, BPA-fructose, BSA-BPA, and BSA-BPA-MnO_2 (with ^{10}B concentrations equiv-alent to 25 μg/ml) were added, respectively. The effect of boron neutron irradiation with these new compounds were investigated with colony formation assay.

Using U87MG tumor-bearing nude mice, two *in vivo* experiments were performed. In first experiment, BPA-fructose solution, BSA-BPA-MnO_2 at boron concentration of 2.5mg/kg and PBS as control and mice injected with each preparation were subdivided into neutron-irradiated and non-irradiated groups. In second experiment, BPA-F&DOTA-Gd solution, BPA-F&DOTA-Gd@LIPO at boron concentration of 2.5mg/kg and PBS as control were used and the mice injected these compoundswere subdivided into neutron-irradiated and non-irradiated groups. Mice in the neutron irradiation group were irradiated with KUR. After that, body weight and tumor volume of mice in six groups were measured every four days. The body weight curve and tumor volume curve were recorded.

RESULTS: U87MG cells were incubated with each preparation at a concentration of 25 μg/mL ^{10}B for 4 hours and then irradiated with thermal neutrons for different times (0, 15, 30, 45 min). According to the colony formation results, it can be seen that the boron drug BSA-BPA and BSA-BPA-MnO_2 can effectively kill tumor cells at the cellular level, and the effect is slightly stronger than BPA-fruc-tose.

Compared with the control group injected with PBS only, mice in the BSA-BPA-MnO_2 + neutron irradiation group showed significant inhibition of tumor growth within 30 days. Meanwhile, the trend of body weight change was basically the same in all groups, illustrating that experimental BNCT treatment did not bring obvious body damage to the animals.

It was observed that in the short term, the tumor growth of the neutron irradiation groups had a significant tendency to be suppressed. Then, we performed the experiment again using the same method and the results are shown in Figure 4. Compared with the control group injected with PBS only, mice in the BPA-F&DOTA-Gd@LIPO + neutron irradiation group showed significant inhibition of tumor growth within 30 days. Meanwhile, the trend of body weight change was basically the same in all groups, illustrating that experimental BNCT treatment did not bring obvious body damage to the animals.

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CO7-37

Analysis of boron compound micro-distribuion in BNCT

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INTRODUCTION:

In boron neutron capture therapy (BNCT), the adverse effects of normal tissues depend on micro-distribution of boron compound. For example, boronophenylalanine (BPA) accumulates mucosal epithelium, which lead to oral mucositis in BNCT for head and neck cancer. In this study, the micro-distribution of BPA in the esophagus was investigated.

EXPERIMENTS

Mice: Ten- to twelve-week-old female C3H/He mice were used. The mice were purchased from Japan SLC, Inc.

Analysis of BPA micro-distribution by autoradiography using CR-39: BPA was administered subcutaneously at the dose of 500 mg/kg. At one hour after the BPA administration, the esophagus was extracted and embedded in Optimal Cutting Temperature (O.C.T) Compound. The samples were sliced 10 μm thickness using cryostat. The detailed procedure for analysis of BPA micro-distribution was referred to the previous published paper from our laboratory [1]

RESELTS

Figure 1 shows the superimposed image of the dots on CR-39 and hematoxylin and eosin (HE) image. The dots on CR-39, to some extent, appears to overlay the basal cells in the esophageal mucosa.

