

CO7-1 Development of Albumin Binding Gadolinium Complexes for MRI-Guided BNCT

S. Okada, K. Nishimura¹, Q. Ainaya¹, I. B. Sivaev², K. Miura, T. Takada³, M. Suzuki³, H. Nakamura

Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology
¹School of Life Science and Technology, Tokyo Institute of Technology
²Russian Academy of Sciences, Russia
³Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a promising cancer treatment of harsh and un-operatable malignant tumors. In order to obtain effective BNCT treatment of patients, it is important to control the biodistribution of the boron-containing drug and its accumulation in tumors. The conventional method to estimate biodistribution is positron emission tomography (PET) based on drugs labeled by radioactive isotopes[1], although ¹⁰B isotope is non-radioactive.

We focused on Gd contrast agents for magnetic resonance imaging (MRI), which is a non-invasive method and one of the most widely used medical diagnostics. The ¹⁵⁷Gd isotope has the highest thermal neutron capture cross section of all stable nuclides in the periodic table. The thermal neutron capture cross section of the ¹⁵⁷Gd isotope exceeds that of the ¹⁰B isotope by more than 60 times. The neutron capture reaction by ¹⁵⁷Gd causes complex inner shell transitions that produce prompt γ -emission displacing an inner-core electron, resulting in internal-conversion electron emission, and finally in the Auger electron emission, together with soft X-ray and photon emission. Therefore, the synthesis of compounds containing both boron and gadolinium can be useful not only to estimate biodistribution of boron drugs under MRI guide but also to develop efficient neutron capture cancer therapy. We have developed maleimide-functionalized *closo*-dodecaborate (MID) albumin conjugates that demonstrated high and selective accumulation in tumor tissue with no toxicity in the absence of thermal neutrons, hence a promising boron delivery system [2].

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

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

study.

CT26 tumor bearing mice (Balb/cCrSlc nu/nu female, 5–6 weeks old, 16–20 g) were injected via the tail vein with 200 μ L of MID-BSA or Gd-MID-BSA (7.5 mg [¹⁰B]/kg). The plasma, liver, lung, kidney, spleen, muscle, brain, and tumor were extracted 6, 12, and 24 hours after the injection and proceeded to ashing process, followed by quantification of B and Gd via ICP-OES.

RESULTS: Previously, we demonstrated that tumor accumulation of MID-BSA was the largest 12 h after the injection[3]. Therefore, we compared the B and Gd concentration in the organs at 6, 12, 24 hours after injection. The results are shown in Fig. 1. The B concentration of Gd-MID-BSA in tumor was comparable to that of MID-BSA in all the time points, although the Gd concentration of Gd-MID-BSA was the largest in liver, followed in order by spleen and tumor. Therefore, the concentration of B was not consistent with that of Gd, indicating the release of Gd from Gd-MID-BSA due to non-specific binding of free Gd to BSA. These results indicate it is necessary to synthesize more stable Gd complexes and remove free Gd ions in reaction solution thoroughly to proceed thermal neutron irradiation experiment.

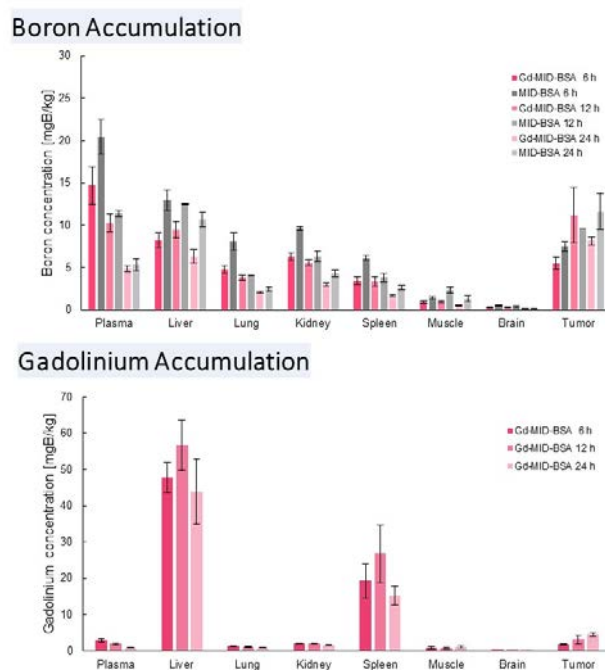


Fig. 1. Biodistribution of boron and gadolinium in colon 26 (CT26) tumor bearing mice. Gd-MID-BSA was intravenously injected at a dose of 7.5 mg [¹⁰B]/kg via the tail vein, and the organs were extracted 6, 12, and 24 hours after injection. The concentration of B and Gd in organs was quantified using ICP-OES after ashing.

REFERENCES:

- [1] V. Tolmachev *et al.*, *Bioconjugate Chem.*, **10** (1999) 338-345.
- [2] K. Kawai *et al.*, *Mol. Pharm.*, **17** (2020) 3740–3747.
- [3] S. Kikuchi *et al.*, *J. Control. Release*, **237** (2016).

CO7-2 Iodophenyl-Conjugated *closo*-Dodecaborate as a Promising Small Boron Agent for BNCT

K. Nishimura, K. Kawai, T. Morita¹, S. Okada¹, K. Miura¹, T. Takada,² M. Suzuki², H. Nakamura¹

School of Life Science and Technology, Tokyo Institute of Technology

¹*Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology*

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

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is attracting attention as a non-invasive radiotherapy in the treatment of cancer. The efficiency of boron agent depends highly on tumor selectivity, sufficient amount of boron agent in tumor site, non-toxicity, tumor/normal tissues ratio (>3) and absorption of thermal neutrons by boron. In March 2020, accelerator-based BNCT for head and neck cancer using 4-borono-L-phenylalanine (L-BPA) was approved by the Pharmaceuticals and Medical Devices Agency in Japan, making BNCT more accessible treatment [1]. L-BPA is known to actively accumulate into tumor cells through L-type amino acid transporter 1 (LAT-1). However, there are still many patients for whom L-BPA is not applicable. Therefore, the development of novel boron carriers applicable to various cancers including BPA-negative tumors is required for further expansion of BNCT.

We recently developed maleimide-functionalized *closo*-dodecaborate sodium form (MID), which conjugates not only the free SH group of a cysteine residue (Cys34) but also several lysine residues in serum albumin under physiological conditions [2,3]. The MID-conjugated albumin selectively accumulated in tumors due to the enhanced permeability and retention effect and significantly inhibited tumor growth in colon 26 tumor-bearing mice subjected to thermal neutron irradiation [2].

In this study, we focused on small molecule albumin ligands that bind noncovalently to albumin. 4-Iodophenylbutanamide (IP) was chosen as the albumin ligand and conjugated with *closo*-dodecaborate to demonstrate in vivo biodistribution.

EXPERIMENTS: Boron-conjugated 4-iodophenylbutanamide (BC-IP) was designed and synthesized from *closo*-dodecaborate. Tumor-bearing mice (female, 5-6 weeks old) were prepared by injecting subcutaneously (s.c.) a suspension of U87MG human glioblastoma cells in PBS. The mice were kept on a regular chow diet and water for a week. The tumor-bearing mice were injected *i.v.* with 200 μ L of BC-IP or MID dissolved in ultrapure (Milli-Q) water at the final dose of 15 μ B/g. At 3, 12, and 24 h after injection, the mice were lightly anesthetized and blood samples were collected from heart. The mice were then sacrificed by cervical dislocation and dissected. Liver, spleen, kidney, brain, and tumor were excised, washed with saline, and weighted. Each tissue was digested with 1 mL of HNO₃ at 90 °C for 3 h, and then the digested samples were diluted with distilled wa-

ter. After filtering through a membrane filter (0.5 μ m ϕ , 13JP050AN, ADVANTEC, Japan), boron concentrations were measured by ICP-OES. All protocols for *in vivo* studies involving the use of mice were approved by the Institutional Animal Care and Use Committee of Tokyo Institute of Technology.

RESULTS: The boron concentration in the tumor showed the highest value of 11 μ B/g at 3 h and gradually decreased to 2.4 and 2.3 μ B/g at 12 and 24 h respectively (Fig. 1A). In contrast, boron concentrations in other tissues remained below 5.0 μ B/g at all time periods, demonstrating the highest BNCT effect can be achieved with neutron irradiation at 3 h post injection. For comparison, we investigated the biodistribution of MID at 12 h post injection, the time point at which MID exhibited the highest accumulation in tumors. MID tended to accumulate in plasma, followed by lung, tumor, and kidney (Fig. 4B). The boron concentration in tumor was about 5 μ B/g and thus much lower than was observed with BC-IP. These results suggest that IP, a non-covalent albumin ligand, can enhance tumor accumulation over the maleimide, covalent ligand. Therefore, BC-IP has a potential to act as a more efficient boron carrier than MID in BNCT.

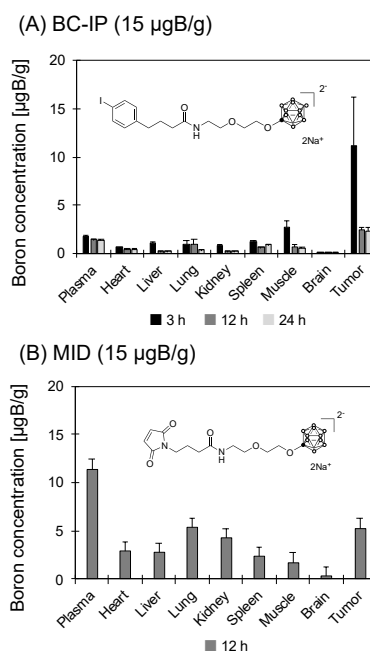


Fig. 1. Biodistribution of (A) BC-IP and (B) MID in U87MG tumor mouse models with *i.v.* injection. Data are expressed as mean \pm SD (n = 3).

REFERENCES:

- [1] H. Kanno *et al.*, *The Oncologist*, **26** (2021) e1250-e1255.
- [2] S. Kikuchi *et al.*, *J. Control. Release*, **237** (2016) 160-167.
- [3] K. Kawai *et al.*, *Mol. Pharm*, **17** (2020) 3740-3747.

CO7-3 Effects of overexpression of *LAT1* in cancer stem cell-like cells on suppression of tumor growth by boron neutron capture therapy

K. Ohnishi¹, T. Tani² and M. Suzuki³

Department of ¹Biology, Ibaraki Prefectural University of Health Sciences

²QST Hospital, National Institutes for Quantum and Radiological Science and Technology

³Institute for Integrated Radiation and Nuclear Science, Kyoto University

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

EXPERIMENTS: We transplanted tumors formed with a clone (T98G/K10, *pCD133-TRE/LAT1-tdTomato/IRES/tTA*-transfected, LAT1-overexpressed cells in CD133 positive cell selectively), or a clone (T98G/KC2, control plasmid-transfected, LAT1-nonoverexpressed cells) into femoral region of nude mice. Accumulated amounts of ¹⁰BPA in blood and tumor were measured using prompt gamma-ray assay (PGA) on 1 h after ¹⁰BPA s.c. injection (100 mg/kg, 1 h before irradiation). The transplanted tumors into mice were irradiated with thermal neutron

beam at the fluences of 3.3×10^{12} n/cm² on 1h after the ¹⁰BPA injection.

RESULTS: Relative tumor volumes in ¹⁰BPA-treated mice are shown in Fig. 1. Tumor growth in ¹⁰BPA-treated T98G/K10 (n=3) and T98G/KC2 mice (n=3) was strongly suppressed for approximately 40 days after neutron irradiation. However, such suppression was not observed in PBS-treated T98G/K10 and T98G/KC2 mice (data not shown). Relative tumor volumes of 2 mice and 1 mouse were approximately 2 and 6, respectively on 57th day after neutron irradiation in T98G/K10 mice. In contrast to T98G/K10 mice, in T98G/KC2 mice, those of 1 mouse and 2 mice were immediately 4 and over 6, respectively on the same day after neutron irradiation. It seemed that tumor regrowth of T98G/K10 mice is delayed compared with that in T98G/KC2 mice. These results suggest that BNCT is more suppressive on tumor regrowth in CD133 expressing cell-selective LAT1 overexpression cells compared with control cells. We plan additional experiments to confirm the effect of BNCT on tumor regrowth using CD133 expressing cell-selective LAT1 overexpression cells and control cells.

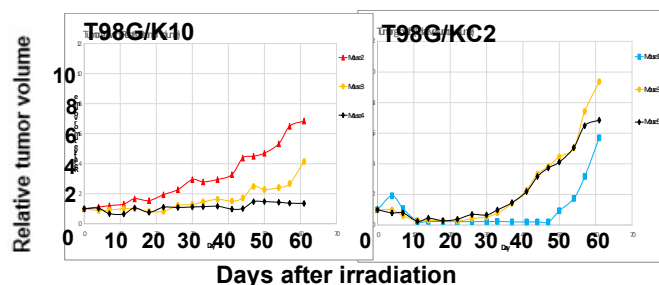


Fig. 1. Tumor growth curves in mice transplanted with CD133 expressing cell-selective LAT1 overexpressing (T98G/K10) or control cells (T98G/KC2).

CONCLUSION: We obtained preliminary data from this study, suggesting that BNCT is more effective by manipulation of cancer stem cell-selective *LAT1* gene expression. On the basis of the present data, we plan to perform further detailed experiments.

REFERENCES:

- [1] Y. Kanai *et al.*, J. Biol. Chem., **273** (1998) 23629-23632.
- [2] K. Kaira *et al.*, Br. J. Cancer, **107** (2012) 632-638.
- [3] K. Ohnishi *et al.*, Radiat. Res., **196** (2021) 17-22.
- [4] K. Ohnishi *et al.*, Biochem. Biophys. Res. Communi., **546** (2021) 150-154

CO7-4 Optimization study of polymer-BPA conjugates for non-clinical study

T. Nomoto^{1,2}, K. Konarita^{1,2}, D. Tokura^{1,2}, M. Suzuki³, N. Nishiyama^{1,2}

¹*Institute of Innovative Research, Tokyo Institute of Technology*

²*School of Life Science and Technology, Tokyo Institute of Technology*

³*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: Boronophenylalanine (BPA) has been clinically used in boron neutron capture therapy (BNCT). Because BPA can be internalized into cells through LAT1 amino acid transporters expressed on many tumor cells, BPA can show selective accumulation within malignant tumors [1]. However, it was also reported that intracellular BPA is sometimes exchanged with extracellular amino acids including tyrosine due to the antiport mechanism of the amino acid transporter, causing short retention time in a target tumor [2]. Since this unfavorable efflux of intracellular BPA is likely to compromise the therapeutic effect, it is important to prolong the intracellular retention of BPA.

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

EXPERIMENTS: PVA-BPA and free BPA were administered by intravenous injection. Three-hour after injection, The chest was irradiated with epi-/thermal neutrons at 1 MW for 50 min using the Kyoto University Research Reactor (KUR).

RESULTS: The result is shown in Fig. 1. The free BPA exhibited obvious therapeutic effects, and PVA further improved the survival rate. The result is consistent with our biodistribution study, in which PVA-BPA prolonged the intratumoral retention. Thus, PVA-BPA may be a promising formulation for BNCT against lung cancers.

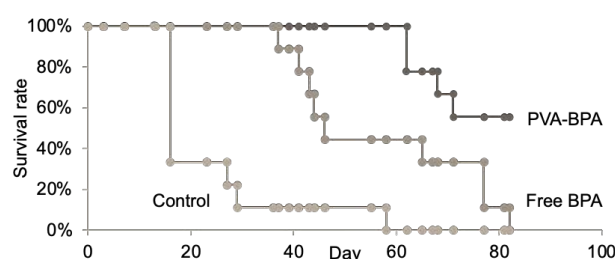


Fig. 1. Survival rate.

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.
- [4] T. Nomoto *et al.*, *J. Control. Release*, **332** (2021) 184-193.

CO7-5 Neutron capture therapy using Gd-chelated polymeric drug delivery systems

Y. Miura^{1,2}, T. Nomoto^{1,2}, K. Konarita^{1,2}, D. Tokura^{1,2}, Y. Sakurai³, M. Suzuki³, N. Nishiyama^{1,2}

¹*Institute of Innovative Research, Tokyo Institute of Technology*

²*School of Life Science and Technology, Tokyo Institute of Technology*

³*Institute for Integrated Radiation and Nuclear Science, Kyoto University*

REFERENCES:

[1] P. Mi *et al.*, ACS Nano, **9** (2015) 5913-5921.

INTRODUCTION: ¹⁵⁷Gd has the high neutron capture cross section and can generate Auger electrons and γ -rays upon thermal neutron irradiation. Thus, Gd has been expected to be a promising atom in neutron capture therapy. We previously synthesized the inorganic-organic hybrid nanoparticle encapsulating Gd and demonstrated strong antitumor efficiency in subcutaneous tumor models [1]. To improve the tumor accumulation and penetration performance of drug carriers, we designed and synthesized new class of Gd-DOTA introduced polymers (polymer-drug) by using controlled polymerization techniques and selective polymer modifications. According to *in vivo* biodistribution study, obtained polymer-drug exhibited selective Gd delivery against targeted tumors, thereby inducing strong antitumor effects upon neutron irradiation. In this year, we compared the therapeutic potential of Gd-chelated polymers with BNCT of boron cluster-conjugated polymers.

EXPERIMENTS: The polymeric drugs or the low-molecular drug as a control were intravenously injected to the BALB/c mice having subcutaneous CT26 tumors. The tumor sites were irradiated with epi-/thermal neutrons 24 or 1 h after injection. using KUR at 5 MW for 10 min. The tumor volume (V) was calculated using the following equation:

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

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

RESULTS: The result indicated that the polymeric drugs could show higher antitumor effects than the low-molecular drugs, which is consistent with our previous results. In addition, the boron cluster-conjugated polymers exhibited strong therapeutic effects comparable to Gd-chelated polymers. In order to elucidate the merit of Gd-NCT, it is important to further investigate detailed mechanism of Gd-NCT. With such study, our results about biodistribution and therapeutic effects may provide significant insights for the development of NCT.

CO7-6 Lipid-coated boronic oxide nanoparticles as a boron agent for BNCT

R. Kawasaki,¹ H. Hirano,¹ K. Yamana,¹ A. Oshige,¹ K. Nishimura,¹ N. Kono,¹ Y. Sanada,² A. Tabata,³ N. Yasukawa,³ H. Azuma,³ T. Takata,² Y. Sakurai,² H. Tanaka,² M. Suzuki,² N. Tarutani,¹ K. Katagiri,¹ T. Nagasaki,³ and A. Ikeda¹

¹Program of Applied Chemistry, Graduate School of Advanced Science and Engineering, Hiroshima University

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

³Department of Chemistry and Bioengineering, Osaka Metropolitan University

INTRODUCTION: With noninvasiveness, boron neutron capture therapy (BNCT) is considered as one of the elegant therapeutic modalities in cancer treatment. In this therapy, cytotoxic particles are generated by nuclear reaction between ^{10}B and neutron, that is boron neutron capture reaction. The effective ranges of the energy from these cytotoxic particles are corresponding to the size of cells, suggesting that deliverability of boron agents to cancer cell with high specificity and efficiencies is the critical to maximize therapeutic benefits from BNCT. Today, we can use two types of boron agents, L-boronophenylalanine (L-BPA) and 1-mercaptoborocaptate (BSH), in clinical. There are still several issues in delivery including poor solubility in water and cancer selectivity. For these points of views, development of boron agents that can overcome these issues are indispensable.

In this study, we developed hybrid nanoparticles comprising boronic oxide nanoparticles and phospholipids (LCB) as a boron agent for BNCT (Figure 1) [1]. Here, phospholipids are one of the promising candidates as drug delivery nanocarrier that is widely used in liposomes and lipid nanoparticles with excellent biocompatibility and excellent water dispersibility. Boronic oxide nanoparticles possesses several advantages as a boron agent for BNCT including their large contents of boron atom in each nanoparticle with large area to enhance the efficiencies to achieve boron neutron capture reaction and their degradability to avoid undesirable side effects in accumulation of inorganic nanoparticles.

RESULTS: Boronic oxide nanoparticles with a diameter of ~ 10 nm were prepared by mechanochemical formulation approach as we previously reported [2]. We prepared LCB using eggPC *via* conventional liposome preparation method and their size were manipulated by extruder. Their hydrodynamic diameter was determined to be 120 nm (polydispersity index, 0.15) by dynamic light scattering measurement (DLS). Agglomerate of boronic oxide nanoparticles were found by transmission electron microscopic observation (TEM), indicating the cluster of boronic oxide nanoparticles were coated with eggPC. Moreover, our systems did not form undesirable precipitate and the boron concentration of the dispersion did not change for 1 week, indicating our systems are

colloidally stable.

We next evaluated appropriateness of our system as a boron agent by estimating biocompatibility. As a result, current system did not induce cytotoxicity toward murine fibroblast cell (L929) and murine colon carcinoma cell (Colon26) even at the highest concentration, indicating our systems are safely applicable as a boron agent. Moreover, our system could efficiently deliver boron atom toward cancer cells compared to clinically available boron agent, L-BPA, resulting in the therapeutic benefit of BNCT were improved by using current system. Moreover, the anti-cancer effects were obtained even in cancer spheroid, which is used as model for 3-dimensional tumor tissues. Our system comprising boronic oxide nanoparticles and phospholipids are potentially applicable as a boron agent for BNCT.

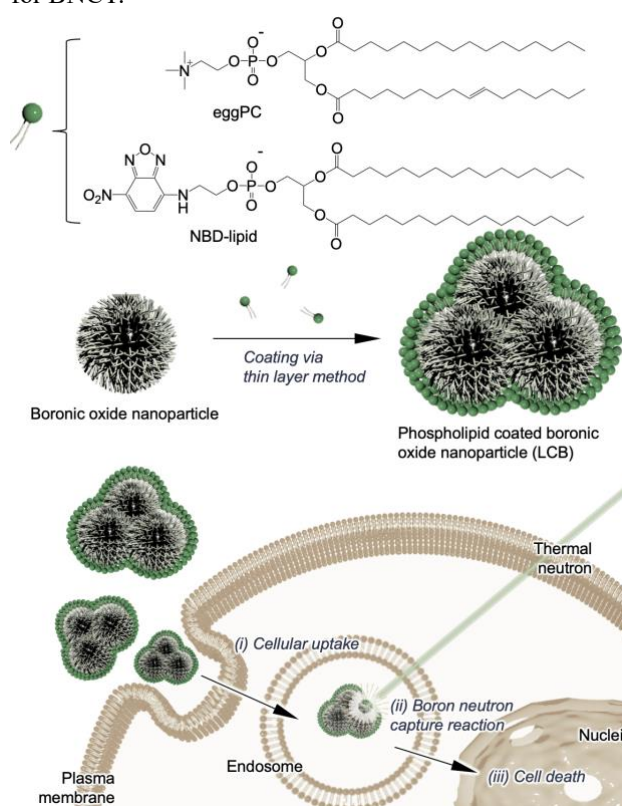


Fig. 1. Schematic illustration of BNCT using LCB.

REFERENCES:

- [1] R. Kawasaki *et al.*, ChemBioChem, **in press** (doi):10.1002/cbic.202300186.
- [2] R. Kawasaki *et al.*, Nanomed. Nanotechnol. Biol.Med., **49** (2023) 102659 .

CO7-7 Combination Effect of Shikonin on BPA-BNCT toward SCCVII Cells-Bearing C3H Mice

K. Bando, A. Tabata, N. Yasukawa, R. Kawasaki¹, Y. Sakurai², Y. Sanada², M. Suzuki², N. Kondo², and T. Nagasaki

Graduate School of Engineering, Osaka Metropolitan University

¹ Graduate School of Advanced Science and Engineering, Hiroshima University

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron neutron capture therapy (BNCT) is a potent cancer therapy that exhibits cancer selectivity at the cellular level and has few side effects. However, in many cases, it will not be completely cured due to recurrence/metastasis (distant dissemination). One of the reasons is the involvement of tumor associated macrophages (TAM) present in the stroma of tumor tissue. It has become a major problem that TAM not only promotes neo-vascularization and tumor regrowth/metastasis, but also suppresses antitumor immunity. In this study, β -1,3-glucan is used as a novel carrier to efficiently and selectively deliver the M2→M1 polarizer to M2 macrophages by Drug Delivery System targeting dectin-1 expressed in M2 macrophages. The complex nanogel of β -1,3-glucan with shikonin as macrophage M2→M1 polarizer was prepared to suppress the malignant transformation of the tumor micro-environment, further increase the cancer immunoreactivity in cancer treatment, and increase BNCT efficiency. Combination effect of shikonin/ β -1,3-glucan nanogel on BPA-BNCT toward SCCVII tumor cells-bearing C3H mice was estimated.

