CO7-1

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

A Toji[1], S Terada[1], S Tsunetoh[1], T Tanaka[1], M Ohmichi[1], J Arima[2], K Taniguchi[2], M Suzuki[3]

[1]*Department of Obstetrics and Gynecology, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*
[2]*Department of general and gastroenterological surgery, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*
[3]*Institute for Integrated Radiation and Nuclear Science, Kyoto University, Asasiro-Nishi, Kumatori-cho, Japan*

INTRODUCTION: In Japan, approximately 10,000 women develop cervical 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 carcinoma (SCC) is the most common histological type at 80%, and adenocarcinoma (Adeno) accounts for about 20%.The main treatment options for cervical cancer are surgery or radiation therapy. However, local and surface lymph nodes recurrence is not uncommon. Thus, new treatment modalities for cervical cancer are needed. In this 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 Chemiphar (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 patients themselves. [1, 2] Treatment was initiated 4-6 weeks after tumor with matrigel injection into the thighs of mice. Mice were divided into hot control (neutron ir-radiation only) and BNCT (peritoneal BPA followed by neutron irradiation) groups. 2.5 hours before neutron irra-diation, 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 and Fig.2 show that the tumor volume in the hot control and BNCT groups for cervical squamous cell carcinoma and adenocarcinoma PDX model. The tumor was suppressed in the BNCT group than in the hot control group ($P<0.05$). No adverse effects were observed in hot control and BNCT groups after irradiation. The body weight was no remarkable change in the both groups.

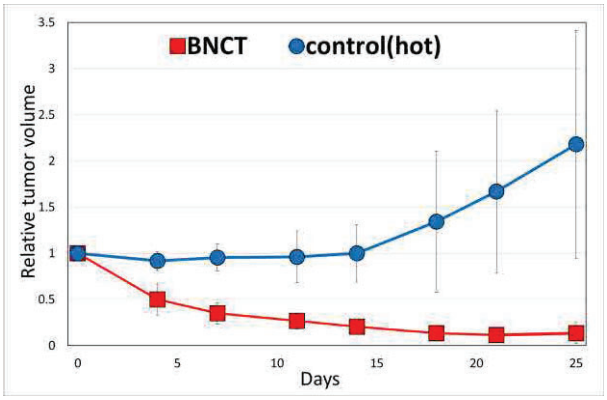


Fig.1 Antitumor effect on subcutaneous PDX (scc) tumor model.
Tumor grows curves in the hot control (irradiation only) and BNCT (irradiation after BPA administration) groups (n=6 in each group).

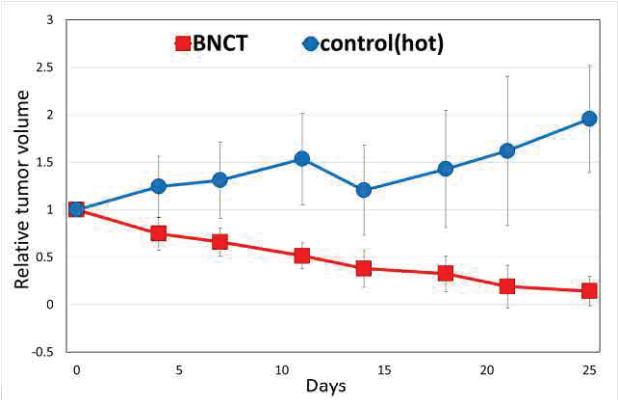


Fig.2 Antitumor effect on subcutaneous PDX (adeno) tumor model.
Tumor grows curves in the hot control (irradiation only) and BNCT (irradiation after BPA administration) groups (n=9 in each group).

REFERENCES:

[1] Shahrokh Abdolahi *et al.*, J. Transl. Med., **20(1)** (2022) 206.
[2] Miyamoto S. *et al.*, Cancers, **14 (12)** (2022) 2969.

CO7-2

The effect of boron neutron capture therapy (BNCT to gastrointestinal stromal tumor cell line GIST-T1

S. Hagihara[1], J. Arima [1], K. Taniguchi[1,2], Y. Inomata[1], A. Miyamoto[1], M. Suzuki[3], and Sang-Woong Lee [1]

Graduate School of Science, Kyoto University
[1]*Department of Physics, Niigata University*
[2]*Accelerator Laboratory, KEK*
[3]*Faculty of Engineering, Toyama University*
[4]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: Gastrointestinal stromal tumors (GIST) are the most fre-quent soft-tissue sarcomas substantially arising from the gastrointestinal tract. Surgery is the first choice of treat-ment 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 re-sistance 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 caused 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 for 2h at 37 ◦C in 5% atmospheric CO2. In vivo study, GIST-T1 cells were concentrated to 2.0×107/100μL in 0.7ml of PBS and 0.3ml of Matrigel and injected into the right leg of each mouse. Animals were divided into three groups; the cold control (no treatment, no neutron irradi-ation), hot control (neutron irradiation only), and BNCT (intraperitoneal BPA administration and neutron irradia-tion) groups.

RESULTS: In vivo study, **Fig.1** showed that tumor volume (mm^3) significantly reduced in the BNCT group compared with that in the cold and hot control groups. In GIST tumors 1h after BNCT, the expression levels of γH2AX significantly increased in the BNCT group. Moreover, in tumor samples collected 72 h after irradiation, the expression levels of cleaved PARP, cleaved caspase-3 and cleaved caspase-8, which are markers of apoptosis, significantly increased in the BNCT group (**Fig.2A, B)** These results indicate that BNCT induces tumor apoptosis through severe DNA damage.

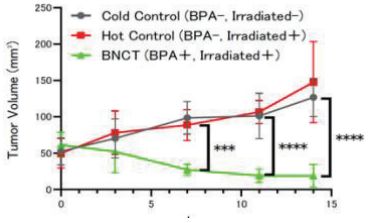


Fig.1 Tumor growth curves of each group after neutron irradiation. (n = 6 for the cold control group; n = 5 for the hot control group; and n = 7 for the BNCT group. Data are presented as the mean ± SD. ***P < 0.001 and ****P ＜ 0.0001).

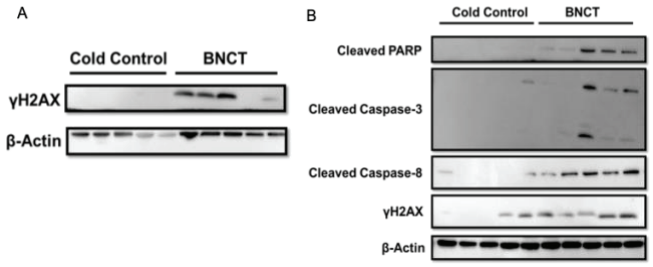


Fig.2 (a) γH2AX protein expression in the cold control (CC) and BNCT groups 1 h after irradiation, as determined by western blotting.(b) Cleaved PARP, Caspase-3, Caspase-8, and γH2AX protein expression levels in the CC and BNCT groups 72 h after irradiation, as determined via western blotting.

****

REFERENCES:

[1] Makiko Sasaki *et al.*, PLOS ONE, Apri 7, 2021 (doi)10.1371/journal.pone.0249650.

CO7-3

The effect of boron neutron capture therapy (BNCT to liver metastasis of colorectal cancer

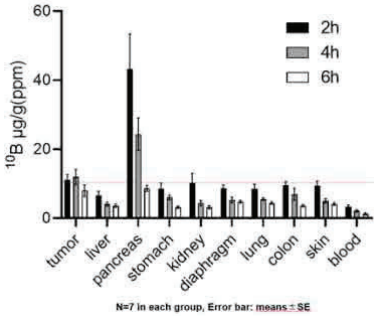
Y. Ueda[1], J. Arima [1], K. Taniguchi[1,2], S. Hagihara[1], Y. Inomata[1], N. Kawaguchi [1], M. Suzuki[3], and Sang-Woong Lee [1]

[1] *Department of General and Gastroenterological Surgery, Osaka Medical and Pharmaceutical College, Osaka, Japan*
[2] *Translational Research Program, Osaka Medical and Pharmaceutical College, Osaka, Japan*
[3] *Department of Particle Radiation Oncology, Research Reactor Institute, Kyoto University, Kumatori, Osaka, Japan.*

INTRODUCTION: Management of liver metastases in colorectal cancer is a clinically important issue. However, only 10–15% of patients are eligible for surgery based on the size and number of metastatic lesions. Furthermore, for patients with multifocal, unresectable, or bilateral liver metastases who do not respond to chemotherapy, palliative care is the only option available. In this context, the development and evaluation of new treatment strategies are justified. In this study, we investigated the effectiveness of boron neutron capture therapy (BNCT) to liver metastasis of colorectal cancer using the mouse model.

EXPERIMENTS: We used Boronophenylalanine (BPA) as a boron compound. Also, we used seven-week-old female BALB/cCrSlc mouse having physiological environment of immunity. Firstly, we established a mouse model of liver metastasis of CRC using DLD-1-Luc cells concentrated to 1.0×10^6/100μL in 0.1ml of PBS. The boron concentrations in DLD-1-Luc tumors or surrounding organs at 2h, 4h, 6h after 500mg/kg BPA administration intraperitoneally. (**Figure.1**) According to this result, we considered the effects on the pancreas and decided to inject BPA intraperitoneally at 6h before irradiation. In BNCT study, animals were divided into three groups; the cold control (no treatment, no neutron irradiation), hot control (neutron irradiation only), and BNCT (intraperitoneal BPA administration and neutron irradiation) groups.

Figure.1 Biodistribution



RESULTS: In the BNCT group, transient weight loss was observed shortly after neutron irradiation, but no significant differences were observed among the groups at the endpoint. (**Figure 2)**
Tumor weight (g) in the BNCT group was significantly reduced compared to the cold control group and showed a decreasing trend compared to the hot control group. Additionally, tumor volume (mm³) in the BNCT group showed a decreasing trend compared to the other two groups, although no significant difference was observed. (**Figure 3)**
No severe adverse effects, including death, were observed in each of the groups.

Figure.2 Body weight

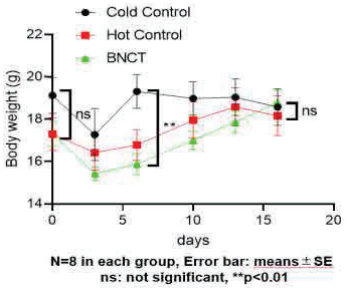
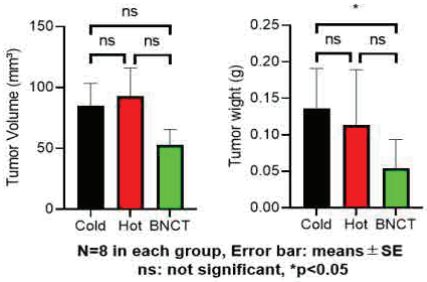


Figure.3 Tumor volume and weight



Ongoing study:
Neutron irradiation will be performed 4 hours after BPA administration to investigate the effects on surrounding organs such as the liver and pancreas.
This study will be continued, and the results will be reported in the future.

REFERENCES:
[1] Wittig A. *et al.*, J Cell Mol Med., Aug 13 (2009) (doi)10.1111/j.1582-4934.2009.00856.
[2] Jun Arima *et al.*, Biomed. Pharmacother., 154 (2022) (doi)10.1016j.biopha.2022.113632.

CO7-4

Development of a Therapeutic Agent for Bone Metastases with Nuclear Imaging and Boron Neutron Capture Therapy

K. Ogawa[1], Y. Akita, K. Tamamura and M. Suzuki[2]

Graduate School of Medical Sciences, Kanazawa University
[1]Institute for Frontier Science Initiative, Kanazawa University
[2]Institute for Integrated Radiation and Nuclear Science, Kyoto University

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 chemical 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), 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]. Here, Ga-DOTA-K(ε-closo-dodecaborate)D_{11} (**1**) (Fig. 1), containing closo-dodecaborate ($[B_{12}H_{12}]^{2-}$), [$^{67/68}$Ga]Ga-DOTA, and an aspartic acid peptide as a carrier molecule to bone metastases sites binding to hydroxyapatite [2] was synthesized and evaluated for theranostics of bone metastases. Although we aimed to develop a ^{68}Ga-labeled agent, we used ^{67}Ga ($T_{1/2}$: 3.3 days), which has a long half-life and is easy to handle, and synthesized and evaluated [^{67}Ga]**1**.

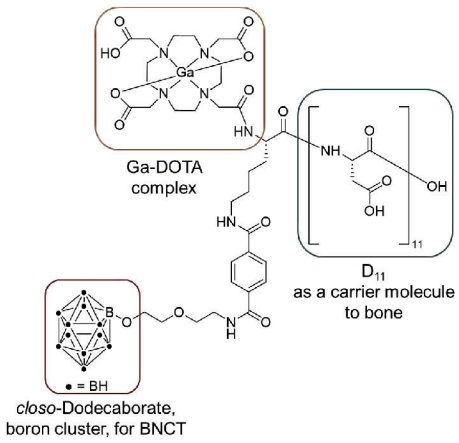


Fig 1. Chemical structure of **1**.

EXPERIMENTS: DOTA-KD_{11} was synthesized by the method of solid-phase peptide synthesis, and the precursor DOTA-K(ε-closo-dodecaborate)D_{11} was synthesized by condensation reaction between DOTA-KD_{11} and N-hydroxysuccinimide ester of closo-dodecaborate-conjugated terephthalic acid. [^{67}Ga]**1** was prepared with 91% radiochemical conversion and over 95% radiochemical purity after HPLC purification. Hydroxyapatite (HA) binding assay, biodistribution experiments in normal mice, and SPECT imaging of [^{67}Ga]**1** in a normal mouse were performed. In addition, boron accumulation in major organs after injection of DOTA-K(ε-closo-dodecaborate)D_{11} was determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

RESULTS: In the HA binding assay, the binding ratio of [^{67}Ga]**1** increased in a HA concentration-dependent manner. In the biodistribution experiments, [^{67}Ga]**1** showed high accumulation in bone and low accumulation in non-target tissues except the kidney. SPECT imaging of [^{67}Ga]**1** visualized the bone joint. Meanwhile, the boron distribution in normal mice after injection of DOTA-K(ε-closo-dodecaborate)D_{11} determined by ICP-OES analysis was equivalent to that of the distribution of radioactivity after injection of [^{67}Ga]**1**.
These results indicate that a combination of PET or SPECT of [$^{67/68}$Ga]**1** and BNCT with **1** could be promising for cancer theranostics.

REFERENCES:
[1] K. Mishiro *et al.,* J. Med. Chem., **65(24)** (2022) 16741-16753.
[2] K. Ogawa *et al.,* PLoS One, **8(12)** (2013) e84335.

CO7-5

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

Y. Tamari[1], R. Saba[2], T. Takata[3], M. Suzuki[3], H. Yamazaki[2]

[1]*Department of Medical Information Technology, School of Medical Scinence, Fujita Health University*
[2]*Department of Radiology, Kyoto Prefectural University of Medicine*
[3]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

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 extracel-lular 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 concentra-tion ratio (T/N ratio) between tumor and normal tissues has become an issue. In this study, we at-tempted to improve the therapeutic effect of BNCT by restricting Phe and improving L-BPA up-take.

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 carcino-ma-bearing mice were fed Phe-free feed, and 500 mg/kg of L-BPA was administered after 24 hours. Carcinoma-bearing mice were fed Phe-free feed for 24 hours, and 2 hours after admin-istration 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 tu-mor size were regularly measured.

RESULTS: A mouse tumor model irradiated for 6 minutes at 5 MW in a heavy water facility showed a decrease in tumor size in the neutron-only group compared to the non-irradiated group. Tumor size was further reduced in the BNCT (Phe+) and BNCT (Phe-) groups and was significantly reduced with BNCT (Phe-) compared to BNCT (Phe+). There was no significant decrease in mouse body weight. we plan to investigate the contribution of other amino acid depletion and preload to L-BPA uptake.

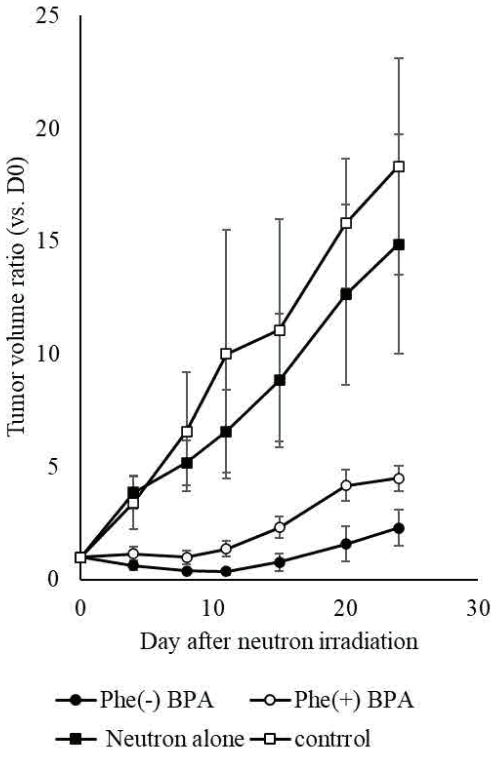


Fig. 1. Change over time in tumor size ratio after Phe-restricted BNCT.

REFERENCES:
[1] A. Wittig *et al.,* Radiat Res., **153** (2000) 173-180.
[2] P. Wongthai *et al.,* Cancer Sci., **106** (2015) 279-286.

CO7-6

Basic research on new BNCT strategies for melanoma

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

[1]*Neutron Therapy Research Center, Okayama University; 2-5-1, Shikata-cho, Kita-ku, Okayama City, Okayama, Japan.*
[2]*Department of Gastroenterological Surgery, Okayama University; 2-5-1, Shikata-cho, Kita-ku, Okayama City, Okayama, Japan.*

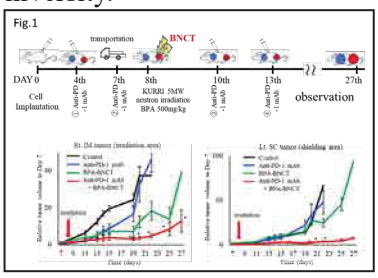
[3]*Institute for Integrated Radiation and Nuclear Science, Kyoto University, 2-1010, Asashiro-Nishi, Kumatori-cho, Sennan-gun, Osaka, Japan.*

INTRODUCTION: The starting point for melanin synthesis in the body is aromatic amino acids such as phenylalanine and tyrosine, and melanin synthesis is promoted in skin malignancies such as malignant melanoma. Boron-substituted phenylalanine (BPA), which contains boron atoms bound to these amino acids, has been developed as a boron-based drug targeting malignant melanoma. Its efficacy was demonstrated in a 1989 Lancet paper by Mishima et al.: "Treatment of malignant melanoma with single-dose thermal neutron capture therapy: Single-dose thermal neutron capture therapy using 10B compounds targeting malignant melanoma" was published in The Lancet in 1989 by Mishima et al. This clinical study demonstrated the efficacy of BNCT using boron-based drugs characterized by cell-specific uptake and neutron irradiation of tumor tissue.While there have been reports of BNCT for skin malignant tumors such as localized melanoma, we have undertaken a project to evaluate the efficacy of BNCT in combination with immune checkpoint inhibitors to establish its applicability for advanced cancers with lesions outside the irradiation field. This is a challenge to explore new possibilities for BNCT, which has developed as a local therapy.