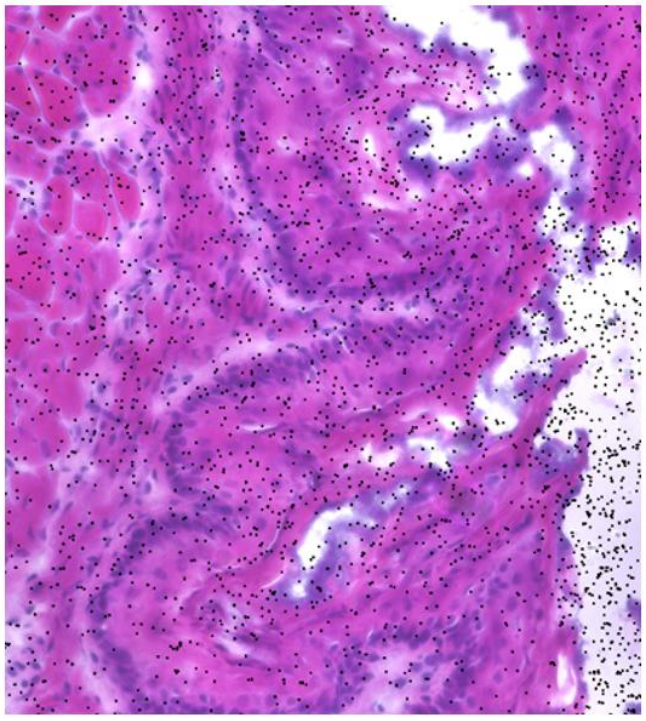

Fig.1. superimposed image of the dots on CR-39 and HE image.

REFERENCES:

[1] S. Takeno, Physica Medica, **82** (2021) 306-320.

CO7-38

Effects of neutron irradiation on immune cells

M. Shirakawa[1,2], A. Zaboronok[2], H. Michiue[1], K. Igawa[1], H. Kumada[2], T. Sakae[2], A. Matsumura[2], T. Takata[3], N. Kondo[3], Y. Sakurai[3], M. Suzuki[3]

[1] *Neutron Therapy Research Center, Okayama University*
[2] *Institute of Medicine, University of Tsukuba*
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INTRODUCTION:

Recent studies have shown that irradiation of normal tissue, such as skin, results in an accumulation of tumor-infiltrating regulatory T cells (Treg cells), which subsequently leads to tumor resistance to radiotherapy [1]. In light of these findings, we sought to determine whether similar effects are observed with neutron irradiation and will present some of the key results here.

EXPERIMENTS:

1. In vivo animal model

Female, 3-week-old C57BL/6J mice were purchased from CLEA Japan Inc., Tokyo, Japan. The mice were acclimatized for one week at the Kyoto University Institute for Integrated Radiation and Nuclear Science (KURNS). A radiation-affected model was established by exposing the mice to thermal neutron irradiation. Irradiation was performed with a flux of 1.0-1.2 × 10^9 neutrons/cm^2/s for durations of 4, 8, and 12 minutes.

2. Injection of tumor cell line.

The left flank skin and right lower limb of irradiated recipient mice were shaved. Subsequently, 5×10^5 B16-F10 cells were injected subcutaneously into the left flank, and 2×10^5 cells were injected intramuscularly into the right lower limb. Tumor size was measured over time with an electronic caliper until day 16 post-irradiation, and tumor volume was calculated using a previously applied formula [2]. On the final measurement day, two mice in each of the 8-minute and 12-minute irradiation groups died spontaneously. Therefore, significant differences in tumor sizes between groups were calculated on day 13 using one-way ANOVA with Tukey's multiple comparison tests.

RESULTS:

As shown in Figure 1, tumor growth was more rapid in the left flank compared to the right lower limb. However, by day 13, no significant differences were observed between the groups exposed to different irradiation times, suggesting that the impact of neutron irradiation on immune cells is very limited. Additionally, no significant weight loss was observed in any of the groups.