EXPERIMENTS: A subcutaneous inoculation of 6×10^5 SCCVII cells in female C3H mice was established for 14 days. Thermal neutron beam irradiation was started from the time point of 120 min after the s.c. injection of BPA (300 mg/kg)-fructose. BNCT effects were evaluated on the basis of the changes in tumor volume of the mice. In order to estimate a combination effect on shikonin, the complex of shikonin/ β -1,3-glucan ([shikonin]=100 μ M, 200 μ L) was administered via the tail vein at the time points of 1, 3, 5 days after irradiation.

RESULTS: Takeya *et al.* reported that triterpenoid derivatives such as soyasapogenol contained in soybeans and corosolic acid in apple pomace inhibited the activation of STAT3, thereby reducing the activity of M2 macrophages [1]. In this study, shikonin, well-known STAT3 inhibitor, was used as a candidate of M2→M1 polarizer [2].

As shown in Fig. 1, clear antitumor effect was found for the combination of shikonin/ β -1,3-glucan nanogel with BPA-BNCT. With shikonin/ β -1,3-glucan treatment alone and neutron irradiation alone, tumors proliferated as well

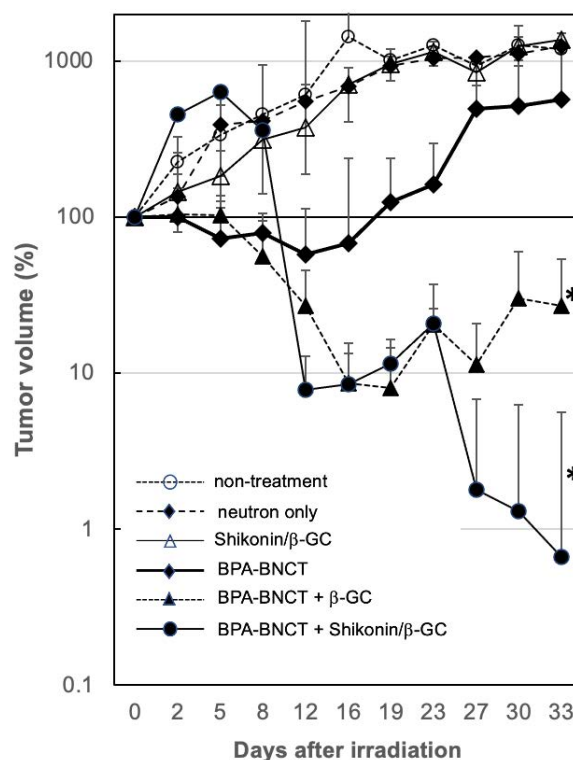


Fig. 1. Tumor volume in SCCVII allograft tumor model mice (C3H female, 6 weeks old, 18–21 g) irradiated with thermal neutron for 30 min (4.8×10^{12} neutrons/cm²) or without irradiation. Data are expressed as means \pm SD (n = 5). Statistical significance: *P < 0.05 compared with BPA-BNCT controls.

as non-treatment. Tumor regrowth was seen after 27 days on BPA-BNCT due to insufficient dose of BPA. β -1,3-glucan is known to have an immunostimulatory effect and is used as a concomitant drug for cancer radiotherapy [3]. Moreover, it has recently been reported that β -1,3-glucan acts as an M2→M1 polarizer [4]. Herein, administration of β -1,3-glucan only after BPA-BNCT also enhanced the BNCT effect. However, its effect is weaker than that of combination of shikonin/ β -1,3-glucan and BPA-BNCT.

These results on the combination of shikonin/ β -1,3-glucan nanogel with BPA-BNCT suggests our hypothesis that shikonin may induce a tumor-killing M1 macrophage in the tumor microenvironment and enhance the antitumor effect of BNCT.

REFERENCES:

- [1] M. Takeya *et al.*, *Can. Sci.*, **102** (1981) 206-211.
- [2] X. Gao *et al.*, *Int. Immunopharmacol.*, **19** (1981) 327-333.
- [3] Y. Shimizu *et al.*, *Cancer*, **69** (1992) 1188-1194.
- [4] J. Yan *et al.*, *J. Immunol.*, **195** (2015) 5055-5065.

CO7-8 The Response of Tumor Cells to BNCT

Y. Tong¹, Gao Zhongming¹, B. Saraswat¹, A. Vadi Velu¹, Y. Sasaki¹, M. Ishiai^{2,3}, S. Imamichi^{1,2,3}, T. Nozaki^{1,5}, M. Suzuki⁴, Y. Sanada⁴, 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 Division of Boron Neutron Capture Therapy, EPOC, National Cancer Center

3 Central Radioisotope Division, National Cancer Center Research Institute

4 Institute for Integrated Radiation and Nuclear Science, Kyoto University

5 Dept. Pharmacology, Osaka Dental Univ.

INTRODUCTION: Boron neutron capture therapy (BNCT) is a novel cancer treatment method that uses high-energy alpha particles and lithium nuclei generated by nuclear reactions. This method is highly precise, short in duration, and has minimal side effects. To better optimize the therapeutic effects of BNCT, it is important to conduct in-depth analysis of its response to cancer cells and therapeutic efficacy. We conducted detailed studies on the effects of BNCT on tumor cells, including cell death and various biological responses. The use of cancer cells and xenograft mouse models can provide powerful support for this effort.

EXPERIMENTS: Neutron irradiations at KUR reactor were operated at 1 MW in all experiments. Irradiation in KUR was carried out with mode : OO-0000F mixed irradiation mode, Dr ratio at ~9.4. Gold foil activation analysis measured thermal neutron fluences and thermoluminescence dosimeter (TLD) measured the γ -ray doses including secondary γ -ray. Total physical dose calculation was carried out using the flux-to-dose conversion factor by the sum of the absorbed doses resulting from ^1H (n, γ) ^2D , ^{14}N (n, p) ^{14}C , and ^{10}B (n, α) ^7Li reactions [1] (Tables 1, 3).

The human squamous cell line SAS and human malignant Melanoma A375 cells were incubated with ^{10}B -boronophenylalanine fructose complex (BPA) for 2.0 hrs. The cell survival of SAS and A375 cells was analyzed by colony formation assay after BNCT with the medium containing fetal bovine serum (FBS). The serum-free medium (SFM) was used to culture SAS and A375 cells after BNCT, and cells and supernatants were harvested at 6 and 24 hours. Cells were harvested for RNA and proteins isolation and molecular analysis was carried out. E-3 port measurement of BPA solution was carried out using teflon tubes (Table 2).

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

and blood, tumors, and other organs were analyzed.

RESULTS: The measurement of thermal neutron fluence and doses for cells were as indicated (Table 1) and for mice were shown in Table 2. Analysis by colony formation assay and RNA and protein expression were carried out to elucidate early cellular and *in vivo* responses to BNCT. A potential role of HMGB1 and proteomic data as early biomarkers for evaluation of cellular and *in vivo* response to BNCT were also indicated [2, 3].

Table 1. Irradiated doses at cells from 6:32-6:42 am and 6:43-7:43 am on Nov. 16, 2022 (Single layer, E-4 rail port) .

Irradiation time [min]	Position	Fluence [cm^{-2}]		[Gy]					
		Thermal neutron [cm^{-2}]	Epi-thermal neutron	Thermal neutron [Gy]	Epi-thermal neutron [Gy]	Fast neutron [Gy]	Gamma-ray [Gy]	Physical Dose [Gy]	B-10* (1ppm)
10	U, Cl	1.1E+12	1.9E+11	1.4E-01	1.5E-02	1.0E-01	9.4E-02	3.6E-01	7.9E-02
	U, F	5.5E+11	9.8E+10	7.4E-02	7.9E-03	5.5E-02	9.4E-02	2.3E-01	4.1E-02
	D, Cl	1.1E+12	2.0E+11	1.5E-01	1.6E-02	1.1E-01	9.4E-02	3.7E-01	8.2E-02
	D, F	6.9E+11	1.2E+11	9.2E-02	9.8E-03	6.8E-02	9.4E-02	2.6E-01	5.1E-02
60	U, Cl	6.4E+12	1.1E+12	8.6E-01	9.2E-02	6.4E-01	6.2E-01	2.2E+00	4.8E-01
	U, F	2.8E+12	5.0E+11	3.7E-01	4.0E-02	2.8E-01	6.2E-01	1.3E+00	2.1E-01
	D, Cl	6.0E+12	1.1E+12	8.0E-01	8.5E-02	5.9E-01	6.2E-01	2.1E+00	4.5E-01
	D, F	3.5E+12	6.2E+11	4.6E-01	4.9E-02	3.4E-01	6.2E-01	1.5E+00	2.6E-01

Table 2. E-3 port measurement result of BPA solution in triplicate on Nov. 16, 2022.

Sample	Number	NET counts H	NET counts B	B/H Ratio	^{10}B conc. (ppm)
Standard ^{10}B	1	1942.0	3721.0	1.9	50.0
	2	698.0	24338.0	34.9	909.9
Samples 2-4	3	666.0	25245.0	37.9	989.1
	4	512.0	18982.0	37.1	967.5
Empty tube	5	97.0	228.0	2.4	61.3

Table 3. Irradiated doses for local irradiation of mice (cart, irradiation room) on 11:17-12:27 am, January 24, 2023 (Cart, irradiation room) .

Irradiation time [min]	Position	Fluence [cm^{-2}]		[Gy]					
		Thermal neutron [cm^{-2}]	Epi-thermal neutron	Thermal neutron [Gy]	Epi-thermal neutron [Gy]	Fast neutron [Gy]	Gamma-ray [Gy]	Physical Dose [Gy]	B-10* (1ppm)
60	Ce1	3.6E+12	6.4E+11	4.8E-01	5.1E-02	3.5E-01	3.2E-01	1.2E+00	2.7E-01
60	Ce2	3.3E+12	5.8E+11	4.3E-01	4.6E-02	3.2E-01	3.3E-01	1.1E+00	2.4E-01

REFERENCES:

- [1] S. Nakamura *et al.*, Proc Jpn Acad Ser B Phys Biol Sci, **93** (2017) 821-831.
- [2] S. Imamichi *et al.*, Biology, **11** (2022) 420.
- [3] D. Perico *et al.*, Cancer Biother Radio-pharm., **38** (2023) 152-159.

CO7-9 Elucidating the effects of boron neutron capture therapy on host immunity in mice tumor models

T. Watanabe¹, Y. Sanada¹, T. Takata¹, G. E. Sato², M. Yoshimura², T. Mizowaki², Y. Sakurai¹, H. Tanaka¹, M. Suzuki¹

¹Institute for Integrated Radiation and Nuclear Science, Kyoto University

²Radiation Oncology and Image-Applied Therapy, Graduate School of Medicine, Kyoto University

INTRODUCTION: Boron neutron capture therapy (BNCT) is a type of radiation therapy that utilizes a reaction in which boron atoms (¹⁰B) capture neutrons and cause them to fission into alpha particles and lithium nuclei [1]. By selectively delivering boron atoms in the form of drugs to tumor cells, subsequent neutron irradiation can selectively induce nuclear reactions in the delivered cancer cells, resulting in the death of the cancer cells. In particular, immune cells are known to be more radiosensitive than normal cells and can be killed even by small doses of radiation [2]. On the other hand, it has also been shown that irradiation of tumor tissue releases the immune escape mechanism, which is designed to prevent tumor tissue from being attacked by the immune system, and that irradiation of tumors makes it easier for immune cells to attack tumor cells [3]. BNCT is also a type of radiation and may have some effect on the number and function of host immune cells after BNCT, but there have been only a few reports on the immune response after BNCT in detail [4]. The purpose of this study is to elucidate the effects of BNCT on host immune cells.

EXPERIMENTS: A tumor model was created in which mouse-derived malignant melanoma cells B16 and mouse-derived squamous cell carcinoma SCCVII were transplanted subcutaneously into the lower leg skin of C57BL/6 and C3H mice, respectively. A tumor model was created in which mouse-derived malignant melanoma cells B16 and mouse-derived squamous cell carcinoma SCCVII were transplanted subcutaneously into the lower leg skin of C57BL/6 and C3H mice, respectively. Each mouse subcutaneous tumor model was treated with BNCT or BNCT plus immunotherapy (anti-PD-1 antibody), and subcutaneous tumor size was measured every 3 days. After X-ray therapy or BNCT was performed on the mouse tumor models, tumor tissue was removed and RNA-seq was performed using a next-generation sequencer. Tumor tissue was removed after BNCT and BNCT + immunotherapy combination treatment, and the tumor tissue was enzymatically treated and separated into single cells.

RESULTS: Using each mouse subcutaneous tumor model, we compared the BNCT group and the combination group of BNCT and immunotherapy (anti-PD-1 antibody), and found that the combination group of immunotherapy and BNCT showed better

long-term tumor growth inhibition in the tumor curve. In addition, RNA-seq analysis of post-treatment tumors in the target group (X-ray therapy group) and the BNCT group was performed using a next-generation sequencer to examine gene expression status at the time of analysis, and revealed that the expression of chemokines (a group of proteins released into the tumor microenvironment to modulate immune function) in the tumor tissue was significantly increased in the BNCT group. The BNCT group showed significantly increased expression of chemokines (a group of proteins released into the tumor microenvironment that modulate immune function). Since the combination of BNCT and immunotherapy showed a long-term inhibition of tumor growth compared to the control group and the BNCT alone group, we focused on the memory function of lymphocytes, which is involved in immune memory and shows a long-term inhibition of tumor growth, and examined the percentage of CD3+ T cells with a memory function among the lymphocytes infiltrating the tumor. However, contrary to the hypothesis, no significant differences were found between the treatment groups or in comparison with the control group.

REFERENCES:

- [1] RF. Barth *et al.*, *Radiat Oncol.*, **7** (2012) 146.
- [2] Guillaume Vogin and Nicolas Foray., *Int J Radiat Biol.*, **89** (2013) 2-8.
- [3] T. Watanabe *et al.*, *Int J Clin Oncol.*, **28** (2023) 201-208.
- [4] A. A. Khan *et al.*, *PLoS One.*, **14** (2019) e0222022.

CO7-10 Development of carborane-containing amino acid derivatives for BNCT

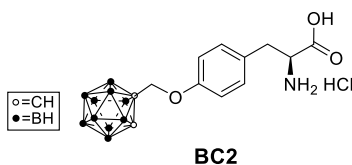
A. Niitsu¹, R. Yamamoto¹, T. Watanabe², Y. Sanada², M. Tsuji¹, T. Hirayama¹, S. Masunaga³, and H. Nagasawa¹

¹ Laboratory of Medicinal & Pharmaceutical Chemistry, Gifu Pharmaceutical University

² Institute for Integrated Radiation and Nuclear Science, Kyoto University

³Hanwha Daini Senboku Hospital

INTRODUCTION: Cancer cells have a greatly increased requirement for amino acids to maintain rapid proliferation and active metabolism, and are particularly dependent on glutamine, which has been termed "glutamine addiction". Glutamine is transported into cells through ASCT2, and the imported glutamine can be used or exchanged through the L-type amino acid transporter (LAT1 or SLC7A5) for hydrophobic or aromatic amino acids such as isoleucine, valine, methionine, tryptophan, and phenylalanine. It is known that these transporters are overexpressed in a variety of tumor cells. Therefore, we are developing a boron carrier that efficiently accumulates ¹⁰B atoms in tumors exploiting these amino acid transporters that is highly expressed in cancer. We have synthesized various amino acid derivatives containing boron clusters as hydrophobic pharmacophore and screened them by boron uptake into cells, and **BC2** was selected as a promising candidate.



In this study, to investigate the mechanism of its cellular uptake, we evaluated the correlation between LAT1 expression and the uptake of boron carriers using genetically modified LAT1-deficient/enhanced cell lines.

EXPERIMENTS: **BC2** is synthesized by a microwave reaction in which the corresponding alkyne reacts with decaborane in the presence of a Lewis base to construct carborane. This study used cell lines generated from the SCC7 tumor cell line, a murine squamous cell carcinoma cell line that obtained spontaneously from a C3H/He mouse. LAT1-deficient (SCC7-ΔLAT1) and LAT1 overexpressing (SCC7-LAT1OE) cells were established from SCC7 cells expressing 6×His-tagged LAT1 protein (SCC7-WT), which were established previously [1]. T98G and LAT1-modified SCC7 cells were added with BPA or BC2 at 1 or 10 μg ¹⁰B/mL, incubated for 15 minutes, then trypsinized and collected (Method A) or washed with cold PBS (Method B) and dissolved in nitric acid. The boron concentration was determined by ICP-AES.

RESULTS: In the collection of cells treated with boron carriers, boron uptake was found to be significantly increased when cells were dissolved in nitric acid immedi-

ately after cold PBS washing without trypsin treatment (Method B), as shown in Fig. 1. This is expected because boron compounds are excreted during trypsinization.

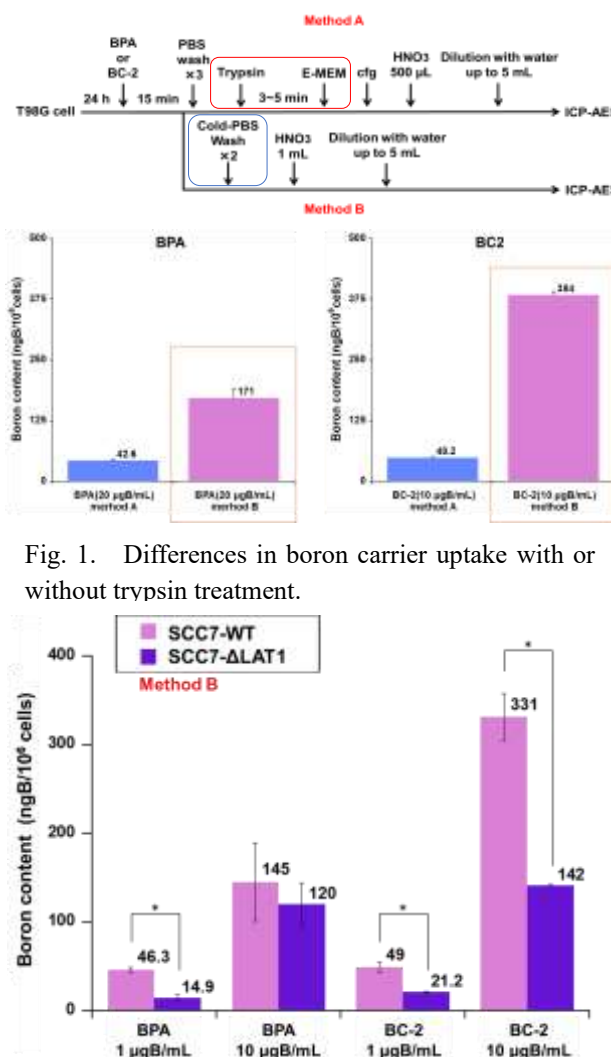


Fig. 1. Differences in boron carrier uptake with or without trypsin treatment.

Fig. 2. Effect of LAT1 knockout on boron uptake. Student t-test, n = 2 or 3, *: p<0.05.

As shown in Fig. 2, boron uptake was significantly reduced in SCC7-ΔLAT1 compared to SCC7-WT, suggesting that BC-2, like BPA, is taken up into the cells via LAT1. The effects of LAT1 inhibitors and the uptake in LAT1 overexpressing cells are also under investigation.

These results indicate that BC-2 is partially taken up into the cell via LAT1 and accumulated and retained in the cell by an unknown mechanism. Its biodistribution will be investigated in the future.

ACKNOWLEDGEMENTS: This research was supported by JSPS Grants-in-Aid for Scientific Research JP19H03352 and JP26670059.

REFERENCES:

[1] T. Watanabe *et al.*, *J. Rad. Res.*, **64** (2023) 91-98.

CO7-11 Development of Nano Carriers Installed with Gd(III)-Thiacalixarene Complex for Gd-NCT

N. Iki¹, K. Ohama, M. Komiya¹, T. Nagasaki², and M. Suzuki³

¹ Graduate School of Environmental Studies, Tohoku University

² Graduate School of Engineering, Osaka City University

³ Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Owing to a large thermal neutron capture cross section and total kinetic energy of $^{157}\text{Gd}(n,g)^{158}\text{Gd}$ larger than that of $^{10}\text{B}(n,\alpha)^7\text{Li}$, gadolinium attracts growing attention as an alternative to boron in neutron capture therapy [1]. Because free gadolinium ($\text{Gd}(\text{OH}_2)_9$) has toxicity, a safe carrier of Gd to tumor not to release free Gd is required. We recently found that thiacalix[4]arene-p-tetrasulfonate (TCAS) self-assembled three lanthanide (Ln) cores including Gd to form a sandwich-type complex, Ln_3TCAS_2 (Fig. 1) [2], the characteristic features of which are high kinetic stability, luminescence signal [3], and 1H relaxation arising from the Ln center [4]. Nano-sized particles are frequently used as a drug carrier toward tumor by enhanced permeability and retention (EPR) effect. We have so far studied nano-carriers for Gd_3TCAS_2 such as silica nano-particle (NP) [5] and albumin NP (AlbNP) [6, 7] aiming at Gd-NCT. This FY, we devised a new AlbNP installed with Gd_3TCAS_2 inside and at the surface of NP (core-shell type) and compared the ability to kill cancer cells upon neutron irradiation with the core- and shell-type AlbNPs.



Fig. 1. Structure of Ln_3TCAS_2 complex.

EXPERIMENTS: *Preparation of core-shell AlbNP.* The trinuclear complex Gd_3TCAS_2 was prepared as reported elsewhere [2]. The AlbNP was prepared by a method reported [8]. Briefly, an aqueous mixture of BSA and Gd_3TCAS_2 solution was added EtOH, followed by addition of glutaraldehyde to afford core-AlbNP. This was further mixed with Gd_3TCAS_2 to provide Gd_3TCAS_2 -installed core-shell AlbNP (denoted as core-shell AlbNP). *Cell experiment.* MCF-7 cells were seeded in a 6-well plate at a cell concentration of 1.0×10^5 cells/mL and incubated for 24 h. After supernatant was removed, RPMI medium and solution containing Gd in a form of 1) shell AlbNP, 2) core AlbNP, 3) free Gd_3TCAS_2 , 4) Gd-DTPA, 5) PBS (as control), and 6) core-shell AlbNP 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 $25 \mu\text{M}$ for 1–3). After washing with PBS, the cells were detached

from the well and transferred to tubes to be irradiated with thermal neutron for 20 min.

Assay. To the wells containing 2 mL of RPMI medium in 6-well plates, irradiated cells were seeded at the concentration of 500 cells/well. After incubation for 14 days, the colony was stained with crystalviolet.

RESULTS:

The colony formation units (CFU) normalized with one for no irradiation is shown in Fig. 2. As can be seen, there seems appreciable difference between CFU for 1) control and others except for 3) free Gd_3TCAS_2 , suggesting that a suitable carrier like AlbNP is essential for delivery of Gd_3TCAS_2 . Regarding the effect of the types of AlbNP, 6) core-shell showed highest ability for cell killing ability at fluence of around $7 \times 10^{11} \text{ cm}^{-2}$. With higher fluence of neutron (at around $1.3 \times 10^{11} \text{ cm}^{-2}$), however, core-shell showed similar ability as shell AlbNP did. On the whole, the core-shell AlbNP is promising at lower dose of neutron. Material design on the basis of Gd_3TCAS_2 and nano-materials enabling more efficient NCT effect is now on the way.