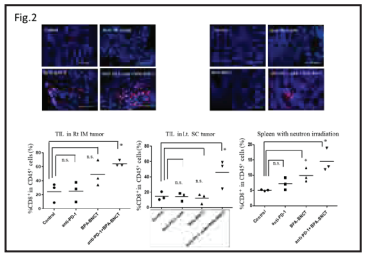
****

EXPERIMENTS: We purchased the B16-F10 mouse melanoma cell line to create a melanoma model, Mouse anti-PD-1 was intraperitoneally administrated on day 4 or 7 after cell implantation. On the following day, mice were anesthetized, and 500 mg/kg BPA was subcutaneously injected. After 1 h, 5 MW neutron irradiation was performed for 12 min. At day 10 and 13 after implantation, mouse anti-PD1 was intraperitoneally administrated on day 3, 5, 9, and 13. Animal experiments were performed after strict approval by the ethics committees for animal experiments at Okayama University and Kyoto University.

.**********



RESULTS: Ex1) We used BPA as an effective boron agent for Pharmacokinetic (PK) evaluation of BPA was performed in a mouse model of advanced-stage melanoma. To establish the model, B16F10 melanoma cells were transplanted intramuscularly in the right thigh and subcutaneously in the left flank. BPA (500 mg/kg) was then administered intraperitoneally or subcutaneously. Subcutaneous administration of BPA resulted in approximately 50 ppm 10B in the right tumor and 12.7 ppm 10B in the left tumor after 1 h. Next, BNCT with immunotherapy (B-NIT) was administered to mice modeling advanced-stage melanoma (Figure 2A). At the neutron-irradiated site, tumor volume ratios on day 22 were 37.6 (control group), 46.4 (anti-PD-1 mAb group), 18.4 (BNCT group), and 3.7 (B-NIT group) (Fig.1). On the other hand, the tumor volume ratio of the shielded remote site was 66.6 (control group), 47.8 (anti-PD-1 mAb group), 24.7 (BNCT group), and 3.9 (B-NIT group), the immune effect was confirmed (Fig.1).



Ex2) In the tumor tissue of the right thigh (irradiation site), a high density of CD8+ T cells was identified in the BNCT and B-NIT on day 22 (Fig.2). Analysis of TILs in the left subcutaneous tumors showed high levels of CD8+ T cells in the B-NIT group compared with other groups (control: 14.4%, anti-PD-1 mAb: 14.1%, BPA-BNCT: 12.1%, B-NIT: 45.6%, Figure 4D). Immunostaining also showed strong intratumor localization of CD8+ cells in the B-NIT (Fig.2).

****

REFERENCES: [1] T. Fujimoto *et al.,* Cancer Sci., **115(10)** (2024) 3231-3247.

CO7-7

Cytosolic Delivery Technology Using Cationic Lipids in BNCT

S. Hirase[1], Y. Araki[1], A. Aoki[1], Y. Hattori[2], M. Kirihata[2], I. Nakase[1]

[1]*Graduate School of Science, Osaka Metropolitan University*
[2]*Research Center for BNCT, Osaka Metropolitan University*

INTRODUCTION: Boron neutron capture therapy (BNCT) is a therapy in which ^{10}B boron is introduced into the target cancer cells, and ^{10}B atoms are transmuted by thermal neutron irradiation, generating α particles and Li recoil nuclei, leading to cell death. BNCT is currently recognized as highly effective in targeting refractory cancers such as brain tumors and head and neck cancer [1]. The ^{10}B compounds such as dodecaborate (BSH) are used in BNCT, however, high concentrations of the boron compounds must be administered continuously to achieve therapeutic effects with current technology (high concentrations of several mM are required in in vitro experiments), and the urgent issue is to improve technology to increase intracellular introduction efficiency and retention rate. In this study, in order to enhance the intracellular delivery of BSH with polyhedral borane anion structure, we evaluate the cellular uptake and BNCT activity using cationic lipids [2] and arginine-rich cell-penetrating peptides (CPPs) [3].

EXPERIMENTS: Lipofectamine LTX as cationic lipid and hexadeca oligoarginine (R16) synthesized by Fmoc-solid phase method were used in the experiments. Fluorescence-labeled BSH (FITC-BSH) was used, and cellular uptake was evaluated by confocal laser microscope and flow cytometer. BNCT activity was examined by irradiating rat C6 glioma cells with thermal neutron beams (Institute for Integrated Radiation and Nuclear Science, Kyoto University) for cancer cell killing activity after internalization of the BSH by the cells.

RESULTS: Mixing FITC-BSH with Lipofectamine LTX markedly increased cellular uptake in C6 glioma cells, and confocal laser microscopy confirmed cytosolic release and nuclear accumulation of the FITC-BSH. In the case of mixing FITC-BSH with R16, the efficacy of cellular uptake was also enhanced, however, only endosomal fluorescent signals were observed in the cells. In the absence of thermal neutron irradiation, no cytotoxicity was observed under each mixing condition. After FITC-BSH was internalized by C6 glioma cells, the cells were irradiated with thermal neutron, and a colony assay was performed. The results showed that a mixture of FITC-BSH and Lipofectamine LTX has superior cancer cell-killing activity. On the other hand, it was also found that when FITC-BSH and R16 were mixed, thermal neutron irradiation induced very low cancer cell-killing activity. The results show that cationic lipids are suitable for intracellular delivery of BSH, and that BSH can be efficiently delivered to the cell nucleus by mixing with lipids, and that its subcellular localization greatly affects the cancer cell-killing activity induced by thermal neutron irradiation [4].

REFERENCES:

[1] Y. Hattori *et al.,* J. Radiat. Res., **64** (2023) 859−869.
[2] S. Futaki, I. Nakase, Acc. Chem. Res., **50** (2017) 2449−2456.
[3] I. Nakase, S. Futaki, Sci. Rep., **5** (2015) 10113.
[4] S. Hirase *et al.,* Biol. Pharm. Bull., **48** (2025) 344−354.

CO7-8

Neutron irradiation experiments using a novel BPA formulation based on ionic liquids

M. Shirakawa[1], K. Banshoya[2], A. Machida[2], M. Ohnishi[2], T. Takata[3], N. Kondo[3], Y. Sakurai[3], T. Hanafusa[1], K. Igawa[1], H. Michiue[1] and M. Suzuki[3]

[1] *Neutron Therapy Research Center, Okayama University*
[2] *Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University*
[3] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: L-p-boronophenylalanine (BPA) exhibits strong antitumor activity following thermal neutron exposure; however, its limited solubility necessitates high dosing to attain therapeutic outcomes. To address this challenge, our research has focused on the application of ionic liquids (ILs), which we have previously explored and reported [1, 2]. In the present study, we synthesized a novel formulation that has the same or better potential as IL and reported its in vivo antitumor effect after thermal neutron irradiation.

EXPERIMENTS: Three-week-old female BALB/cA mice were obtained from CLEA Japan Inc. (Tokyo, Japan). To establish the tumor model, 2×10^6 murine colon carcinoma CT26 cells were subcutaneously inoculated into the right thigh of 4-week-old mice (body weight: 16–20 g), resulting in tumors measuring 6–8 mm in diameter.
Ten days after inoculation, a novel BPA formulation (MS01; 20 μL, equivalent to 14 mg ^{10}B/kg) was administered intravenously prior to neutron irradiation. For comparison, a BPA–sorbitol complex (BPA-Sor) was administered intravenously at a volume of 200 μL. In a separate group, MS01 (200 μL, equivalent to 140 mg ^{10}B/kg) was also administered intravenously.
Two hours after administration, thermal neutron irradiation was performed for 12 minutes at a flux of 5.5–6.1 $\times 10^9$ neutrons/cm^2/s. Tumor growth was monitored until day 26 post-irradiation, and tumor volumes were calculated using a previously described formula [3].

RESULTS: MS01 suppressed tumor growth to a level comparable to that achieved with BPA-Sor, despite being administered in a substantially smaller volume. Furthermore, in the group that received MS01 at the same volume as the BPA-Sor group, a more pronounced antitumor effect was observed, including cases of complete tumor regression.
In addition, no significant adverse effects, such as body weight loss, were observed following the administration of either MS01 or BPA-Sor.

REFERENCES:
[1] M. Shirakawa *et al.,* Patent No. 7525094.
[2] M. Shirakawa *et al.,* Patent No. 7525095.
[3] M. Shirakawa *et. al.,* KURNS PROGRESS REPORT 2021 (2022) 208.

CO7-9

Investigation of boron drug using albumin-binding polymer modified with boron clusters

K. Banshoya[1], A. Machida[1], Y. Kaneo[1], T. Tanaka[1], T. Takata[2], N. Kondo[2], Y. Sakurai[2], M. Suzuki[2], M. Ohnishi[1] and M. Shirakawa[3]

[1] *Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University*
[2] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[3] *Neutron Therapy Research Center, Okayama University*

INTRODUCTION: Boron neutron capture therapy (BNCT) is theoretically a cell-selective radiotherapy. The anti-tumor effect of BNCT depends on the accumulated concentration of non-radioactive boron isotopes in tumor cells. We developed a new polymerized boron drug, BSH-AB polymer, by combining mercaptoundecahydrododecaborate (BSH) with a serum albumin-binding polymer (AB polymer), which targets malignant tissues via the EPR effect. This study examines the anti-tumor effects of BSH-AB polymers.

EXPERIMENTS: The BSH-AB polymer was synthesized by reacting the thiol group of BSH with the carboxyl group of the AB polymer using a condensation agent. Unreacted BSH was removed by dialysis against distilled water. After dialysis, the BSH-AB polymer was obtained as a powder by lyophilization. To evaluate the antitumor effect, an experiment was conducted using BALB/c mice bearing subcutaneous xenografts of the CT26 mouse colon carcinoma cell line. A dose of 7 mg ^{10}B/kg of BSH-AB polymer was administered via intravenous injection prior to neutron irradiation. Twenty-four hours after injection, thermal neutron irradiation was performed for 12 minutes at a flux of 5.0-5.7 × 10^9 neutrons/cm^2/s. Tumor size was monitored over time following irradiation, and tumor volume was calculated using a previously established formula, continuing until day 24.

RESULTS: As shown in Figure 1, compared to the irradiate-only group, tumor growth tended to be suppressed when thermal neutron irradiation was performed 24 hours after BSH-AB polymer administration. However, this antitumor effect was less pronounced than that observed with the BPA–sorbitol complex administered 2 hours before irradiation. Further research is needed to optimize the timing of thermal neutron irradiation. As shown in Figure 2, no significant side effects (e.g., weight loss) were observed after using BSH-AB polymer, similar to BPA-sorbitol complex.

REFERENCES:

[1] K. Maruyama *et al.,* J. Controlled. Release. **98** (2004) 195-207.

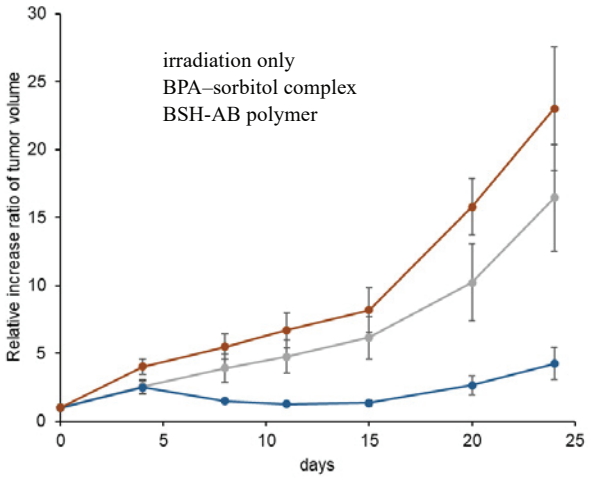


Fig. 1. BNCT effects of BSH-AB polymer.

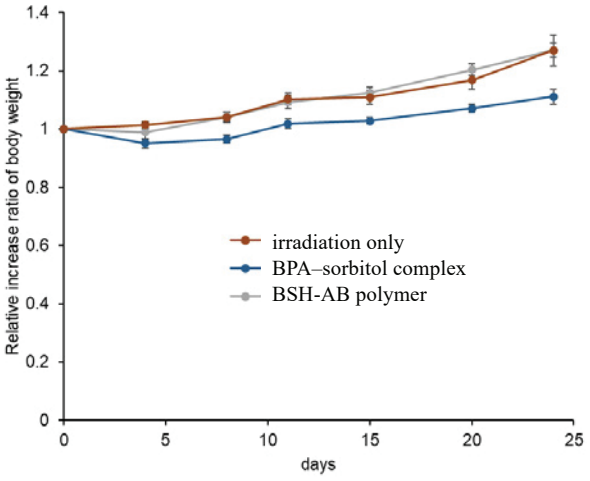


Fig. 2. The ratio of post-BNCT weight changes.

CO7-10

Development of boron rich nanostructure with amphiphilic block polymers as boron agents for BNCT

R. Kawasaki,[1] A. Oshige,[1] K. Yamana,[1] Y. Miura,[1] T. Saito,[1] Y. Sanada,[2] Y. Sakurai,[2] H. Tanaka,[2] M. Suzuki,[2] M. Y. Hattori,[3] M. Kirihata,[3] T. Nishimura,[4] N. Miyamoto,[5] T. Nagasaki,[6] and A. Ikeda[1]

[1]*Applied Chemistry Program, Department of Advanced Science and Engineering, Graduate School of Advanced Science and Engineering, Hiroshima University*

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

[3]*Research center for BNCT, Osaka Metropolitan University*

[4]*Department of Chemistry and Materials, Faculty of Textile Science, Shinshu University*

[5]*Faculty of Engineering, Aichi Institute of Technology*

[6]*Department of Orthopedic Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University*

[7]*Department of Applied Chemistry and Bioengineering, Graduate School of Engineering, Osaka Metropolitan University*

INTRODUCTION: Amphiphilic block polymers which possess both hydrophilic and hydrophobic segments in a polymer spontaneously formulate supramolecular nanostructure including polymer micelles and polymer vesicles via self-assembly in aqueous media. These submicron-sized assembly can trap large amounts of pharmaceuticals within their polymer matrixes and their size are advantageous in enhancing deliverability of pharmaceuticals to tumor tissue via EPR effect. In addition, the easiness to fabricate functionality including imaging, targeting, and controlled release properties encouraged to use these nanosystems as drug delivery systems [1]. We designed and synthesized amphiphilic block polymers comprising phenylboronic ester groups as hydrophobic segment and demonstrated the performance of the nanosystem as boron agents for BNCT. Herein, the hydrophobic core or membrane-like structure consisting large amounts of boron atom are advantageous in enhancing the efficiency of boron neutron capture reaction, which can contribute to improve therapeutic efficacy of BNCT.

RESULTS AND DISCUSSION: The amphiphilic polymers comprising phenylboronic ester groups were synthesized by RAFT polymerization using activated polyethylene glycol as a RAFT initiator. To address the effects of polymerization degree of phenylboronic ester group containing monomers to the structure of nanoassembly, we prepared the series of polymers with varying the polymerization degree of the monomers. Both systems formulated nanoassembly with a diameter of 80-150 nm within aqueous media and spherical morphology were found by transmission electron microscopy. To clarify the structure, we measured small angle X-ray scattering for the polymers. Scattering profiles of nanoassembly using polymers with low and high polymerization degree exhibited representative peaks found in polymer vesicles and polymer micelles, respectively.

We next investigated the performance of polymer micelles and polymer vesicles as boron agents for BNCT and L-BPA-sorbitol complex, which is used as clinical drugs, were employed as control. As a result, our polymer systems exhibited higher BNCT activity than L-BPA-sorbitol complex toward SCC VII. For these results, our systems are potentially applicable as boron delivery system for BNCT.

REFERENCES:

[1] W. Y. Fu *et al.,* Biomacromolecules, **25** (2024) 4215-4232.

CO7-11

Development of a Water-Soluble Small Molecule Boron Carrier Targeting Biotin Receptors for Neutron Capture Therapy

H. Nakamura K. Nishimura[1], S. Tanaka[1], K. Miura, S. Okada, M. Suzuki[2]

Laboratory for Chemistry and Life Science, Institute of Integrated Research, Institute of Science Tokyo
[1] School of Life Science and Technology, Institute of Science Tokyo
[2] Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) has emerged as a promising non-invasive radiotherapeutic modality for the treatment of cancer. 4-Borono-L-phenylalanine (BPA) is known to preferentially accumulate in tumor cells via the L-type amino acid transporter 1 (LAT1). However, the therapeutic efficacy of BPA-based BNCT is limited in tumors with low LAT1 expression, due to its dependency on this transporter. Therefore, the development of novel boron delivery agents, independent of LAT1 expression, is essential for expanding the clinical applicability and efficacy of BNCT. We recently developed a novel boron carrier, pteroyl-closo-dodecaborate-conjugated 4-(p-iodophenyl) butyric acid (PBC-IP) [1]. PBC-IP demonstrated selective uptake by glioma cells at levels 10–20 times higher than those of BPA and exhibited excellent BNCT efficacy in a glioblastoma xenograft mouse model. In this study, we designed a biotinyl-closo-dodecaborate conjugate with an iodophenyl moiety (BBC-IP) [2]. Biotin receptors (BRs) are known to be overexpressed in a variety of cancer cells.

EXPERIMENTS: BBC-IP was designed and synthesized. Tumor-bearing mice (female, 5-6 weeks old) were prepared by injecting subcutaneously (s.c.) a suspension of human head and neck squamous cell carcinoma SAS cells. The tumor-bearing mice were injected i.v. with 200 µL of BBC-IP and BPA in PBS at a 25 mg [^{10}B]/kg dose. At 3 h after injection, the tumors of mice were irradiated with neutrons in the nuclear reactor at a dose range of 3.0–4.2 × 10^{12} neutrons/cm^2. The tumor volume and body weight of the mice were measured after neutron irradiation.

RESULTS: Biodistribution studies revealed that BBC-IP achieved enhanced tumor accumulation (9.7 µg [B]/g, 3 h) in mouse colon tumors, surpassing BPA's accumulation levels (7.2 µg [B]/g, 3 h) at a dose of 15 mg [B]/kg. In contrast, BPA showed significantly higher antitumor efficacy than BBC-IP-HSA (Fig.1A). This observation suggested one possibility that the observed antitumor effects can be attributed to differences in intracellular localization between BBC-IP and BPA. Body weight remained stable across the hot control, BBC-IP, and BPA groups, suggesting minimal systemic toxicity (Fig. 1B).

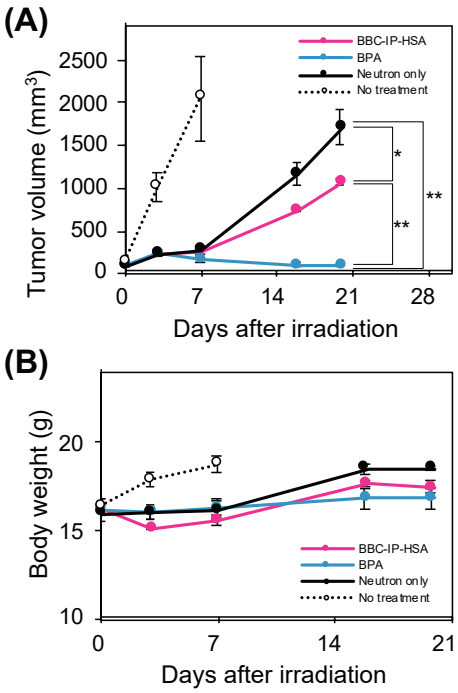


Fig. 1. Anti-tumor effects of BNCT in CT26 tumor-bearing mice. (A) Tumor volume in mice after BNCT with BBC-IP and BPA (25 mg [^{10}B]/kg i.v.). Data are expressed as mean ± SD (n = 4–6). Significance was determined as *p < 0.05 and **p < 0.0001. (B) Body weight changes of mice after BNCT.