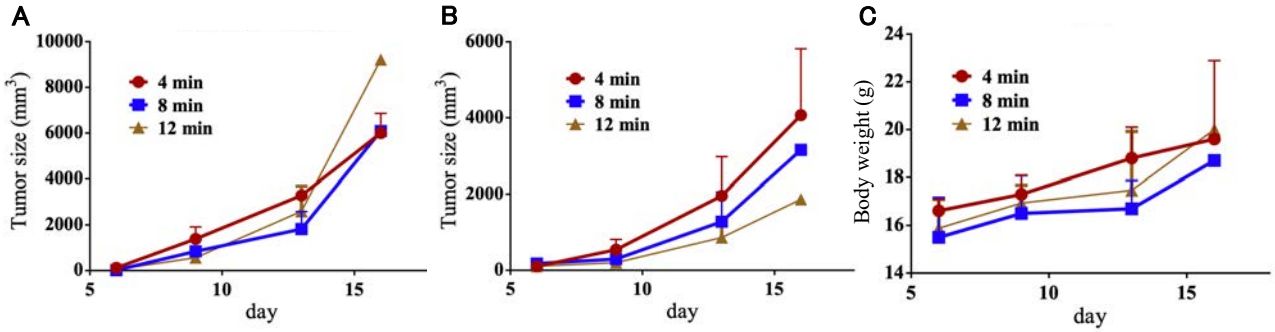

Fig.1) In vivo effect of neutron irradiation (n=3). (A) Tumor growth at the injected/non-irradiated sites. (B) Tumor growth at the injected/irradiated sites. (C) Body weight.

REFERENCES:

[1] Jeremy G Price *et al.*, Nature Immunology, **16** (2015) 1060-1068.
[2] M. Shirakawa *et. al.,* KURRI PROGRESS REPORT 2017 (2018) 69 .

CO7-39

Attempts to sensitize tumor cells by exploiting the tumor microenvironment

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a radiotherapy that kills tumor cells via the $^{10}B(n, \alpha)^{7}Li$ reaction [1]. Especially for BNCT with BPA, SLC7A5 (LAT1) protein expression level is an important factor for the anti-tumor efficacy, since the LAT1 is a major transporter for intracellular uptake of BPA. Recent studies suggest that tumor microenvironment, such as hypoxia, may influence the expression profiles of SLC7A5 and hinder the intracellular uptake of BPA. However, it has not been fully understood how the disturbance of SLC7A5 expression affects the anti-tumor efficacy of BPA-BNCT. In the present study, we generated multiple tumor cell lines that lack the function of SLC7A5 and that overexpress SLC7A5 proteins, and compared the sensitivity of these cells to BPA-BNCT.

EXPERIMENTS: Like previously established SCCVII (mouse squamous cell carcinoma cell lines)-SLC7A5 deficient cells [2], A2058 (human melanoma cell lines)-SLC7A5 deficient cells were generated using CRISPR-Cas9 system. Since two SLC7A5 pseudogenes have been found in the human genomes, the exon 2 of human SLC7A5 locus were chosen as the CRISPR-Cas9 target-site in order to minimize the off-target effects. To generate SCCVII that overexpress SLC7A5 proteins, the SCC VII cells were transfected with the SLC7A5 expression vector generated by modifying pBApo-EF1α vector. To examine the sensitivity to BPA-BNCT, these cells were exposed to neutron beams (KUR Heavy Water Facility) and then clonogenic cell survival assays were performed.

RESULTS: We examined the sensitivity of SCC VII-ΔSLC7A5 (SLC7A5 deficient) cells and SCC VII cells overexpressing recombinant SLC7A5 proteins to BPA-BNCT. As shown Fig. 1, the result suggested that the expression level of SLC7A5 proteins is slightly, but not significantly, related to the cellular survival. We also measured the cellular survival of A2058-ΔSLC7A5 (SLC7A5 deficient) cells. A2058-ΔSLC7A5 was more resistant to BPA-BNCT than the parental A2058 cells. Although a further investigation is needed, changes in SLC7A5 expression levels likely affect the cellular survival after BPA-BNCT to an extent that depends on cell type.

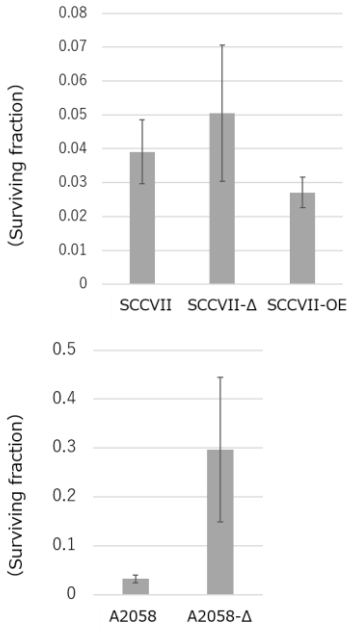

Fig.1 Survival fractions of neutron-irradiated cells. Cell suspensions prepared with BPA containing medium were exposed to neutrons for 20 min (thermal neutrons at a fluency of 2.1×10^{12} n/cm^2).