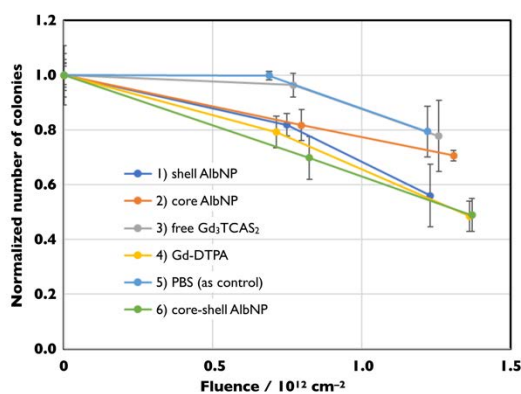


Fig. 2. Dependence of neutron fluence on the colony formation rate ($n = 3$).

REFERENCES:

- [1] M. Takagaki *et al.*, Future Application of Boron and Gadolinium Neutron Capture Therapy, 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] R. Karashimada *et al.*, Chem. Commun. **52** (2016) 3139-3142.
- [4] N. Iki *et al.*, Inorg. Chem. **55** (2016) 4000-4005.
- [5] T. Yamatoya *et al.*, KURNS Progress Report 2019. 31P11-8, p.105,
- [6] N. Iki *et al.*, KURNS Progress Report 2020. R2P5-2.
- [7] N. Iki *et al.*, KURNS Progress Report 2021. R3P2-2.
- [8] C. Weber *et al.*, Int. J. Pharm., **194** (2000) 91-102.

CO7-12 International Screening study of Boron / Gadolinium Compounds for NCT of malignant tumors, 2022

RR. Zairov¹, M. Neikter², M. Takagaki³, N. Kondo⁴, Y. Sakurai⁴ and M. Suzuki⁴

¹Kazan Federal University, Russia

²University West, Sweden

³RCNP, Osaka University, Japan,

⁴IIRNS, Kyoto University, Japan

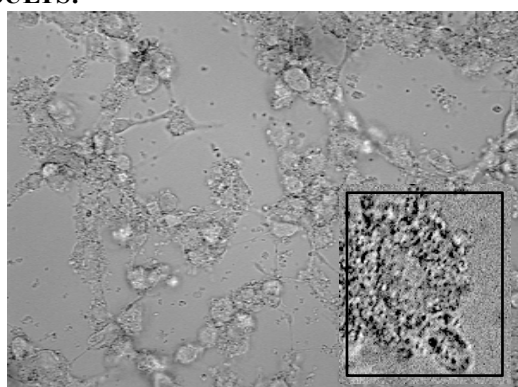
INTRODUCTION:

The possibility of gadolinium neutron capture therapy is controversial, but the total trajectory range of heavy-charged particles generated by the $^{10}\text{B} (n, \alpha) ^7\text{Li}$ reaction close to the cell diameter, and the total dose distribution is probably too selective. In GdNCT, it is expected that the dose distribution will be improved by the higher total kinetic energy of γ rays, electron beams, Auger electrons, etc., by a single neutron capture reaction of $^{167}\text{Gd} (n, \gamma) ^{168}\text{Gd}$. Zairov and Neikter have prepared gadolinium inclusion novel nanoparticles, and we repeated GdNCT experiments in vitro, and have confirmed their low toxicity, tumor retention and concentration-dependent GdNCT effect in this research program.

EXPERIMENTS:

SCCVII and/or rat glioma cell line C6 were incubated with an aliquot of nano particle micelles containing natural gadolinium for 5 hrs, then their survival property has been investigated with variety of GdNCT modality; Gd-free, Gd-preincubation+, Gd-preincubation+Gd+. The survival character of GdNCT was compared with BNCT using BPA.

RESULTS:



Gd micelles +

Fig. 1. Microscopic view of rat glioma cell line C6 incubating with the Gd nano micelles.

Tiny micelles are well visualized and reveals their high tumor affinity. No significant tumor damage was con-

firmed via microscopical observation and toxic study of IC₅₀.

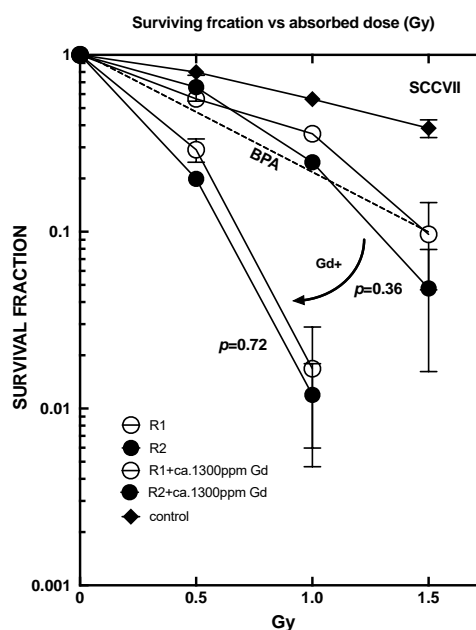


Fig. 2. The survival fraction of SCCVII after GdNCT using the Gd nano compounds of R1 and R2.

The absorbed dose yielding the D₃₇ (dose used to inhibit 63% colony formation) values were 0.55 Gy for R1, 0.56 Gy for R2, and 1.50 Gy for control.

As far as the comparison under this experimental condition is concerned, the GdNCT effect was comparable to the standard BNCT effect.

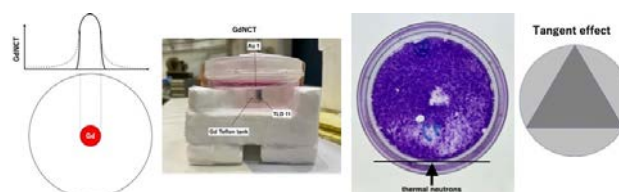


Fig. 3. Macro dose distribution of GdNCT.

Our in-vitro preliminary study using rat glioma cell line C6 for estimation of GdNCT dose distribution shows the macro lethal dose caused by GdNCT might distribute close to the tumor nest without serious damage onto the surrounding tumor cells. These data will be reported details in next time.

We will continue to investigate the possibility of GdNCT.

REFERENCES:

- [1] S. Fedorenko *et al.*, Colloids Surf. A Physicochem. Eng. Asp., **559** (2018) 60–67.
- [2] R. Zairov *et al.*, Sci. Rep. **7** (2017) 14010. (doi) 10.1038/s41598-017-14409-6.

CO7-13 Fluorescent Dodecaborate to Development of Theranostic Type Boron Carrier

Y. Hattori, M. Ishimura, I. Nakase¹, M. Kirihata

Research Center of Boron Neutron Capture Therapy,
Osaka Metropolitan University

¹ Graduate School of Science, Osaka Metropolitan University

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

BSH, a class of water-soluble boron cluster compounds with low toxicity, is clinically used as boron carrier for the treatment of brain tumors, although, tumor selectivity and cell membrane permeability of BSH is slightly low. In the course of our developing studies on new boron carrier for BNCT, we have designed and synthesized thiododecaborate ($[B_{12}H_{11}S]^{2-}$) unit-containing tumor seeking compounds such as amino acids, peptides and antibodies [1-3]. Furthermore, we reported the bifunctional type thiododecaborate containing compounds which $B_{12}H_{11}S^{2-}$ cluster is linked two kinds of organic moiety through S^+ sulfaneyl groups ($B_{12}H_{11}S^+(-R_1)-R_2$) [4].

Recently, theranostic drug delivery is strong criteria for effective cancer treatment. In BNCT, development of theranostic type boron carrier is highly noted, because visualization of the boron distribution and determination of tumor/normal ratio by non or minimally invasive examination are very important for the planning of BNCT. To develop the theranostic type boron carrier for BNCT, we present the design and synthesis of novel bifunctional boron cluster containing compounds which linked fluorescent dye (fluorescein, BODIPY, Aza-BODIPY etc.) and alkyl linker ($-COOSu$, maleimide, etc.) to conjugate with tumor seeking compounds such as peptides, proteins and antibodies. Furthermore, we report the conjugation of novel bifunctional boron compounds with antibody, and the biological evaluation of boronated antibody as boron carrier for BNCT.

RESULTS and Discussion: The synthetic route of bifunctional boron cluster containing compounds was illustrated in Fig. 1. The S-alkylation of cyanoethyl BSH (**1**) with 6-bromohexanoic acid or N-Boc-3-bromopropyl amine was achieved by previously reported method in good yield [5]. After deprotection of S-cyanoethyl group, the brominated fluorescent dye (fluorescein, BODIPY,

Aza-BODIPY and Cy5.5) was treated to give fluorescent sulfoniododecaborate followed by treatment with an ion-exchange resin. The succinimide ester type compound (**4**) was prepared by the reaction of compound **3** with di(N-succinimidyl)carbonate (DSC). The alkyl amine type compound **6** was reacted with N-methoxycarbonylmaleimide to give maleimide type fluorescent sulfoniododecaborate **7**.

The conjugation of novel bifunctional boron compounds with peptides, protein or antibody, and the evaluation of boronated compounds as boron carrier for BNCT and is now under investigation.

References:

- [1] Y. Hattori *et al.*, J. Med. Chem., **55** (2012) 6980-6984.
- [2] I. Nakase *et al.*, Chemm. Commun., **55** (2019) 13955-13958.
- [3] I. Nakase *et al.*, ACS Omega, **5** (2020) 22731-22738.
- [4] Y. Hattori *et al.*, ACS. Med. Chem. Lett., **13** (2022) 50-54.
- [5] S. Kusaka *et al.*, Appl. Radiat. Isot., **69** (2011) 1768-1770.

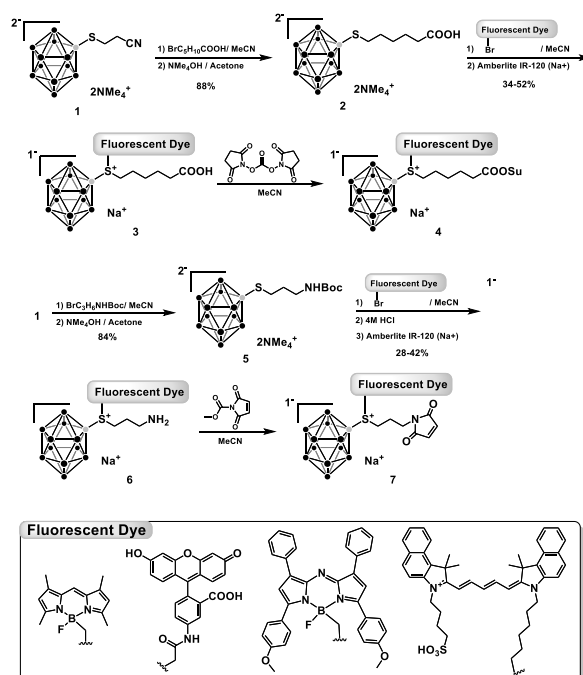


Fig. 1. Synthesis of bifunctional sulfoniododecaborate.

CO7-14 Evaluation of gadolinium biodistribution and tumor-killing effects of surface-modified gadolinium-loaded chitosan nanoparticles for gadolinium neutron capture therapy

T. Andoh¹, T. Fujimoto², M. Suzuki³, T. Takata⁴, Y. Sakurai⁴ and H. Ichikawa¹.

¹Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Japan.

²Department of Orthopaedic Surgery, Hyogo Cancer Center, Japan.

³Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University, Japan.

⁴Division of Radiation Life Science, Institute for Integrated Radiation and Nuclear Science, Kyoto University, Japan

INTRODUCTION: Neutron-capture therapy using nonradioactive ¹⁵⁷Gd (Gd-NCT) is currently under development as a potential radiation therapy option for cancer. Gd-NCT with ¹⁵⁷Gd has several potential advantages over boron (¹⁰B) neutron capture therapy (BNCT). The deep tissue penetration (100 μm) of γ-rays from the ¹⁵⁷Gd (n, γ) ¹⁵⁸Gd reaction is expected to provide tumor-killing efficacy within bulky tumors such as head and neck cancers. Furthermore, oral mucositis caused by BNCT using *p*-boronophenylalanine could be a potential dose-limiting consideration for head and neck tumors [1]. We have previously developed gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs) for controlled Gd delivery in GdNCT. These nanoparticles comprised Gd-diethylenetriaminepentaacetic acid (Gd-DTPA, an MRI contrast agent), and chitosan (a naturally abundant biodegradable polysaccharide with good biocompatibility and bioadhesive characteristics). The present study investigates the *in vivo* gadolinium biodistribution and tumor-killing effects after NCT with intra-tumoral injected nanoparticulate formulations.

EXPERIMENTS: Gd-nanoCP was prepared using chitosan and Gd-DTPA through the previously developed w/o emulsion-droplet coalescence technique [2]. The condensation reaction of the amino group present in intact Gd-nanoCP and NHS-activated ester-PEG prepared direct PEG-modified Gd-nanoCP (PEG-Gd-nanoCP). The thin-film hydration method carried out surface-modification of Gd-nanoCPs with Soybean Lecithin and PEG-lipid (PEG-SL-Gd-nanoCP). Mean particle size and zeta potential of the resultant Gd-nanoCPs were measured by Zetasizer[®] (Malvern). Gd concentration of tumor tissue was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, SPS3100) followed by incineration of each sample. In the NCT trial, male 5-week-old C3H/HeN mice were used. SCC-VII (1 × 10⁶ cells/mouse) were injected into the left masseter muscle [3]. The mice were divided into NCT group and HOT control group. Before injection, centrifugation concentrated Gd-nanoCPs to 6000 μg Gd/mL.

Gd-nanoCPs incorporating 1.2 mg of natural Gd were injected intratumorally twice to the mice. The tumors were exposed to thermal neutron irradiation at the Institute for Integrated Radiation and Nuclear Science, Kyoto University. The tumor volume ratio before and after neutron irradiation assessed the tumor-growth suppressing effect.

RESULTS: Mean particle diameter and zeta potential of the Gd-nanoCP, PEG-Gd-nanoCP, and PEG-SL-Gd-nanoCP were 171, 168, and 179 nm, 15, 20, and -6 mV, respectively. In the intratumoral injection test, the Gd concentrations of Gd-nanoCP, PEG-Gd-nanoCP, and PEG-SL-Gd-nanoCP in the tumor tissues were 2337, 1551, and 1533 ppm, respectively. PEG-SL-Gd-nanoCP wasn't used in the GdNCT because it's shown good permeation, leaked out the tumor tissue, and accumulated to normal tissues. In GdNCT, the NCT group significantly suppressed tumor growth relative to that observed in the HOT control group. Although the Gd concentration in the tumor tissue of the PEG-Gd-nanoCP group was approximately 2/3 lower than that in the Gd-nanoCP group, the PEG-Gd-nanoCP group showed similar tumor growth suppression as the Gd-nanoCP group. This result was found to be related to the distribution behavior of Gd preparations within the tumor after administration. Unlike extensive delivery via tumor vasculature, the intratumoral injection causes particle diffusion and distribution from the injection site. Therefore, improving the permeability and diffusion of Gd preparations within the tumor tissue after injection is crucial for achieving uniform Gd distribution in the tumor tissue. The improvement in dispersion stability with surface modification possibly led to the homogenization of Gd distribution in the tumor tissue because the particle sizes of the two types of Gd formulations did not differ significantly.

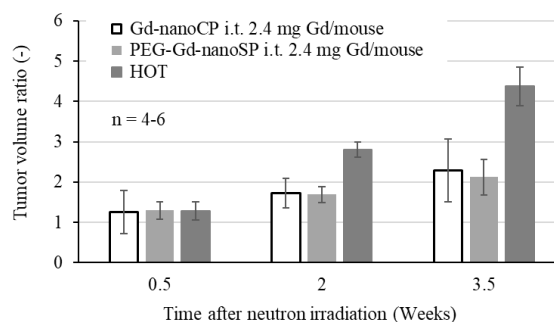


Fig. 1. Tumor volumes after thermal neutron beam irradiation of NCT and HOT control groups.

REFERENCES:

- [1] J. A. Coderre *et al.*, *Radiat. Res.*, **152** (1999) 113-118.
- [2] H. Tokumitsu *et al.*, *Pharm. Res.*, **16** (1999) 1830-1835.
- [3] T. Nomura *et al.*, 5th Asian Conference on Oral and Maxillofacial Surgery, (2002) 111-115.

CO7-15 Basic research to expand the indication of boron neutron capture therapy to non-neoplastic diseases

T. Watanabe¹, Y. Hattori³, Y. Sanada¹, T. Takata¹, G. E. Sato², M. Yoshimura², Y. Sakurai¹, H. Tanaka¹, T. Mizowaki², M. Kirihata³, M. Suzuki¹

¹Institute for Integrated Radiation and Nuclear Science, Kyoto University

²Radiation Oncology and Image-Applied Therapy, Graduate School of Medicine, Kyoto University

³Research Center for Boron Neutron Capture Therapy, Organization for Research Promotion, Osaka Metropolitan University

INTRODUCTION: The purpose of this study is to explore the possibility of applying BNCT, which has been developed as a cancer therapy [1], to intractable diseases other than cancer (intractable non-tumor diseases) using mouse models, in order to further develop BNCT as a medical field and to discover potential indications for diseases other than cancer [2]. In this study, we will develop boron agents for the above purposes based on antibodies and examine their efficacy using disease-specific mouse models.

EXPERIMENTS: It is known that intraperitoneal administration of β -glucan to mice (Balb/c or Balb/c-derived offsprings) induces an immune response and may induce symptoms similar to collagen disease [3, 4]. In this study, we used a mouse model of inflammation induced by intraperitoneal administration of β -glucan to induce symptoms such as arthritis and enteritis, which are commonly observed in human collagen diseases. When 30 mg of laminarin, a β -glucan, was administered intraperitoneally to mice, swelling of bilateral wrist joints was observed from 7 days after administration, and 10-14 days after administration, redness of the wrist joints was observed, suggesting clear inflammation. No obvious diarrhea was observed in this mouse model or at the β -glucan dose.

To bind a boron atom to the stationary portion (Fc portion) of a commercially available IgG antibody, a boronated module was prepared by attaching a functional group that reacts against an amino group to a boron cluster. The number of boron atoms (relative ratio) was quantified by binding the FITC-bound boronated module to the isotype antibody and measuring the fluorescence intensity per antibody molecule.

To determine whether the antigen recognition ability of the antibody is maintained after binding of the boronated module, the boronated module conjugated with FITC was conjugated to a commercially available anti-mouse CD8a antibody (BioLegend), and mouse-derived splenocytes were measured together with BV421-anti-mouse CD8b antibody. The IL17 receptor was targeted by the method described above.

A new boron drug targeting the IL17 receptor was prepared by the above method, and its therapeutic effect was

examined by comparing it with that of the neutron alone irradiation group, the L-BPA administration group, and the boron drug targeting the immune response group.

RESULTS: FITC-conjugated boronated anti-CD8a and BV421-aCD8b antibodies co-stained CD3+CD8+ T cells in splenocytes, indicating that the antigen recognition capacity of the antibodies is maintained within the protocols used in this study even when the boronated modules are bound to the antibodies. After binding a boronated module to a commercially available anti-mouse IL17 receptor (BioLegend) and administering 200 μ g to a mouse model of arthritis created by intraperitoneal administration of β -glucan, the therapeutic effect on arthritis was observed by irradiating bilateral wrist joints with neutrons. Mice with arthritis were prepared under the same conditions, and the same neutron irradiation was performed on the following two control groups; control group 1: Neutron irradiated without boronated antibody, control group 2: Neutron irradiated with boronated derivatives of amino acids, which are commonly used as boron drugs for BNCT. No obvious improvement in arthritis was observed when arthritis was evaluated by scoring over time after neutron irradiation.

REFERENCES:
[1] RF Barth *et al.*, *Radiat Oncol.*, **7** (2012) 146.
[2] TD Malouff *et al.*, *Front Oncol.*, **11** (2021) 601820.
[3] S. Sakaguchi *et al.*, *FEBS Letters*, **585** (2011) 3633-9.
[4] K. Hirota *et al.*, *Immunity*, **48** (2018) 1220-1232.e5.

CO7-16 In Vivo Efficacy of BPA-Ionic Liquid as a Novel Compound for BNCT (2)

M. Shirakawa^{1,2}, T. Sakai¹, R. Terada¹, Y. Sato¹, N. Kamegawa³, R. Takeuchi³, H. Hori³, K. Nakai², A. Zaboronok², F. Yoshida², T. Tsurubuchi², T. Sakae², A. Matsumura², T. Takata⁴, N. Kondo⁴, Y. Sakurai⁴, M. Suzuki⁴

¹Department of Pharmaceutical Sciences, Fukuyama University

²Department of Neurosurgery, Faculty of Medicine, University of Tsukuba

³Morita Pharmaceutical Ind., Ltd.

⁴Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION:

L-p-boronophenylalanine (BPA) shows excellent anti-tumor effects after thermal neutron irradiation but is known to require large doses to achieve clinical efficacy because of its low solubility. Therefore, we have been investigating and reporting the use of ionic liquids (ILs) [1, 2]. Here, we have gone further and synthesized a more stable BPA-based IL (BPA-IL) and reported its in vivo antitumor effects after thermal neutron irradiation.

EXPERIMENTS:

1. Synthesis of BPA-IL

BPA-IL applied in this study was synthesized using meglumine as the cation substance and BPA as the anion substance, as in the previous study [3]. However, meglumine and BPA were mixed 2:1 and stirred at room temperature for 24 hours. After that, the water content of the IL was reduced to about 20 wt% by rotary evaporator at 80°C.

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

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

Ten days later, 34 μ L of BPA-IL was administrated via intravenous injection before irradiation was delivered at a dose of 24mg¹⁰B/kg. Similarly, 200 μ L of BPA-Fru was administered. In addition, 29 μ L of BPA-IL was administered intravenously over 30 minutes. Two hours after injection, thermal neutron irradiation was performed with a flux of $1.1-1.2 \times 10^9$ neutrons/cm²/s over 60 min. The tumor size was measured over time after the irradiation until day 27, and the volume was calculated using the previously applied formula [4].

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

RESULTS:

As shown in Figure 1, BPA-IL (Meg-BPA) significantly inhibited tumor growth compared to the control group despite the lower dose volume compared to BPA-Fru. However, the intravenous administration of BPA-IL (Meg-BPA i.v.D) did not produce excellent therapeutic effects. As shown in Figure 2, no significant side effects (e.g., weight loss) were observed after using BPA-IL, similar to BPA-Fru.

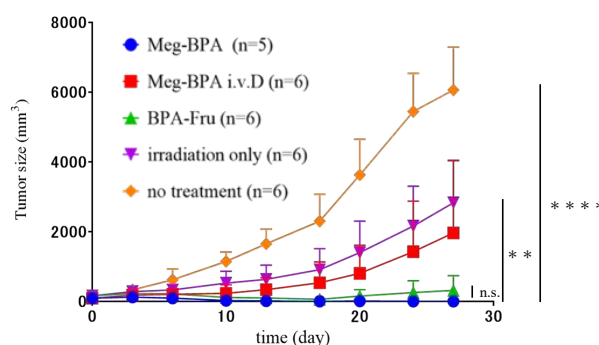


Fig.1) Meg-BPA anti-tumor effect after BNCT.

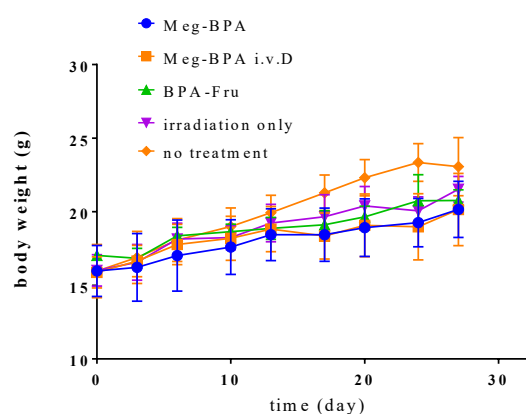


Fig.2) Mice body weight after Meg-BPA injection with further neutron irradiation.