REFERENCES:

[1] K. Nishimura *et al.,* J. Control. Release, **360** (2023) 249–259.
[2] K. Nishimura *et al.,* ACS Omega, **9** (2024) 51631−51640.

CO7-12

BNCT Effect of PBC-IP on Head and Neck Squamous Cell Carcinoma Mouse Model

K. Miura, K. Nishimura[1], M. Suzuki[2], H. Nakamura

Laboratory for Chemistry and Life Science, Institute of Integrated Research, Institute of Science Tokyo
[1]School of Life Science and Technology, Institute of Science Tokyo
[2]Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) has emerged as a promising non-invasive radiotherapeutic modality for the treatment of cancer. 4-Borono-L-phenylalanine (BPA) is known to preferentially accumulate in tumor cells via the L-type amino acid transporter 1 (LAT1). However, the therapeutic efficacy of BPA-based BNCT is limited in tumors with low LAT1 expression, due to its dependency on this transporter. Therefore, the development of novel boron delivery agents, independent of LAT1 expression, is essential for expanding the clinical applicability and efficacy of BNCT. We recently developed a novel boron carrier, pteroyl-closo-dodecaborate-conjugated 4-(p-iodophenyl) butyric acid (PBC-IP) [1]. PBC-IP demonstrated selective uptake by glioma cells at levels 10–20 times higher than those of BPA and exhibited excellent BNCT efficacy in a glioblastoma xenograft mouse model. Nevertheless, it re-mains to be clarified whether PBC-IP can also be selectively taken up by other cancer cell lines and retain its therapeutic efficacy in BNCT. In this study, we investigated the potential of PBC-IP for the treatment of head and neck cancer, with the aim of expanding its therapeutic applicability. **EXPERIMENTS:** PBC-IP was designed and synthesized according to the previous report [2]. Tumor-bearing mice (female, 5-6 weeks old) were prepared by injecting subcutaneously (s.c.) a suspension of human head and neck squamous cell carcinoma SAS cells. The tumor-bearing mice were injected i.v. with 200 μL of PBC-IP and BPA in PBS at a 25 mg [^{10}B]/kg dose. At 3 h after in-jection, the tumors of mice were irradiated with neutrons in
the nuclear reactor at a dose range of 3.0–4.2 × 10^{12} neutrons/cm^2. The tumor volume and body weight of the mice were measured after neutron irradiation.

RESULTS: The therapeutic efficacy of BNCT was evaluated by monitoring tumor volume and body weight following thermal neutron irradiation. Significant tumor suppression was observed in mice treated with either PBC-IP or BPA, whereas rapid tumor progression occurred in both cold and hot control groups. BPA exhibited a more pronounced anti-tumor effect than PBC-IP. Body weight remained stable across the hot control, PBC-IP, and BPA groups, suggesting minimal systemic toxicity [2].
In conclusion, although PBC-IP demonstrates potential as an alternative boron carrier for BNCT, particularly in FR-expressing tumors, BPA remains the preferred agent for BNCT in head and neck cancer due to its higher tumor accumulation and superior therapeutic efficacy.

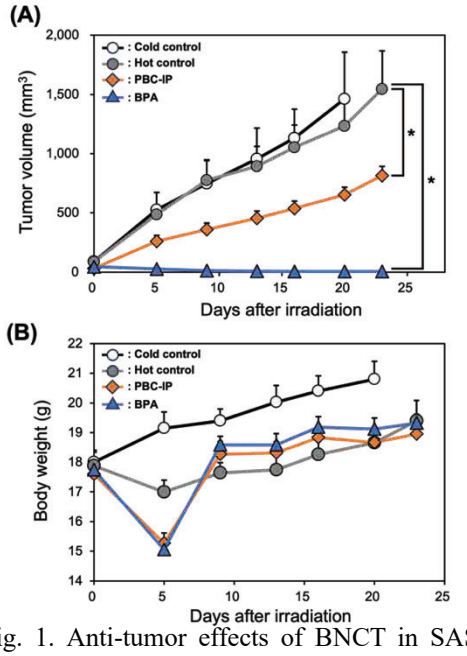


Fig. 1. Anti-tumor effects of BNCT in SAS xenograft model mice. (A) Tumor volume in mice after BNCT with PBC-IP and BPA (25 mg [^{10}B]/kg i.v.). Data are expressed as mean ± SD (n = 4–6). Significance was determined as *p < 0.05 using the two-sided Student's t-test. (B) Body weight changes of mice after BNCT.

REFERENCES:

[1] K. Nishimura *et al.,* J. Control. Release, **360** (2023) 249–259.
[2] K. Miura *et al.,* App. Radiat. Isotop., **221** (2025) 111837.

CO7-13

Construction of novel Boron-containing silica nanoparticles and BNCT experiments

F. Tamanoi[1], K. Matsumoto[1], A. Komatsu[1], Y. Higashi[1] and M. Suzuki[2]

[1]*Institute for Advanced Study, Institute for Integrated Cell-Materials Sciences, Kyoto University*
[2]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: Two type of boron compounds which are boron phenyl alanine (BPA) and borocaptate (BSH) have been mainly used for BNCT. But, these compounds have problems such as BSH having low uptake into cancer cell and BPA having short retention in tumor. Thus, novel boron compounds which can overcome these problems need to be developed for future BNCT.

We used a new type of mesoporous silica-based nanoparticle (MSN) that is modified with poly-ethylene glycol (PEG) and tetramethylammonium chloride (TMAC) to positively charged on sur-face. This nanoparticle has a large surface area where a large quantity of boron compounds which include BPA or BSH can be loaded. As we previously reported, our silica-based nanoparticle does have the ability to be effectively taken up in cancer cells by endocytosis system. It is also accumu-lated by EPR effect and retained in the tumor after intravenously injected to animal body. In this study, we evaluated the biodistribution of new MSN which has positively charged surface and di-ameter of less than 50 nm.

EXPERIMENTS: We used MSN which was synthesized by sol-gel method using tetraethox-ysilane (TEOS) and modified with PEG and TMAC to positively charge on surface. It has the size of less than 50 nm. This MSN was also labeled with Rodamine B dye to trace where it accumulates in mouse body after injection. The MSN was characterized by using TEM, DLS, nitrogen adsorp-tion-desorption analysis and zeta potential. For biodistribution analysis, MSN was intravenously injected to CT26 mouse colon cancer- mouse xenografts at 5 mg/mouse or 2 mg/mouse, and we dissected tumor and organs which include liver, lung and kidney at 24 hours after injection. Tumor and organs were made into thin sections and the fluorescence of MSN was detected with a confocal microscope.

RESULTS: The analysis showed that the size of MSN was less than 50 nm diameter and had homogenous shapes examined by DLS and TEM microscopy. And then, the surface of MSN was positively charged due to modification with PEG and TMAC. The zeta potential of MSN was 38.29 $\pm$0.77 mV in water. MSN accumulation in the CT26-transplanted mouse was investigated with a confocal microscope after making thin sections. MSN accumulated in the tumor at 24 hours after injection. A maximum level of red fluorescence of MSN was detected in the tumor, whereas it was weakly detected in other organs including liver, kidney and lung.

We are currently attaching boron to MSN. These results suggest that MSN may be an effective boron carrier for BNCT. This MSN may be able to become a new boron reagent for BNCT beyond BPA and BSH if boron compounds are grafted to it.

CO7-14

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

F. Tamanoi[1], K. Matsumoto[1], A. Komatsu[1], Y. Higashi[1] and M. Suzuki[2]

[1]*Institute for Advanced Study, Institute for Integrated Cell-Materials Sciences, Kyoto University*
[2]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: Peptide transporter 1 (PEPT1) has been noticed because it is expressed on various type of cancer cells. Especially, it has been reported that PEPT1was highly expressed pancreatic cancer PDX model.

We used some of dipeptide which contained ^{10}B that has higher solubility, longer retention ability in the tumor than BPA. These dipeptides are actively taken up into cancer cells by PEPT1 and LAT1 (weakly).

We investigated BNCT efficacy of these ^{10}B-dipeptides or BTS and the vaccine-like effect to distant tumor (uniraddiated tumor) when dipeptides are used.

EXPERIMENTS: We used two types of dipeptides. Dipeptides were intravenously injected to CT26-tansplanted BALB/c mice or FaDu-transplanted nude mice 2 hours before neutron irradiation. Our aim was to investigate whether the BNCT efficacy of dipeptides can be improved compared with BPA by neutron irradiation at KUR. The thermal neutron was irradiated for 6 minnutes at 5MW. After neutron irradiation, tumor volume and body weight measured for 6 weeks after irradiation (up to 42 days after irradiation) to evaluate the BNCT efficacy of dipeptides. For vaccine-like effect evaluation, the CT26-transplanted BALB/C mouse was injected with dipeptides and irradiated with neutron for 6 minutes at 5MW. Two weeks after irradiation, fresh tumor was transplanted on the left leg and was examined the engraftment to evaluate vaccine-like effect induction.

RESULTS:

In previous experiments for tumor accumulation of boron, it has been shown that dipeptides are effective boron carriers. We then investigated BNCT efficacy of these boron compounds. Dipeptides were intravenously injected to CT26-transplanted mice 2 hours before neutron irradiation. These mice were held to 12 mouse holder and placed in front of KUR, and neutron was irradiated. Tumor was almost disappeared on dipeptides-injected mice and tumor regrowth was not observed up to 27 days after BNCT, whereas regrowth was observed on BPA-injected mice. Similar results were observed with FaDu-transplanted nude mice. These results indicate that dipeptides have the potential to strongly cure cancer by BNCT.

We have further carried out vaccine-like effect induction on CT26-transplanted mice which were injected with dipeptides by neutron irradiation. Mice which were transplanted CT26 on right leg was irradiated with neutron, and the tumor was completely eradicated 2 weeks after irradiation. These mice were transplanted CT26 again on left leg for vaccine-like effect observation. As a result, no tumors were observed in the left reg on each mouse, and tumors were completely disappeared from both legs.

We are now preparing the paper to describe these experiments.

In the future, we will investigate the abscopal effect related to BNCT using dipeptides.

CO7-15

Sensitization of BPA-BNCT by Regulating the Polarity of Tumor-Associated Macrophage Using Shikonin

N. Takabayashi, N. Yasukawa, K. Matsuda, K. Bando, R. Kawasaki[1], N. Kondo[2], Y. Sanada[2], Y. Sakurai[2], M. Suzuki[2]and T. Nagasaki

Graduate School of Engineering, Osaka Metropolitan University
[1] Graduate School of Advanced Science and Engineering, Hiroshima University
[2] Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Although boron neutron capture therapy (BNCT) has excellent cancer cell killing ability, in many clinical cases it is ultimately incurable due to recurrence and metastasis. One of the factors for the cancer malignancy is the influence of the cancer microenvironment, represented by tumor-associated macrophages (TAMs). TAMs are broadly divided into M1 and M2 types, with M1 being tumoricidal and M2 being involved in suppressing cancer immunity and cancer cell proliferation and metastasis. In order to enhance the therapeutic effect of BNCT, it is important to control the cancer microenvironment as well as improve boron drugs.[1] It is known that macrophages express dectin-1 as β-1,3-glucan receptor on plasma membrane. In this study, we aim to efficiently and selectively deliver a TAM polarizer to TAM using β-1,3-glucan as a carrier, and evaluate the BNCT sensitization effect by the control of TAM polarity. When M2 macrophage is activated, STAT3, a transcription factor involved in anti-inflammatory responses and cell proliferation, is increased. Shikonin, a naphthoquinone compound produced by plants of the Boraginaceae family, functions as a STAT3 inhibitor and is therefore expected to act as a TAM polarizer.

EXPERIMENTS: To solubilize poorly water-soluble shikonin with β-1,3-glucan, we prepared shikonin/β-1,3-glucan composite nanogels (SKN/GC nanogels) using a dialysis complexation. To examine the inhibitory effect of SKN/GC nanogel on M2 induction in vitro, we used Raw264.7 cells and analyzed the mRNA expression levels of M1 (iNOS) and M2 (Arg-1, CD206) markers in the presence or absence of SKN/GC nanogel upon stimulation with IL-4 and IL-13 by RT-PCR. In vivo, SCC-VII cells (a mouse squamous cell carcinoma cell line) were subcutaneously implanted into C3H mice to prepare tumor-bearing mice. After that, neutron irradiation was performed with BPA-BNCT (250 mg/kg), and SKN/GC nanogel ([SKN] = 100 μM, 200 μl) was administered via the tail vein on the 2nd, 5th, and 7th days after irradiation to evaluate the combined effect. In addition, tumor tissue was excised on the 2nd, 5th, and 14th days. Total RNA was extracted from the excised tumor, and the M1/M2 ratio was analyzed by RT-PCR.

RESULTS: In vitro results showed that the presence of SKN/GC nanogel reduced the expression of M2 markers (Arg-1 and CD206), suppressing M2 progression. In vivo results showed that the combination of BPA-BNCT and SKN/GC nanogel suppressed tumor regrowth from 3 weeks after irradiation. RT-PCR analysis confirmed an increase in M1 markers and suppression of M2 markers from 4 days after neutron irradiation.

These results demonstrated that delivery of shikonin, a STAT3 inhibitor, with a β-1,3-glucan carrier, to dectin-1 cells enhances the anticancer effect of BPA-BNCT by controlling the polarity of TAMs in the cancer microenvironment toward M1-rich conditions.

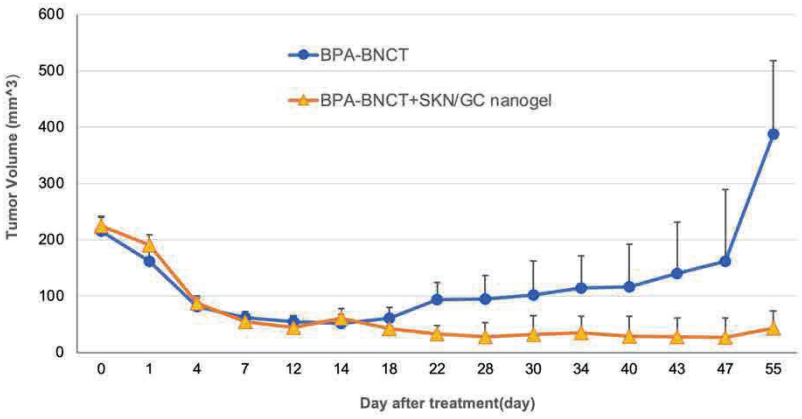


Fig. 1. Tumor suppression effect of BPA-BNCT in the presence or absence of SKN/GC nanogel.

REFERENCES:

[1] N. Yasukawa *et al.*, KURNS Prog. Rep. 2023 (2024) R5151.

CO7-16

Boosting Antitumour Efficacy and Immunity by BNCT with Size-Controlled Nanoparticles

Weian Huang[1], Heon Gyu Kang[1], Xu Han[1], Jie Yu[1], Xiaoxiao Chen[1], Takushi Takata[2], Yoshinori Sakurai[2], Minoru Suzuki[2] & Naoki Komatsu[1*]

[1] Graduate School of Human and Environmental Studies, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.

[2]Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University, 2-1010, Asashiro-nishi, Kumatori-cho, Sennan-gun, Osaka 590-0494, Japan.

Nanoparticles (NPs) hold significant promise in clinical practice especially as drug carriers to cancer. Although various lipid- and protein-based "soft" NPs have been approved in clinical cancer therapies, "hard" inorganic NPs have faced challenges such as low tumour selectivity resulting in poor therapeutic efficacy and potential safety concerns. In BNCT, on the other hand, BPA and BSH have been used clinically, yet they have inherent issues such as low tumour targetability and retentivity. Especially, BPA–BNCT suffers from the long-time infusion of high doses. To overcome these challenges, these boron agents have been conjugated with polymers, encapsulated in micelles or liposomes, and incorporated in inorganic NPs[1],[2], which still required high dosage.

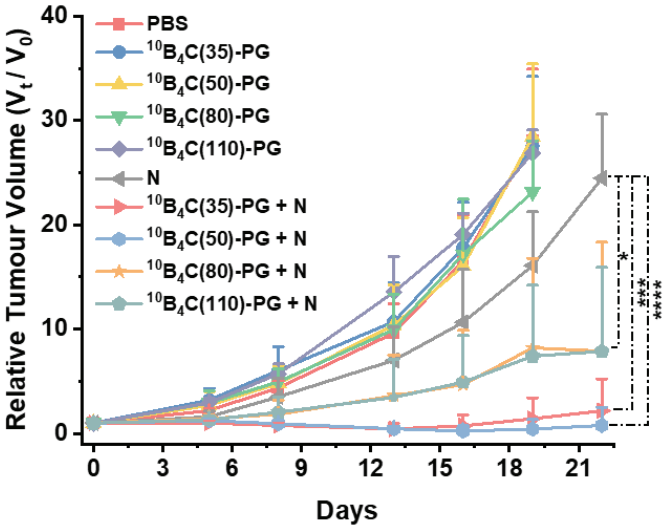


Figure 1. Relative CT26 tumor volume monitored for 22 days at a dosage of 5.1 mg [^{10}B]/kg (mouse) for in vivo BNCT (n = 5), where N indicates neutron irradiation. Statistical analysis of relative tumor volume with one-way ANOVA post Bonferroni test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Herein, we firstly establish size-controllable mechanochemical synthesis of $^{10}B_4C$ NPs, which are grafted with poly(glycerol) (PG). Then, the resulting $^{10}B_4C$-PGs are evaluated in their size effect to find that 50 nm core size, or $^{10}B_4C$(50)-PG, shows the higher ^{10}B delivery efficiency than the other sizes; $^{10}B_4C$(Y)-PG (Y = 35, 80 and 110), realizing 88% complete regression (CR) by single bolus intravenous injection at much lower dosage (12 mg / kg (mouse)) than BPA in preclinical studies. Since millions of boron-10 atoms are densely packed in one $^{10}B_4C$(50)-PG particle, its ^{10}B dosage is about five times less than that of BPA. The dosage is further reduced to half by twice neutron irradiation. Besides, antitumour immunity is boosted by $^{10}B_4C$(50)-PG–BNCT to exhibit abscopal ef-fect, in which distant tumours are suppressed or even eradicated.

Overall, $^{10}B_4C$(50)-PG demonstrates great promise as ^{10}B carrier in BNCT for clinical trials.[3]

REFERENCES:

[1] Zhang, Y. *et al.,* Adv. Mater., **35** (2023) e2301479. (doi)10.1002/adma.202301479.
[2] Wang, Y. *et al.,* Small, **18** (2022) e2204044. (doi)10.1002/smll.202204044.
[3] Huang, W. *et al.,* bioRxiv, (2025) 646946. (doi)10.1101/2025.04.02.646946.

CO7-17

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

R. Yamamoto[1], S. Inoue[1], T. Watanabe[2], Y. Sanada[2], M. Tsuji[1], T. Hirayama[1], and H. Nagasawa[1]

[1] *Laboratory of Medicinal & Pharmaceutical Chemistry, Gifu Pharmaceutical University*
[2] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: Amino acid transporters such as LAT1 and ASCT2 are highly expressed in cancer cells and promote tumor growth by mediating the uptake of glutamine and essential amino acids. Given their metabolic role, they are being explored as targets for boron delivery in BNCT. We are developing boron cluster–conjugated amino acid analogues that mimic LAT1 substrates to achieve tumor-selective accumulation. Among these, BC2 and BCY2 showed high cellular uptake and greater BNCT efficacy in vitro than the clinical LAT1-targeting agent BPA. However, their limited solubility and physicochemical properties hinder further evaluation in animal models.
In this study, we examined the cellular uptake mechanisms of BC2 and BCY2 using LAT1-deficient cells and a LAT1-specific inhibitor, and additionally initiated an investigation into prodrug strategies to improve their physicochemical properties for in vivo application.

