CO7-1 Establichment of a novel mutation breeding using Boron Neutron Capture Reaction (BNCR)

M.Kirihata, S. Segami[1], Y. Hattori, T. Kinouchi, Y. Kinashi[2]

Research Center of BNCT, Osaka Prefecture University

[1]Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture

[2]KURNS

INTRODUCTION: Boron Neutron Capture Reaction (BNCR) is based on the nuclear reaction of ^{10}B atom with thermal/epithermal neutron already applied to cancer treatment (BNCT) [1, 2]. As a new utilization method of BNCR, the purpose of this study is to establish a novel mutation breeding using BNCR.

This method uses the principle of inducing mutation by an alpha particle and ^{7}Li recoil nuclei high linear energy transfer and short range when irradiated with neutrons (low energy thermal neutrons (< 0.5 eV) can be absorbed the ^{10}B atoms, leading to generating high linier energy transfer alpha particles (~ 150 keV/μm) and ^{7}Li nuclei (~ 175 keV/μm)) that are produced by BNCR of ^{10}B selectively taken into the meristematic cell with thermal neutron. This principle is different from both chemical mutagens, such as EMS and MNU, and physical mutagens, such as gamma rays and ion beams, used for mutation breeding so far. In other words, the mutagenic effect depends on chemical and physical factors, such as ^{10}B concentration, thermal neutron intensity, and irradiation time.

The germination rate is used as one of the traits to verify the effect of mutagenesis[3]. In previous report, the seeds were immersed into different concentrations (0, 10, 100, 200 ppm) of ^{10}B-enriched *p*-boronophenylalanine (BPA) [4] for 16 h, re-dried seeds were irradiated with thermal neutron for 90 minutes. The germination rate of these irradiated seeds was investigated, and no BPA concentration-dependent effect on germination rate was observed. In other words, in order to study the optimal treatment conditions for mutagenesis, need to try a stronger process. This time, new treatment conditions were investigated by increasing the ^{10}B concentration of the treatment solution by using BPA as a fructose complex and by extending the immersion time.

EXPERIMENTS: The experimental material used *Oryza sativa* L. cv. Nipponbare. The dry seeds were immersed into different concentrations (0, 10, 100 mM) of ^{10}B-enriched *p*-boronophenylalanine-fructose complex (BPA-Fc) for 24 h or 48 h. The samples were washed with water and re-dried. The seeds in 2-mL tubes were irradiated with thermal neutron for 90 minutes in the Kyoto University Research Reactor (KUR). The irradiated seeds were sown in cell trays on May 31, 2020, germinated in a germination machine, grown outdoors, and the germination rate was examined 20 days after sowing.

RESULTS: The germination rate not decreased with BPA-Fc concentrations. But the germination rate decreased with immersion time (Table 1). Although there was a significant decrease in germination rate in the strongest treatment, the 100 mM BPA-Fc for 48h condition, the germination rate was similar to the control 48 h condition. Therefore, the decrease in germination rate in 48h immersing was not due to BNCR, but simply due to the effect of longer immersion time. In this experiment, we used BPA-Fc to increase the ^{10}B concentration, but again, we could not confirm any decrease in germination rate due to the BNCR under the examined conditions. It is unclear at this time whether the accumulation of ^{10}B in the meristematic cell of the seeds is not going well or whether this method is less likely to cause a decrease in germination rate. However, considering the solubility of BPA, the concentration of ^{10}B in BPA is considered to be at the upper limit of the current conditions, and there may be differences in the uptake by plant seeds depending on the compound. Therefore, we are planning to study the treatment with boron compounds other than BPA.

Table 1. The relationship each treatment conditions and germination rate.

Concentrations of BPA-Fc (mM)	Immersion time (hour)	No. of seeds	No of seeds germinated	germination rate (%)
0	24	40	28	70.0
10 (100 ppm)		80	72	90.0
100 (1000 ppm)		80	66	82.5
0	48	40	23	57.5
10 (100 ppm)		80	55	68.8
100 (1000 ppm)		80	45	56.3

* Numbers in ( ) indicate ^{10}B concentrations.