REFERENCES:

- [1] M. Shirakawa *et al.*, Patent application 2020-067196 and patent publication 2021-161093.
- [2] M. Shirakawa *et al.*, Patent application 2020-136911 and patent publication 2022-032754.
- [3] M. Shirakawa *et al.*, KURNS PROGRESS REPORT 2021, (2022) 208.
- [4] M. Shirakawa *et al.*, KURRI PROGRESS REPORT 2017, (2018) 69.

CO7-17 Synthesis of a Novel Boron Compound with Potential Peptide-Related Nuclear Import (2)

M. Shirakawa^{1,2}, T. Nakano¹, A. Okuda³, A. Shigenaga¹
and M. Sugiyama³

¹Department of Pharmaceutical Sciences, Fukuyama University

²Department of Radiation Oncology, Faculty of Medicine, University of Tsukuba

³Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION:

The therapeutic effect of BNCT is provided by the alpha-rays generated in the ¹⁰B neutron capture reaction, which leads to double-strand DNA breaks in tumor cells. Based on its principle, the anti-tumor effect of BNCT is presumed to be maximized when ¹⁰B is present in or near the nucleus of the tumor cell [1].

Therefore, we have been investigating and reported the peptide-BSH (TAT-GALA-BSH) [2]. Here, we have gone synthesized a novel boron peptide of other amino acid sequence and reported the obtained experimental results.

EXPERIMENTS:

Novel boron peptides were combined with mercaptododecaborate (BSH) and three functional peptides, and the synthesis was performed on Rink Amide resin (100~200 mesh) by the Fmoc solid-phase method [3]. As amino acids sequence of the peptide, we selected S19 (PFVIGAGVVLGALGTGIGGI), H16 (HHHHHHHHHHHHHHHHHH), NLS (RREKYGIPEPPEKRRK), and synthesized them. After that, BSH was conjugated with the peptide N-terminal region by binding chloroacetic acid. Each amino acid coupling reaction was carried out for 60 min at room temperature. De-protection and cleavage of resin were accomplished with a cleavage cocktail at room temperature, then precipitated by adding a large amount of diethyl ether. After the freeze-drying procedure, we obtained the desired compound.

RESULTS:

As shown in Fig. 1, we succeeded in synthesizing the novel boron peptide (BSH-S19-H16-NLS). In the identification of this boron peptide, ESI-TOFMS (Xevo G2-XS QToF) showed m/z 1576.1 of the tetravalent peak. However, the yield and purity were low, and not enough yield was obtained to be used in other experiments.

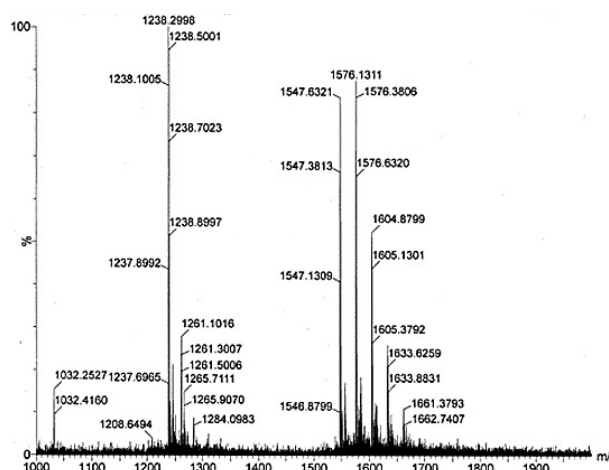


Fig.1) Identification of BSH-S19-H16-NLS by ESI-TOFMS

REFERENCES:

- [1] T. Sato *et al.*, *Sci. Rep.*, **8** (2018) 988.
- [2] M. Shirakawa *et al.*, *KURNS PROGRESS REPORT 2020*, (2021) 198.
- [3] M. Gongora-Benitez *et al.*, *ACS Comb. Sci.*, **15**(5) (2013) 217-228.

CO7-18 Construction of novel Boron-containing silica nanoparticles and BNCT experiments

F. Tamanoi¹, M. Laird¹, K. Matsumoto¹, A. Komatsu¹, Y. Higashi¹, A. Kubota and M. Suzuki²

¹Institute for Advanced Study

Institute for Integrated Cell-Materials Sciences, Kyoto University

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Borocaptate (BSH) has been developed as useful boron compound which is available for the Boron neutron capture therapy (BNCT) therapy of brain tumor. But, BSH has poorly accumulation capability into cell, so it is necessary to develop of novel type of boron compound that can effectively be transported BSH into cells. We have recently developed a mesoporous silica-based nanoparticle which has biodegradable bonds in the framework, named biodegradable periodic mesoporous organosilica (BPMO), that are coupled with BSH by thiol-ene reaction. These nanoparticles have a large surface area where BSH can be coupled for BNCT application. In this study, we investigated BSH-BPMO uptake into OVCAR8 ovarian cancer cells and spheroid, and evaluated spheroid destruction efficacy.

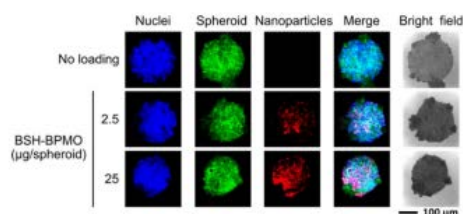
EXPERIMENTS: BPMO was synthesized by sol-gel synthesis of two precursors, bis[3-(triethoxysilyl) propyl] tetrasulfide and 1, 2-bis(triethoxysilyl) ethane. This resulted in the incorporation of tetrasulfide bonds into the framework of the nanoparticles. BPMO was then processed to modify with triethoxyvinylsilane that contain vinyl groups. After vinyl group modification on BPMO surface, BPMO was phosphonated and coupled with BSH by overnight mixing. The synthesized nanoparticles were characterized by using SEM, TEM, FT-IR, NMR, nitrogen adsorption-desorption analysis and zeta potential. The amount of boron attached on the nanoparticles was examined by ICP, and boron content was determined.

GFP-expressed OVCAR8 cells (5,000 cells/well) were inoculated on U-bottom 96-well plate to form cancer spheroid for 7 days. After spheroid formation, BSH-BPMO was loaded to spheroid and evaluation of BSH-BPMO uptake by confocal microscopy. Finally, we investigated BSH-BPMO-loaded spheroid destruction with thermal neutron for 1 hour at an operating power of 1MW. After the irradiation, spheroids were cultured for 24h with 5%-humified CO₂ and evaluated the size.

RESULTS: Nanoparticles synthesized had approximately 300 nm of diameter and homogenous shapes examined by SEM and TEM microscopy. FT-IR analysis of BPMO showed diagnostic peaks of typical Si-O-Si, -(CH₂)₂- and -CH₂- vibrations. After coupling of BSH to BPMO, we analyzed surface charge of BSH-BPMO which was negative due to modification with phosphonate. The zeta potential of BPA-BPMO was -51.5 mV.

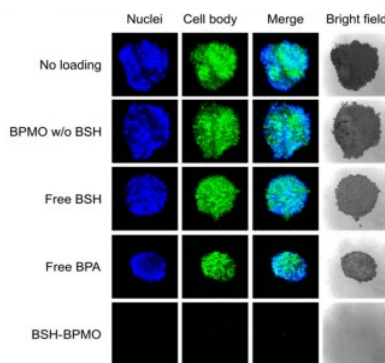
BSH-BPMO accumulation in the OVCAR8 cell was investigated with a confocal microscope. BSH-BPMO was effectively able to take up into cells and localized at perinuclear region. We also evaluated the amount of boron which was taken into cancer cells by ICP. $19.15 \pm 0.39\%$ of boron were detected from cell lysate, whereas normal BPA was $0.33 \pm 0.02\%$. And then, we investigated spheroid uptake of BSH-BPMO by confocal microscopy. As seen in Fig.1, The red fluorescence of BSH-BPMO was overlapped with GFP fluorescence at each focal plane, and it indicated BSH-BPMO was effectively taken up into spheroid. These results indicated that BSH can effectively improve the cellular uptake and accumulation by BPMO nanoparticles.

Investigation of the spheroid destruction of cancer spheroid loaded BSH-BPMO was carried out with Kyoto University Nuclear Reactor and evaluated the spheroid size after neutron irradiation. Following solutions were loaded to spheroid with 24h incubation at 37°C in humidified CO₂: 500 µg/ml of BSH-BPMO, 500 µg/ml of BPMO, 15.6 µg/ml of free BSH and 186 µg/ml of free BPA corresponding to 89 ng of ¹⁰B. These spheroids were irradiated with thermal neutron. As seen in Fig.2, the spheroid which was loaded BSH-BPMO was completely destructed compared with free BPA-loaded cancer spheroid. We demonstrated that BPMO can be useful for effectively carrier of BSH into cells, and BSH-BPMO could be a novel boron compound with the potential to dramatically improve the BNCT efficacy.



BSH-BPMO uptake in OVCAR8 spheroids with 10⁴ cells: Hoechst-dyed nuclei observed at 405 nm, GFP-expressed OVCAR8 cell at 488 nm and Rhodamine B containing nanoparticles at 561 nm.

Fig.1. Spheroid uptake of BSH-BPMO.



Neutron exposure results of (a) OVCAR8 spheroids observed by confocal microscopy after 1 h of neutron irradiation and three days of incubation: Hoechst-dyed nuclei observed at 405 nm and GFP modified OVCAR8 cell at 488 nm.

Fig.2. Spheroid destruction by neutron irradiation.

CO7-19 Development of Gadolinium-loaded mesoporous silica-based nanoparticles and application to cancer radiotherapy

F. Tamanoi¹, K. Matsumoto¹ and M. Suzuki²

¹*Institute for Advanced Study
Institute for Integrated Cell-Materials Sciences, Kyoto
University*

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

INTRODUCTION: Gadolinium neutron capture therapy (GNCT) has emerged as an attractive neutron-based cancer therapy different from BNCT. Neutron exposure leads to the generation of gamma-rays and Auger electrons. We aim to develop a novel type of nanoparticles for GNCT. We have previously developed silica-based nanoparticles for BNCT [1]. Our approach for GNCT reagents is to utilize a similar approach. Mesoporous silica-based nanoparticles have the advantage of well-established synthesis methods as well as a large surface area. In addition, the nanoparticles are stable enabling various chemical modifications to be carried out.

EXPERIMENTS:

BPMO synthesis was carried out by sol-gel synthesis of two precursors, bis[3-(triethoxysilyl) propyl] tetrasulfide and 1, 2-bis(triethoxysilyl) ethane. The nanoparticles synthesized contain tetrasulfide bonds within their framework. In order to bind gadopentetic acid, we modified the nanoparticle surface with amino groups.

Gadolinium was coupled onto BPMO surface using gadolinium diethylenetriamine penta-acetic acid (DTPA). The nanoparticles synthesized were characterized by SEM and EDX-TEM. ICP-AES was used to determine the amount of Gd attached on the nanoparticles.

Uptake of Gd-BPMO into human cancer cells was examined. In addition, CAM model established by transplanting human ovarian cancer cells OVAR8 on the CAM membrane in fertilized chicken egg was used to check the efficacy of Gd-BPMO.

After intravenously injection of Gd-BPMO, the eggs were placed at the center of emerging neutron beam and were irradiated with thermal neutron for 1 h at an operating power of 1MW. After the irradiation, eggs were incubated for 3 days at 37°C with 65% humidity. Tumors were then cut out to evaluate the tumor size.

RESULTS: We have developed a reproducible method for Gd-BPMO. The nanoparticles had a diameter of approximately 80-100 nm, as determined by SEM and TEM microscopy. The amount of Gd that was coupled onto BPMO was 2.6% of the total weight, as determined by ICP. Scaling-up of the method was attempted.

We have established a convenient assay to examine the efficacy of Gd-BPMO for GNCT. This assay utilizes the

CAM assay and examines tumor growth inhibition upon neutron exposure after injection of the nanoparticles. This assay can be used to test a variety of nanoparticles and the results obtained can be used to further improve the nanoparticles. Evaluation of Gd-BPMO efficacy using a mouse model needs to be carried out in the future.

REFERENCES:

[1] F. Tamanoi *et al.*, *Int. J. Mol. Sci.*, **22** (2021) 2251.

CO7-20 Is the boron neutron capture reaction captured using CR-39 related to boron concentration and cell viability?

K. Nakai¹, Y. Yoshitaka¹, Y. Sugawara² and Y. Sanada³

¹Department of Radiation Oncology, Proton medical research center, Faculty of Medicine, University of Tsukuba.

²Doctorial program in Medical Sciences, Graduate School of Comprehensive human Sciences, University of Tsukuba.

³Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron neutron capture therapy is a particle therapy that produces alpha rays and lithium nuclei in vivo for anticancer effect. Alpha autoradiography is also used in the BNCT field as a simple method to show the localization of alpha radiation.

As a solid range detector, CR-39 is insensitive to X-rays and gamma rays and is also a material used for neutron detection by incorporating proton radiators and alpha converters.

In the present experiment, we aimed to investigate the internal alpha distribution of tubes irradiated with a horizontal neutron beam in a cell survival experiment system using cell suspensions, which are commonly used in BNCT experiments.

MATERIAL AND METHODS:

The CR-39, which is a passive Solid-state Nuclear Track Detector cut into the shape shown in Figure 1(a) by a carbon dioxide laser machine. The CR-39 treated with UV sterilization was placed vertically inside the sample tube at a 90-degree angle as shown in Figure 1(b). The sample was filled with 0.5 mL of solution containing cell suspension and used as the irradiation sample.

Cells: CHO, Neutron source: KUR thermal neutron beam.

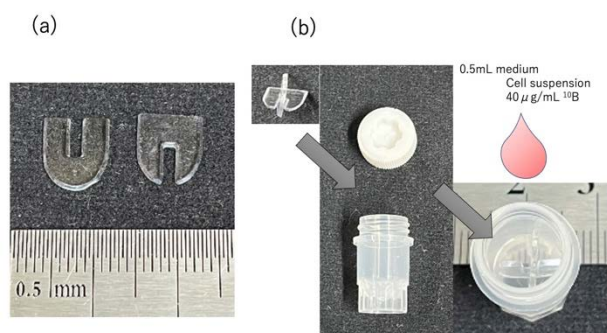


Figure 1. Schema of CR-39 shape (a) and placed inside the sample tube (b).

EXPERIMENTS: The irradiation set-up is the same as the cell survival experiments performed in KUR. Cell suspension and samples with medium were irradiated in the reactor for 18 and 36 min. CHO cells were

maintained at a boron concentration of 40 µg/ml in the medium for 2 h before irradiation and diluted with medium immediately before irradiation to 1/10 the boron concentration. Cell suspensions and samples with medium were irradiated in the reactor for 18 minutes and 36 minutes, respectively. After the irradiation, for the cell suspension samples, a colony formation assay was performed at each irradiation time, and the boron neutron capture reaction was confirmed to have occurred Figure 2.

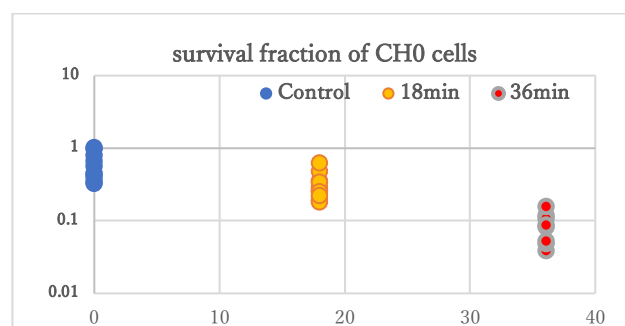


Figure 2. Survival Fraction of CHO, pretreated with 40 µg/ml ¹⁰B 2hrs before irradiation. Y axis showing irradiation time of KUR.

RESULTS: The CR-39 samples inserted in the same tube, were etched in a 6N NaOH solution at 60 °C for 60min, images of the pits were acquired with an optical microscope (Figure 3).

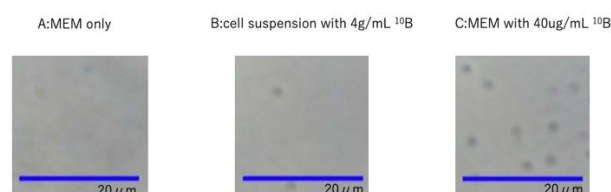


Figure 3. Blue bars are 20 µm; A: no pits observed in culture medium only, no boron; B: approximately 1-2 pits observed in 20 µm x 20 µm in CR-39 irradiated with cells; C: approximately 10 pits observed in culture medium only sample with 40 µg/mL of boron.

We can now relate the correlation between boron concentration, beam direction, and viability in colony formation tests with cell suspensions when the liquid surface is in contact with CD-39 with respect to alpha autoradiography. We plan to verify this.

REFERENCES:

- [1] I. Postuma *et al.*, Appl Radiat Isot., **167** (2021) 109353.
- [2] H. Tanaka *et al.*, J Radiat Res., **55** (2014) 373-380.

CO7-21 L-Phenylalanine Deficiency in Human Tumor Cells Improves BNCT Therapeutic Efficiency

Y. Tamari^{1,2}, R. Saba¹, T. Takata², M. Suzuki², K. Yamada¹ and H. Yamazaki¹

¹Department of Radiology, Kyoto Prefectural University of Medicine

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Boron neutron capture therapy (BNCT) is a type of radiotherapy that utilizes the nuclear reaction between ^{10}B and neutrons, and is an excellent method of selectively destroying cancer cells. In Japan, BNCT is now covered by insurance for head and neck tumors, but one problem is the insufficient concentration of ^{10}B , and the tumor tissue/ normal tissue (T/N) ratio of ^{10}B concentration is often small in some patients. L-Boronophenylalanine (L-BPA), used as a boron drug, is a boronated isotope of phenylalanine and is taken up through increased amino acid metabolism in tumors. L-type amino acid transporter 1 (LAT1) is known to be the uptake pathway for L-BPA. LAT1 is an exchange transporter that releases one molecule of amino acid to the extracellular space for uptake of one molecule of amino acid into the cell [1]. Although LAT1 expression in normal cells is rather limited, with only a small amount of expression at the blood-brain barrier and placental barrier, L-BPA can be taken up by normal cells via LAT2 and other pathways, which is a major problem when BNCT is performed [2, 3]. In this study, we attempted to improve the therapeutic effect of BNCT by restricting phenylalanine and improving L-BPA uptake.

EXPERIMENTS: The cell lines used were the human tongue cancer-derived cell line SAS, the human glioblastoma-derived cell line U87MG, and the human pancreatic cancer-derived cell line Panc-1. Phenylalanine-free medium was used for phenylalanine restriction, and dialyzed fetal bovine serum was used. Intracellular boron concentration was determined for each cell line using inductively coupled plasma atomic emission spectrometry (ICP-AES). In addition, the viability of each cell line was determined by the colony formation method after neutron irradiation in a research reactor at the Institute for Integrated Radiation and Nuclear Science, Kyoto University.

RESULTS: The results of the study on L-BPA uptake by phenylalanine restriction showed that the change in uptake efficiency was greatest in SAS, with an over 4-fold increase in L-BPA uptake in the group cultured on L-Phe-free medium compared to the group cultured on normal medium. U87MG and Panc-1 also showed a significant increase in BPA uptake under phenylalanine restriction compared to the group cultured in normal medium. This increase in L-BPA uptake due to phenylalanine restriction was greatest at 24 hours after phenylala-

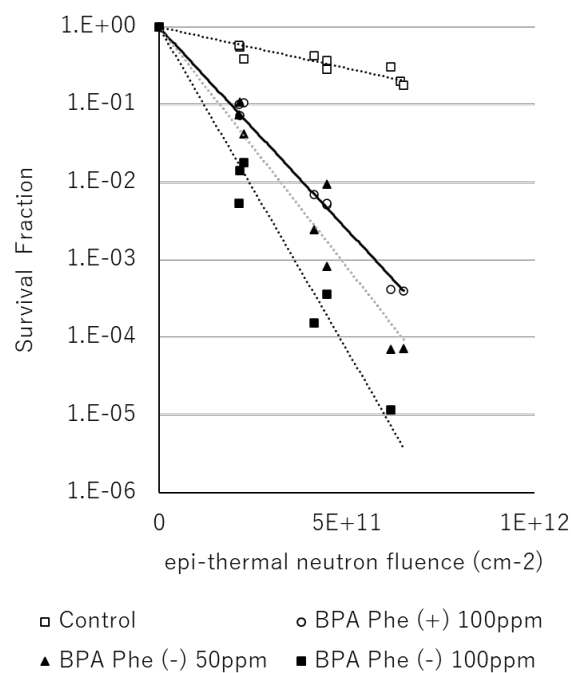


Figure 1. Effect of BNCT on phenylalanine deficiency in human tongue cancer-derived cell line SAS.

nine restriction, and was greatest when the phenylalanine concentration in the medium of SAS/Panc-1 and U87MG was 0 μM and 2 μM , respectively. The survival of cells after neutron irradiation was investigated. In all cell lines, the survival rate of the neutron-irradiated group treated with L-BPA without phenylalanine restriction was significantly lower than that of the neutron-irradiated group treated with neutrons alone. In addition, the survival rate of the L-BPA-neutron irradiated group under phenylalanine-restricted conditions was greatly reduced compared to the L-BPA-neutron irradiated group without phenylalanine restriction.

This study shows that phenylalanine restriction may improve the efficiency of L-BPA uptake in cancer cell lines and increase the therapeutic efficacy of BNCT. We will continue to investigate the improvement of therapeutic efficacy of BNCT by phenylalanine restriction in tumor-bearing mice.

REFERENCES:

- [1] A. Wittig *et al.*, *Radiat. Res.*, **153** (2000)173-180.
- [2] T. Watanabe *et al.*, *BMC Cancer*, **16** (2016) 859.
- [3] P. Wongthai *et al.*, *Cancer Sci.*, **106** (2015) 279-286.

CO7-22 Mechanism of Glioma Resistance After BNCT Conferred by Glioma Niche

N. Kondo¹, E. Hirata², Y. Sakurai¹, T. Takata¹, T. Kinouchi¹ and M. Suzuki¹

¹ Institute for Integrated Radiation and Nuclear Science, Kyoto University (KURNS)

²Division 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 immunotherapy. With BNCT, we achieved better local control and survival benefit in malignant glioma using thermal neutrons produced by the reactor in Kyoto University. However, the recurrence is inevitable after BNCT. Reasons for recurrence after BNCT have not been fully elucidated.

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

EXPERIMENTS:

Cell culture: We used murine glioma cell line G261 and astrocytes which was established from a brain of baby mouse. Both cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum at 37 °C in CO₂ incubator.

Boronophenylalanine (BPA) Treatment and Thermal Neutron Irradiation: We treated G261 cells with medium containing BPA at the concentration of 20 ppm for 30 minutes. The BPA was formulated and its concentration was measured as previously described [2]. After we trypsinized and rinsed the cells, cells were collected in plastic tubes and irradiated with thermal neutron for 20 minutes.

Co-culture system: After thermal neutron irradiation, 10⁴-7.5x10⁴ GL261 cells per well were disseminated into the bottom dish of 6 well plates. And 10⁴-1.5x10⁵ astrocytes per well were disseminated into the insert (pore size, 0.4 μm), and co-cultured with GL261 cells in 6 well plates. Three and eleven days after co-culture, GL261 cell numbers were counted and compared with mono-culture of GL261 cells.

RESULTS: On day 3, the cell numbers of BNCT mono-culture groups were smaller than those of non-irradiated mono-culture. There was no significant difference between mono and co-cultures both in non-irradiated group and BNCT group. On day 11, the

cell numbers of co-culture were higher than mono-culture in non-irradiated group. In BNCT groups, there were no significant change between mono-culture and co-culture.

We are continuing these experiments and collecting cell samples and soluble factors in medium.

REFERENCES:

- [1] N. Kondo *et al.*, *Cancers*, **12** (2020) 3040. (doi) 10.3390/cancers12103040.
- [2] N. Kondo *et al.* *Radiat. Environ. Biophys.*, **55** (2016) 89-94.

CO7-23 Antitumor effect of boron neutron capture therapy in vulvar cancer mouse model.