REFERENCES:
[1] S. Masunaga *et al.,* Int. J. Rad. Biol., **92** (2016) 187-194.
[2] Y. Sanada *et al.,* Int. J. Rad. Biol., **97** (2021) 1441-1449.

CO7-40

Sensitization of BPA-BNCT by Regulating the Polarity of Tumor-Associated Macrophage Using All-*Trans* Retinoic Acid

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INTRODUCTION: In the tumor microenvironment (TME), various stromal cells such as immune cells, fibroblasts, and vascular endothelial cells interact with cancer cells and are involved in the proliferation and progression of cancer cells. Tumor-associated macrophages (TAM) are attracting attention as major players in the TME. TAMs are broadly divided into tumor-suppressive type M1 and tumor-promoting type M2, and TAMs mainly exist as an M2-like phenotype in the TME [1]. M2-like macrophages are involved in promoting angiogenesis and suppressing antitumor immunity. Therefore, if the phenotype of tumor-infiltrating macrophages can be induced from M2 to M1, it will lead to the novel method for cancer prevention and treatment. Since the predominance of STAT3 activation results in M2 macrophage polarization, STAT 3 inhibitors are promising as TAM polarity regulators. Although all-*trans* retinoic acid (ATRA) shifts the polarity of macrophages from M2 type to M1 type by suppressing the STAT3 signal transduction pathway [2], it has a problem of poor water solubility as a drug. Therefore, we attempted to create a TAM-targeted nanogel formulation by including β-1,3-glucan, which functions as a ligand for Dectin-1, a membrane protein of macrophages, as a solubilizer. Furthermore, we confirmed the combination effects of ATRA/β-1,3-glucan nanogel on BPA-BNCT toward SCCVII tumor cells-bearing C3H mice.

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EXPERIMENTS: A subcutaneous inoculation of 6 x 10^5 SCCVII cells in female C3H mice was established for 14 days. Thermal neutron beam irradiation (1MW, 70 min) was started from the time point of 120 min after the s.c. injection of BPA (250 mg/kg)-fructose. BNCT effects were evaluated on the basis of the changes in tumor volume. In order to estimate a combination effect on ATRA, the complex of ATRA/ β-1,3-glucan ([ATRA]=100 μM, 200 μL) was administered via the tail vein at the time points of 1, 5, 7 days after irradiation. Treated mice were sacrificed 8 days after thermal neutron beam irradiation, tumor tissues were removed, and frozen sections were prepared. The samples were observed using a microscope BZ-X800 (KEYENCE), and the percentage of positive cells within the tumor was analyzed using a BZ-X Analyzer.

****

RESULTS: In the combination experiment of BPA-BNCT with ATRA treatment, the combination treatment group effectively suppressed tumor volume increase compared to BPA alone. Additionally, tumor recurrence was observed in both groups from around day 20, however, the combined treatment group maintained a relatively low increase in tumor volume. As shown in Fig. 1, On day 8, the rate of iNOS-positive cells, which are M1 phenotype macrophages, were 4.93% and 0.58% in the ATRA/β-GC nanogel and BPA-BNCT combination treatment group and the BPA-BNCT alone treatment group, respectively.

These results suggest that the presence of a TAM polarity regulator during BNCT treatment affects the TME, increases M1-type TAMs, and sensitized the BPA-BNCT effect.