EXPERIMENTS: For boron uptake studies, LAT1-modified SCCVII cells and human glioblastoma T98G cells were treated with boron-containing compounds (BC2, BCY2; 10 μg B/mL) at 37°C for 15 minutes. In LAT1 inhibition studies, T98G cells were pre-incubated with the LAT1-specific inhibitor JPH-203 (10 μM) for 5 minutes before compound treatment. After exposure, cells were washed with cold PBS, lysed in nitric acid, and heated at 75°C for 1 hour to ensure complete dissolution. Boron concentrations in the lysates were then measured by ICP-AES.
As part of our prodrug development efforts, we also synthesized a series of model compounds designed to enable tumor-selective release of the boron carrier via cathepsin B–cleavable linkers. Phenylalanine was used as a model payload, and derivatives with varying linker structures and cleavage sequences were prepared.

RESULTS: As shown in Fig. 1, BC2 uptake was significantly reduced both in LAT1-KO SCCVII cells and in T98G cells treated with JPH-203, whereas BCY2 uptake remained unchanged under either condition. These findings indicate that BC2 is transported in a LAT1-dependent manner, while BCY2 uptake is largely LAT1-independent.

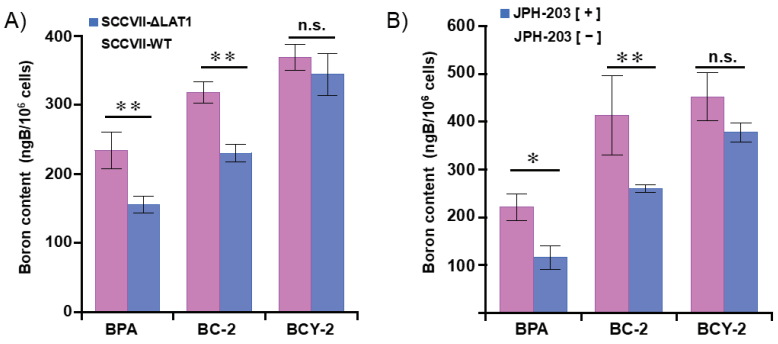
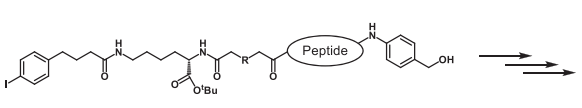


Fig. 1. Effects of boron carriers on cellular boron uptake
A) LAT1-knockout SCCVII cells B) LAT1 inhibition in T98G cells (JPH-203)

As shown in Scheme 1, we successfully synthesized several albumin-binding prodrug derivatives featuring different cathepsin B–responsive linkers. The prodrug function is being assessed using model compounds with phenylalanine as the payload, with the next step being the synthesis of derivatives incorporating BC2 or BCY2.



S.M.	Peptide	R	result
1a	Val-Cit	c	1aF, 22% (two steps)
2a	Val-Cit	c	2bF, 68% (two steps)
2b	Phe-Lys	o	2bF, 9% (two steps)

Scheme 1. Synthesis of albumin-binding prodrug model compounds with phenylalanine payload

CO7-18

Tumour Growth Suppression by Neutron Capture Therapy using Intratumoral Administration of ^{10}Boro-plex encapsulated Water-in-Oil-in-Water Emulsion

H. Yanagie [1, 2, 3], M. Yanagawa [4], T. Matsukawa [5], A. Kubota [5], Y. Yanagie [6], M. Suzuki [7], Y. Sakurai[7], T. Takata [7], H. Tanaka [7], M. Nashimoto [3], H. Yamauchi[2, 8], M. Ono [2, 8], T. Sugihara [3], H.Takahashi [1, 2, 9]

[1]Institute of Engineering Innovation, School of Engineering, The University of Tokyo, [2]Cooperative Unit of Medicine & Engineering, The University of Tokyo Hospital, [3]Niigata Univ of Pharmacy & Applied Life Sciences, [4]Joint Faculty of Veterinary Medicine, Kagoshima University, [5]Department of Hygiene, Faculty of Medicine, Juntendo University, [6]Faculty of Medicine, Teikyo University, [7]Kyoto University Institute for Integrated Radiation & Nuclear Science, [8]Department of Cardiac Surgery, The University of Tokyo Hospital, [9]Department of Bioengineering, School of Engineering, The University of Tokyo

INTRODUCTION: We have been continuously developing boron-containing water-in-oil-in-water (WOW) emulsions for application in the multidisciplinary treatment of primary liver cancer using boron neutron capture therapy (BNCT). For effective boron delivery, a two-step targeting process is essential: first targeting involves delivering the drug delivery system (DDS) to the tumor tissue, followed by second targeting, which transports the boron compound into the cancer cells—particularly into the nucleus—via receptors on the cancer cell membrane. Our previously developed boron-containing WOW emulsion has shown the ability to increase boron concen-tration in tumor tissue and achieve tumor shrinkage upon thermal neutron irradiation. However, complete tu-mor eradication has not been achieved, indicating the need for further development of novel boron compounds and DDS strategies. In this study, we report on the basic research conducted prior to hepatic arterial infusion, in which ^{10}Boro-plex was encapsulated in a WOW emulsion, administered intratumorally, and its tumor growth inhibition effect following thermal neutron irradiation was evaluated.

EXPERIMENTS: The internal aqueous phase consisted of the following: 1.2 mL of ^{10}BSH solution (350 mg/mL), 0.12 mL of Lipofectamine 2000, 0.09 mL of sodium hyaluronate (0.25 mL; 10 mg/mL), and 0.09 mL of protamine (0.125 mL; 20 mg/mL). The oil phase consisted of 2 mL of Lipiodol, with surfactants added to each phase. Using our originally developed mixing device, a WOW emulsion was prepared. We prepared mouse colon cancer Colon 26 (5×10^5) model by transplanting to right lower leg. Following the intratumoral injection of 0.2 mL of the emulsion, thermal neutrons at a dose of 3×10^{12} n/cm² were irradiated at Institute for Integrated Radiation and Nuclear Science, Kyoto University. Tumor size was measured post-irradiation to evaluate the tumor growth inhibition effect.

RESULTS: Both the WOW emulsion containing ^{10}BSH and that containing the ^{10}Boro-plex complex demonstrated tumor growth inhibition after thermal neutron irradiation compared with non-irradiated group. As the irradiation was performed two hours after intra-tumoral injection in this study, no significant difference in therapeutic effect was observed between the two formulations.

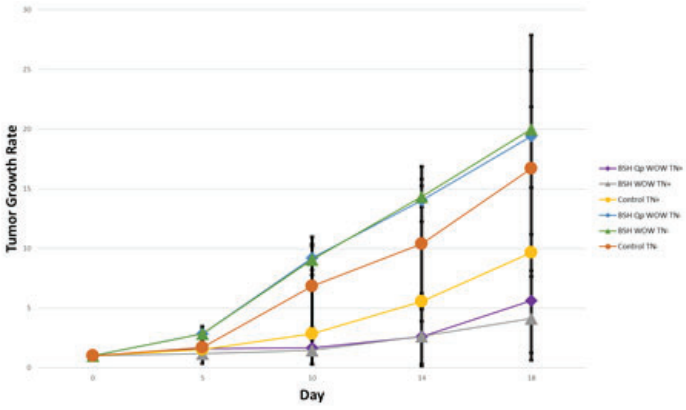


Figure1. Tumor growth suppression in ^{10}BSH Qp-WOW group by NCT was superior compared with non-irradiated group.

REFERENCES:

[1] Yanagie H. *et al.*, Br J Radiol., **90(1074)** (2017) 20170004.

[2] Yanagie H. *et al.,* Appl Radiat Isot., **163** (2020) 109202.

CO7-19

Optimization of polymer-BPA conjugates for non-clinical studies

T. Nomoto, K. Konarita[1,2], D. Tokura[1,2], Shota Tanaka, M. Suzuki[3]

Graduate School of Arts and Sciences, the University of Tokyo
[1]Institute of Integrated Research, Institute of Science Tokyo
[2]School of Life Science and Technology, Institute of Science Tokyo
[3]Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: 4-Borono-L-phenylalanine (L-BPA), the most potent drug in boron neutron capture therapy (BNCT), selectively accumulates within tumors through the LAT1 transporter [1]. However, L-BPA is sometimes exchanged with extracellular amino acids, such as tyrosine, due to the antiport mechanism of LAT1, resulting in short-term retention in the target tumor and compromising therapeutic efficacy [2]. To address this issue, we discovered that poly(vinyl alcohol) (PVA) can form complexes with L-BPA through boronate esters in aqueous solution. These PVA-L-BPA complexes can then be internalized within tumor cells via LAT1-mediated endocytosis, thereby extending the retention time of BPA [3]. In this study, we prepared PVA formulations with 4-borono-D-phenylalanine (D-BPA) as well as L-BPA and evaluated their BNCT effects.

EXPERIMENTS: PVA formulations with different compositions were administered intravenously to mice bearing subcutaneous CT26 tumors. The tumor was irradiated with epi-/thermal neutrons at 1 MW for 50 minutes using the Kyoto University Research Reactor (KUR) 3 h after injection.

RESULTS: All the PVA formulations demonstrated higher BNCT effects compared to the conventional L-BPA (Fig. 1). Since the PVA formulations exhibited almost complete cure, it was difficult to observe a significant difference in antitumor efficacy between the racemic composition (L:D = 1:1 or 1:2) and L-BPA (L:D = 1:0) in this experimental condition.

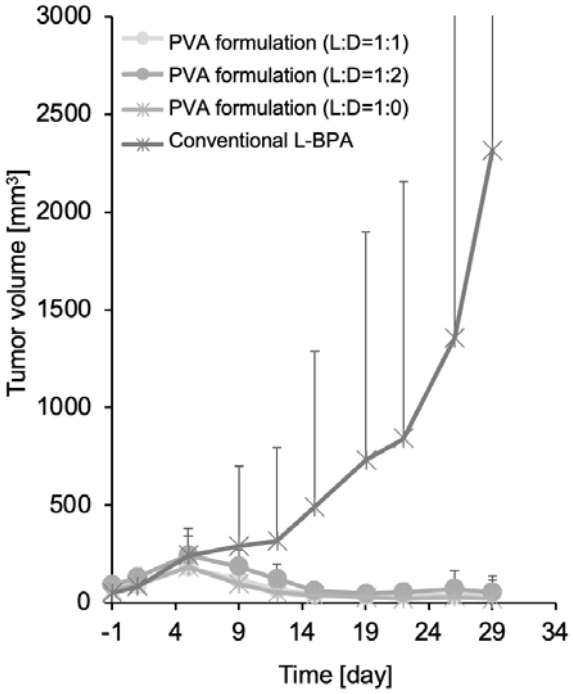


Fig. 1. BNCT effects on subcutaneous CT26 tumors.

REFERENCES:
[1] P. Wongthai *et al.,* Cancer Science, **106** (2015) 279-286.
[2] A. Wittig *et al.,* Radiat. Res., **153** (2000) 173-180.
[3] T. Nomoto *et al.,* Sci. Adv., **6** (2020) eaaz1722.

CO7-20

Development of novel boron delivery systems improving accumulation contrast

T. Nomoto, D. Tokura[1,2], K. Konarita[1,2], Shota Tanaka, M. Suzuki[3]

Graduate School of Arts and Sciences, the University of Tokyo
[1]Institute of Integrated Research, Institute of Science Tokyo
[2]School of Life Science and Technology, Institute of Science Tokyo
[3]Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron neutron capture therapy (BNCT) is a treatment that uses nuclear reactions between thermal neutrons and boron atoms (^{10}B) to kill cancer cells. In clinical settings, the boron concentration in the tumor must be at least 25 ppm, and the ratio of boron in the tumor to blood (T/B ratio) and the surrounding normal organs (T/N ratio) must be at least 2.5 to achieve an efficient therapeutic effect while minimizing radiation exposure to normal tissue. While many studies have developed drug delivery systems (DDSs) such as polymeric micelles [1] and liposomes [2], these conventional DDSs should exhibit significantly prolonged retention in the bloodstream to enhance the likelihood of leakage from tumor vessels into tumor tissue and subsequent tumor accumulation. Therefore, simply applying conventional DDSs to BNCT does not guarantee a high T/B ratio, and even if high tumor accumulation is achieved, the dose of thermal neutron irradiation is limited and does not lead to improved therapeutic outcomes. In this study, we developed a DDS that can achieve the increased T/B ratio.

EXPERIMENTS: Two types of DDSs were administered to mice bearing subcutaneous CT26 tumors, and thermal neutrons were irradiated to the tumors.

RESULTS: As shown in Fig. 1, the DDS (type II) exhibited antitumor efficacy comparable to conventional L-BPA. The DDS (type II) may be a promising candidate to induce efficient BNCT effects with high T/B and T/N ratios, while advantages from the high ratios needs to be clarified in a future study.

REFERENCES:

[1] H.Cabral *et al.,* Chem. Rev., **118(14)** (2018) 6844-6892.
[2] Theresa M. Allen *et al.,* Advanced Drug Delivery Reviews **65** (2013) 36-48.

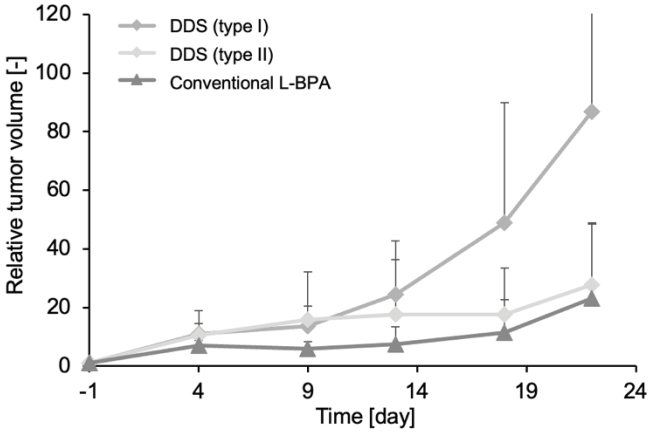


Fig. 1. BNCT effects on subcutaneous CT26 tumors.

CO7-21

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

S. Kanai[1], J. Tsunetomi[1], M. Suzuki[2], Y. Urano[1,3]

[1] *Graduate School of Pharmaceutical Sciences, The University of Tokyo*
[2] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[3] *Graduate School of Medicine, The University of Tokyo*

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. By last year, we have developed 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, and conducted BNCT experiments with tumor bearing mice by intratumoral or intravenous injection. 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. This probe worked well by the intratumoral injection, however, by systemical injection only a small and not enough amount of probe was accumulated in cancer cells, possibly due to the short blood half-life. We also tried to inject higher amount of the probe, however, some side effects were observed due to the toxicity originated from the quinone methide intermediate produced in normal tissues. So this year, we started the development of other probes based on the different molecular mechanisms, Protide-based mechanism [1] and azaquinone methide bearing electron-withdrawing group-based molecular design, which might be safer and show longer blood half-life.

EXPERIMENTS and RESULTS: CB-C2-pSoul-AR was designed and synthesized based on the Protide chemistry to show the reactivity toward carboxypeptidase M (CPM) which is upregulated in some types of cancer cells. By being hydrolyzed by CPM, it yields the carborane derivative bearing phosphate group which show accumulation in cells due to the relatively high hydrophilicity to achieve enough high amount of boron atom. CB-pF-(mCF3)-gGlu was designed based on the azaquinone methide chemistry to have the reactivity toward gamma-glutamyltranspeptidase (GGT) which is known as a good biomarker of breast, oral and hepatic cancers. By the reaction with GGT, it yields azaquinone methide intermediate with electron-withdrawing trifluoromethyl group which shows much lower cytotoxicity than a usual azaquinone methide. After incubating each probe with living target cells, MDCK and MCF-7 for CB-C2-pSoul-AR, A549 and SKOV3 for CB-pF-(mCF3)-gGlu, boron concentration was evaluated. It was found that CB-pF-(mCF3)-gGlu showed enough concentration of boron atoms for BNCT, so we conducted BNCT experiment with this probe, firstly by the intratumoral injection. As a result it was successfully found that tumor growth was suppressed in a drug- and neutron-irradiation-dependent manner. So we now plan to conduct BNCT experiment with intravenous injection next year.

REFERENCES: [1] Y. Kuriki *et al.,* J. Am. Chem. Soc., **146** (2024) 521-531.

CO7-22

Synthesis and evaluation of a novel boron neutron capture therapy agent

H. Kimura[1], M. Suzuki[2]

[1]*Research Center for Experimental Modeling of Human Disease, Kanazawa University*
[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.

EXPERIMENTS: A BSH-labeled SSTR2 agonist-type drug (BSH-TATE) was designed and synthesized. To evaluate the therapeutic effect in BSH-TATE, 400 μg/100 μL of BSH-TATE was administered by tail vein to AR42J cell-bearing mouse models and irradiated with neutron beams 6 hours after administration.

RESULTS: In the saline group, tumor size became too large after 7 days of treatment, and the experiment was terminated due to humane endpoints; in the BSH-TATE group, tumor re-growth was observed after 14 days, but the results suggest that tumor growth can be inhibited (Figure). Multiple neutron irradiation is one possibility to achieve long-term tumor growth inhibition, but since multiple neutron irradiation is not currently allowed in principle in BNCT, we will explore this possibility while evaluating efficacy and safety. The dose administered this time was 400 μg/100 μL, but the dose can be increased with the use of appropriate solubilizing agent, and we consider the dose to be another option to suppress tumor re-growth. In addition, we believe that if the time between administration and neutron irradiation is extended to about 24 hours, the neutron irradiation may be more effective in inhibiting tumor growth because the BSH-TATE is internalized sufficiently.

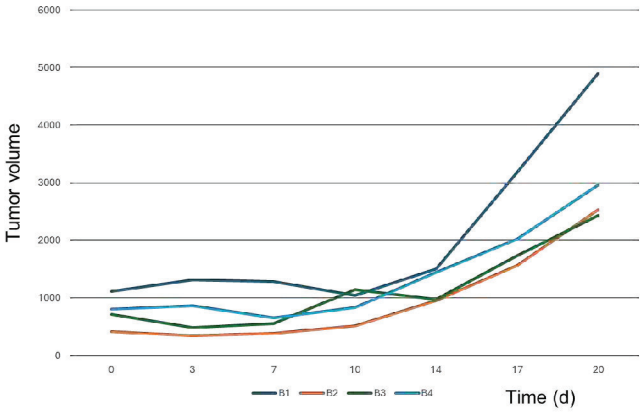


Figure. Tumor Volume Changes after BNCT

REFERENCES:

[1] Ozge Keskin *et al.,* Onco Targets Ther., **6** (2013) 471-483.
[2] Thomas Gunther *et al.,* Phar-macological Reviews, **70** (2018) 763-835.

CO7-23

Measurements of two kinds of thimble-type ionization chambers for an intense epi-thermal neutron beam

T. Matsumoto[1], A. Masuda[1], S. Manabe[1], H. Harano[1], K. Terada[2], H. Tanka[2] and J. Hori[2]

[1]*National Metrology Institute of Japan , National Institute of Advanced Industrial Science and Technology*
[2]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: The need to ensure traceability to neutron standards at AIST for hospitals performing boron neutron capture therapy (BNCT) has been discussed. For this purpose, we are developing a real-time detector that can measure high-intensity neutrons used in BNCT. It is required that the detector can measure neutrons in appropriate measurement time at standard neutron fields whose fluxes are 4 to 5 orders of magnitude lower than those at BNCT as well.