S. Terada¹, S. Tsunetoh¹, A. Toji¹, S Miyamoto¹, T. Tanaka¹, M. Ohmichi¹, J. Arima², K. Taniguchi², M. Suzuki³

¹Department of Obstetrics and Gynecology, Osaka Medical and Pharmaceutical University, Takatsuki, Japan

²Department of general and gastroenterological surgery, Osaka Medical and Pharmaceutical University, Takatsuki, Japan

³Institute for Integrated Radiation and Nuclear Science, Kyoto University, Asasiro-Nishi, Kumatori-cho, Japan

INTRODUCTION:

Vulvar cancer is an uncommon cancer in gynecologic cancer worldwide. Squamous cell carcinoma (SCC) is the most common histologic type. The primary treatment for vulvar cancer is surgical or radiation therapy. However, the recurrences involving local and inguinal node is not uncommon. [1] Therefore, the new treatment for the vulva cancer is needed. In this study we investigated the effectiveness and safety of boron neutron capture therapy (BNCT) for vulvar cancer using mouse model.

EXPERIMENTS:

Stella Chemifa (Osaka, Japan) supplied BPA (L-isomer), which was converted into a fructose complex. Female 4–6-week-old athymic nude mice (BALB/c Slc-nu/nu) were purchased from Japan SLC. 5×10^6 A431 cells (Vulvar squamous cell line) were injected subcutaneously around the genital area of each mouse. Treatment was initiated 4-6-weeks after injection of A431 cells. The mice were divided into the hot control (neutron irradiation only) and BNCT (neutron irradiation after peritoneal BPA administration) groups. BPA (250 mg/kg) was injected intraperitoneally into mice 2.5hr before neutron irradiation in the BNCT group. After irradiation, the tumor size and the mice weight was measured, and the tumor volume was calculated as follows.

$$V=ab^2/2$$

RESULTS: Fig.1 shows that the tumor volume in the hot control and BNCT groups. 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 group,

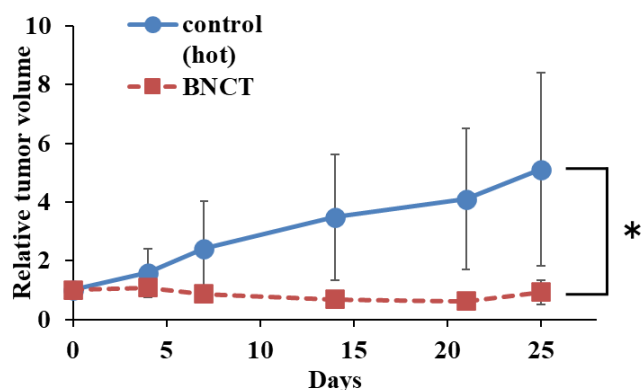


Fig.1. Antitumor effect on subcutaneous A431 tumor model.

Tumor grows curves in the hot control (irradiation only) and BNCT (irradiation after BPA administration) groups (n=7). The results are expressed as means \pm SD. (* $P<0.05$).

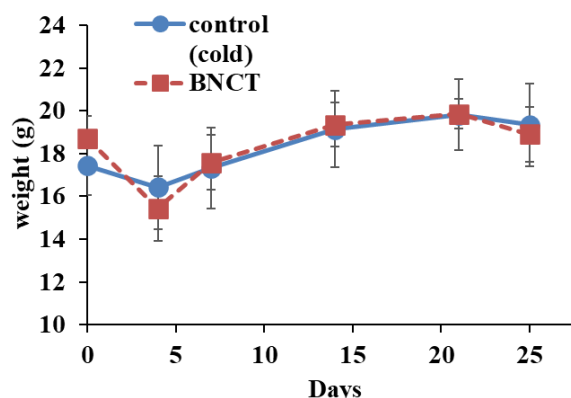


Fig.2. Body weight.

Body weight in the hot control (irradiation only) and BNCT (irradiation after BPA administration) groups (n=7).

REFERENCES:

[1] T. Maggino *et al.*, Gynecol oncol., **89** (2000) 116-122.

CO7-24 Anti-tumor effect of boron neutron capture therapy in pelvic human colorectal cancer in a mouse model

J. Arima, K. Taniguchi¹, M. Yamamoto, T. Watanabe³, Y. Suzuki, H. Hamamoto, Y. Inomata; H. Kashiwagi², S. Kawabata², K. Tanaka, K. Uchiyama, M. Suzuki³ and S-W. Lee

Department of general and gastroenterological surgery, osaka medical and pharmaceutical university, 2-7 daigaku-machi, takatsuki, osaka 569-8686, japan

¹Translational research program, osaka medical and pharmaceutical university, 2-7 daigaku-machi, takatsuki, osaka 569-8686, japan

²Department of neurosurgery, osaka medical and pharmaceutical university, 2-7 daigaku-machi, takatsuki, osaka 569-8686, japan

³Department of particle radiation oncology, institute for integrated radiation and nuclear science, kyoto university, 2 asashiro-nishi, kumatori-cho, sennan-gun, osaka 590-0494, japan.

INTRODUCTION:

Colorectal cancer is the most common cancer worldwide. Surgical resection is the mainstay treatment of colorectal cancer. However, local recurrence still occurs in 5% to 13% of patients after curative resection. When the tumor is unresectable, it needs alternative therapeutic strategy. In this study, we investigated the effectiveness of boron neutron capture therapy (BNCT) to pelvic colorectal cancer using the mouse model of pelvic recurrence of colorectal cancer. [1]

EXPERIMENTS:

¹⁰B-enriched BPA was provided by Stella Pharma Corporation (Osaka, Japan). An aqueous solution of the BPA D-fructose complex (250 mg/ml, 21.28 mg ¹⁰B/ml) was prepared for use in the experiments. Seven-week-old female BALB/c nude mice were purchased from Japan SLC, Inc (Hamamatsu, Japan). DLD-1 cells (1.0×10⁶/100 μL in 0.1 ml PBS) were injected into the pelvic retroperitoneum of each mouse, and the mice were divided into the cold control (no treatment, no neutron irradiation), hot control (neutron irradiation only), and BNCT (intra-peritoneal BPA administration and neutron irradiation) groups. Irradiation was performed 7 days after the injection of DLD-1 cells. BPA was injected intraperitoneally at a dose of 250 mg/kg 4 h before irradiation.

RESULTS:

Kaplan-Meier analysis showed that survival was significantly prolonged in the BNCT group compared with that in the cold and hot control groups (vs. cold control group, P<0.0001; vs. hot control group, P<0.05). (Fig.1) Moreover, survival was significantly prolonged in the hot control group compared with that in the cold control group (P<0.05). In the BNCT group, there was no evidence of

diarrhea, intestinal hemorrhage, or intestinal perforation after irradiation. Remarkable weight loss was observed in the cold and hot control groups (Fig.2).

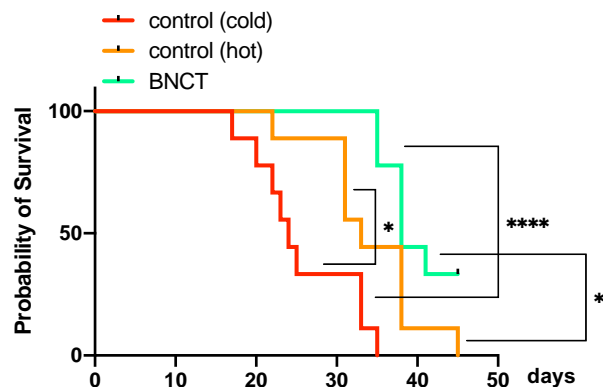


Fig.1. Kaplan-Meier plots showing the rate of survival in the cold control (red line), hot control (orange line), and BNCT (green line) groups (n=9). Survival duration was analyzed for significance using log-rank survival analysis (****P<0.0001, *P<0.05).

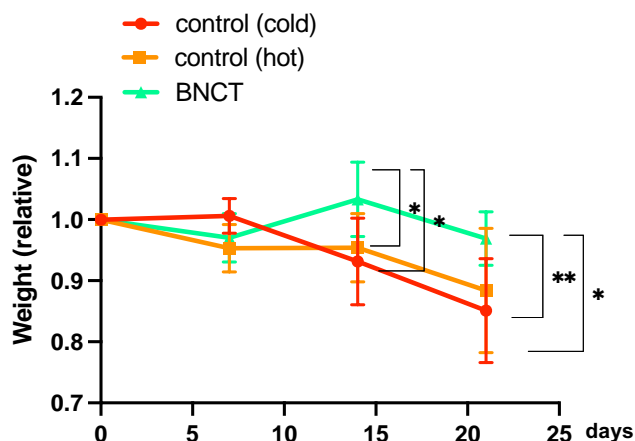


Fig.2. Body weight in the cold control (red line), hot control (orange line), and BNCT (green line) groups. Data is shown as the mean ± SEM. (**P<0.01, *P<0.05)

[1] M. Yamamoto *et al.*, Sci. Rep., 9 (2019) 19630.

CO7-25 Research and Development of New Technology for Boron Neutron Capture Therapy

M. Xu¹, T. Watanabe³, X. Zhou¹, R. Ohgaki^{1,2}, C. Jin¹, H. Okanishi¹, H. Tanaka³, M. Suzuki³, and Y. Kanai^{1,2}

¹Department of Bio-system Pharmacology, Graduate School of Medicine, Osaka University

³Integrated Frontier Research for Medical Science Division, Institute for Open and Transdisciplinary Research Initiatives (OTRI), Osaka University

³Institute for Integrated Radiation and Nuclear Science, Kyoto University

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

EXPERIMENTS: For the cell irradiation experiment, the human-derived tumor cell line MCF-7 was treated with L-BPA by adding it to the culture medium. Following a washing step, cells were incubated for 60 minutes in a medium with or without inhibitors targeting transporters responsible for L-BPA release. Subsequently, cells were harvested in Hanks' Balanced Salt Solution (HBSS) and transferred to 1.5 mL tubes, serving as neutron irradiation samples. A control sample was prepared by irradiating cells without L-BPA loading. Post-neutron irradiation, the cell numbers were adjusted, and cells were seeded in 10 cm dishes. Viable cells were then collected and assessed for colony formation to measure cell survival.

For *in vivo* mouse irradiation experiment, the mouse-derived tumor cell lines 4T1 and CT26 were subcutaneously implanted into Balb/c mice to establish syngeneic tumor models. Mice were administered 8 mg of L-BPA (equivalent to 400 mg/kg) via tail vein injection, followed by the administration of transporter inhibitor responsible for L-BPA release at 1 hour and 1.5 hours post-L-BPA injection. As a control, neutron-irradiated mice were prepared without L-BPA loading but received the inhibitor treatment alone. Neutron irradiation was

conducted at 5 MW for 15 minutes. The therapeutic effects on tumor regression were compared between the experimental and control groups following neutron irradiation.

RESULTS: In cell irradiation experiments, MCF-7 cells were treated with L-BPA, and after washing, the cells were divided into two groups: Group 1 was incubated for 60 minutes in a cell culture medium (RPMI1640) containing an inhibitor of the transporter responsible for L-BPA release. Group 2 was incubated for 60 minutes in a culture medium without the inhibitor. Additionally, Group 3 consisted of cells without L-BPA treatment was prepared. All groups were then subjected to neutron irradiation. After irradiation, the number of viable cells was compared by assessing colony formation in low-density cultures. The results showed no significant difference in cell survival between Group 2 and Group 3 (non-BNCT), suggesting that the intracellular L-BPA content had decreased to a level undetectable for BNCT effects within 60 minutes following L-BPA treatment. In contrast, the cell survival rate in Group 1 was significantly less than that in Group 2, indicating a significant enhancement of BNCT efficacy by inhibiting the transporter responsible for L-BPA release.

In *in vivo* mouse irradiation experiment, mouse triple-negative breast cancer-derived 4T1 cells and mouse colon cancer-derived CT26 cells were implanted into the hind limbs of mice to form allograft tumors. Mice were then administered L-BPA intravenously and divided into two groups. Group 1 received an intravenous injection of a transporter inhibitor responsible for L-BPA release 1 hour and 1.5 hours after L-BPA administration. Group 2 did not receive any inhibitor treatment following L-BPA administration. Additionally, Group 3 was prepared by administering saline instead of L-BPA, followed by injections of the transporter inhibitor at 1 hour and 1.5 hours post-saline administration. For each group, mice were anesthetized 2.5 hours after L-BPA or saline administration and subjected to neutron irradiation. Tumors were excised two weeks after irradiation, and their sizes were measured. The results indicated that, for both 4T1 and CT26 tumors, there was no significant difference between Group 2 and Group 3 (non-BNCT), suggesting that tumor L-BPA levels had decreased to an extent undetectable for BNCT effects within 2.5 hours post-administration. In contrast, tumor sizes in Group 1 were significantly smaller for both 4T1 and CT26 than Group 2, demonstrating that inhibiting the transporter responsible for L-BPA release significantly enhanced BNCT efficacy.

The results obtained in this study provide us with clues for optimizing L-BPA-based BNCT.

REFERENCES:

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

CO7-26 Observation of Intracellular Boron Neutron Capture Reaction with a Novel Boron Compound

R. Nozaki,¹ Y. Takamura,¹ K. Igawa,² N. Kondo,³ Sakurai,³ and H. Kakuta¹

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

² Neutron Therapy Research Center, Okayama University.

³ Institute for Integrated Radiation and Nuclear Science, Kyoto University.

INTRODUCTION: A cancer treatment known as “boron neutron capture therapy (BNCT)” is based on the nuclear interaction between the element boron-10 (¹⁰B) and neutrons. Since the effectiveness of this treatment depends on the collision of ¹⁰B with neutrons, the amount of boron present in the tumor tissue is essential. The blood boron level for borofaran (1), the only treatment currently approved for BNCT, is used to assess the amount of boron in cancer tissue. There are no boron agents available that can quantify boron concentrations in cancer tissue. Thus, we aimed to create a BNCT drug that could identify boron levels in malignant tissue.

We focused on iodine contrast agents for X-ray CT because they can detect iodine concentrations noninvasively by X-ray CT and are given in similar large doses to BNCT agents. This idea led us to propose that boron content in tissues may be determined using this concept. We created and synthesized BS-DIP-OEF (2) (Figure 1A) to test the aforementioned concept. We then used this compound to evaluate intra-cellular concentration and a colony assay utilizing B16BL6 after neutron irradiation with the drug.

EXPERIMENTS: (Boron concentration in compound-treated cells) Compound 2 was synthesized by our group. The boron concentration in boron compound-treated cells was measured using ICP-MS in mouse melanoma B16/BL6 cells. B16/BL6 cells were seeded at 6×10^5 cells/2 mL in 60 mm petri dishes. BSH is treated at 2 mM in many references. However, this concentration is extremely high and requires large amounts of the compound. Therefore, we set an upper limit of 500 μ M for the exposure concentration of 2. In addition, borofaran exposure time is less than 3 hours in actual clinical practice. Based on the above, the compound exposure time was set to 2 hours. After compound exposure, cells were washed with PBS, collected with a scraper, and counted for live and dead cells using trypan blue. All cells were then lysed using RIPA buffer, and the boron (¹⁰B) concentration in the resulting samples was measured by ICP-MS (Agilent 7900/MassHunter).

(Neutron irradiation experiment) B16/BL6 cells prepared as in the previous section were exposed to 2 at a final concentration of 500 μ M for 2 hours. The medium was aspirated off, and after trypsin treatment, 1 mL of cell suspension was prepared in 1.5 mL Eppendorf tubes to a concentration of 5.0×10^3 cells/mL. The samples were irradiated with neutrons at 5 MW for 1

hour at a thermal neutron fluence of 1.2×10^{12} cm⁻². After that, cells were seeded at 250 cells/well on 12 well plates and cultured for 7 days, cells were fixed and stained with crystal violet, and the number of colonies was counted. The number of colonies obtained was divided by the number of seeded cells to obtain the colony formation ratio.

RESULTS: Figure 1B shows the data obtained by the above experiment. Compound 2 was found to give an intracellular boron concentration of 1.02 μ g ¹⁰B/10⁷ cells. This is higher than the boron concentration obtained for B16 cells exposed to 2 mM BSH for 24 h (0.75 μ g ¹⁰B/10⁷ cells).¹ Since the cell proliferation inhibition of neutron irradiation has been expected, we applied neutron irradiation toward 2-treated at a final concentration of 500 μ M for 2 hours and performed a colony assay. As a result, a clear inhibition of cell proliferation was observed (Figure 2B).

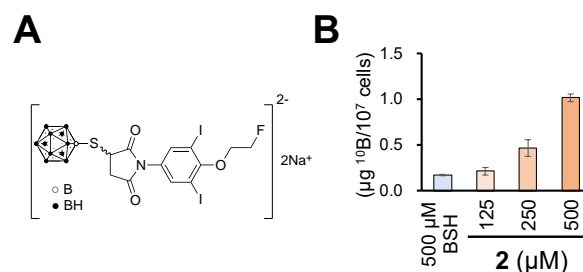


Figure 1. (A) Chemical structure of 2. (B) Intracellular boron concentration measured by ICP-MS at the indicated concentration after 2 hr exposure. Mean \pm SD (n = 3).

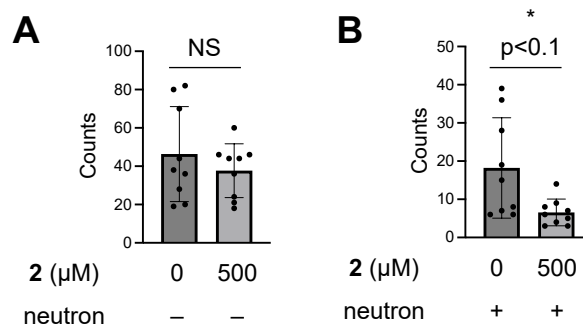


Figure 2. (A) Comparison of colony counts after compound treatment without neutron irradiation. (B) Comparison of colony counts after neutron irradiation.

DISCUSSIONS AND FUTURE PLAN:

Neutron irradiation toward B16/BL6 cells treated with 2 at 500 μ M for 2 hours inhibited the colony formation. Though BNCT of 2 in cancer model mice was planned (R4136), it has not yet been performed. Future in vivo studies are expected to be conducted to elucidate the compound potentials.

REFERENCE:

[1] Y. Hattori *et al.*, *J. Med. Chem.*, **55** (2012) 6980-6984.

CO7-27 Observation of Tumor Tissue Destruction with Boron Neutron Capture Reaction

R. Nozaki,¹ Y. Takamura,¹ K. Igawa,² T. Sasaki,²
N. Kondo,⁴ Y. Sakurai,⁴ and H. Kakuta¹

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

² Neutron Therapy Research Center, Okayama University.

³ Institute for Integrated Radiation and Nuclear Science, Kyoto University.

INTRODUCTION: CR-39 is known as a plastic material that can visualize and quantify the boron neutron capture reaction (BNCR) (Ref.1). Alpha rays generated by BNCR on CR-39 form small holes called "etch-pits". These are enlarged by alkali treatment. In this study, we attempted to visualize the BNCR in boron compound-treated cells and tumor tissue sections. In this experiment, BSH (1) and our boron compound 2 were used as boron agents. Compound 2 is an iodine-containing compound that can be used for X-ray CT contrast and was created to directly measure the boron concentration in cancer tissue. Exposure of mouse melanoma cells B16BL6 to 2 at 500 μ M for 2 hours has been found to give a boron concentration of 1.0 μ g ¹⁰B/10⁷ cells.

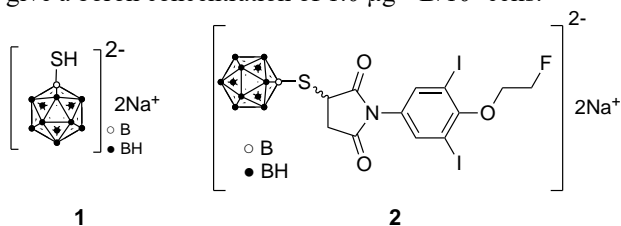


Figure 1. Chemical structures of 1 and 2.

EXPERIMENTS: BNCR detection was performed by seeding cells on CR-39 as follows: cells were cultured in 35 mm petri dishes submerged in 2 cm squares of CR-39, replaced with medium containing the test compound, and exposed to the medium for 2 hours. The CR-39 cells were irradiated with neutrons. Neutron irradiation was performed at KUR at 5 MW for 1 hour (a thermal neutron fluence of 2×10^{12} cm⁻²). The CR-39 was stirred in an alkaline solution (15 wt% KOH, 40 wt% MilliQ, 45% wt% ethanol) at 50°C for 16 min to widen the etch pit and make it quantifiable. Five fields of view of the treated CR-39 were arbitrarily imaged using a microscope, and the resulting images were analyzed using imageJ.

RESULTS: Figure 2A shows a representative example of the obtained image and 2B shows the number of etch pits analyzed using image J. As a result, 2 shows a significantly higher number of etch pits than 1. This relationship corresponds to the intracellular boron concentration. Therefore, 2 was found to generate BNCR in the cell by neutron irradiation.

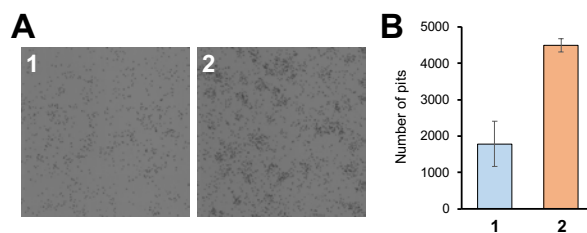


Figure 2. (A) CR-39 images after alkali treatment and (B) comparison of the number of etch pits counted using Image J.

DISCUSSIONS AND FUTURE PLAN: Compound 2, which was found to migrate into B16BL6 cells, showed more pronounced BNCR on CR-39 as etch pits than 1, which was less cell-migrating. The number of etch pits was also successfully quantified by imageJ. We had planned to perform the same experiment with sections of tumor-bearing mice, but we could not prepare the experimental data in time and had to give up the experiment this time. In the future, we hope to observe BNCR on CR-39 using tumor sections of tumor-bearing mice treated with 2.

REFERENCES:

[1] T. Kusumoto *et al.*, Radiat. Res., **191** (2019) 460-465.

CO7-28 Study on Intracellular Protein Destruction by Boron Neutron Capture Reaction

Y. Takamura,¹ R. Nozaki,¹ K. Igawa,² M. Fujihara,¹ N. Kondo,³ Y. Sakurai³ and H. Kakuta¹

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

² Neutron Therapy Research Center, Okayama University.

³ Institute for Integrated Radiation and Nuclear Science, Kyoto University.

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

EXPERIMENTS: The compounds shown in Figure 1A and bexarotene were synthesized by the authors. The RXR ligand binding domain (RXR-LBD) was kindly gifted by Prof. Nakano, University of Shizuoka. RXR-LBD binding assay and reporter gene assay were performed according to references 1 and 2. Neutron beam was irradiated at 1 MW for 60–240 minutes. The irradiated sample (100 μL) was prepared as below; RXR-LBD (10 μM), CBTF-EE-BODIPY (200 μM, converted to ¹⁰B concentration 0.1 ppm) or CBTF-EE-BSH (200, 100, 50 μM, converted to ¹⁰B concentration 24, 12, 6 ppm), bexarotene (20 μM or not), buffer (10 mM HEPES, 150 mM NaCl, 2 mM MgCl₂, 5 mM DTT, 5% DMSO). The irradiated sample was diluted 10 times with above buffer and mixed with BPB-containing buffer in a 4:1 composition, and electrophoresed at 250 V, 20 mA for 70 minutes. The concentration of RXR-LBD was estimated using CBB staining.