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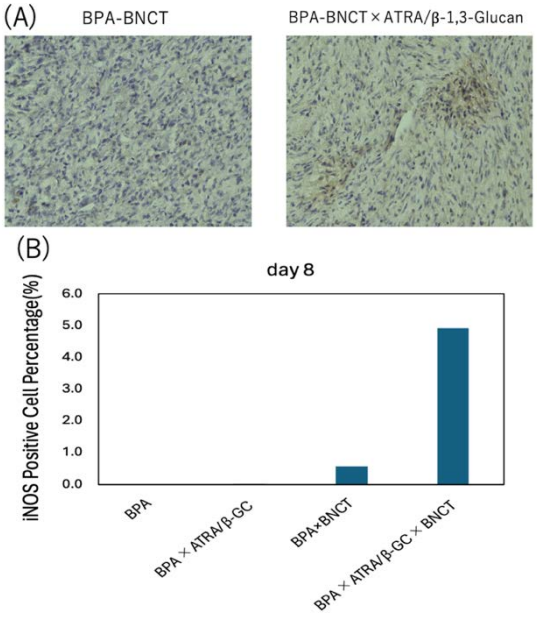

Fig. 1. Examples of immunostains used to identify M1 macrophages (A). Percentage of iNOS-positive cells that are M1 phenotype macrophages on day 8 (B).

REFERENCES:

[1] A. Sica *et al.*, J. Clin. Invest., **117** (2007) 1155.
[2] A. Alatshan *et al*., Cells, **9** (2020) 1591.

CO7-41

Synthesis and evaluation of a novel boron neutron capture therapy agent

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

INTRODUCTION: Neuroendocrine Tumor (NET) is known to be characterized by high expression of somatostatin receptors (SSTRs) on the tumor cell membrane. Somatostatin is a cyclic peptide discovered as a hypothalamic factor that potently inhibits growth hormone secretion from the pituitary gland. There are five subtypes of somatostatin receptors (SSTR1-5), and SSTR2 is highly expressed in NETs. Therefore, somatostatin analogs with high binding to SSTR2 are used for treatment[1) - 2)]. Furthermore, Peptide Receptor-mediated Radionuclide Therapy (PRRT), which uses somatostatin analogs as nuclear medicine drugs for NET patients, has recently been implemented in Europe, and LUTATHERA® was approved for manufacturing and marketing in Japan for the first time on June 23, 2021 as a drug for PRRT in Japan. However, there is a need for the development of new therapies with greater therapeutic efficacy. Therefore, in this study, we conducted a basic study on the potential of BNCT for NETs. In addition, we developed BNCT agents targeting EphA2, which is expressed in cancer. Erythropoietin-producing hepatocellular receptor A2 (EphA2) is overexpressed in cancer cells and causes abnormal cell proliferation[3)].

EXPERIMENTS: This year, since I was unable to conduct animal experiments due to transfer to another university, I focused on *in vitro* evaluations.
SSTR: BSH-conjugated somatostatin derivatives were synthesized. Binding of BSH-conjugated somatostatin derivatives to SSTR was evaluated using AR42J cells.
EphA2: BSH reacted with EphA2-230-1 antibody. BSH-conjugated EphA2-230-1 antibody was used to evaluate the proliferative potential of U87MG cells (human brain glioblastoma cells). After adding the drug to the cells, the cells were irradiated with neutrons at 5 MW for 3 minutes of 1 MW for 15 minutes.

RESULTS: *SSTR:* Binding of BSH-conjugated somatostatin derivatives to SSTR was evaluated using AR42J cells, and it was found that 30% of the amount of drug added was bound to the cells. Next year, we would like to evaluate cell killing by neutron irradiation.
EphA2: The number of BSH bound to the EphA2-230-1 antibody was evaluated using MALDI-TOF MS. As a result, 10 molecules of BSH were introduced into one molecule of EphA2-230-1antibody. Binding of BSH-conjugated EphA2-230-1 antibody to EphA2 was confirmed. The proliferative capacity of cancer cells was evaluated by colony formation assay. Although we have not completed all the analysis, we think that the irradiation conditions need to be optimized. In addition, we think that the cellular internalization rate of BSH-bound EphA2-230-1 antibodies should also be determined.