EXPERIMENTS: Thimble type ionization chambers have been developed as a neutron detector for high intensity neutrons. The ionization chamber is made of aluminum and has a dome shape with a diameter of 13 mm and a length of 15 mm. An aluminum needle-shaped electrode with a diameter of 1 mm is set in the center. We prepared two kinds of chamber. One of chamber was filled with a mixture of ^{3}He gas (0.1 atm) and Kr gas (1.9 atm). Another chamber had 6LiF evaporated on the inner wall and was filled with air as the ionizing gas. Characteristics of the ionization chambers were evaluated using a pulsed white neutron source from an electron linac at the Institute for Integrated Radiation and Nuclear Science of the Kyoto University. The ionization chambers measured neutrons by two-dimensional measurements of neutron time-of-flight and pulse height (PH). In addition, in the current output mode, the measurement was performed by varying the applied voltage for the neutrons in the heavy water facility at the Kyoto University Reactor.

RESULTS: Figure 1 shows a PH spectra obtained from the ionization chambers. Neutron components were clearly observed compared to measurements with a chamber with 3He and Ar gases last year. Figure 2 shows the relations between the applied voltage and output current from the center electrode in the current mode. The applied voltage below 1000 V was the ionization chamber region. In the future, we will evaluate the detection efficiency and stability.

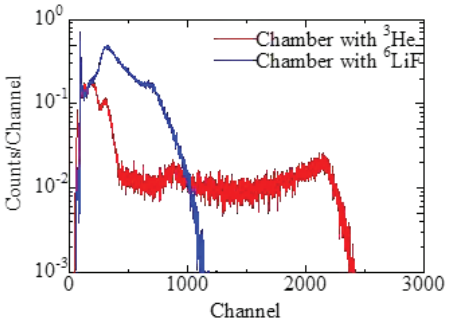


Fig. 1. TOF spectrum for the pulsed neutron source obtained from the electron linac. The thermal bump was clearly observed.

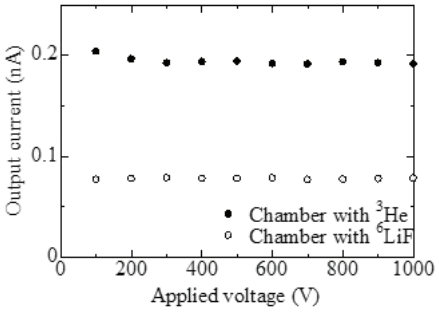


Fig. 2. Relation between applied voltage and output current.

REFERENCES:

[1] K. Kobayashi *et al.,* Annu. Rep. Res. Reactorinst. Kyoto Univ., **22 (**1989)142 .

This work was supported by JSPS KAKENHI (21H03755 and 19K12638).

CO7-24

Development of Nanogels Loaded with Gd(III-Thiacalixarene Complex for Gd-NCT

N. Iki[1], N. Shindo[1], and M. Suzuki[2]

[1]*Graduate School of Environmental Studies, Tohoku University*
[2]*Research Reactor Institute, Kyoto University*

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 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) [5–6] aiming at Gd-NCT. This FY, we introduced a new agent Gd-Nanogel (NG) comprising of Gd_3TCAS_2 and polyethylene imine (PEI) coated with poly(styrene sulfonate) (PSS) and studied the NCT effect.

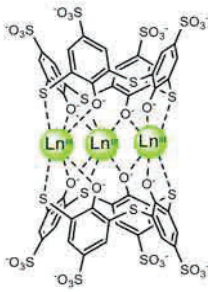


Fig. 1 Ln_3TCAS_2 complex.

EXPERIMENTS: Preparation of NGs. Gd_3TCAS_2-PEI NG [7] was coated with PSS by simply mixing the solutions. 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 agents 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 12 and 25 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.

RESULTS: The largest amount of Gd delivered to MCF-7 cells with the Gd_3TCAS_2-PEI-PSS NG was 3.88 ± 1.57 nmol/10^6 cells. The cytotoxicity was not observed up to 100 μM. Cell viability after neutron irradiation suggests that the NCT effect was smaller than that of obtained with Gd_3TCAS_2-PEI NG (Fig. 2), suggesting that the negative charge of Gd_3TCAS_2-PEI-PSS NG led to lower cellular uptake. When incubated with the NG with higher concentration, NCT effect was observed. Studies to increase the cellular uptake by changing the coating materials is now underway.

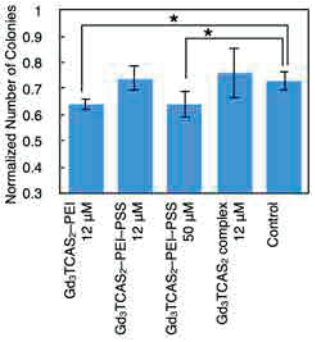


Fig. 2 Cell viability of MCF-7 cells incubated in the presence of Gd agents followed by washing out of free Gd agents and irradiation with thermal neutron. Incubation: 50 μM Gd for 24 h. Neutron fluence: 1.1×10^{12} thermal neutrons cm^{-2}, 1.9×10^{11} epithermal neutrons cm^{-2}.

REFERENCES:

[1] M. Takagaki *et al.,* Future Application of Boron and Gadolinium Neutron Capture Thrapy, in Boron Science, Ed N. S. Hosmane (CRC Press) (2012) 243.
[2] N. Iki *et al.,* Eur. J. Inorg. Chem., **2016** (2016) 5020-5027.
[3] N. Iki *et al.,* Inorg. Chem. **55** (2016) 4000-4005.
[4] T. Yamatoya *et al.,* 31P11-8, p. 105, KURNS Progress Report 2019.
[5] N. Iki *et al.*, R5012, KURNS Progress Report 2024.
[6] N. Iki *et al.,* Colloids Surf. A Physicochem. Eng. Asp., **699** (2024) 134579.
[7] N. Iki *et al.,* The 40th Annual Meeting of the Japan Society of Drug Delivery System, **2024**, Tsukuba.

CO7-25

Research and Development of New Technology for Boron Neutron Capture Therapy

M. Xu[1], T. Watanabe[4], X. Zhou[1], C. Jin[1], R. Ohgaki[1,2], H. Okanishi[1], H. Tanaka[4], M. Suzuki[4], and Y. Kanai[1,2,3]

[1]*Department of Bio-system Pharmacology, Graduate Schoo l of Medicine, The University of Osaka*
[2]*Integrated Frontie r Research for Medical Science Division, Institute for Open and Transdisciplinary Research Initiatives (OTRI), , The University of Osaka*
[3]*Premium Research Institute for Human Metaverse Medicine, The University of Osaka*
[4]*Institute for Integrated Radiation a nd Nuclear Science, Kyoto University*

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a cancer treatment modality that involves the selective accumulation of boron-containing compounds within tumor cells, followed by exposure to a neutron beam. Achieving a high intracellular concentration of the boron compound is essential for this nuclear reaction between ^{10}B and thermal neutrons to occur efficiently within cancer cells. Among various boron carriers, L-p-boronophenylalanine (L-BPA) is widely used owing to its preferential uptake by cancer cells through upregulated amino acid transport systems [1]. A major limitation, however, is the swift decline of intracellular L-BPA levels, primarily due to its efflux via specific transporters expressed in cancer cells. This study experimentally verifies a strategy to boost the therapeutic impact of neutron irradiation by inhibiting those transporters mediating L-BPA efflux. Using both in vitro cultured cells and in vivo tumor-bearing mouse models, we assessed whether blocking L-BPA efflux could enhance the effectiveness of BNCT While the studies conducted in FY2022–2023 focused on experimentally validating the underlying concept, in FY2024, we aim to advance toward clinical development by employing the optimized inhibitor's active stereoisomer.

EXPERIMENTS: In the FY2024 study, tumor cells with high expression of transporters responsible for BPA uptake were selected, and the human pancreatic cancer cell lines T3M4 and Suit-2 were used. In the cell-based irradia-tion experiments, T3M4 and Suit-2 cells were first incubated with L-BPA by supplementing it into the culture medium. Following a washing procedure, the cells were further cultured for 60 minutes in the presence or absence of transporter inhibitors that block the efflux of L-BPA. After incubation, cells were collected using Hanks' Balanced Salt Solution (HBSS) and transferred into 1.5 mL microtubes to be used as samples for neutron exposure. As a control, additional samples were prepared by irradiating cells not treated with L-BPA. Cell suspensions were adjusted to appropriate densi-ties post-irradiation and plated onto 10 cm dishes to assess clonogenic survival through colony formation assays.

For the in vivo irradiation studies, tumor models were established by subcutaneous implantation of T3M4 and Suit-2 cell lines into nude mice. L-BPA was administered intravenously via the tail vein at 300 mg/kg. Subsequently, an active stereoisomer of a transporter inhibitor (50 mg/kg), designed to suppress L-BPA efflux, was administered at 1 hour and again at 1.5 hours after L-BPA injection. Control mice received the inhibitor alone, without prior L-BPA administration, before undergoing neutron irradiation. Neutron exposure was performed at a reactor output of 5 MW, with irradiation times set at 30 minutes. Tumor response following neutron treatment was monitored and compared between the inhibitor-treated groups with or without prior L-BPA loading to evaluate therapeutic efficacy.

RESULTS: In the cell-based irradiation studies, T3M4 and Suit-2 cells were exposed to L-BPA and washed. The cells were then divided into three groups. Group 1 was incubated for 60 minutes in RPMI 1640 medium supplemented with an inhibitor targeting the transporter responsible for L-BPA efflux. Group 2 was incubated under identical conditions but without the inhibitor. Group 3 served as a control and consisted of cells not exposed to L-BPA. All groups were then subjected to neutron irradiation. Post-irradiation, colony formation assays were performed under low-density conditions to evaluate cell viability. Results showed no notable difference in survival between Group 2 and Group 3 (non-BNCT), implying that L-BPA was rapidly expelled from the cells and had diminished to subtherapeutic levels within 60 minutes. In contrast, Group 1 exhibited a significantly reduced survival rate compared to Group 2, confirming that inhibition of the efflux transporter enhanced the therapeutic efficacy of BNCT.

For the in vivo studies, T3M4 cells were subcutaneously implanted in the hind limbs of mice to establish tumor-bearing models. Following intravenous L-BPA administration, the mice were allocated into three groups. Group 1 received additional injections of a transporter inhibitor at 1 and 1.5 hours post-L-BPA administration. Group 2 received no inhibitor following L-BPA injection. Group 3 received saline instead of L-BPA, followed by inhibitor injections at the same time points as Group 1. All mice underwent neutron irradiation 2.5 hours after the initial L-BPA or saline administration. Tumor volumes were measured for 18 days post-irradiation. Group 1 displayed a notable reduction in tumor size relative to Group 2, indicating that transporter inhibition substantially improved BNCT efficacy. This supports the effectiveness of the proposed strategy to enhance the therapeutic impact of L–BPA–based BNCT.

REFERENCES: [1] P. Wongthai *et al.,* Cancer Sci., **106** (2015) 279-286.

CO7-26

Mechanism of Glioma Resistance After BNCT via Small Extracellular Vesicles

Natsuko Kondo[1], Tadatoshi Kinouchi[2], Manabu Natsumeda[3], Eishu Hirata[4], Yoshinori Sakurai[1], and Minoru Suzuki[1]

Graduate School of Science, Kyoto University
[1] Particle Radiation Oncology Research Center, and [2]Division of Radiation Biochemistry, Institute for Integrated Radiation and Nuclear Science, Kyoto University
[3]Department of Neurosurgery, Brain Research Institute, Niigata University,
[4] Division of Tumor Cell Biology and Bioimaging, Cancer Research Institute of Kanazawa University

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. We investigated miRNAs in sEVs secreted from glioblastoma cells after BNCT using microarray.

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.

sEVs collection

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.

Measurement of size and concentration of the sEVs

The size distribution profile and concentrations of the sEVs were analyzed with 6. Tunable resistive pulse sensing (TRPS).

Total RNA extraction from sEVs and miRNA microarray analysis

Total RNA was extracted from the sEVs using Toray's 3D-Gene RNA extraction reagent (Toray Industries, Inc., Tokyo, Japan). Comprehensive miRNA expression analysis was performed using a 3D-Gene miRNA Labeling kit and 3D-Gene Human miRNA Oligo Chip Ver. 22 (Toray Industries, Inc.), according to the manufacturer's protocol to detect 2,565 human miRNA sequences.

RESULTS: We detected an increase in 21 individual miRNAs (ratio>2) and a decrease in 2 individual miRNAs (ratio<0.5) in BNCT cells compared to non-irradiated cells. Also, more than 20 miRNAs that associate with poor prognostic markers in glioma were produced or increased after BNCT.

CO7-27

Characterization of Solar Cell-Based Radiation Detectors for BNCT Applications

Y. Okuno, H. Tanaka[1], Y. Sakurai[1], N. Matsubayashi[1]
RAP, RIKEN
[1]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: This study focuses on the development of solar cell-based radiation detectors for applications in Boron Neutron Capture Therapy (BNCT), with particular emphasis on the use of hybrid organic–inorganic perovskite (HOIP) solar cells. These devices are investigated for their potential to detect neutrons through the measurement of radiation-induced currents. HOIP solar cells offer unique advantages, including high carrier mobility, strong absorption coefficients, and tunable electronic properties, which make them promising candidates for neutron detection under high-radiation environments. The research aims to characterize the neutron response of these detectors, evaluate their sensitivity and stability, and explore the integration of neutron converter layers such as Boron-10 to enhance their performance. This work contributes to the advancement of compact, low-cost, and real-time radiation monitoring systems for next-generation radiotherapeutic applications.

****

EXPERIMENTS: A neutron detection device was fabricated by coating a 30 μm-thick layer of boron nitride (BN) powder onto a hybrid organic–inorganic perovskite solar cell with the same structural configuration as previously reported in Ref. [1]. Neutron irradiation experiments were conducted using the heavy water neutron beamline of the Kyoto University Research Reactor (KUR). The detector was positioned immediately downstream of the neutron beam extraction port. To measure the radiation-induced current during neutron exposure, the device was connected to a source measure unit (SMU; B2912A, Keysight) located outside the irradiation room via a 10 m-long BNC cable. The measurement was performed under zero applied bias (0 V), with a sampling time of 1 second per data point.

****

RESULTS: Irradiation was performed under conditions equivalent to those used in BNCT treatment. A neutron-induced current of 8.5 × 10^{-9} A/cm² was measured, which is approximately three orders of magnitude higher than the offset current. This confirms a clear response to neutron flux. During one hour of continuous irradiation, no significant degradation in the current was observed, indicating good stability of the device under neutron exposure. Sharp changes in the output signal were detected during the measurement. These fluctuations coincided with the movement of the reactor control rods, as confirmed by operational records. This correlation demonstrates that the device is capable of detecting neutron flux variations with high temporal resolution. These results show that the device can accurately measure neutron flux under BNCT-equivalent conditions and is suitable for high-precision neutron dosimetry.

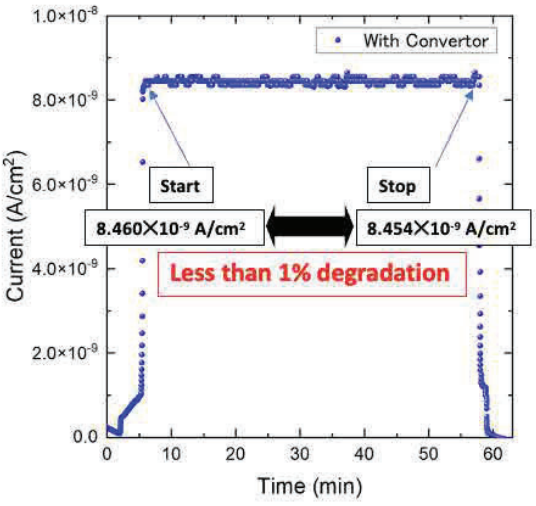


Fig.1. Neutron-induced current characteristics of perovskite solar cells during irradiation

****

REFERENCES:

[1] Y. Okuno *et al.*, ACS Appl. Electron. Mater., **4**, (2022) 3411.

CO7-28

Establishment of innovative BNCT treatment method for intractable bladder cancer

P. Huang[1], K. Igawa[1], Y. Sakurai[2], H. Michiue[1] and M. Suzuki[2]

[1]*Neutron Therapy Research Center, Okayama University*
[2]*Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: Bladder cancer treatment remains a challenge to every urologist. The current first-line treatment for non-muscle invasive bladder cancer is transurethral resection of bladder tumors followed by intravesical Mycobacterium Bovis Bacillus Calmette-Guérin (BCG) immunotherapy. In case of BCG failure, radical cystectomy is the standard of care in high - risk patients. However, many of them are unfit or they refuse to undergo such an intervention; therefore, other treatment options are required. The usefulness of BNCT to urothelial cancer remains unknown. Here we aimed to investigate whether BNCT suppresses in a previously established mouse model of orthotropic bladder cancer.

****

EXPERIMENTS: We constructed BCG-resistant bladder cancer cell lines T24/BCG. In vitro, we investigated the uptake of Boron in various bladder cancer cell lines and normal cell lines through Inductively coupled plasma (ICP). Furthermore, luciferase-expressing T24/BCG cells were implanted in the bladder of nu/nu mice, Neutron irradiation experiments on mouse models of orthotropic bladder cancer.

****

RESULTS: The uptake of boron by various cells is different, but all of them can be effectively taken up and expressed for BNCT treatment. As shown in Fig.1 In Vivo, through intravesical administration of small doses, decreased tumor weight, compared with intravenous systemic administration, intravesical administration has basically no effect on other tissues, organs, liver function, and renal function* *(Data No Shown)*

****

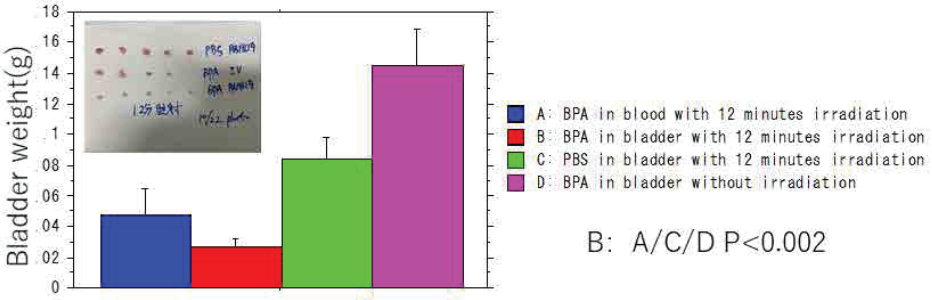


Fig.1 The bladder weight assay of 14 days after Neutron irradiation.