RESULTS: We have reported CBTF-EE (**1a**, Figure 1) as an RXR antagonist.^[1] Also, it has been confirmed that CBTF-EE-BODIPY (**1b**, Figure 1), which was designed by inducing a fluorescent group at the end of the alkoxy chain of **1a**, functions as an RXR antagonist.^[2] Compound **1b** has been confirmed to bind to RXR-ligand binding domain (RXR-LBD). In addition, the fluorescence group of **1b** contains a boron atom. Thus, we have interested in RXR-LBD destruction using **1b**. When neutron beam was irradiated to 200 μM of **1b** (0.1 ppm as ¹⁰B)

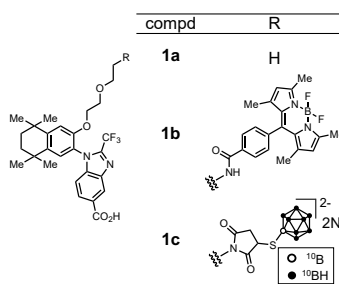


Figure 1. Chemical structures of **1a**–**1c**.

and RXR-LBD, the destruction could not be confirmed (Figure 2A). From the point of which BNCT requires 20 ppm of ¹⁰B in the tumor tissue, we determined that the ¹⁰B concentration was insufficient in **1b**. Thus, we designed and synthesized CBTF-EE-BSH (**1c**, Figure 1), which has ¹⁰B cluster molecule BSH, and evaluated whether **1c** will function as an RXR ligand. As a result of RXR-LBD binding assessment, it has been revealed that a *K_i* value of **1c** was 3.31 μM (Figure 2B). In addition, a reporter assay using COS-1 cells revealed that **1c** inhibits the transcriptional activity of RXR agonist bexarotene in a dose-dependent manner and functions as a non-competitive RXR antagonist (Figure 2C). Then, neutron irradiation was performed on each solution in which **1c** and RXR-LBD coexisted, and in which bexarotene, which is an RXR agonist and inhibits the binding of **1c** to RXR-LBD, was further added. After irradiation, the amount of RXR-LBD was estimated by SDS-PAGE. However, 240 minutes of irradiation at 1 MW did not show the destruction of RXR-LBD (Figure 2D). On the other hand, we next interested in the use of **1c** as a ¹⁰B-carrier for BNCT, so that the ¹⁰B introduction ability of **1c** have being evaluated.

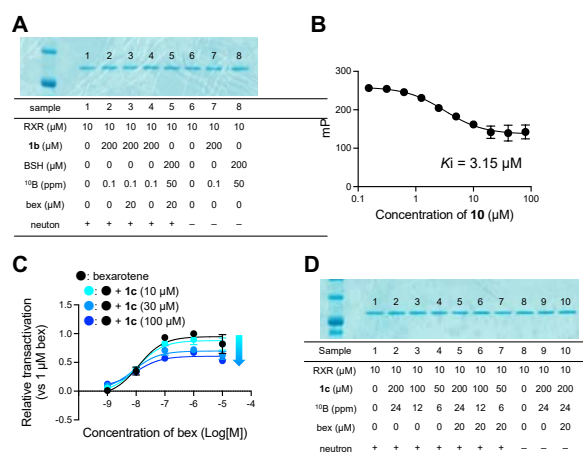


Figure 2. (A) SDS-PAGE of **1b**. (B) Binding ability and (C) antagonistic activity of **1c**. (D) SDS-PAGE of **1c**.

DISCUSSIONS AND FUTURE PLAN: The neutron irradiation to the combination of **1b** or **1c** and RXR-LBD could not induce RXR-LBD degradation. On the other hand, the intracellular introduction and its non-competitive antagonistic activity of **1c** has been confirmed. These results suggest the potential use of **1c** as an intracellular ¹⁰B-carrier. In recent years, various fields have been challenging to produce boron delivery agents which could replace L-boronophenylalanine (BPA). Given that the BNCT targets tumor DNA cleavage, it is of interest to use nuclear receptor ligands as boron delivery agents. The results of this research may provide useful knowledge for the development of ¹⁰B-carriers for BNCT, which bind to the nuclear receptors.

REFERENCES:

- [1] M. Watanabe *et al.*, *J. Med. Chem.*, **64** (2021) 430-439.
- [2] M. Takioku, *ACS Med. Chem. Lett.*, **12** (2021) 1024-1029.

CO7-29 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: In the boron neutron capture therapy with BPA, SLC7A5 (LAT1) protein expression level of tumor cells is an important factor for the anti-tumor efficacy, since LAT1 is a major transporter for intracellular uptake of BPA. We previously examined the SLC7A5 expression profiles of murine squamous cell carcinoma (SCC VII) cells, and reported that its expression can be affected by hypoxia and the changes in SLC7A5 levels is likely linked to the function of Hif-1 α [1]. In the present study, we examined whether the presence of SLC7A5 affected cellular survival after BNCT, using SCC VII SLC7A5-knockout cells.

EXPERIMENTS: We previously established SCC VII cells expressing SLC7A5-6xHis proteins, SCC VII-SH cells. In the present study, SLC7A5 knockout cells (SCC VII-SH- Δ SLC7A5 cells) were generated using CRISPR-Cas9 system.

In order to examine the sensitivity to BPA-BNCT and gamma-ray, these cells were exposed to neutron beams (KUR Heavy Water Facility) and gamma-ray (Co-60 Gamma-ray Irradiation Facility), respectively, and then clonogenic cell survival assays were performed.

RESULTS: In the present study, SLC7A5 deficient cells (SCC VII-SH- Δ SLC7A5 cells) were generated, and the phenotype of these cells were examined. Compared with SCC VII-SH cells, the SLC7A5 deficient cells are characterized by a relatively low proliferation rate, as previously described. Similarly, the colonies of SCC VII-SH- Δ SLC7A5 cells were smaller than those of SCC VII-SH cells.

We next examined the sensitivity of SCC VII-SH- Δ SLC7A5 cells to BPA-BNCT. When exposed to neutron-beams after treatment of BPA, SCC VII-SH- Δ SLC7A5 cells exhibited higher survival rate than SCC VII-SH cells.

We should note that the genetic disruption of SLC7A5 protein may differ from its temporal downregulation by hypoxia. For examples, knock out cells often undergo reprogramming during selection, and several pathways can permanently up- or down-regulated.

We also performed gamma-irradiation experiments for SCC VII-SH- Δ SLC7A5 cells. Surprisingly, the disruption of SLC7A5 increased the survival after gamma-ray irradiation.

One possible explanation for these results is that slow growth phenotype of these cells affected the sensitivity to radiation. It is well-known that slowly proliferating cells were radio-resistant, because the delayed cell cycle provides the cells with enough time to repair DNA damages. Therefore, it is now difficult to conclude whether the

BNCT-resistance of SCC VII-SH- Δ SLC7A5 cells was caused by lower BPA uptake capacity or lower proliferation rate.

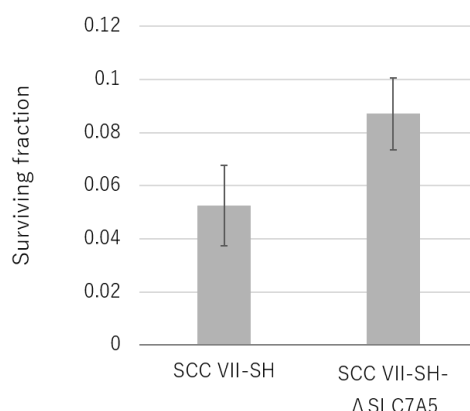


Fig. 1. Cell survival rate for SCC VII-SH or SCC VII-SH- Δ SLC7A5 cells. Cells were exposed to neutron beams (thermal neutrons at a fluence of 2.0×10^{12} n/cm²) after treatment with BPA (20 ppm).

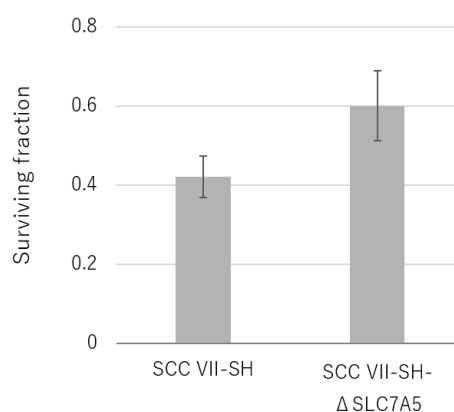


Fig. 2. Cell survival rate for SCC VII-SH or SCC VII-SH- Δ SLC7A5 cells. Cells were exposed to gamma-ray (2.5 Gy).

REFERENCES:

- [1] Y. Sanada *et al.*, *Int. J. Rad. Biol.* **97** (2021) 1441-1449.

CO7-30 Enhancement of Tumour Growth Suppression by Bubble Liposome / Ultrasound Stimulation on Intravenous Injection of ^{10}B SH entrapped PEG Liposome for Boron-Neutron Capture Therapy to Pancreatic Cancer Model *in vivo*

H. Yanagie^{1,2,3}, X. Hou⁴, S. Kageyama⁵, K. Maruyama⁵, T. Takata⁶, T. Watanabe⁶, M. Suzuki⁶, Y. Sakurai⁶, H. Tanaka⁶, M. Nashimoto³, J. Nakajima^{2,7}, M. Ono^{2,8}, T. Sugihara³, and H. Takahashi^{1,2,4}

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 University of Pharmacy & Applied Life Sciences,

⁴ Dept of Bioengineering, School of Engineering, The University of Tokyo,

⁵ Faculty of Pharma-Science, Teikyo University,

⁶ Kyoto University Institute for Integrated Radiation & Nuclear Science,

⁷ Dept. of Pulmo-nary Surgery, The University of Tokyo Hospital,

⁸ Dept. of Cardiac Surgery, The University of Tokyo Hospital, JA-PAN

INTRODUCTION:

For achieving effective Boron neutron capture therapy (BNCT), we performed the experiments of boron delivery systems for BNCT using boronododecaborane ($^{10}\text{B}_{12}\text{H}_{11}\text{SH}$; ^{10}B SH) [1, 2, 3].

Recently, for effective chemotherapy, drug delivery using a combination of ultrasound and microbubbles has been reported [4]. The interaction of microbubbles and ultrasound increases the local permeability of blood vessels, allowing the injected drug to move easily out of the vessel and increasing the drug concentration in the tissue. The combination of liposomal doxorubicin, microbubbles, and ultrasound has been reported to inhibit growth of tumors [5].

In this study, we evaluated the tumor growth suppression by intravenous injection of ^{10}B SH-encapsulated PEG liposomes combined with perfluorocarbon microbubbles / Ultrasound external stimulation system to AsPC-1 human pancreatic tumor-bearing mice.

EXPERIMENTS:

Human pancreatic cancer AsPC-1 cell was used for the *in vivo* anti-tumor effect evaluation. We prepared AsPC-1 (5×10^5) model by transplanting to right lower leg.

^{10}B SH-encapsulated PEG liposomes (mean ^{10}B concentration : 4466ppm) and bubble liposomes were injected via the tail vein, and then ultrasound was applied to the tumors. The construction of Bubble liposome is DSPC : DSPG : DSPE-PEG2000-OMe = 30 : 60 : 10. The Ultrasound condition is below ; Machine : Sonitron2000, Intensity : 2 W/cm², Frequency : 1MHz. We performed thermal neutron irradiation at Institute for Integrated Radiation and Nuclear Science, Kyoto University (average neutron fluence of 2.0×10^{12} n/cm²). The change in tumor growth and survival rate of the mice reflected the anti-tumor effect of ^{10}B SH-encapsulated PEG liposomes.

While measuring the size of tumor, the weight change was also recorded for evaluation of the toxicity of these samples.

RESULTS:

The experimental results showed that tumor growth suppression in the group treated by ^{10}B SH-encapsulated PEG liposomes combined with perfluorocarbon microbubbles / Ultrasound external stimulation system and NCT was 3 times superior compared with non-irradiated group (only treated with ^{10}B SH PEG Lip / Bubble Lip / Ultrasound). No significant weight loss were observed after treatment suggesting low systemic toxicity of this system.

It is thought that the Bubble Liposome / Ultrasound stimulation is effective to increase the concentration of the compounds into the cytoplasm of cancer cells. We hope to apply these techniques from the surface of the body to the tumour site in the body actually using in clinical to BNCT for local advanced cancers [6,7].

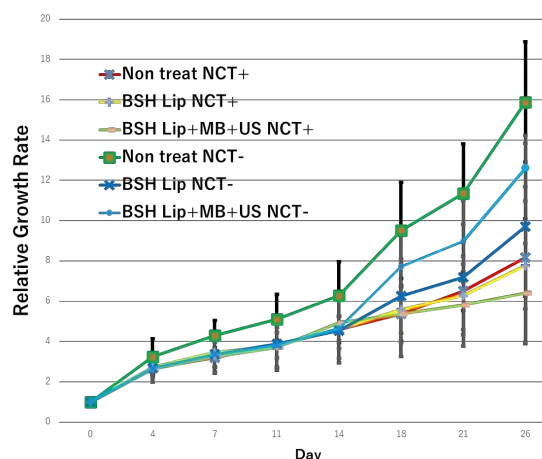


Figure 1. Tumor growth suppression by intravenous injection of ^{10}B SH entrapped PEG Liposome combined with perfluorocarbon microbubbles / Ultrasound external stimulation system with thermal neutron irradiation on AsPC-1 model *in vivo*

REFERENCES:

- [1] H. Yanagie *et al.*, Br J Cancer, **75**(5) (1997) 660-665.
- [2] K. Maruyama *et al.*, J Control Release, **98**(2) (2004) 195-207.
- [3] H. Yanagie *et al.*, In Vivo, **35**(6) (2021) 3125-3135.
- [4] I. Yokoe *et al.*, Drug Deliv., **28**(1) (2021) 530-541.
- [5] I. Yokoe *et al.*, Cancers, **12**(9) (2020) 2423.
- [6] L. Lambrecht *et al.*, Expert Opin Drug Deliv., **13**(2) (2016) 295-310.
- [7] B. Trotošek *et al.*, World J Gastroenterol, **27**(48) (2021) 8216-8226.

CO7-31 The basic research of boron neutron capture therapy for spinal cord gliomas in rat spinal cord glioma models

K. Tsujino¹, S. Kawabata¹, R. Kayama¹, H. Kashiwagi¹, K. Yoshimura¹, Y. Fukuo¹, H. Shiba¹, R. Hiramatsu¹, T. Takata², H. Tanaka², M. Suzuki², S-I, Miyatake³, T. Takami¹ and M. Wanibuchi¹

¹Department of Neurosurgery, Osaka Medical and Pharmaceutical University

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

³Kansai BNCT Medical Center, Osaka Medical and Pharmaceutical University

INTRODUCTION: Boron neutron capture therapy (BNCT) is a particle therapy that can be targeted at the cellular level. So far, BNCT has been shown to be effective for intracranial malignant gliomas [1] and high-grade meningiomas [2], which are invasive cancers. Spinal cord gliomas, due to their infrequent occurrence [3-5], have no established treatment and are mostly mimicked by standard treatment for intracranial malignant gliomas. Surgery is limited to removal of the tumor mass, and the presence of numerous nerve travel routes in the spinal cord parenchyma makes it difficult to treat for spinal cord tumors including the tumor invasion area. As a result, recurrence from residual disease is frequent and the prognosis is poor. There is also a tolerated dose to the spinal cord, which limits radiotherapy to recurrent lesions. In the face of these numerous problems, we focused on BNCT, which can target tumors at the cellular level. In the past, BNCT to the spinal cord has been used to test the safety of BNCT for intracranial malignant gliomas. In the present study, a rat spinal glioma model was used to evaluate the efficacy of BNCT for spinal cord gliomas.

EXPERIMENTS: F98 rat glioma cells were used to create a rat spinal cord glioma model. The F98 rat spinal cord glioma models were implanted 10⁴ F98 rat glioma cells in 3μl Dulbecco's Modified Eagle Medium (DMEM) into the spinal cord at thoracic 9/10 level. Intravenous administration (i.v.) of BPA (12 mg B/b.w.) to the F98 rat spinal glioma model was performed to assess in vivo boron distribution. After creating the models, lower limb function was assessed using the Basso, Bresnahan and Beattie (BBB) scale. The rats were euthanized, when the BBB scale was less than or equal to 5, or severe bladder and rectal obstruction was observed. The effectiveness of BNCT for rat spinal cord glioma models was also tested by neutron irradiation experiments with the following experimental design prepared. The control group was prepared as the sham-operated group (implanted 3μL DMEM).

Study 1 for the sham-operated models

1. Sham-untreated group (n=5)
2. Sham-neutron only group (n=4)
3. Sham-BNCT BPA 2.5 h (n=3)

Study 2 for the F98 rat spinal cord tumor models

1. Untreated group (n=8)
2. Neutron only group (n=6)
3. BNCT BPA 2.5 h (n=8)

Study 3 for the normal rats

BNCT BPA 2.5 h (n=3)

RESULTS: The boron concentration in the spinal cord tumor at 2.5 h after i.v. was sufficient for BNCT, and more than 20 μg B/g. The results of the present in vivo neutron irradiation experiment are currently being analysed and cannot be shown, but the BNCT group with BPA significantly prolonged survival compared to the untreated group. The decrease in BBB scale was also significantly reduced compared to the untreated group. BNCT for normal rats did not show an obvious decrease in BBB scale after neutron irradiation. These findings suggest that BNCT for spinal gliomas may have a potential therapeutic effect, including safety. Most importantly, careful consideration is needed in terms of radiation dose in clinical application.

REFERENCES:

- [1] S. Kawabata *et al.*, *Neurooncol Adv.*, **3**(1) (2021) vdab067.
- [2] S. Kawabata *et al.*, *J Neurosurg.*, **119**(4) (2013) 837-844.
- [3] A. Raco *et al.*, *Neurosurgery*, **56** (2005) 972-981.
- [4] Parsa AT *et al.*, *Clin Neurosurg.*, **52** (2005) 76-84.
- [5] M. C. Chamberlain *et al.*, *Curr Neurol Neurosci Rep.*, **11** (2011) 320-328.

CO7-32 Boron neutron capture therapy using folate receptor targeted novel boron carrier for F98 rat brain tumor models

K. Tsujino¹, S. Kawabata¹, R. Kayama¹, H. Kashiwagi¹, K. Yoshimura¹, Y. Fukuo¹, H. Shiba¹, R. Hiramatsu¹, T. Takata², H. Tanaka², M. Suzuki², S-I. Miyatake³, K. Nishimura⁴, H. Nakamura⁴ and M. Wanibuchi¹

¹Department of Neurosurgery, Osaka Medical and Pharmaceutical University

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

³Kansai BNCT Medical Center, Osaka Medical and Pharmaceutical University

⁴Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology

INTRODUCTION: Boron neutron capture therapy (BNCT) is a particle therapy that can be targeted at the cellular level and is considered an effective treatment for malignant gliomas in which tumor cells have invaded the normal brain parenchyma [1]. Clinical trials of reactor-based BNCT for malignant gliomas have been performed and their efficacy has been reported. BNCT using an accelerator-based neutron generator has also been shown to be effective for the treatment of malignant gliomas [2,3]. Although boronophenylalanine (BPA) is commonly used in BNCT for head and neck cancer, there is an urgent need to develop novel boron carrier to improve therapeutic efficiency of BNCT, and our research group has reported various type novel boron carrier [4,5,6]. We previously reported that Pteroyl-Boron-conjugated 4-iodophenylbutanamide (PBC-IP) is effective in the F98 rat brain tumor models. PBC-IP targeted to the folate receptors which is highly expressed in malignant glioma [7]. Neutron irradiation experiments using PBC-IP administered by Convection enhanced delivery (CED), a local drug delivery system, in the F98 rat brain tumor models showed 50% long-term survival (>90 days) in the irradiation group using PBC-IP only, and even better results when PBC-IP was used in combination with BPA. With these results, we considered PBC-IP to be a promising novel boron carrier and conducted further experimental studies with a view to preclinical trials.

EXPERIMENTS: The boron concentrations in each organ were measured following by PBC-IP CED administration at 1, 3, 6, 12, 24 and 48 h after CED 24 h administration. Neutron irradiation was performed on F98 rat brain tumor models in which PBC-IP was administered by CED, and the therapeutic effect was evaluated by survival time. Neutron irradiation experiments were performed to evaluate the therapeutic effect of different catheter tip positions of the CED in consideration of the nature of the penetrating tissue, and the maintenance of drug effect by BNCT at different time points (3, 6, and 24 h after of CED 24 h administration). In the in vivo neutron irradiation experiment to evaluate the therapeutic effect of different catheter tip positions of CED, four groups were prepared as follows: group 1, untreated; group 2, BNCT PBC-IP CED to the contralateral brain; group 3, BNCT PBC-IP CED to the peritumoral brain; group 4, BNCT

PBC-IP CED to the tumor body (Fig. 2.). On the other hand, in the in vivo neutron irradiation experiment to evaluate the maintenance of drug effect by BNCT at different time points, six groups were prepared as follows: group 1, PBS CED; group 2, PBC-IP CED drug only; group 3, NCT PBS CED; group 4, BNCT PBC-IP CED 24 + 3 h; group 5, BNCT PBC-IP CED 24 + 6 h; group 6, BNCT PBC-IP CED 24 + 24 h.

RESULTS: The boron concentrations in each organ shows in Fig.1. The boron concentration in almost all of the organs decreased gradually. The results of neutron irradiation have not yet been finalized and analyzed. However, in the in vivo neutron irradiation experiment to evaluate the therapeutic effect of different catheter tip positions of the CED, long-time survivors were observed in the group of BNCT PBC-IP CED to the tumor body, and BNCT PBC-IP CED to the peritumoral brain. In addition, in the in vivo neutron irradiation experiment to evaluate the maintenance of drug effect by BNCT, significantly difference was observed between the control group and all the BNCT groups through the log-rank test.

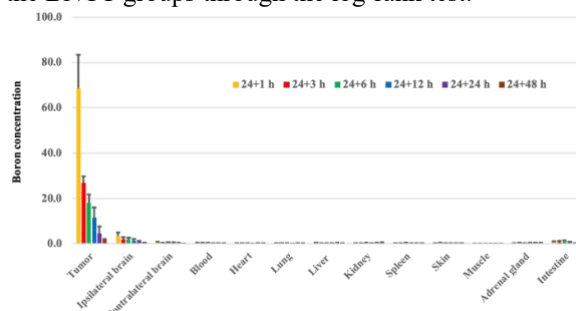


Fig. 1. The boron concentration in each organ was shown at 1, 3, 6, 12, 24 and 48 h after CED 24 h administration.

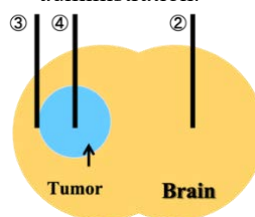


Fig. 2. This figure shows the schema of a different catheter tip positions of CED. ② shows the position of CED at the contralateral brain. ③ shows the position of CED at the peritumoral brain. ④ shows the position of CED at the tumor body.

REFERENCES:

- [1] R.F. Barth *et al.*, Radiation Oncology, **7** (2012)146.
- [2] S. Kawabata *et al.*, Neurooncol Adv., **3**(1)(2021) vdab067.
- [3] S. Arami *et al.*, Cancer Biother Radiopharm. **38**(3) (2023) 201-207.
- [4] T. Kanemitsu *et al.*, Radiat. Environ. Biophys., **58** (2019) 59-67.
- [5] H. Kashiwagi *et al.*, Investig. New Drugs, **10** (2022) 255-264.
- [6] K. Tsujino *et al.*, Biology., **12** (2023) 377.
- [7] F. Nakagawa *et al.*, Cells, **9**(7) (2022) 1615.

CO7-33 Development of a Nanomaterial-based Boron Delivery System for BNCT

G. Choi¹, K. Nishimura², J.-H. Choy¹, Y. Sakurai³, H. Nakamura^{2,4}, M. Suzuki³

¹Institute of Tissue Regeneration Engineering, Dankook University

²School of Life Science and Technology, Tokyo Institute of Technology

³Institute for Integrated Radiation and Nuclear Science, Kyoto University

⁴Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is attracting attention as a non-invasive radiotherapy in the treatment of cancer. In order to achieve efficient BNCT effect, the boron concentration in tumor tissue needs to be more than 20 $\mu\text{gB/g}$. Moreover, tumor/normal tissues ratio of boron concentration should be more than three to decrease undesirable side effects. In March 2020, accelerator-based BNCT for head and neck cancer using 4-borono-L-phenylalanine (L-BPA) was approved by the Pharmaceuticals and Medical Devices Agency in Japan, making BNCT more accessible treatment [1]. L-BPA is known to actively accumulate into tumor cells through L-type amino acid transporter 1 (LAT-1). On the other hand, mercaptoundecahydrodecaborate (BSH) is a boron cluster containing 12 boron atoms although BSH does not show efficient tumor-selective pharmacokinetics. Therefore, the development of tumor-selective BSH delivery system is required for further expansion of BNCT, especially for the treatment of BPA-negative tumors.