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[3] Pasquale E. B., Nat. Rev. Cancer, **10** (2010)165–180.

CO7-42

Estimation of Macro Leathal Distribution Around Tumor Mass in GdNCT

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

INTRODUCTION: Gadolinium neutron capture therapy is not necessarily unrealistic. For Gd neutron capture therapy (Gd-NCT) to be successful, sufficient amounts of Gd must be delivered and maintained within the tumor. In recent years, it has become possible to deliver and maintain in tumor high concentrations of Gd using nano micelles［1］.
Therefore, considering the distribution of the lethal effect of GdNCT is an important step for practical application, and is also of great radiobiological interest.
The peritumoral dose distribution of GdNCT is very complex depending on the tumor shape and the Gd concentration within the tumor［2］. In this experiment, we investigated the practicality of GdNCT using a simple model to investigate the distribution of lethal effects depending on dose near the tumor.

EXPERIMENTS and RESULTS:

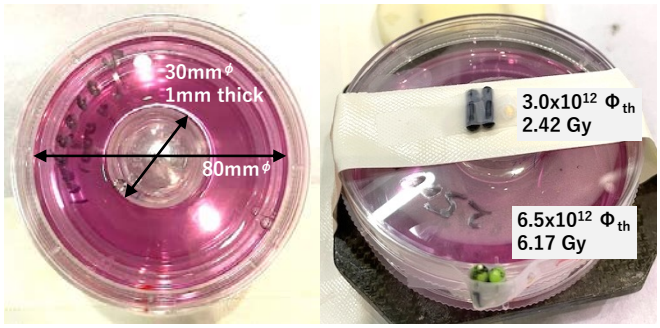

Fig.1 A model for estimating the lethal effect of tumor margins for GdNCT; Concentrical double structured petri dish.

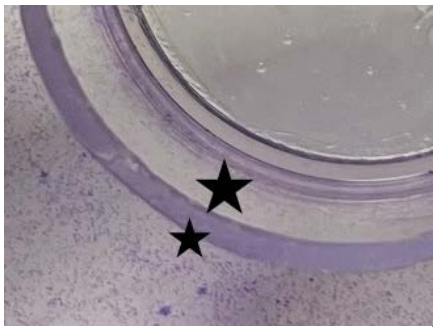

Fig.2 Paucity of surviving cells was observed at 2-3 mm rim (★) around the petri dish.

A gas-sterilized double-walled petri dish was created as shown in Fig.1(*left*). A 3 cm diameter Petri dish was fixed concentrically in the center of an 8 cm Petri dish. After filling the periphery with 1x10E5 cell suspension of U87 and allowing the periphery with 1x10E5 cell suspension of U87 and allowing the cells to adhere to the Petri dish in a usual incubator, a small Petri dish in the center was filled with a maximum concentration of 500 mmol/L of GdDTPA (approximately 12,285 ppm ^{157}Gd) and thermal neutrons were horizontally irradiated Thermal neutron fluence and Gy dose are shown to the *right*.
A high degree of cell death was observed within 2-3 mm rim of the Petri dish, and significant cell death was observed in the fur peripheral area in accordance with Gd concentration, which will lead to a safety indication of GdNCT. For detailed analysis, we will improve and conduct further experiments this year to confirm reproducibility and reevaluate.

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CO7-43

Establishment of innovative BNCT treatment method for intractable bladder cancer

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INTRODUCTION: Bladder cancer generally has a high survival rate, but it has a poor prognosis and tends to repeat recurrence, which accounts for 70% of cases. In order to verify this new boron neutron capture therapy for refractory bladder cancer, we will use cell lines and the mouse bladder orthotopic cancer model for the neutron irra-diation necessary for BNCT at Kyoto University's Institute for Integrated Radiation and Nuclear Science. The results obtained in this experiment are expected to expand the indications of BNCT and contribute to improving the results of bladder cancer treatment.