Neutron irradiation for 12 minutes after Boron added for 1 hour

REFERENCES:

[1] T. Fujimoto *et al.,* Biomaterials., **309** (2024)

[2] T. Fujimto *et al.,* Cancer Sci., **115**(2024)

[3] R. Kayama *et al.,* Sci Rep., **14** (2024)

CO7-29

Evaluation of a novel cyclodextrin-based polyrotaxane boron compound for BNCT

Y. Matsumoto[1,2], Y. Sugawara[2], M. Suzuki[3], H. Takeuchi[2], A. Nakaie[2], H. Arase[4], H. Ishiki[4], T. Taharabaru[4], R. Onodera[4], H. Motoyama[4], H. Sakurai[1,2] and T Higashi[4]

[1]*Institute of Medicine, University of Tsukuba*
[2]*Proton Medical Research Center, University of Tsukuba Hospital*
[3]*Institute for Integrated Radiation and Nuclear Science , Kyoto University*
[4]*Graduate School of Pharmaceutical Sciences, Kumamoto University*

INTRODUCTION: Boron neutron capture therapy (BNCT) is an emerging cancer treatment that utilizes the nuclear reaction between boron-10 and thermal neutrons to selectively destroy tumor cells while minimizing damage to the surrounding normal tissue. The effectiveness of BNCT de-pends heavily on the selective delivery and retention of boron compounds within the tumor micro-environment. Among the clinically used boron agents, boronophenylalanine (BPA) is known for its tumor selectivity but has limitations owing to its rapid efflux from tumor cells, necessitating high dosing. In this study, we developed a novel supramolecular boron compound, FPBA-PRX, which is composed of a cyclodextrin-based polyrotaxane (PRX) modified with 4-fluorophenylboronic acid (FPBA). FPBA selectively binds to sialic acid residues that are overexpressed on the surfaces of many tumor cells. Importantly, the FPBA moieties on the PRX backbone are mobile, allowing more efficient and multivalent interactions with tumor cell membranes. We hypothesized that this design would improve tumor-specific accumulation and intracellular uptake of boron compounds, thereby enhancing the therapeutic efficacy of BNCT.

EXPERIMENTS: FPBA-PRX was synthesized via a multi-step process involving α-cyclodextrin, PEG-based axial chains, and FPBA as the boron-containing ligand. A comparison compound, FPBA-CEL (FPBA-modified cellulose), was also synthesized with a similar boron content but without the mobility provided by the polyrotaxane structure. We characterized the physicochemical properties (particle size and ζ-potential) and sialic acid-binding affinity using alizarin red S displacement assays. Cellular uptake was evaluated using TRITC-labeled compounds and analyzed by flow cytometry and confocal microscopy in HeLa and Colon-26 cancer cell lines. For in vivo experiments, Colon-26 tumor-bearing BALB/c mice were intravenously or subcutaneously administered FPBA-PRX or FPBA-CEL. Boron accumulation in tumors and blood was measured using ICP-AES. BNCT efficacy was assessed following thermal neutron irradiation using the Kyoto University Research Reactor, and tumor growth was monitored for several weeks post-treatment.

RESULTS: FPBA-PRX exhibited favorable physicochemical properties, including a particle size of approximately 15 nm and a neutral surface charge, making it suitable for passive tumor targeting via the enhanced permeability and retention (EPR) effect. This compound exhibited strong sialic acid-binding ability and significantly higher cellular uptake in tumor cells than FPBA-CEL or un-modified PRX, attributable to the mobility of the FPBA ligands on the PRX framework. In vivo studies demonstrated superior tumor accumulation of FPBA-PRX compared with that of FPBA-CEL. Importantly, when administered before neutron irradiation, FPBA-PRX resulted in greater boron accumulation in tumors than BPA and significantly inhibited tumor growth compared to BPA. No adverse effects on body weight or blood biochemistry were observed, indicating the safety of this compound. These findings support the use of FPBA-PRX as a promising candidate for tumor-targeted boron delivery in BNCT.

REFERENCES:
[1] Wang J. *et al.,* Sci. Adv., **5** (2019) eaaw4357.
[2] Obayashi S. *et al.,* Oral Oncol., **40** (2004) 474-482.

CO7-30

Attempts to sensitize tumor cells by exploiting the tumor microenvironment

Y. Sanada, T. Takata, Y. Sakurai, H. Tanaka and T. Watanabe

Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron neutron capture therapy (BNCT) is a radiotherapy that kills tumor cells via the 10B(n, α)7Li reaction [1]. As reported previously, these high-LET particles produce highly complex DNA damages[2], which can activate cytosolic DNA-mediated signaling pathways, such as interferon response. Since tumor tissues contain various types of cells, it is necessary to take advantages of reporter genes to detect tumor cells-specific signaling. In the present study, we established a targeting vector containing two reporter genes that allows us to monitor expression of Ifnb and ActB genes.

EXPERIMENTS: Targeting vector #1 was generated as described in Fig 1. The targeting vector contained mouse Ifnb promoter, EGFP, mouse Actb promoter, mOrange2 and a neo-resistance marker. SCC VII cells were transfected with targeting vector #1 and CRISPR/Cas9 expression vector, the G418-resistant cell clone was obtained (SCC VII-pIfnb-EGFP/pActb-mOr2 cells).

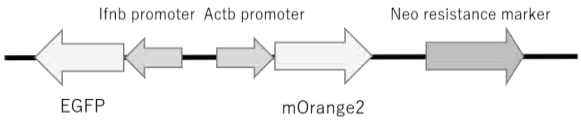


Fig. 1. Targeting vector #1 used for the establishment of SCC VII-pIfnb-EGFP/pActb-mOr2 cells.

RESULTS: Transcriptional induction of Ifnb gene was examined using HT-DNA as a positive control (Fig 2). The upregulation of Ifnb mRNA levels was observed 6 hours after transfection of HT-DNA. There was no increase in Ifnb mRNA level 3 or 24 hours after transfection. Next, we tested if two transgenes are functional. Similar to Ifnb, the reporter EGFP mRNA level was increased 6 hours after transfection of HT-DNA, while the other reporter mOrange2 level was not greatly increased. In this study, we used ActB as an internal control, but also found that HT-DNA transfection possibly influenced ActB mRNA levels. Therefore, mRNA expression level analysis should be performed using multiple internal controls.

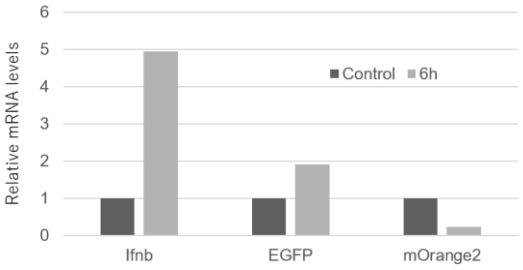


Fig. 2. Relative mRNA levels of Ifnb, and two transfenes EGFP and mOrange2 after HT-DNA transfection. The values are normalized to the mRNA level of Actb at each time point.

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.

CO7-31

Tumor responses after BNCT at early stages

Y. Tong[1, 2], Gao Zhongming[1], B. Saraswat[1], M. Ishiai[2,3], Y. Sanada[4], Y. Sasaki[1,5], T. Nozaki[1,5], and M. Masutani[1,2,3]

[1] *Dept. of Molecular and Genomic Biomedicine, Center for Bioinformatics and Molecular Medicine, Nagasaki University Graduate School of Biomedical Sciences*
[2] *Central Radioisotope Division, National Cancer Center Research Institute*
[3] *Division of Boron Neutron Capture Therapy, EPOC, National Cancer Center*
[4] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[5] *Dept. Pharmacology, Osaka Dental Univ.*

INTRODUCTION: Boron neutron capture therapy (BNCT) is a molecular-targeted cancer therapy that employs high-energy alpha particles and lithium nuclei generated by nuclear reactions and ^{10}B carrier drug that could be preferentially incorporated into cancer cells. We reported the role of HMGB1 and the early effect on proteome after BNCT [1-2]. To optimize the effectiveness of BNCT and to find biomarkers for treatment, we analyzed the response of cancer cells to BNCT further.

EXPERIMENTS: Neutron irradiation experiments at the KUR reactor were carried out at a constant power level of 1 MW in all cases as described [1]. The total physical dose was calculated using the flux-to-dose conversion factor [1]. The relevant data is presented in Tables 1-3.

The cancer cells including human squamous cell line SAS, HSC3 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 protein isolation and molecular analysis.

Mouse melanoma cell lines B16F10 and the variant were grafted to the hind legs and were locally irradiated for 60 min using ^{6}LiF containing shield for thermal neutron. Mice were injected with BPA at 500 mg/kg bodyweight approximately 30 min before irradiation. Mice were euthanized on day 7-14 after irradiation, and blood, tumors, and other organs were analyzed.

RESULTS: The measurement of thermal neutron fluence and doses for cells (Table 1) and mice (Tables 2 & 3 and Fig. 1) were indicated. Modulation of the expression level of SNHG12 caused changes in the early responses of cancer cells to BNCT. The cellular responses of mice to BNCT are being analyzed.

Table 1. Irradiated doses of cells on Dec. 4, 2024 (Rail, irradiation room).

Irradiation time[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** (1ppm)
10	Center	1.2E+12	2.1E+11	1.5E-01	1.7E-02	1.1E-01	1.2E-01	4.0E-01	8.6E-02
60	Center	6.8E+12	1.2E+12	9.1E-01	9.7E-02	6.7E-01	1.1E+00	2.8E+00	5.1E-01
2	Center	2.3E+11	4.1E+10	3.1E-02	3.3E-03	2.3E-02	2.2E-02	7.9E-02	1.7E-02
4	Center	3.8E+11	6.8E+10	5.1E-02	5.4E-03	3.8E-02	2.3E-02	1.2E-01	2.8E-02
6	Center	6.8E+11	1.2E+11	9.0E-02	9.6E-03	6.7E-02	3.8E-02	2.0E-01	5.0E-02
8	Center	8.8E+11	1.6E+11	1.2E-01	1.2E-02	8.7E-02	8.5E-02	3.0E-01	6.5E-02

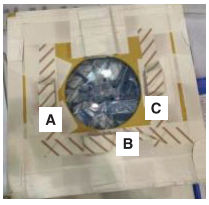
Table 2. Irradiated doses for local irradiation of mice on Dec.10, 2024 (Cart, irradiation room).

Irradiation time [min]	Position	Fuence [/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** (1ppm)
60	Center	3.5E+12	6.2E+11	4.6E-01	4.9E-02	3.4E-01	2.3E-01	1.1E+00	2.6E-01
60	Center	3.9E+12	7.0E+11	5.2E-01	5.6E-02	3.9E-01	2.5E-01	1.2E+00	2.9E-01

Table 3. Irradiated doses for local irradiation of mice on February 5, 2025 (Cart, irradiation room). Positions A-C were indicated in Fig. 1.

Irradiation time[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** (1ppm)
60	Center	3.8E+12	6.8E+11	5.1E-01	5.4E-02	3.8E-01	2.5E-01	1.2E+00	2.8E-01
60	Center	3.9E+12	6.9E+11	5.2E-01	5.5E-02	3.8E-01	2.3E-01	1.2E+00	2.9E-01
	Position A	3.8E+12	6.7E+11	5.0E-01	5.3E-02	3.7E-01	2.3E-01	1.2E+00	2.8E-01
	Position B	3.8E+12	6.7E+11	5.0E-01	5.3E-02	3.7E-01	2.3E-01	1.2E+00	2.8E-01
	Position C	3.8E+12	6.8E+11	5.1E-01	5.4E-02	3.8E-01	2.3E-01	1.2E+00	2.8E-01

Fig. 1. Positions A-C for February 5, 2024. (Cart, irradiation room)



REFERENCES:

[1] Imamichi S. *et al.,* Biology, **11** (2022) 420.
[2] Perico D., *et al.,* Cells, **12(12)** (2023) 1562.
[3] Tong Y. *et al.,* J Oncology., **4(2)** (2024) 1163.
[4] Imamichi S. *et al.,* Appl Radiat Isot., **218** (2025)111648.

CO7-32

BNCT with a novel boron drug, BBCIP, for a rat brain tumor model

K. Eza, S. Kawabata, Y. Fujikawa ,H. Kashiwagi, H. Tanaka[1], M. Suzuki[1], S-I. Miyatake[2], K.nishimura[3],H.nakamura[3] and M. Wanibuchi

Department of Neurosurgery, Osaka Medical and Pharmaceutical University
[1]Institute for Integrated Radiation and Nuclear Science, Kyoto University
[2]Kansai BNCT Medical Center, Osaka Medical and Pharmaceutical University
[3]School of Life Science and Technology, Tokyo Institute of Technology

INTRODUCTION:

Malignant gliomas diffusely invade normal brain parenchyma, leaving residual tumor cells even after surgical resection. Combination therapy with radiation chemotherapy is the standard of care, however, despite this approach, gliomas remain highly aggressive and and prone to recurrence, leading poor prognosis. Boron neutron capture therapy (BNCT) is a form of particle therapy that selectively destroys tumor cells by incorporating boron compounds and irradiating neutrons.
BNCT is being researched and developed as a treatment for malignant gliomas because of its strong anti-tumor effect at the cellular level, but it has not yet been approved by insurance for malignant brain tumors. The company aims to expand the indication of malignant brain tumors with BNCT using a novel boron-based drug.

****

EXPERIMENTS:

In this study, we used a novel boron compound called BBCIP, designed to target the biotin receptor, specifically the Sodium-dependent Multivitamin Transporter (SMVT). BBCIP is a low-molecular-weight boron carrier that incorporates biotin as a ligand for the biotin receptor, along with an albumin ligand and a boron source. In vivo biodistribution study using a rat brain tumor model, we confirmed sufficient boron accumulation in the tumor when BBCIP was administered via convection-enhanced delivery (CED) **[1]**, a method our group has previously reported. Furthermore, neutron irradiation was performed on the rat brain tumor model after CED administration of this compound. The rat tumor models were randomly as
signed into five groups: a control group receiving no treatment (untreated), a group subjected to neutron irradiation alone (neutron only), a BPA-BNCT group (The BNCT group received an intravenous injection of BPA at a dose of 250mg/kg, followed by neutron irradiation 2.5 h later),a CED BBCIP-BNCT group(The BNCT group received a 200 μL solution of BBC-IP was directly injected into the interstitial space of the brain over 24 h under continuous low positive pressure, followed by neutron irradiation 3h later),and a combination BNCT(BPA and CED BBCIP group) In overall survival and any adverse events were assessed post-irradiation.

****

RESULTS:

CED BBCIP BNCT significantly prolonged overall survival in brain tumor rats. However, the effect was insufficient compared to BPA-BNCT. Conbination BNCT showed prolonged survival, but no significant difference compared to BPA-BNCT.

****

REFERENCES:

[1] K. Tsujino *et al.,* Neurooncol Adv., **22** (2024) vdae062.

CO7-33

Pathological Assessment of Boron Neutron Capture Therapy with CED-Based Delivery of FRα-Targeting PBC-IP in Non-Tumor-Bearing Rats

K. Tsujino[1], K. Nishimura[2], K. Eza[1,] Y. Fujikawa[1], H. Kashiwagi[1], H. Tanaka[3], M. Suzuki[3], S-I. Miyatake[4], M. Wanibuchi[1], H.Nakamura[2] and S. Kawabata[1]

[1]*Department of Neurosurgery, Osaka Medical and Pharmaceutical University*
[2]*School of Life Science and Technology, Institute of Science Tokyo*
[3]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[4]*Kansai BNCT Medical Center, Osaka Medical and Pharmaceutical University*

INTRODUCTION

Malignant gliomas diffusely infiltrate brain tissue, making complete resection difficult and leading to recurrence despite standard treatments. We evaluated the safety of boron neutron capture therapy (BNCT) using PBC-IP [1], a boron compound targeting folate receptor alpha (FRα), which is highly expressed in gliomas [2]. PBC-IP was delivered via convection-enhanced delivery (CED), enabling direct distribution into the brain. While its therapeutic potential in tumor models has been reported [3], safety under neutron irradiation in normal brain tissue remains unclear. We therefore assessed pathological effects of CED-administered PBC-IP with BNCT in healthy rats.

EXPERIMENTS

PBC-IP at concentrations of 500 or 1500 μg B/mL (200 μL total volume) was administered into the brain of normal Fischer rats via CED at a rate of 8 μL/h. Neutron irradiation was conducted at 1 and 3 hours after the end of administration to examine the effects at different time points post-delivery. Animals were sacrificed at 2 and 4 weeks after irradiation for pathological examination of the brain and other major organs.

RESULTS

Histopathological evaluation revealed no evident abnormalities in brain tissue or peripheral organs attributable to neutron irradiation in either dose group. These findings suggest that BNCT using PBC-IP administered via CED can be safely performed without inducing discernible tissue damage in non-tumor-bearing models.

DISCUSSION

The safety of boron delivery is crucial for BNCT's clinical use. Our results show that intracerebral PBC-IP via CED causes no significant pathological changes, supporting its tolerability. The lack of damage in brain and peripheral organs suggests a favorable safety profile for future use in tumor-bearing models and clinical studies.

FRα is a promising target due to its high glioma-specific expression and minimal presence in normal tissue. CED effectively bypasses the blood–brain barrier, and the 1–3 hour window for irradiation allows practical flexibility. Further studies should quantify boron distribution at the cellular level and assess long-term safety, including neurobehavioral outcomes. These findings support the development of safer, more targeted BNCT in neuro-oncology.

****

REFERENCES:

[1] Kanemitsu, T. *et al.*, Radiat. Environ. Biophys., **58** (2019) 59–67.
[2] Nishimura, K. *et al.*, J. Control. Release., **360** (2023) 24.
[3] K. Tsujino *et al.,* Neurooncol Adv., **22** (2024) vdae062.

CO7-34

Exploring Boron Neutron Capture Therapy for Chordoma: Experimental Study

Y. Fujikawa, S. Kawabata, H. Yamada, K. Eza, T. Takata[1], H. Tanaka[1], M. Suzuki[1], N. Hu[2], S-I. Miyatake[2], T. Takami, and M. Wanibuchi

Department of Neurosurgery, Osaka Medical and Pharmaceutical University
[1]Institute for Integrated Radiation and Nuclear Science, Kyoto University
[2]Kansai BNCT Medical Center, Osaka Medical and Pharmaceutical University

INTRODUCTION: Chordomas are rare bone tumors characterized by local invasiveness, high recurrence rates, and relative radioresistance. While treatment modalities such as proton and carbon ion irradiation have been developed in recent years, definitive evidence supporting their clinical efficacy remains limited [1]. This study aimed to evaluate the potential effectiveness of boron neutron capture therapy (BNCT) as a treatment for chordoma through experimental investigations.
****

EXPERIMENTS: ***In vitro:*** U-CH1 and JHC7 human chordoma cell lines were employed in this study. Neutron irradiation was then applied to these two cell lines, BNCT with BPA (10 μg Boron/mL for a 24h exposure before irradiation) (BNCT group), and neutron irradiation without BPA (hot control group), for 0-, 10-, 20- and 30-min (1MW). Assessment of the cell-killing effect was carried out using a colony forming assay. ***In vivo:*** Subcutaneous U-CH1-bearing mice were intravenously administered BPA. After 1 and 3 h, the mice were sacrificed, and the boron concentrations in both the tumor and each organ were measured using Inductively Coupled Plasma Atomic Emission Spectroscopy. The tumor-bearing mice were randomly assigned into four groups: a control group receiving no treatment (untreated; n = 4), a group that was administered BPA intravenously (BPA iv; n = 4), a group subjected to neutron irradiation alone (neutron only; n = 4), and a BNCT group (n = 4). The BNCT group received an intravenous injection of BPA, followed by neutron irradiation (5MW, 15min) 2h later. Relative tumor volume ratios were assessed post-irradiation for 3 months. More detailed information related these methodologies can be obtained by our previous study [2].
****

RESULTS: In the in vitro study, neutron irradiation revealed that the BNCT group demonstrated a more pronounced cell-killing effect than the hot control group in both cell lines. In the in vivo biodistribution of boron, the tumor accumulation was 5.1 μg B/g with a tumor-to-blood ratio (T/Bl) of 1.04 at 1h and 3.6 μg B/g with a tumor-to-blood ratio (T/Bl) of 1.77 at 3h, respectively. Neutron irradiation, especially with i.v. BPA (BNCT), significantly suppressed the tumor growth compared to the untreated or BPA iv group.
****

CONCLUSION: Despite relatively lower boron uptake compared to other malignant tumors, these findings suggest that BNCT could be an effective therapeutic option for chordoma.
****

REFERENCES:

[1] A. Iannalfi *et al.,* Neuro Oncol., **29** (2020) 1348-1358.
[2] Y. Fujikawa *et al.,* Cancer Sci., **115** (2024) 2774-2785.