We recently applied layered double hydroxide (LDH) to develop a nanohybrid of BSH and LDH (BSH-LDH) as a boron delivery carrier [2]. The tumor-to-blood ratio of BSH in the BSH-LDH-treated-group is found to be 4.4-fold higher than that in the intact BSH, demonstrating BSH-LDH is a promising integrative therapeutic platform for BNCT.

In this study, we estimated BNCT effect of BSH-LDH in glioblastoma mouse models.

EXPERIMENTS: BSH-LDH 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 U87MG human glioblastoma cells in PBS. The mice were kept on a regular chow diet and water for a week. The tumor-bearing mice were injected *i.v.* with 200 μL of BSH-LDH ($n = 6$), BSH ($n = 6$), BPA ($n = 4$) and PBS ($n = 4$) (5.7 mg/kg and 30 mg/kg for BSH and BSH-LDH, respectively). At 2 h after injection, the tumors of mice were irradiated with thermal neutrons in the nuclear reactor for 15 min. The tumor volume and body weight of the mice were measured at 0, 4, 8, and 12 days after neutron irradiation.

RESULTS: The BNCT effect was evaluated by measuring tumor volume and body weight after neutron irradiation.

The tumor volume slightly increased in all the groups 12 days after irradiation (Fig. 1A). There was no significant difference in all the groups, demonstrating that the tumor did not grow appropriately. The body weight of the mice did not change significantly in all the groups 12 days after irradiation (Fig. 1B). Therefore, BSH-LDH nanohybrid did not have critical toxicity.

These results indicate that BSH-LDH can be a promising boron carrier with lower toxicity even in high dose of administration. Further BNCT experiment is necessary to evaluate anti-cancer effect of BSH-LDH compared with BSH and BPA. For this purpose, the U87MG mice models should be constructed properly by optimizing the tumor bearing conditions such as the number of the injected cells.

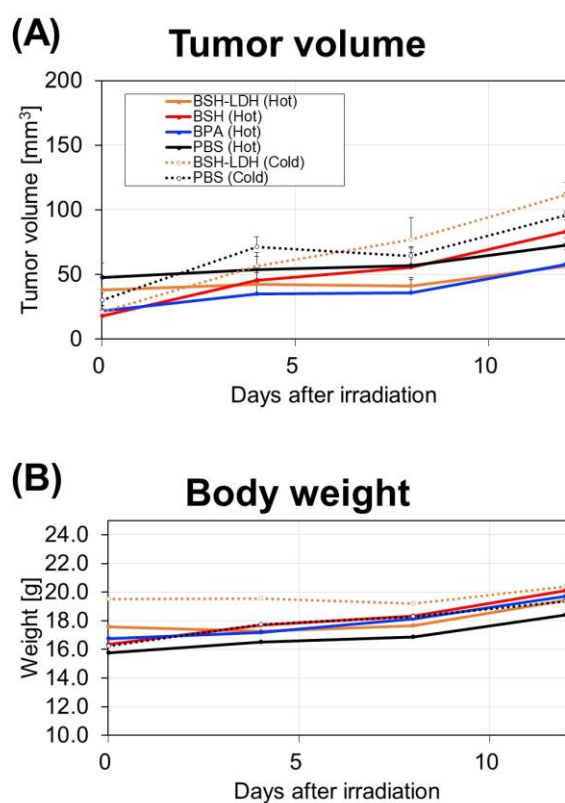


Fig. 1. (A) Tumor volume in mice after BNCT with BSH-LDH ($n = 6$), BSH ($n = 6$), BPA ($n = 4$) and PBS ($n = 4$) (5.7 mg/kg and 30 mg/kg for BSH and BSH-LDH, respectively). The tumors were irradiated with thermal neutron for 15 min at 2 h after administration. Hot and cold mean with and without irradiation of thermal neutron, respectively. (B) Body weight changes of mice after BNCT. The data are shown as mean \pm sem.

REFERENCES:

- [1] H. Kanno *et al.*, *The Oncologist*, **26** (2021) e1250-e1255.
- [2] G. Choi *et al.*, *Adv. Func. Mater.*, **28** (2018) 1704470.

CO7-34 Gadolinium-containing nanoparticles grafted with polyglycerol for magnetic resonance imaging and gadolinium neutron capture therapy of cancer

L. Zhao¹, H.G. Kang², W. Huang², J. Yu², Y. Yoshino³, F. Yoshino⁴, M. Suzuki⁵ and N. Komatsu²

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 Department of Radiology, Kyoto Prefectural University of Medicine, Japan

⁴ Department of Obstetrics and Gynecology, Shiga University of Medical Science, Japan

⁵ Institute for Integrated Radiation and Nuclear Science, Kyoto University, Japan

Cancer is one of leading causes of death worldwide, accounting for nearly 10 million deaths in 2020. Gadolinium neutron capture therapy (GdNCT) is an alternative to BNCT using Gd-containing agents as a sensitizer, killing cancer cells with Auger electrons of high linear energy transfer (LET) as well as low-LET γ photons.^[1] The Gd-containing agents can also serve as an efficient tracer for magnetic resonance imaging (MRI), enabling MRI-guided GdNCT of cancer (Figure 1)^[2]. This can not only help optimize the GdNCT plan, but also enhance the accuracy and efficacy of GdNCT. In this context, developing efficient Gd-containing agents is crucial. We have recently developed Gd-containing inorganic nanoparticles grafted with poly(glycerol) (GdNP-PG), showing excellent dispersibility (> 10 mg Gd/mL in saline) and colloidal stability in physiological media. Upon intravenous administration, GdNP-PG nanoparticles accumulated in subcutaneous CT26 mouse colon tumor through enhanced permeability and retention (EPR) effect, reaching a high Gd concentration of about 150 $\mu\text{g/g}$ in the tumor at 24 h postinjection.

Preliminary MRI measurements of the mice bearing CT26 tumor were carried out on a Bruker Biospec 47/40[®] system before and after intravenous injection with saline dispersion of GdNP-PG. A brightened T_1 -weighted image (T_1 WI) of the tumor was observed at 1 h postinjection as compared to that before injection, due to the T_1 -shortening effect of GdNP-PG. The T_1 WI of the tumor became brighter at 24 h postinjection, suggesting that GdNP-PG gradually penetrated into the tumor tissue with time.

Encouraged by the MRI results, we conducted preliminary GdNCT of cancer mediated by GdNP-PG. After intravenous injection with the saline dispersion of GdNP-PG for 24 h, the mice bearing CT26 tumor were irradiated with thermal neutrons at a reactor power of 5

MW for 12 min. The results show that GdNP-PG-mediated GdNCT significantly suppressed the growth of CT26 tumor as compared to the control groups. In particular, two out of the three mice subjected to the GdNCT displayed a shrinking tumor size after the neutron irradiation. In addition, no obvious difference was observed in body weight between the GdNCT and the control groups, suggesting the low in vivo toxicity of GdNP-PG.

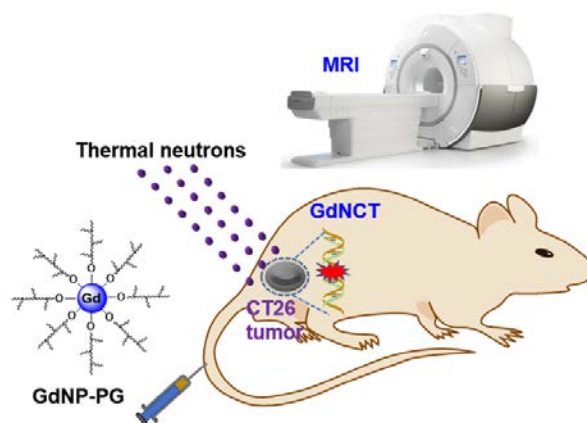


Figure 1. MRI-guided GdNCT using GdNP-PG as a theranostic agent.

To sum up, preliminary MRI and GdNCT experiments collectively indicate that GdNP-PG should be a promising theranostic agent for cancer treatment. However, more detailed MRI measurements are required to monitor the GdNP-PG's accumulation in the tumor tissue, according to which the neutron irradiation timing for GdNCT can be adjusted to achieve better efficacy. In addition, the therapeutic mechanism of GdNP-PG-mediated GdNCT will also be explored in the future.

Reference:

- [1] D. Duan *et al.*, *CCS Chemistry*, (2023) (doi) 10.31635/ccschem.023.202202488.
- [2] P. Mi *et al.*, *ACS Nano*, **9** (6) (2015) 5913-5921.

CO7-35 Development of theranostic agents for boron neutron capture therapy and its companion diagnostics

K. Ogawa¹, S. Imai and M. Suzuki²

Graduate School of Medical Sciences, Kanazawa University

¹Institute for Frontier Science Initiative, Kanazawa University

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION:

Boron neutron capture therapy (BNCT) using ¹⁰B-labeled agents with companion diagnostics is promising for cancer theranostics. In current clinical practice, companion diagnostics using positron emission tomography (PET) with 4-borono-2-[¹⁸F]fluoro-L-phenyl alanine ([¹⁸F]FBPA) can be performed before BNCT with 4-[¹⁰B]borono-L-phenylalanine ([¹⁰B]BPA) [1]. If BNCT and companion PET imaging can be performed with compounds of the same structure, it will be possible to predict the therapeutic and side effects of compounds for BNCT with higher accuracy. For the establishment of a new BNCT system, we synthesized and evaluated a probe [⁶⁷Ga]1 containing *closo*-dodecaborate ([B₁₂H₁₂]²⁻) as a boron cluster, a [⁶⁷Ga]Ga-DOTA derivative for imaging, and an RGD (arginine-glycine-aspartic acid) peptide for tumor-targeting. Recently, we demonstrated that [⁶⁷Ga]1 highly accumulated in U-87MG tumors with high expression of $\alpha_v\beta_3$ integrin. In this study, we evaluated the therapeutic effect of [¹⁰B]1 with neutron irradiation using U-87MG cells.

EXPERIMENTS:

WST assay

U-87MG human glioma cells were cultured in EMEM medium containing 10% FBS and 1% penicillin-streptomycin at 37°C under 5% CO₂. [¹⁰B]BPA (192 μ M, ¹⁰B 40 ppm) and [¹⁰B]1 (16 μ M, ¹⁰B 192 ppm) were prepared in FBS-free EMEM medium. In a microtube, U-87MG cells (1 \times 10⁵ cells) were suspended in 1 mL of the prepared medium and shaken at room temperature at 550 rpm for 1 h. After removing the drug-containing medium, the cells were seeded on 96-well plates at 1 \times 10⁴ cells/well in FBS-free EMEM medium. Neutron irradiation (1 MW) was performed for 15 or 30 min. The irradiated cells were incubated at 37°C under 5% CO₂ for 24 h. After adding WST-8 and incubating for 90 min at 37°C under 5% CO₂, the absorbance at 450 nm was measured using a plate reader.

Colony formation assay

U-87MG cells were seeded on a 96-well plate at 1 \times 10⁴ cells/well in EMEM medium and pre-incubated at 37°C under 5% CO₂ for 24 h. FBS-free EMEM medium containing [¹⁰B]BPA (192 μ M, ¹⁰B 40 ppm) and [¹⁰B]1 (1 μ M, ¹⁰B 12 ppm) were added and incubated for 3 h. Neutron irradiation (1 MW) was performed for 15 or 30 min. The drug-containing medium was removed, and the cells

were harvested. The collected cells were seeded on a 6-well plate and incubated at 37°C under 5% CO₂ for 6 days. After staining and fixing with 0.1% crystal violet in 70% EtOH (1 mL/well), the cells were dissolved in 30% acetic acid (1 mL/well), and the absorbance at 570 nm was measured using a plate reader.

RESULTS:

In WST assay and colony formation assay using U-87MG cells, [¹⁰B]1 showed significantly lower cell viability than control group when irradiated for 15 and 30 min. In the colony formation assay, [¹⁰B]1 showed a higher therapeutic effect than [¹⁰B]BPA. These results suggest that [¹⁰B]1 would be useful as a drug for BNCT.

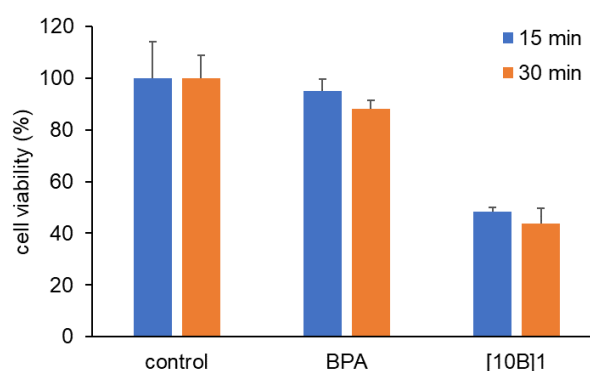


Fig. 1. BNCT effects on cell viability after thermal neutron irradiation by WST assay

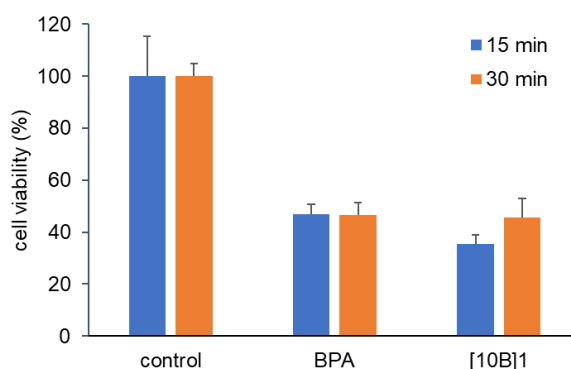


Fig. 2. BNCT effects on cell viability after thermal neutron irradiation by colony formation assay

REFERENCES:

- [1] N. Fukumitsu and Y. Matsumoto, *Cells*, **10**(8) (2021) 2135.

CO7-36 Development of Novel Small-molecule Boron Neutron Capture Therapy Drugs Targeting Tumor-specific Enzymatic Activity

J. Tsunetomi¹, M. Suzuki², M. Kamiya³, Y. Urano^{1,4}

¹ Graduate School of Pharmaceutical Sciences, The University of Tokyo

² Institute for Integrated Radiation and Nuclear Science, Kyoto University

³ Departments of Life Science and Technology, Tokyo Institute of Technology

⁴ 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 overexpressed 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.

We have developed a library of fluorescence probes that can detect various enzymatic activities, and by applying these probes to living cells and clinical specimens, we have established a technique that enables comprehensive evaluation of tumor cell-specific enzymatic activities[1]. By utilizing the enzymatic activities found by the above mentioned screening as a biomarker of certain cancers, we considered that it would be possible to develop novel BNCT drugs that accumulate in tumor cells with high selectivity.

We focused on an aminopeptidase, dipeptidyl peptidase 4 (DPP-4), which is specifically highly expressed in esophageal cancer sites[2]. We designed and synthesized a novel small-molecule carborane-containing drug candidate targeting DPP-4 activity, EP-4OCB-FMA. 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.

EXPERIMENTS and RESULTS:

Cellular uptake assay

EP-4OCB-FMA was administered to live cells at a concentration of 10 μ M and the intracellular boron concentration was measured by MP-AES after 3 hours of incubation. The results showed that the drug retained in DPP-4 highly expressing cells selectively and that sufficient intracellular boron concentration could be achieved.

Neutron capture therapy for H226 / Caco-2 cells

Based on the above results, we next performed BNCT on cultured cells to evaluate the therapeutic effect of EP-4OCB-FMA. After administering EP-4OCB-FMA (10

or 20 μ M), which was not ¹⁰B-enriched, with or without sitagliptin (DPP-4 inhibitor), or ¹⁰BPA-fructose complex (3 mM and 7.7 mM each) to live cells, the cells were detached with trypsin after 2.5 or 3 hours-incubation. After thermal neutron irradiation, cells were seeded on 100 mm dish and cell viability was evaluated by colony formation assay after 2 weeks incubation. As a result, a high cell killing effect was observed in DPP-4 highly expressing cells, such as H226 cells and Caco-2 cells. This effect was cancelled by co-incubation of DPP-4 inhibitor sitagliptin, indicating that EP-4OCB-FMA has a selective effect depending on DPP-4 activity of cells. Furthermore, in Caco-2 cells, which express high levels of LAT1 as well as DPP-4, EP-4OCB-FMA showed a superior cell-killing effect compared to ¹⁰BPA-fructose complex.

Biodistribution

Tumor-bearing BALB/c mice (female, 8-9 weeks old) were prepared by injecting subcutaneously (s.c.) a suspension of H226 cells (1.0×10^6 cells/ 100 μ L/ mouse, 50 % Matrigel in PBS). The mice were injected intratumorally with EP-4OCB-FMA (0.1 mg/mouse, \sim 5 mg/kg), which was not ¹⁰B-enriched, dissolved in saline containing < 5 % DMSO. The mice were euthanized 1, 6, or 24 hours after injection, and blood was obtained from heart, followed by collection of tumors and organs. The samples were put into Teflon tubes. The amount of boron in the samples was quantified by prompt gamma-ray analysis (PGA). The results suggested that high concentrations of boron remained in the tumor even after 24 hours post administration.

Neutron capture therapy to H226 tumor mouse models

Tumor-bearing BALB/c mice (female, 6-7 weeks old) were prepared by injecting subcutaneously (s.c.) a suspension of H226 cells (1.5×10^6 cells/ 100 μ L/ mouse, 50 % Matrigel in PBS). EP-4OCB-FMA (0, 20 or 40 mg/kg), which was not ¹⁰B-enriched, was intratumorally injected, with or without sitagliptin (40 mg/kg) in saline containing < 20 % DMSO. The mice were placed in acrylic holders, which were secured on a 5-mm-thick thermoplastic plate that contained 40 weight % (wt %) of ⁶LiF (96% ⁶Li) to block thermal neutrons and had a circular hole in the center. The thigh containing the tumor was stretched over the hole, and the tumor was irradiated with epi-/thermal neutrons for 15 min (fluence: 4.7×10^{11} to 2.6×10^{12} neutrons/ cm²) 2.5 hours after injection. The results showed that tumor growth was suppressed in a drug-, enzyme-, and neutron-irradiation-dependent manner. These results indicate that EP-4OCB-FMA is a useful BNCT drug that targets tumor cell-selective enzymatic activity with an intracellular retention ability.

REFERENCES:

- [1] Y. Kuriki *et al.*, Chem.Sci., **13**(2022) 4474-4481.
- [2] H. Onoyama *et al.*, Sci Rep., **6** (2016) 26399.

CO7-37 Quantitative Evaluation Method of Elemental Contents Related to Activation in Radiation Shielding Concrete

T. Takata, K. Kimura¹, Y. Sakurai, H. Tanaka, and K. Takamiya

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

INTRODUCTION: Concrete is a very useful material for all aspects of construction, including infrastructure, office buildings, and facilities. In addition, concrete is widely used as radiation shield in nuclear reactors and irradiation facilities because of its flexibility, sufficient supply and low cost. On the other hand, once these facilities start operating, the concrete for shielding is affected by the radiation and becomes radioactive in facilities where neutrons are generated. For the above situation, low activation concrete is the one of the ways to solve the problem [1-3]. Especially, Boron Neutron Capture Therapy (BNCT) should be effective facilities to apply the low activation concrete.

To estimate the level of activation in shielding concrete, we have performed neutron activation analyses (NAA) on more than several hundred samples of shielding concrete and raw materials using KUR facilities. The results of the NAA are reported in detail in Ref. 4. We describe a summary of the method including sample preparation, irradiation, and post-irradiation process for gamma-ray spectroscopy.

MATERIALS ANDS METHODS: Three nuclides produced by reactions of $^{151}\text{Eu}(n,\gamma)^{152}\text{Eu}$, $^{59}\text{Co}(n,\gamma)^{60}\text{Co}$ and $^{133}\text{Cs}(n,\gamma)^{134}\text{Cs}$ in concrete and raw materials were subject to quantitative evaluation. Since FY2018 we have been exploring various experimental conditions; since FY2019 we have been conducting a series of analyses under almost constant conditions. The procedure is described below.

Samples were prepared as powder or small grain by Fujita Corporation. Each sample of about 0.7 to 1 g was sealed in double plastic bags to prevent radioactive contamination. Commercially available Rock reference materials and an in-house reference powder sample with known concentrations of Eu, Co and Cs were prepared in the same way as internal standards. Approximately 7 to 10 samples, including the two internal standards, were encapsulated in a polyethylene irradiation capsule.

Using Pn-2 system, each capsule was irradiated for 60 minutes, 8 capsules per session, 2 or 3 sessions per year for a total of 16 to 24 capsules. The irradiated capsules were opened sequentially for measurement after about two months or more of radioactive cooling. The samples were individually sealed in additional outer plastic bags to prevent radioactive contamination.

The gamma-ray spectrum of each sample was measured one by one with an HP-Ge detector with automatic measurement system equipped with sample changer. In the quantitative analysis of nuclide concentrations, we focused on the peaks at 1408 keV for ^{152}Eu , 1333 keV for

^{60}Co , and 795 keV for ^{134}Cs , respectively. The target uncertainty of the peak quantification analysis was set to less than 10%. Measurements of 18,000 to 20,000 s were performed for all samples, and longer measurements were performed for samples with large uncertainties.

RESULTS: The content of each nuclide was calculated by comparison with the measured values of internal standards. Figure 1 shows the frequency distribution of elemental concentrations for 568 samples measured in FY2019-2021. For ^{60}Co , the target uncertainty was achieved for almost all samples by measuring for about 20,000 seconds. For ^{152}Eu and ^{134}Cs , there were samples with low content, for which measurements were performed for up to about 160,000 seconds. The mean radioactivity immediately after irradiation, estimated from the content of nuclides, was 1.7 kBq/g for ^{152}Eu , 0.9 kBq/g for ^{60}Co , and 0.45 kBq/g for ^{134}Cs , respectively.

In FY2022, 236 new samples were irradiated. We will conduct a detailed analysis of the data acquired to date and continue to accumulate data to build a materials database.

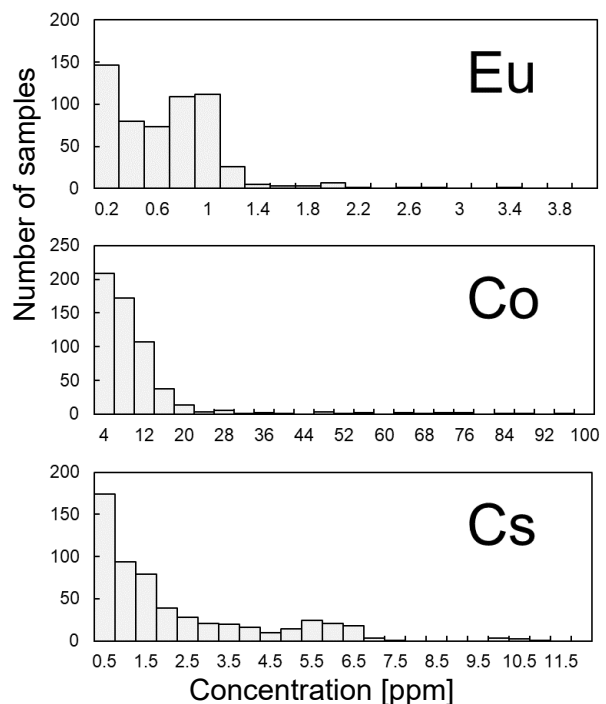


Fig. 1. Frequency distributions of elemental contents for samples analyzed in FY2019-2021.

REFERENCES:

- [1] K. Kimura *et al.*, Proc. of 8th Int. Conf. on Radiation Shielding, ANS inc, Arlington, USA.,1(1994) 5-42.
- [2] M. Kinno *et al.*, ANS Radiation Protection & Shielding Conference, Spokane, USA, (2022) 673-678.
- [3] K. Kimura *et al.*, Proc. of Int. Conf. on Nuclear Engineering (ICONE16), Florida, USA, (2008) 4848.
- [4] K. Kimura *et al.*, KURNS Progress Report 2018-2021.