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EXPERIMENTS: We used human bladder cancer T24 cells, BCG-resistant T24 cells, and the human bladder transitional epithelial cell line hBMSC, and perform neutron irradiation after adding boron chemicals.
After irradiation, analyze cell viability using XTT.
Boron addition concentration is (0, 5, 25, 50, 100, 500)
Cells were cultured at 96W and analyzed for XTT for 72 hours after irradiation
(each concentration N>20)

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RESULTS: As shown in Fig. 1, The effects of BNCT on bladder cancer T24 cells and BCG-resistant T24 cells were dose- and time-dependent.
No effect in human bladder transitional epithelial cell line hBMSC at boron concentrations of less than 50ug/ml.

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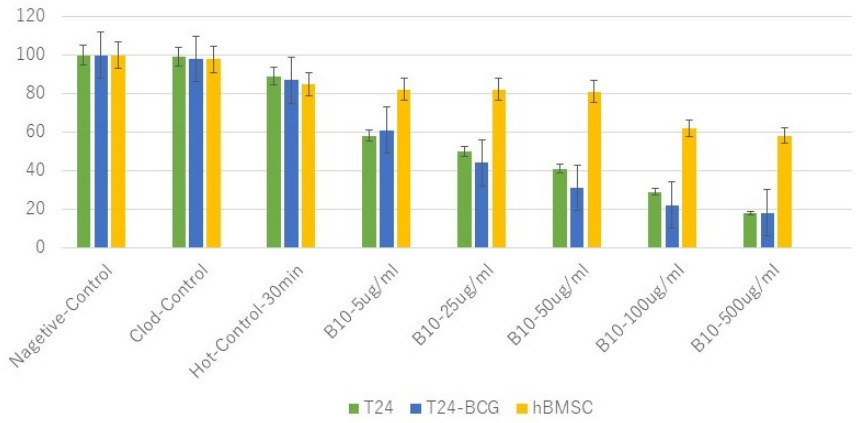

Fig. 1. The cell viability assay of 72 hours after Neutron irradiation.

Neutron irradiation for 30 minutes after Boron added for 3 hours; Boron addition concentration is (0, 5, 25, 50, 100, 500ug/ml) **(N>20)**

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CO7-44

Analysis of the Structural Change of Boronophenylalanine by Boron Neutron Capture Reaction

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INTRODUCTION:

Boron neutron capture therapy (BNCT) is one of the potent radiotherapies based on the combination of boron-containing molecules and epi-/thermal neutron irradiation. In this therapy, high linear energy (LET) particles, alpha (^{4}He) and lithium (^{7}Li) nucleus, are generated from boron-10 (^{10}B) atom due to the nuclear reaction between ^{10}B and neutrons. In 2020, L-4-boronophenylalanine (BPA) is approved as a BNCT agent for the treatment of recurrent head and neck cancer in Japan [1]. Although BPA have been used in BNCT research for decades, the effect of boron neutron capture reaction on the molecular structure of BPA is unclear. In this work, we attempted the initial studies to identify the structural change of BPA in aqueous solution after epi-/thermal neutron irradiation.

EXPERIMENTS and RESULTS

The aqueous solution of BPA was prepared by using ^{10}B-enriched BPA purchased from Inter-Pharma (Prague, Czech Republic). The boron concentration was determined by Prompt Gamma-ray Analysis (PGA) and/or Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using the same standard solutions (1, 2, 5, 10, 20, 50 ppm ^{10}B). Teflon tubes containing boron solution were irradiated with epi-/thermal neutrons using the Heavy Water Neutron Irradiation Facility of the Kyoto University Research Reactor (KUR) operated at 1 MW and/or 5 MW for several times. The activation rate of samples was confirmed by the measurement of gamma-ray from the irradiated samples, and the change of BPA was analyzed by some methods. In this study, a preliminary investigation of structural change analysis was carried out. However, the change of boron concentrations was not observed possibly due to deficiency of neutron irradiation. We need further studies and optimization of conditions such as sample preparation, irradiation time, and evaluation methods.

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