CO7-35

Deviation of Important Elements for Activation in Three Types of Ordinary Concrete for Radiation Shielding

K. Kimura, T. Takata[1], Y. Sakurai[1], H. Tanaka[1] and K. Takamiya[1]

Fujita Corporation
[1] Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: In several years, we performed neutron activation analyses (NAA) to more than several hundreds of samples for radiation shield concrete and raw materials by KUR facilities, including a few types of ordinary concrete and several types of low-activation concrete. Concrete is widely used as radiation shield in nuclear reactors and accelerator facilities because of its flexibility, sufficient supply and low cost. Once these facilities start operating, however, the shielding concrete becomes radioactive by nuclear reaction with neutrons generated. Therefore, it is very important to know their activation property. To estimate the level of activation, we have performed NAA on more than several hundred concrete samples using KUR facilities [1]. In this report, we describe the deviation of important rare elements in eight types of concrete manufactured by different plants.
METHODS: Three types of ordinary concrete by three different JIS (JIS A 5308) qualified concrete plants (Toei, Yoko, and Line, referring plant name, respectively) were prepared and irradiated in KUR, focusing to estimate Eu, Co and Cs (detail procedure was described in the last reports [1]). Eight kinds of concrete are symbolized by type of plant and type of cement in figures (First character indicate name of plant, such as "Toe" meaning Toei concrete, "Yok" meaning Yoko concrete, and "Line" meaning Line concrete. Second character indicate kind of cement, such as "Ord" as Ordinary Portland cement, "Med" as Moderate heat cement, and "Low" as Low heat cement).
RESULTS: Figures show the distribution of deviation for the contents of Eu, Co, and Cs as ratio for the average value, respectively. The ratios of Eu and Co, describing in left figure, are existing in limited ranges within ±10% for Eu and within ±20% for Co, while the ratio of Cs widely disperse up to ± 80%. Considering that past measurements of Cs content have shown large uncertainty, these results indicate that the deviation of Eu and Co is enough small in the concrete with JIS qualified manufacturer plants, under the proper measurement procedure of NAA

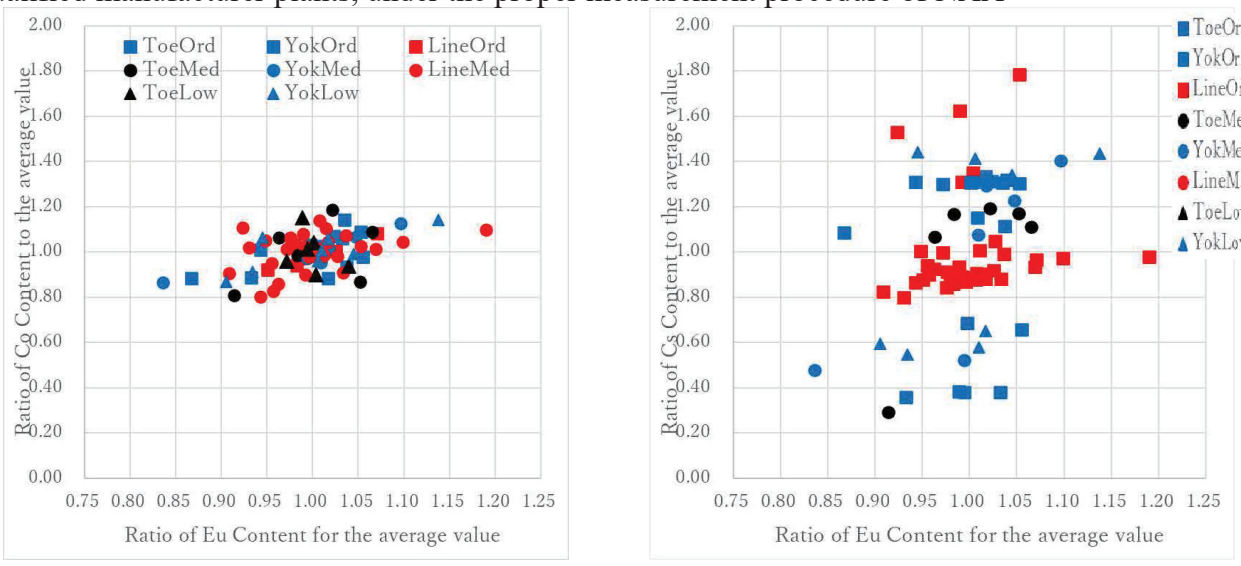


Fig. 1 Ratio of Eu between Co content (Left) and Eu between Cs content (Right) to the average value in several types of ordinary concrete.

REFERENCES:

[1] K. Kimura *et al.,* KURNS Progress Report 2018–2023.

CO7-36

Detection of *p*-Borono-L-phenylalanine (BPA Absorbed in Rice Seeds Using a Boron Neutron Capture Reaction.

T. Kinouchi

I*nstitute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: To cope with the decline in paddy rice yields due to global warming, efforts are underway to develop new rice varieties that are resistant to high-temperature injury. However, the frequency of beneficial rice varieties resulting from crossbreeding is relatively low, highlighting the need for breeding methods that can artificially and efficiently produce new varieties. Recently, the application of genome editing technology in breeding has gained attention. Although this method can accurately target specific genes and introduce mutations, it cannot create new varieties that exceed expectations, leading to a reevaluation of methods that induce random mutations on a genome-wide scale. Conversely, the conventional radiation breeding method, which employs gamma rays or fast neutrons, can damage biopolymers and intracellular organelles beyond DNA due to their high energy. This significantly affects the survival rate and physiological functions of rice seeds and their embryonic tissues, thus reducing efficiency. We have developed a novel breeding method to introduce mutations into the rice genome using the Boron Neutron Capture (BNC) reaction, a technique also utilized in cancer therapy. The BNC reaction aims to minimize damage by irradiating rice with low-energy epithermal neutrons, thus efficiently producing new rice varieties. Consequently, we have decided to investigate whether BPA, a neutron-sensitizing reagent, is absorbed by actual rice seeds.

EXPERIMENTS: Materials> Rice seeds (Oryza sativa L. cv. Hinohikari) were kindly gifted from Dr. Segami, Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture. Boron-10 (^{10}B)-containing neutron-sensitizing reagent, BPA (p-borono-L-phenylalanine) was kindly gifted from Dr. Hattori, Research Center for BNCT, Osaka Metropolitan University.
In situ visualization of BPA in rice seeds> Rice seeds were immersed in BPA solution. (100 ppm) 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 BPA solution (100 ppm). Fig. 1(A) is a bright-field image. Fig. 1(B) is an α-tracking autoradiograph generated by the BNC reaction and reveals the distribution of BPA in the cross-section. The areas enclosed by the circle indicate the embryo. Many etch-pits derived from BPA were imaged throughout the section as small black spots. It was observed that BPA accumulated in the embryo compared to the area not circled (endosperm).

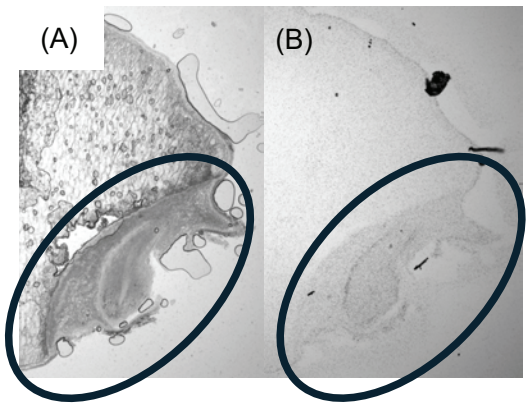


Fig. 1. Detection of BPA absorbed into the rice seed.

CO7-37

Investigation of Potential Adverse Effects of Boron Neutron Capture Therapy on Host Immunity

Tsubasa Watanabe[1], Takushi Takata[2], Yoshinori Sakurai[2], Hiroki Tanaka[2], Minoru Suzuki[1]

Institute for Integrated Radiation and Nuclear Science, Kyoto University
[1]Particle Radiation Oncology Research Center, Particle Radiation Oncology
[2]Particle Radiation Oncology Research Center, Particle Radiation Medical Physics

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a form of radiation therapy for cancer that utilizes neutron capture reactions, wherein boron-10 atoms capture thermal neutrons, subsequently undergoing nuclear fission to produce alpha particles and lithium nuclei [1]. Recent studies have revealed that X-ray irradiation does not suppress anti-tumor immune function but rather activates it. To investigate whether BNCT similarly enhances anti-tumor immunity, we have explored the relationship between BNCT and tumor immunomodulation. The purpose of this study is twofold: (1) to determine whether BNCT induces host immune-activating effects, and (2) to evaluate the composition of infiltrating immune cells within tumors post-BNCT, thereby assessing potential adverse impacts on tumor immunity.

****

EXPERIMENTS: Tumor cells were subcutaneously implanted into the hind limbs of C3H/He mice. At 12 days post-implantation, radiation treatment was administered. The control group received X-ray irradiation (20 Gy), while the BNCT group was injected subcutaneously with 500 mg/kg boronophenylalanine (BPA). One hour post-BPA administration, neutron irradiation was delivered to the tumor site using a 5 MW reactor for 12 minutes. Tumor dimensions (height, width, and length) were serially measured under isoflurane anesthesia using digital calipers.

At 72 hours post-irradiation, tumor tissues were excised from three experimental cohorts: untreated controls, X-ray-treated, and BNCT-treated groups. Single-cell suspensions were prepared using the BD Tumor Dissociation Kit (BD Biosciences, #130-096-730) per manufacturer protocol. Cells were stained with fluorochrome-conjugated antibodies against CD45 (leukocyte common antigen), CD3 (T-cells), CD4 (helper T-cells), CD8 (cytotoxic T-cells), Foxp3, CD25, and CD11b (myeloid cells). Flow cytometric analysis (Cytek) quantified immune cell infiltration ratios, with data processed using SpectroFlo software.
****

RESULTS: Initial post-BNCT analysis revealed a transient 32% reduction in anti-tumor immune cell populations compared to untreated controls. However, these populations exhibited time-dependent recovery, reaching baseline levels by Day 21 post-treatment. Tumor volume reduction was significantly greater in BNCT-treated mice versus X-ray cohorts, with no correlation observed between tumor size regression and immune cell depletion. Notably, both BNCT and X-ray groups showed increased proportions of immunosuppressive cells: Inter-group comparison revealed no significant differences in immunosuppressive cell expansion between BNCT and X-ray modalities.
****

REFERENCES:

[1] Rolf F. Barth *et al.*, Cancer Communications (2024) (doi)10.1002/cac2.12581.

CO7-38

Investigation of Potential Adverse Effects of Boron Neutron Capture Therapy on Host Immunity

Tsubasa Watanabe[1], Takafumi Shiagehira[1], Takushi Takata[2], Yoshinori Sakurai[2], Hiroki Tanaka[2], Minoru Suzuki[1]

Institute for Integrated Radiation and Nuclear Science, Kyoto University
[1]Particle Radiation Oncology Research Center, Particle Radiation Oncology
[2]Particle Radiation Oncology Research Center, Particle Radiation Medical Physics

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a targeted radiotherapy that utilizes the nuclear capture reaction of thermal neutrons by boron-10, producing high-linear energy transfer (LET) alpha particles and lithium-7 nuclei. The primary boron delivery agent, boronophenylalanine (BPA), is an amino acid analog transported into cells via L-type amino acid transporters LAT1 and LAT2. While cancer cells exhibit elevated LAT1 expression due to metabolic reprogramming, normal tissues also express LAT2, enabling BPA uptake in healthy cells. This unintended accumulation contributes to adverse effects post-BNCT, despite the therapy's theoretical tumor specificity. This study hypothesizes that selective inhibition of LAT1 (tumor-predominant) and LAT2 (normal tissue-expressed) could improve the tumor-to-normal tissue boron concentration ratio. By pre-administering transporter-specific inhibitors prior to BPA infusion, we aim to suppress normal tissue boron uptake while preserving tumor targeting, thereby enhancing BNCT's therapeutic index.

****

EXPERIMENTS: Tumor Model and Irradiation Protocol: Subcutaneous tumors were established in mice using non-genetically modified cancer cells. At a tumor volume of 150–200 mm, BPA (500 mg/kg) was intravenously administered. Neutron irradiation was performed using a heavy water-based facility, with comparative X-ray cohorts receiving equivalent physical doses (20 Gy). Mice were sacrificed at 7, 14, and 21 days post-irradiation for histopathological analysis of tumor and normal tissues (tongue, liver, skin). Pharmacokinetic Modulation: To assess BPA biodistribution modulation, anticholinergic (atropine, 2 mg/kg) or cholinergic (pilocarpine, 5 mg/kg) agents were administered intraperitoneally 30 minutes prior to BPA. Tissues were digested in a 3:1 mixture of perchloric acid and hydrogen peroxide, followed by boron quantification via inductively coupled plasma atomic emission spectroscopy (ICP-AES). Abdominal Irradiation Study: A separate cohort of tumor-free mice received abdominal X-ray or BNCT irradiation to evaluate gastrointestinal and hepatic toxicity, with organ-specific boron concentrations measured as above.

****

RESULTS: Biodistribution Analysis: Pre-administration of anticholinergic or cholinergic agents failed to alter boron concentrations in normal tissues. No significant differences in tumor boron retention were observed between groups, indicating the tested agents did not modulate LAT1/LAT2-mediated transport under these conditions. Abdominal Toxicity: BNCT induced severe duodenal edema, leading to the irradiated mice death vs. X-ray, correlating with elevated boron levels in intestinal mucosa (14.2 ± 2.3 ppm). Hepatic boron accumulation remained low, consistent with low CBE and minimal hepatotoxicity compared to the CBE of the mucosa.

****

REFERENCES:

[1] Rolf F. Barth*et al.* , Cancer Communications (2024) (doi)10.1002/cac2.12581.

CO7-39

Combining GdNCT and anti-PD-1 immunotherapy to boost abscopal effect

Li Zhao[1], Weian Huang[2], Minoru Suzuki[3] and Naoki Komatsu[2]

[1] School for Radiological and Interdisciplinary Sciences (RAD-X) and Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Soochow University, China

[2] Graduate School of Human and Environmental Studies, Kyoto University, Japan [3] Institute for Integrated Radiation and Nuclear Science, Kyoto University, Japan

INTRODUCTION: Gadolinium neutron capture therapy (GdNCT) is a promising binary radiotherapy for cancer treatment, leveraging the nuclear reaction between thermal neutrons and ^{157}Gd, which exhibits an exceptionally high neutron capture cross-section of 254,000 barns. This reaction generates high-LET Auger electrons and low-LET γ-photons, both of which contribute to targeted cancer cell destruction.[1]

The abscopal effect—a phenomenon in which non-irradiated tumors regress alongside directly irradiated lesions following radiotherapy—has shown significant potential in enhancing systemic antitumor efficacy. With the widespread clinical adoption of PD-1/PD-L1 immune checkpoint inhibitors, cancer therapy has entered the era of immunotherapy. Notably, the combination of radiotherapy and immunotherapy has been demonstrated to amplify the antitumor effects of radiation.[2]

Recently, our group has developed poly(glycerol) functionalized Gd nanoparticle (GdNP-PG) that can mediate effective GdNCT of cancer upon intravenous administration. The therapeutic outcomes were further improved when combined with anti-PD-1 immunotherapy.

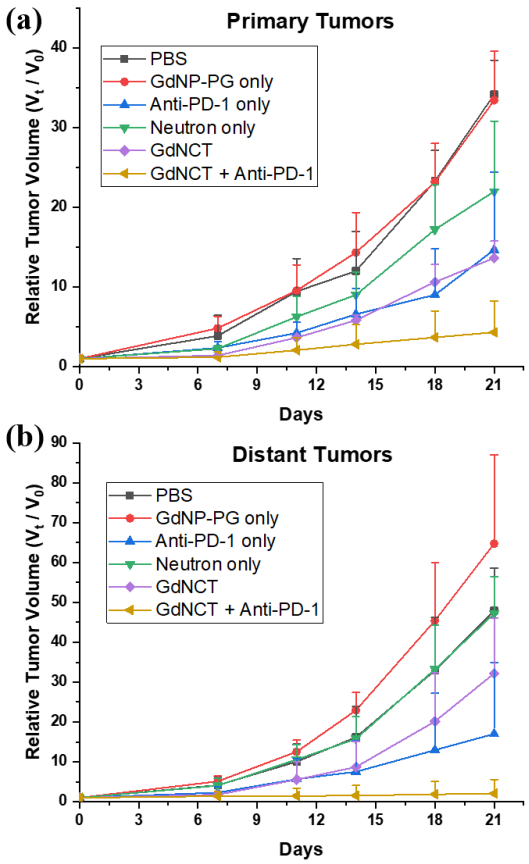


Figure 1. Tumor growth curves of (a) primary tumors inoculated on the right legs; (b) distant tumors inoculated on the right backs. (n = 3)

EXPRIMENTS and RESULTS: In this study, we investigated the abscopal effect induced by the combination of GdNCT and anti-PD-1 therapy in a dual-tumor mouse model (CT26 colon carcinoma). Each mouse bears two tumors: a primary tumor (inoculated on the right leg and subjected to irradiation) and a distant tumor (on the right back but left untreated). For the GdNCT + anti-PD-1 group, mice received thermal neutron irradiation 24 h after GdNP-PG injection, followed by intraperitoneal anti-PD-1 administration on days 1, 5, 8, and 11. As illustrated in Figure 1, the combined treatment significantly suppressed growth in both primary and distant tumors compared to control groups. Remarkably, two-thirds of distant tumors were completely eradicated, demonstrating a pronounced abscopal effect. Further studies are underway to elucidate the underlying mechanisms, including potential modulation of the tumor immune microenvironment.

Reference:

[1] Y-H. Lai *et al.,* Nat. Commun., **14(1)** (2023) 285.
[2] D. Duan *et al.,* CCS Chemistry, **5** (2023) 2589–2602.