CO7-38 Synthesis and evaluation of a novel boron neutron capture therapy agent

H. Kimura¹ and M. Suzuki²

¹ Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University

²Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION:

Neuroendocrine Neoplasms (NEN) is a general term for tumors that arise from cells of the neuroendocrine system and have developed an abnormal growth. Because the neuroendocrine system is distributed throughout the body, neuroendocrine tumors can occur anywhere in the systemic organs. The incidence is particularly high in the pancreas, gastrointestinal tract, and lungs. It is a relatively rare disease with an incidence of approximately 5 per 100,000 people, and has been considered a "rare cancer." In recent years, however, its incidence has been increasing due to improvements in diagnostic techniques and other factors clinically, NEN is classified into functional NEN, which presents with specific symptoms depending on the hormone-producing capacity of the tumor, and nonfunctional NEN, which does not present with hormone-specific symptoms.¹⁾

Pathologically, pancreatic NETs were classified into three categories in the 2010 WHO classification (digestive organs) based on the Ki-67 index and fission images: Grade 1 and 2 NETs with low mitotic activity were classified as Neuroendocrine Tumor (NET) and Grade 3 NETs with high mitotic activity as Neuroendocrine Carcinoma (NEC). For pancreatic NETs, the 2017 WHO Classification of Endocrine Organs subdivided the NEC part and incorporated a new definition, NET-G3, which classifies the disease into four categories: NET-G1, G2, G3, and NEC-G3. Furthermore, the WHO Classification (Gastrointestinal) was revised in 2019 and the definition of pancreatic NET G3 was incorporated directly in the gastrointestinal tract, unifying the same grading system for both pancreatic and gastrointestinal tract.

NETs are 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.^{2) - 4)} 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:

Cell culture: AR42J cells, a cell type that highly expresses SSTR2, were cultured. The cells were cultured as in Ham's F-12K medium supplemented with 20% fetal bovine serum, glutamine, and antibiotics (penicillin/streptomycin) in a humidified CO₂ incubator (37°C/5% CO₂).

Mouse Tumor-Bearing Models: Cell suspensions prepared by suspending AR42J cells in D-PBS (-) were injected subcutaneously into the legs of male BALB/c Slc-nu/nu mice (5 weeks old) at 100 μ L (5.0 \times 10⁶ cells) per animal under isoflurane anesthesia.

Neutron irradiation: A newly developed boron drug was administered by tail vein and subcutaneously. Two hours after drug administration, tumor-bearing mice were neutron irradiated at 5 MW for 15 minutes.

RESULTS:

Tumor volume increased over time in mice that were exposed to neither drugs nor neutron radiation. On the other hand, the tumor volume of mice irradiated with both the drug and neutrons showed no increase in tumor volume up to 10 days after neutron irradiation. No significant differences were observed between subcutaneous and tail vein administration. In the future, we plan to conduct detailed studies including reproducibility, such as examining the time from drug administration to neutron irradiation and the amount of drug administered.

REFERENCES:

- [1] T. Masui *et al.*, BMC Cancer, **20** (2020) 11-04.
- [2] O. Keskin *et al.*, Onco Targets Ther., **6** (2013) 471-483.
- [3] T. Gunther *et al.*, Pharmacol Rev. **70** (2018) 763-835.
- [4] E. Pauwels *et al.*, Am J Nucl Med Mol Imaging., **8**(5) (2018) 311-331.

CO7-39 Investigating Gd-EDTMP as a Neutron Capture Therapy Agent for Mammary Tumor Bone Metastasis in Mouse Models

T. Matsukawa¹, M. Suzuki², A. Kubota¹, A. Shinohara^{1,3}, K. Yokoyama^{1,4}

¹ Juntendo University Faculty of Medicine

² Institute for Integrated Radiation and Nuclear Science, Kyoto University

³ Research Institute for Cultural Studies, Seisen University

⁴ International University of Health and Welfare

INTRODUCTION: In pursuit of advancing cancer radiation therapy, this study evaluated the tissue distribution and effects of Gd tetra (methylene phosphonic acid) chelate (Gd-EDTMP) as a potential neutron capture therapy agent. Prior experiments using Gd-EDTMP in young mice indicated a high dose distribution in femur regions, particularly in bones with an epiphyseal line. To further investigate the basis for neutron capture therapy, we developed a mouse model of carcinogenesis in elderly mice with closed epiphyseal lines. After neutron irradiation, the distribution of ¹⁵⁷Gd in and around the bone was analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

EXPERIMENTS: Gd-EDTMP solution was prepared from gadolinium chloride and EDTMP. Female BALB/cAJcl mice (12 weeks old) were acclimated for one week and then transplanted with the Luciferase expressing 4T1 mouse mammary tumor cell line JCRB1447 into their right tibia. After another week of acclimation and tumor formation confirmation, mice were divided into three groups (n=3 or 4): Gd-EDTMP treated and neutron irradiated (Gd+/Nu+), Gd-EDTMP treated and not neutron irradiated (Gd+/Nu-), and phosphate buffered saline treated and neutron irradiated (Gd-/Nu+). Gd+/Nu+ and Gd-/Nu- mice received a single intraperitoneal dose of 20 mg/kg body weight Gd-EDTMP (1 mg Gd/ml PBS) (n=3). Gd-/Nu+ mice (n=4) received an equivalent dose of PBS. Twenty four hours after Gd-EDTMP or PBS administration, Gd+/Nu+ and Gd-/Nu+ mice's lower limbs were irradiated with thermal neutrons for 15 minutes at the Kyoto University Research Reactor (KUR, 5MW), with an irradiation fluence of $3.6 \times 10^{12} \text{ cm}^{-2}$. Following irradiation, mice were monitored for 14 days with free access to food and water. After euthanasia, right thighs and shins were harvested, weighed for tumor weight, and prepared as non-decalcified frozen sections using the Kawamoto method. The distribution of ¹⁵⁷Gd was imaged by LA-ICP-MS (LA: NWR213, ICP-MS: Agilent 8800) according to previously established methods [1]. The tumor status was further assessed using micrographs of Hematoxylin Eosin (H.E.) stained sections of the lower limbs.

RESULTS and DISCUSSION: The ICP-MS analysis revealed that administering a 20 mg/kg intraperitoneal dose of Gd-EDTMP led to a concentration of 1000-2000 ppm of Gd in the bone after 24 hours. In a mouse model of bone metastasis originating from a 4T1 mammary tumor, a tumor shrinking effect was observed following neutron irradiation, although it was not statistically significant [2]. This finding suggests potential for future therapeutic applications. LA-ICP-MS imaging results showed that Gd accumulated in the bone. Interestingly, by muting the Gd signal in the bone distribution area, the distribution of Gd concentration outside the bone became observable. This image processing revealed that Gd was also present in the tumor cells themselves, which may contribute to the tumor shrinking effect.

To achieve a tumor suppressive effect suitable for therapy, it is necessary to further enrich Gd in the tumor area. Analyzing the accumulation mechanism of Gd in the 4T1 cells themselves, as observed in this study, is essential. Additionally, exploring more effective methods of administration could lead to improve therapeutic outcomes.

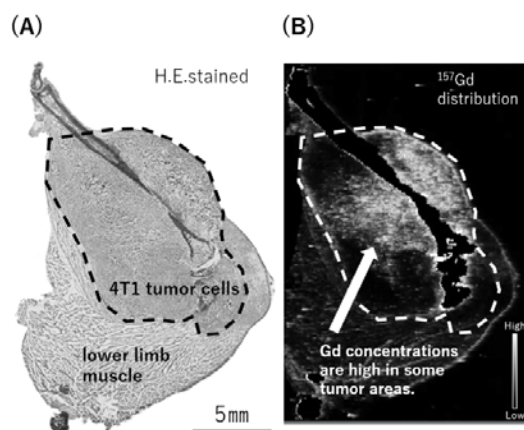


Fig.1 A leg section of a mouse with a 4T1 mammary tumor cell transplant after administering Gd tetra (methylene phosphonic acid) chelate (Gd-EDTMP) (20 mg/kg) for 14 days. Figure A shows the Hematoxylin and Eosin (H&E) stained image, and Figure B displays the ¹⁵⁷Gd distribution image obtained through Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS), excluding the bone area. The area within the dotted line contains a high concentration of tumor cells. In Figure B, a concentration of Gd can be observed surrounding the 4T1 tumor cells that are in contact with the bone.

REFERENCES:

- [1] A. Kubota *et al.*, Juntendo Medical Journal., **65** (2019) 461-467.
- [2] T. Matsukawa *et al.*, 8th International symposium of Metallomics, Kanazawa, 7/11-14 (2022).

CO7-40 Tumour Growth Suppression by Intra-Tumoural Injection of Gadolinium-Polyplex with Bubble Liposome / Ultrasound Stimulation for Gadolinium-Neutron Capture Therapy to Pancreatic Cancer Model *inVivo*

H. Yanagie^{1,2,3}, X. Hou⁴, S. Kageyama⁵, K. Maruyama⁵, T. Takata⁶, M. Suzuki⁶, T. Watanabe⁶, Y. Sakurai⁶, H. Tanaka⁶, M. Nashimoto³, J. Nakajima^{2,7}, M. Ono^{2,8}, T. Sugihara³, and H. Takahashi^{1,2,4}

¹Institute of Engineering Innovation, School of Engineering, The University of Tokyo,

²Cooperative Unit of Medicine & Engineering, The University of Tokyo Hos-pital,

³Niigata University of Pharmacy & Applied Life Sciences,

⁴Dept of Bioengineering, School of Engineering, The University of Tokyo,

⁵Faculty of Pharma-Science, Teikyo University,

⁶Kyoto University Institute for Integrated Radiation & Nuclear Science,

⁷Dept. of Pulmo-nary Surgery, The University of Tokyo Hospital,

⁸Dept. of Cardiac Surgery, The University of Tokyo Hospital, JA-PAN

INTRODUCTION:

The cytotoxic effect by 1µm-range high LET Auger electron, and long-range gamma rays was estimated on Gadolinium-neutron capture therapy(GdNCT) [1, 2, 3]. For effective GdNCT, it is necessary to accumulate Gadolinium atoms into the tumor tissues selectively. We performed the experiments of Gadolinium delivery systems for GdNCT using meglumine gadoterate (Magnescope; C₁₆H₂₅GdN₄O₈ - C₇H₁₇NO₅; MW:753.86). The Magnescope is difficult to be kept in the cytoplasm and nucleus in the cancer cells, so we need to develop some functional delivery systems.

Recently, for effective gene transfectins, drug delivery using a combination of ultrasound and microbubbles has been reported [4, 5]. The interaction of microbubbles and US increases the local permeability of cancer cell membrane, allowing the injected plasmid DNA to move easily into the cells and increasing the concentration of plasmid DNA in the cancer cells.

In this study, we evaluated Magnescope / hyaluronic acid / protamine-mixed with cationic liposome (¹⁵⁷Gd-plex) as neutron capture therapy agent by *in vivo* experiment on AsPC-1 human pancreatic tumor-bearing mice. We also use perfluorocarbon microbubbles / Ultrasound external stimulation system combined cationic liposome as a method for selective delivery

of ¹⁵⁷Gd compound into the cells.

EXPERIMENTS:

¹⁵⁷Gd-plex were prepared mixed with 1.2mL of Magnescope (0.38 mg/mL), 0.24mL of a solution of 10mg/mL hyaluronic acid sodium, and 0.12mL of 10mg/mL of protamine incubating at room temperature for 30min, then, these mixing solutions were poured into 0.24mL of cationic Liposome; Lipofectamine3000. Human pancreatic cancer AsPC-1 cell was used for the *in vivo* anti-tumor

effect evaluation. We prepared AsPC-1(5x10⁵) model by transplanting to right lower leg.

Ultrasound stimulation was performed after intra-tumoral injection of 0.1mL of ¹⁵⁷Gd-plex and 0.04mL of bubble liposomes, then, we performed thermal neutron irradiation at Institute for Integrated Radiation and Nuclear Science, Kyoto University (average neutron fluence of 3.0 × 10¹² n/cm²). The change in tumor growth and survival rate of the mice reflected the anti-tumor effect of ¹⁵⁷Gd-plex. While measuring the size of tumor, the weight change was also recorded for evaluation of the toxicity of these samples.

RESULTS:

The experimental results showed that tumor growth suppression in the treated group of ¹⁵⁷Gd-plex / Bubble Liposome / Ultrasound stimulation on NCT was 2 times superior compared with non-irradiated group. No significant weight loss were observed after treatment.

We attempted to enhance of retention of ¹⁵⁷Gd atoms by mixing ¹⁵⁷Gd-plex. It is thought that the Bubble Liposome / Ultrasound stimulation is effective to increase the ratio of transfection of the genes and compounds into the cytoplasm of cancer cells. We hope to apply these techniques including gene therapy and electrochemotherapy acutually using in clinical to GdNCT for local advanced cancers [6,7].

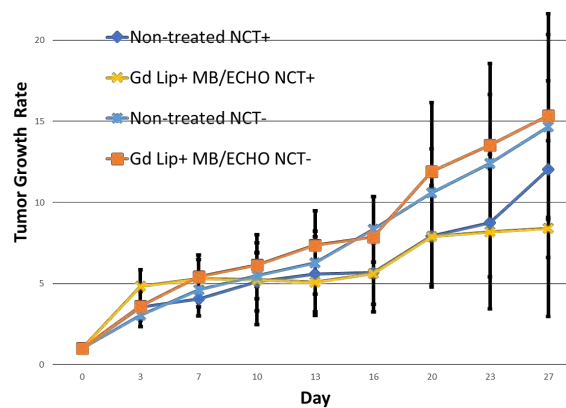


Figure 1. Tumor Growth Suppression by NCT with Intra Tumoral Injection of ¹⁵⁷Gd-plex /Bubble Liposome and Ultrasound Stimulation

REFERENCES:

- [1] N. Dewi *et al.*, Biomed & Pharmacother, **67** (2013) 451-7.
- [2] N. Dewi *et al.*, J Can.Res.Clin.Oncol., **142**(4) (2016) 767-75.
- [3] P. Miet *et al.*, J Cont. Release, **174** (2014) 63-71.
- [4] I. Yokoe *et al.*, Drug Deliv., **28**(1) (2021) 530-541.
- [5] I. Yokoe *et al.*, Cancers, **12**(9) (2020) 2423.
- [6] L. Lambricht *et al.*, Expert Opin Drug Deliv., **13**(2) (2016) 295-310.
- [7] B. Trotovšek *et al.*, World J Gastroenterol, **27**(48) (2021) 8216-8226.

CO7-41 Basic research on new BNCT strategies for melanoma

H. Michiue¹, T. Fujimoto^{1,2}, N. Kanehira^{1,2}, K. Igawa¹, Y. Sakurai³, N. Kondo⁴, T. Takata³, and M. Suzuki³

¹Neutron Therapy Research Center, Okayama University; 2-5-1, Shikata-cho, Kita-ku, Okayama City, Okayama, Japan.

²Department of Gastroenterological Surgery, Okayama University; 2-5-1, Shikata-cho, Kita-ku, Okayama City, Okayama, Japan.

³Institute 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 vivo is aromatic amino acids such as phenylalanine and tyrosine, and melanin synthesis is enhanced in cutaneous malignancies such as malignant melanoma. Boron-phenylalanine (BPA), a boron atom bonded to these amino acids, was created as a melanoma-targeted boron drug, and the success of BPA-BNCT was demonstrated in the 1989 Lancet article by Dr Mishima et al: "Treatment of malignant melanoma by single Malignant melanoma by single thermal neutron capture therapy with melanoma-seeking 10B-compound", published by Dr Mishima et al in the Lancet in 1989 [1]. This clinical study paved the way for the effectiveness of BNCT with boron drugs with cell-specific uptake and neutron irradiation of the cancer tissue area.

Melanoma is a cutaneous malignancy with an incidence of 1-2 per 100 000 people and is considered a rare cancer. In Australia, it is a disease with regional and racial variation, with an incidence of around 35 per 100 000 people. Surgery is the standard treatment of first choice for localized melanoma, and the prognosis is very good for Stage I melanomas, which have a low likelihood of spreading to the regional lymph nodes. The prognosis, mainly surgery, for localized melanoma is very good with a 5-year survival rate of 95-100% and the disease is reported to be curable by surgery. The usefulness of BNCT for localized melanoma is that there is no pain or functional impairment associated with surgery, which makes BNCT highly useful for melanoma patients, many of whom are elderly.

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

While the efficacy of mainly surgical therapies in localized melanoma has been established, the development of treatments in inoperable advanced-stage melanoma is

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

EXPERIMENTS:

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

Experiments were conducted while giving due consideration to the intensity of neutron radiation at the neutron irradiation site and at the shielded site.

RESULTS:

We used BPA as an effective boron agent for melanoma and confirmed the anti-tumor effect of neutron irradiation. The results were favorable, and are useful for the development of BPA-BNCT for melanoma in the future. We would like to express our deepest gratitude to the many collaborators who assisted in this project.

REFERENCES:

[1] Y. Mishima *et al.*, Lancet, **334** (1989) 388–9.

CO7-42 New boron drug development research targeting pancreatic cancer

H. Michiue¹, T. Fujimoto^{1,2}, N. Kanehira^{1,2}, K. Igawa¹, Y. Sakurai³, N. Kondo⁴, T. Takata³, and M. Suzuki³

¹Neutron Therapy Research Center, Okayama University; 2-5-1, Shikata-cho, Kita-ku, Okayama City, Okayama, Japan.

²Department of Gastroenterological Surgery, Okayama University; 2-5-1, Shikata-cho, Kita-ku, Okayama City, Okayama, Japan.

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

INTRODUCTION:

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

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

Ultrasonography, CT, MRI, endoscopic pancreatography, and angiography are used to diagnose pancreatic cancer. If pancreatic cancer is suspected, the pancreas cannot be seen from the surface of the body, so an ultrasound or CT scan is first performed to check for the presence of a mass in the pancreas. CT scan can also be used to check for metastasis of pancreatic cancer to other organs such as the lungs and liver.

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

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

DDS (Drug Delivery System) is a research field that delivers drugs such as anticancer agents to such malignant tumors. It has been reported that when a liposomal formulation containing a drug is administered to a tumor-bearing model, such a macromolecular drug accu-

mulates specifically in the tumor by leaking from the tumor blood vessels. This effect is called the EPR effect (Enhanced Permeability and Retention effect) and has been proposed as a theory that minimizes drug damage to normal tissue and maximizes the effect on tumor tissue.

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

EXPERIMENTS:

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

RESULTS:

In this study we have successfully synthesized a glucose-binding boron drug and have filed a patent application. The glucose boron drug was found to be efficiently introduced into cells via glucose transporters (GLUTs), which are highly expressed in pancreatic cancer. The anti-tumor effect of a novel drug agent in a mouse model of pancreatic cancer was confirmed in vitro and in vivo by neutron irradiation in a nuclear reactor, CA19-9, at the Institute for Complex Nuclear Science, Kyoto University, where a high therapeutic effect was obtained in a pre-experiment. The results are further developed and reported as a novel boron drug for targeting pancreatic cancer with high CA19-9 levels. We thank the many collaborators for their cooperation.

REFERENCES:

[1] RD Leone *et al.*, Science. **366** (6468) (2019)1013-1021.

CO7-43 Boron-compound diagnostics in the plant using a neutron capture reaction

T. Kinouchi

*Institute for Integrated Radiation and Nuclear Science,
Kyoto University*

INTRODUCTION: Mutation breeding of plants is a method of expanding genetic variation by artificially inducing mutations into the target DNA. The process starts by exposing seeds to chemicals (e.g., ethyl methanesulfonate, dimethyl sulfate) or radiation (e.g., γ -ray, ion-beam, neutron) in order to improve the new crops with desirable characters or phenotypes. More than 3,000 mutagenic plant varieties have already been released around the world, and more than 450 of these varieties were produced in Japan. Approximately 80% of them are caused by γ -ray radiation. On the other hand, γ -rays have a low linear energy transfer (LET), suggesting that they cannot deliver high energy locally to the target DNA, resulting in low relative biological effectiveness (RBE) and low mutation rates. We therefore focused on the phenomenon that a stable isotope of boron (^{10}B) irradiated by low-energy neutrons undergo a nuclear reaction into a high-energy helium nucleus (α -particle) and a lithium nucleus. Since those heavily charged particles can efficiently generate DNA double-strand breaks in the target cells, they would mutate or induce cell death. In fact, this reaction has been applied in combination with ^{10}B -compounds, such as *p*-boronophenylalanine (BPA), in a therapy to selectively destroy cancer cells, known as the boron neutron capture therapy (BNCT). However, BPA has not been well characterized in terms of mutagen for the mutation breeding. Therefore, to determine how much BPA added externally is absorbed by the seeds and where absorbed BPA localizes in them, *in situ* high-resolution visualization technique capable of detecting BPA in the seeds was examined with a solid-state nuclear tracking detector, CR-39.

EXPERIMENTS: Plant materials and growth conditions> Seeds used in analysis were harvested from a dwarf tomato cultivar, 'Micro-Tom' (*Solanum lycopersicum* L). Micro-Tom was originally derived for home gardens as the dwarf cultivar, but is now used as a major material for various plant research. The original seeds were obtained from Inplanta Innovations Inc. and seeded on the moderately moisturized vermiculite and cultivated at 23°C under a 16-h light/8-h dark cycle in a 60%-humidified growth chamber. A week later, their seedlings were transferred to the hydroponic media containing major nutrients (1 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM KH_2PO_4 , 0.5 mM K_2SO_4 , 1 mM MgSO_4 , and 1.5 mM NH_4NO_3) and micronutrients (75 μM

EDTA-Fe , 46 μM $\text{H}_3^{11}\text{BO}_3$, 9 μM MnSO_4 , 0.8 μM ZnSO_4 , 0.3 μM CuSO_4 , and 0.8 μM Na_2MoO_4) under the same conditions.

In situ visualization of BPA in a Micro-Tom seed> Micro-Tom seeds were immersed in BPA solution (100 mM) for 24 hrs. A mounted slice (10- μm thickness) of the seeds onto a solid-state nuclear tracking detector, CR-39 (20 mm \times 30 mm) was irradiated with epithermal neutron for 20 min by applying 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 as small black spots.

RESULTS: Both Figs. 1(A) and (B) show a cross section prepared from the same Micro-Tom seed, which was harvested from a parental strain cultivated in the ^{10}B -free hydroponic media. Fig. 1(A) is an optical microscopic image of the cross section of the seed. Black and white arrows indicate albumen and embryo, respectively. On the other hand, Fig. 1(B) is *in situ* visualization of BPA-distribution in the cross section. A large number of etch-pits derived from ^{10}B contained in BPA were concentrated on the outer seed coat and were barely detectable inside the seed. This strongly suggests that it is very difficult for BPA alone to pass through the seed coat. Since prolonged immersion (over 24 hrs) of seeds in a BPA solution may turn on germination, in order to increase BPA penetration into the seed, BPA concentration and carriers such as surfactants would need to be considered.

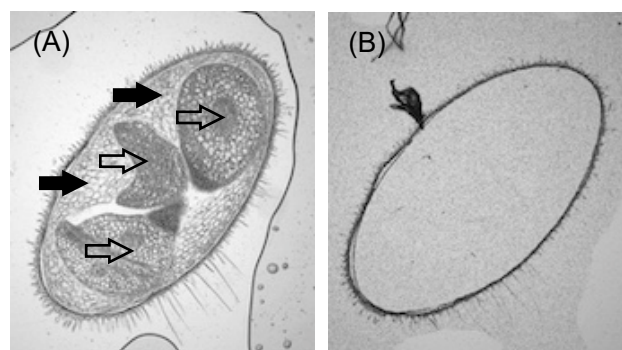


Fig.1 Detection of BPA in the Micro-Tom seed.

(A): Optical microscopic images of the cross section of the seed. Black and white arrows indicate albumen and embryo, respectively. (B): *In situ* visualization of BPA.