CO7-40

Investigation of nano-boron drugs for BNCT

ZhiCheng Zhang[1,2], Qi Dai [1,2], Yaxin Qin [2], Yufan Yang [2], Xiaoyan Sun [2], Qiyao Yang[1], Lihong Liu[1], Minoru Suzuki [3], Min Han[2], Qichun Wei[1]

[1] *Department of Radiation Oncology, Key Laboratory of Cancer Prevention and Intervention, The Second Affiliated Hospital, College of Medicine, Zhejiang University*

[2] *College of Pharmaceutical Sciences, Zhejiang University*

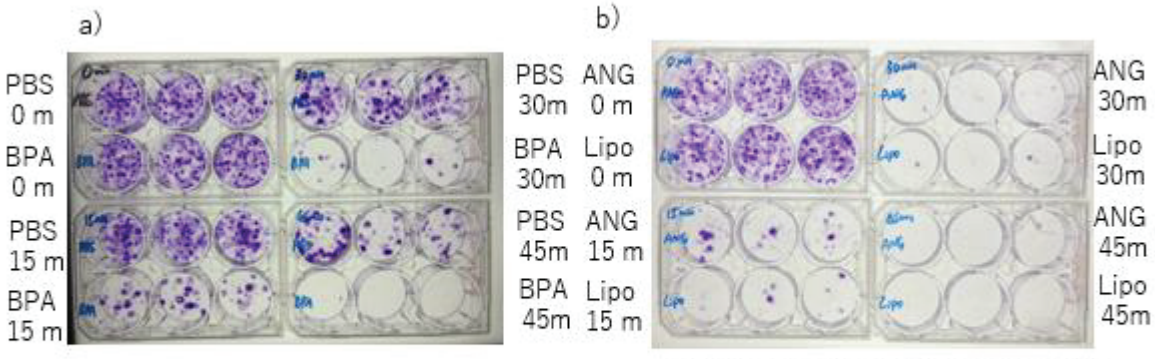
[3] *Division of Particle Radiation Oncology, Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: Boron neutron capture therapy (BNCT) has shown significant efficacy for malignant glioma. However, the boron neutron capturing agent currently used, p-boronophenylalanine (BPA), suffers from poor water-solubility, short tumor retention time, and an inability to monitor real-time boron distribution and concentration within the body. Meanwhile, we constructed nanodrugs including boron agents which were combined with liposome. The therapeutic effect of these nano-drugs 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, boronophenylalanine (BPA)-fructose, and two nano-drugs (ANG and Lipo), were added, respectively. After 4 hours of further incubation, the supernatant was discarded, and the cells were collected and counted using trypsin digestion. The cells were then transferred to four 2 mL centrifuge tubes (each tube containing 2×10^4 cells). Each set of four tubes were subjected to neutron irradiation for 0, 15, 30, and 45 minutes, respectively. Following BNCT, cells from each tube were then seeded onto a 6-well plate (1,500 cells/well), with three replicates for each cell sample. After 14 days of further culture, the cells were fixed with 70% ethanol and then stained with crystal violet solution, allowing for the calculation of cell colony formation number.

RESULTS: According to the colony formation results (Figure 1), it can be seen that two nano-drugs (ANG and Lipo) can effectively kill tumor cells at the cellular level, and the effect is slightly stronger than BPA-fructose. Further investigation of the two nano-drugs is warranted. We plan to conduct an in-vivo assay using cancer-baring mice.

Fig.1 Results of colony formation assay



a): Colonies following PBS and BPA exposure (4h) and thermal neutron irradiation (0, 15, 30, and 45 mi).

b): Colonies following two nano-drugs (ANG and Lipo) exposure (4h) and thermal neutron irradiation (0, 15, 30, and 45 mi).

CO7-41

Analysis of the Structural Change of Boron Compounds after Boron Neutron Capture Reaction

H. Ueda, M. Suzuki, Y. Sakurai, T. Takata

Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION:

Boron neutron capture therapy (BNCT) is one of the unique radiotherapies based on the combination of boron compounds and epi-/thermal neutron irradiation. In this treatment, high linear energy transfer (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 atom 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 has been used in BNCT research for decades, the effect of boron neutron capture reaction on the molecular structure of boron compounds is unclear. In this work, we attempted the investigation of structural change of boron-containing molecules after epi-/thermal neutron irradiation.

EXPERIMENTS and RESULTS

The aqueous solution of boron compound was prepared using ^{10}B-enriched BPA purchased from InterPharma (Prague, Czech Republic). The boron concentration was measured 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). Boron solution was poured into Teflon tubes and 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 the samples was evaluated by gamma-ray measurement. The structural change of BPA was analyzed by electrospray ionization mass spectrometry (ESI-MS) after derivatization of the analytes. As a result, tyrosine was observed in the solution of BPA, possibly due to oxidative degradation by hydrogen peroxide which produced by gamma ray irradiation in neutron-mixed beam.

****

REFERENCES:

[1] M. Suzuki, Int. J. Clin. Oncol., **155** (2021) 182-187.
[2] D.D. Pierro *et al.*, Anal. Biochem. **284** (2000) 301-306.
[3] S. Caquani *et al.*, Int. J. Radiat. Oncol. Bioll. Phys. **72** (2008) 562-567.
[4] L. Dick *et al.*, J. Pharm. Biomed. Anal. **56** (2011) 633-636.
[5] G. Halbert *et al.*, Eur. J. Pharm. **48** (2013) 735-739.

CO7-42

Evaluation of a Retinoid X Receptor-Binding BSH Derivative for Inhibition of Cell Proliferation Under Neutron Irradiation

Atsushi Mashimo[1], Ryuya Otani[2], Natsuko Kondo[3], Yoshinori Sakurai[3], Hiroki Kakuta[1]

[1]*Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.*

[2]*Okayama University, School of Pharmaceutical Sciences.*
[3]*Institute for Integrated Radiation and Nuclear Science, Kyoto University.*

INTRODUCTION: The only ^{10}B boron drug approved for BNCT is borofaran, which is licensed in Japan. To maximize the likelihood of collisions between ^{10}B and thermal neutron beams within cancer cells, borofaran must be administered continuously during neutron irradiation in large doses, such as 500 mg/kg of body weight. Consequently, lower dosages of boron delivery agents are necessary. A recent report indicates that, the dosage of boron medications can be significantly reduced by delivering boron agents directly into the nucleus.[1] Therefore, we focused on boron compounds that bind to nuclear receptors. Although boron compounds targeting nuclear receptors have been reported, there is currently no information regarding the use of these compounds in neutron irradiation. Having previously developed ligands that bind to the retinoid X receptor (RXR), one of the nuclear receptors, we investigated compounds containing ^{10}B that also bind to RXRs. We created CBTF-BSH (**1**) that exhibits RXR-binding characteristics by substituting the fluorescent BODIPY moiety of CBTF-BODIPY (**2**) [2] to BSH, a water soluble boron cluster. The exposure at 100 μM to the human breast cancer cell line MCF-7 gave 1 fmol/cell. In this study, we performed colony formation assay for cells treated with **2**.

****

EXPERIMENTS: Compound **1** was synthesized by authors. The MCF-7 cell line was obtained from RIKEN BioResource. MCF-7 cell were treated with **1** at 100 μM for 24 hours. Subsequently, the cells were collected after washing with PBS and treating with trypsin-EDTA. All resultant cells were then centrifuged in a 15 mL centrifuge tube, the supernatant was aspirated off, and 2 mL of media was added to count the number of cells. After preparing cell suspensions at 5×10^4 cells/mL and the suspension was transferred to one milliliter of them into 1.5 mL Eppendorf tubes, and the samples were exposed to a thermal neutron fluence of 1.0×10^{12} cm^{-2} (1 MW, 10 min). Following this, the cells were seeded at a density of 5×10^3 cells/well in 12-well plates and cultivated for 7 days at 37°C with 5% CO_2. After removing the medium, the cells were fixed in 80% EtOH, stained with crystal violet, washed with Milli-Q water, and the number of colonies was counted. The stained colony area was calculated using the "ImageJ-plugin Colony Area" software [3] and normalized by comparing it with the results of non-irradiated and unexposed cell samples.

****

RESULTS: The combination of **1** and neutron irradiation significantly decreased cell proliferative activity, while exposure to **1** alone had a minimal effect on cell proliferation (Figure 1). These results suggest that boron compounds that bind to nuclear receptors are effective as boron delivery carriers for BNCT and indicate that **1** may be utilized as an intracellular ^{10}B carrier.

****

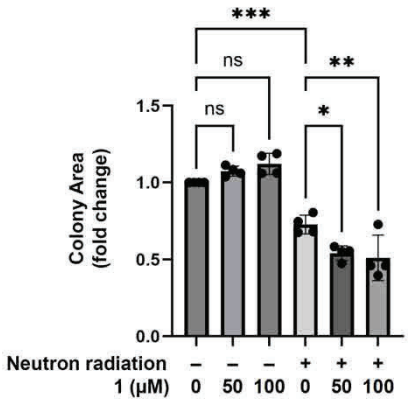


Figure 1. Comparison of the cell proliferative activity of MCF-7 cells following exposure to **1** and neutron irradiation.

REFERENCES: [1] *Adv Theory Simul*. **2023**, *6*, 2300163., [2] *ACS Med. Chem. Lett.* **2021** *12*, 1024–9., [3] *PLoS One.* **2014,** *19*, e92444.

CO7-43

Investigation of Boron Neutron Capture Reaction by Iodine-containing BSH Derivatives

Atsushi Mashimo[1], Rui Nozaki[2], Mayuri Inoue[3], Natsuko Kondo[4], Yoshinori Sakurai[4], Hiroki Kakuta[1]

[1]*Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.*

[2]*Okayama University, School of Pharmaceutical Sciences.*

[3]*Division of Natural Science, Okayama University, Graduate School of Environmental, Life, Natural Science and Technology.*

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

INTRODUCTION: Since BNCT relies on the interaction of ^{10}B with neutrons, the concentration of ^{10}B in tumor tissue is critical for the treatment's effectiveness. There are currently no boron agents available that can accurately quantify boron levels in cancerous tissue. We aimed to develop a BNCT drug capable of identifying boron concentrations in malignant tissues. We focused on iodine contrast agents used in X-ray computed tomography because they can noninvasively detect iodine concentrations and are administered in similar large doses to BNCT agents. To test this hypothesis, we designed and synthesized **1**. Additionally, we conducted experiments to evaluate the effects of combining it with the existing drug **A**, which enhances intracellular delivery capacity.

****

EXPERIMENTS: Compound **1** was synthesized by our group. Following 2-hour chemical exposure, cells were harvested by washing with PBS and treated with trypsin-EDTA. All cells were then lysed using RIPA buffer, and the ^{10}B concentration in the resulting samples was measured by ICP-MS. In 6 well plates, cells were cultivated on CR-39 pieces (2 cm square) in the medium and then exposed to a medium containing test compound. Following a wash with PBS, the CR-39 pieces were irradiated. After neutron irradiation, the CR-39 pieces were treated with alkali, and photographed using an optical microscope, and processed.[1][2] Image J was used to analyze the photographs. Colony assays were performed as described in R6163, with samples irradiated. In this study, samples was irradiated with a thermal neutron fluence of 1.1×10^{12} cm^{-2} (1 MW, 10 min).

****

RESULTS: An intracellular boron concentration in MCF7 cells, following a 2-hour exposure to 500 μM of **1** alone or in combination with drug **A**−which enhances the intracellular delivery of BSH −resulted in approximately 3.6-fold and 6-fold higher levels than BSH, respectively (Figure 1A, left four columns). This preference was further supported by the results obtained from CR-39. Additionally, under the combined conditions with drug **A**, **1** (250 μM) yielded similar intracellular boron levels as BSH at half its concentration (500 μM). The effects of compound exposure and neutron irradiation on cell proliferative activity were assessed using a colony formation assay, which demonstrated that the combination of **1** and neutron irradiation significantly reduced cell proliferative activity (Figure 1B). Furthermore, the cell proliferative activity of **1** was decreased in a concentration-dependent manner when combined with drug **A**.

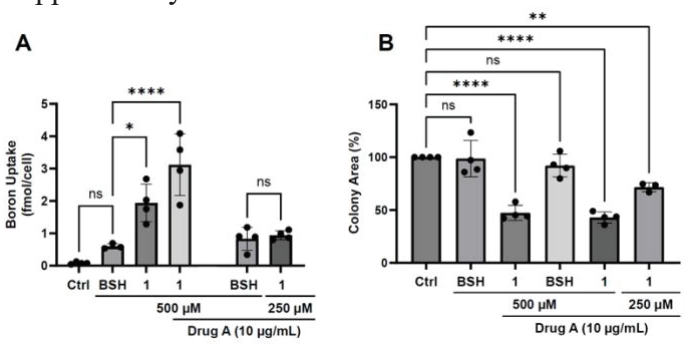


Figure 1. 1 and drug A exposure experiments with MCF7 cells. (A) Intracellular boron concentration. (B) Comparison of cell proliferation ability following neutron

****

REFERENCES: [1] J. Control Release, **330** (2021) 788-796.
[2] Radiat. Res., **191** (2019) 460-465.

CO7-44

Safety of Boronated matrix metalloproteinase ligand 1 *in vivo*

Natsuko Kondo[1], Jonathon Kane[2], Masao Takagaki[3], Yoshinori Sakurai[1] and Daniel P. Becker[2]

[1] *Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University (KURNS)*
[2] *Depar tment of Chemistry and Biochemistry, Loyola University Chicago*
[3] *Research Center for Nuclear Physics, Osaka University*

INTRODUCTION: BNCT has shown great promise in clinical trials for treatment of glioblas-toma multiforme, malignant gliomas, melanoma, as well as head and neck cancers. Currently, there are only two clinically investigated BNCT drugs, L-boronophenylalanine (L-BPA) and sodium borocaptate (BSH), which are neither tumor-specific, nor do they accumulate in high concentrations within the tumor cells. The effectiveness of BNCT therapy is governed by the selectivity of the drug and the specific accumulation of ^{10}B atoms on tumor cells**.** We indicated the efficacy of new boro-nated matrix metalloproteinase (MMP) ligands for BNCT in vitro [1]. In this study, we proceeded the MMP ligand 1 (B1) to in vivo study, and investigated safety dose.

EXPERIMENTS:

Cells:

We used mouse glioma GL261 cells. They were cultured in DMEM medium with 10% heat-inactivated fetal bovine serum in 5 % CO_2 incubator.

Tests for safety dose of B1:

We dissolved the B1 in DMSO at the dose of 0.886mg/ml (2.5 mmol/L). We mixed this B1 solution with beta cyclodextrin (10mmol/L) and water at the ratio of 1:1:0.5. We evaluated the mixed solu-tion at the dose of 400 and 800 µl (n=3, each). The mice weighted 20g/body.

Mouse orthotopic glioma model:

We used C57BL/6 mice. The GL261 cells were inoculated into the right brain of mice. In brief, 2 x 10^5 GL 261 cells were injected in the location of 2mm right from the bregma, and 3mm depth from the surface of the brain using Hamilton syringe. We created non-irradiated and BNCT groups (n=6 in each group).

BNCT treatment:

Three weeks later, we administrated B1 intraperitoneally and one hour later, irradiated thermal neu-tron to the mouse brains using the heavy water neutron irradiation facility in KUR. After irradiation, the survival time was observed.

RESULTS:

Safety dose of B1 solution: The mice which were administered the 800 µl B1 solution lied down at prone position soon after administration, and they recovered next day. The mice which were admin-istered the 400 µl B1 solution behaved normally after administration. We set the safety dose for the B1 solution at 400 µl/20g body weight. With this safety dose, we treated BNCT to the mouse ortho-topic glioma model and under observation.

REFERENCES:

[1] Flieger S. *et al.,* Carborane-Containing Hydroxamate MMP Ligands for the Treatment of Tumors Using Boron Neu-tron Capture Therapy (BNCT): Efficacy without Tumor Cell Entry, Int J Mol Sci.,**24(8)** (2023) 6973.

CO7-45

Investigation of cellular senescence by BNCT

M. Suzuki[1], M. Sakamoto[2]

[1]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[2]*Graduate School of Biostudies, Kyoto University*

INTRODUCTION:

Boron neutrons capture therapy (BNCT) is expected to be a novel type of cancer radiotherapy. The mechanism of BNCT is based on the generation of α-particles and lithium nucleus by the nuclear fission reaction between thermal neutron and boron-10 atom. When the thermal neutron is absorbed into the boron-10 atom, the complex is split into helium and lithium nucleus. The two heavy particles have the ability to break double-strand DNA and induce apoptosis of cancer cells [1].

Cell senescence is known to be another cellular physiological status induced by various cytotoxic treatments. Cell senescence is cell growth-inhibited status caused by cell cycle stopping. Radiotherapy has been reported to induce cell senescence in tumor cells. However, the relationship between BNCT and cell senescence has not been reported. The aim of this study is to make it clear that the induction of cell senescence by BNCT and its mechanism.

EXPERIMENTS:

B16F10 cells were incubated in 10cm dishes with 70% confluence and exposed by boronophenylalanine (BPA) 24 hours before thermal neutron irradiation. Before the thermal neutron irradiation, Cells were washed with phosphate-buffered saline (PBS) and collected within the tube after trypsinization, and then, a cell number in each tube was adjusted to 10^6/mL. The suspensions of B16F10 cells were irradiated with thermal neutron beam for 0, 10, 20, 30 min at 1MW power at the Heavy Water Neutron Irradiation Facility (HWNIF). The cells after neutron irradiation were dispersed into 6well plates and incubated 37 ℃ for 8 days. SA β Gal assay and colony formation assay were carried out to examine whether BNCT induced the cell senescence in the irradiated cells [2]. Next, whole proteins in B16F10 cell were collected at 6, 12, 24 hours after neutron irradiation to examine an expression level of the cell senescence-related proteins such as p21, phosphorylated p21, p53, phosphorylated p53, p38, and phosphorylated p38 by western blotting [3].

RESULTS:

We have performed SA β Gal assay, Western blotting, colony formation assays to evaluate whether BNCT induced cellular senescence in the irradiated cells. The analysis is currently on going.

REFERENCE:

[1] Locher, G. L., Biological effects and therapeutic possibilities of neutrons, American Journal of Roentgenology and Radium Therapy, **36** (1936)1-13.

[2] Dimri GP, Lee X, Basile G, *et al.,* A biomarker that identifies senescent human cells in culture and in aging skin in vivo., Proc. Natl. Acad. Sci. U S A., **92(20)** (1995) 9363-9367. (doi)10.1073/pnas.92.20.9363.

[3] Sun X, Shi B, Zheng H, *et al.,* Senescence-associated secretory factors induced by cisplatin in mela-noma cells promote non-senescent melanoma cell growth through activation of the ERK1/2-RSK1 pathway, Cell Death Dis., **9(3)** (2018) 260. (doi)10.1038/s41419-018-0303-9.

CO7-46

Clarification of the normal cell fractionation as a trigger for radiation-induced liver injury

M.Suzuki[1] , T. Shigehira[2]

[1]*Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[2]*Graduate School of Science, Kyoto University*

INTRODUCTION: Even though radiation-induced liver injury is one of the fatal adverse events in radiation therapy, normal cell fractionation, which causes radiation-induced liver injury, is still not clear. By delivering the boron atoms in a particular normal cell fraction and irradiating them with thermal neutrons, it is possible to destroy the targeted normal cell fractions, specifically. The key in this study is the device recognizing a particular normal cell fraction. We used a linker (Linker X) which recognized non parenchymal liver cells and a boron-nanoparticles as a carrier of LinkerX in this study.

EXPERIMENTS: We applied the boron-nanoparticles conjugated with LinkerX and boronophenyl-alanine (BPA) to Balb/c mice intravenously. The mice were sacrificed 3 hours later, and the livers were resected from the mice. The resected liver was processed to the thin sections (10 μm) and put on CR-39 (solid state nuclear track detector) and irradiated with thermal neutron beam (5MW power) at the Heavy Water Neutron Irradiation Facility (HWNIF). Then, the spatial distribution of boron atoms was analyzed using autoradiography technique described in our previous study [1].

RESULTS: The distributions of the boron-nanoparticles conjugated with LinkerX and boronophenylalanine (BPA) were successfully depicted as dots which were observed with an optical microscope. In the case of the boron-nanoparticles conjugated with LinkerX, the dots seem distributed linearly along the side of hepatocytes, which indicate that the boron-nanoparticles conjugated with LinkerX distributed in non-parenchymal cells. On the other hand, in the case of BPA, the dots distributed homogeneously in the liver.

REFERENCE:

[1] Takeno S, Tanaka H, Watanabe T, Mizowaki T, Suzuki M., Quantitative autoradiography in boron neutron capture therapy considering the particle ranges in the samples., Phys. Medica, **82** (2021) 306–20.