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CO7-2 Development of antibody-tagged boron compounds using Fc-binding peptide for on-demand receptor target in boron neutron capture therapy

I. Nakase[1,2], A. Aoki[1,2], Y. Sakai[3], S. Hirase[1,2], M. Ishimura[3], T. Takatani-Nakase[4,5], Y. Hattori[3], and M. Kirihata[3]

[1]*Graduate School of Science, Osaka Prefecture University, Japan*
[2]*NanoSquare Research Institute, Osaka Prefecture University, Japan*
[3]*Research Center of BNCT, Osaka Prefecture University, Japan*
[4]*School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Japan*
[5]*Institute for Bioscience, Mukogawa Women's University, Japan*

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a radiation therapeutic method for cancer therapy. Cancer cellular uptake of boron-10 (^{10}B) atoms induces the cell death by the generation of alpha particles and recoiling lithium-7 (^{7}Li) nuclei when irradiated with low-energy thermal neutrons. Current BNCT technology shows effective therapeutic benefits in refractory cancers such as brain tumor and head and neck cancer. However, improvement of insufficient cancer targeting and cellular uptake efficacy of boron compounds, and expansion of disease coverage in BNCT are strongly desired. In this research, we aimed to develop antibody-based drug delivery technology for BNCT using Z33 peptide [1], which shows specific interaction recognition with the Fc of human IgG antibody, for on-demand receptor target. In addition, we found that macropinocytosis induction during the antibody-based drug delivery is important for the biological activity in BNCT *in vitro* assay.

EXPERIMENTS: Z33 peptides were synthesized via Fmoc solid-phase synthesis methods. For preparation of dodecaborate-Z33 peptide conjugate, Z33 peptides was subjected to react with bismaleimide ethane and then to react with mercaptoundecahydrododecaborate (BSH).

RESULTS: We designed Z33 peptide-conjugated boron compounds (Z33-BSH), and we examined the cell membrane accumulation and cellular uptake of Z33-BSH with or without complex of cetuximab antibody, which specifically target epidermal growth factor receptor (EGFR). As a result, in A431 cells (highly expressing EGFR), complex of cetuximab antibody (100 nM) with Z33-BSH (200 nM) highly enhanced their accumulation on the cancer cell plasma membranes. However, only their attachment on plasma membranes with very low cellular uptake were observed. Macropinocytosis (accompanied by actin reorganization, ruffling of plasma membrane, and engulfment of large volumes of extracellular fluid) [2] is considered to be important cellular uptake pathway, and activation of EGFR leads to macropinocytosis induction. Therefore, we next checked effects of treatment of EGF on their cellular uptake. As our results, co-treatment of EGF with cetuximab antibody/Z33-BSH complex significantly increased their cellular uptake, and in the thermal neutron irradiation experiment under the same conditions, the cell killing effect of cetuximab antibody/Z33-BSH complex was enhanced through macropinocytosis induction by EGFR activation [3].

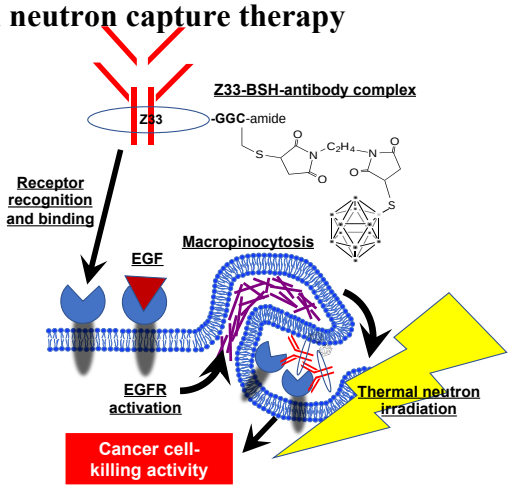

Figure 1. Schematic representation of the receptor targeted delivery of boron compounds. Z33-BSH-antibody recognizes the targeted receptors on cancer cells. EGFR activation induces macropinocytotic cellular uptake of the BSH, leading to increased efficacy of the cancer cell-killing activity after thermal neutron irradiation.

CONCLUSION: In this research, we developed antibody-based receptor target system and found importance of macropinocytosis induction in BNCT. These results provide fundamental knowledge for the further development of receptor target system in BNCT.

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CO7-3 Development of cyclic RGD-functionalized *closo*-dodecaborate albumin conjugates for boron neutron capture therapy

Hiroyuki Nakamura[1,2], Kai Nishimura[2], Kazuki Kawai[2], Satoshi Okada[1,2], Takushi Tanaka[3], Minoru Suzuki[3]⇑

[1]Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan
[2]School of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan
[3]Institute for Integrated Radiation and Nuclear Science, Kyoto University, Osaka 590-0494, Japan

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is an expecting cancer therapy for the treatment of harsh and un-operatable malignant tumors. The efficiency of boron agent depends highly on tumor selectivity, sufficient amount of boron agent in tumor site, non-toxicity, tumour/normal tissues ratio (>3) and absorption of thermal neutrons by boron. In 2020, accelerator-based BNCT for head and neck cancer using L-BPA was approved by the Pharmaceuticals and Medical Devices Agency in Japan, making BNCT more accessible treatment. L-BPA is known to actively accumulate into tumor cells thorough L-type amino acid transporter 1 (LAT-1). However, there are many cancers with low L-BPA accumulation, therefore there is a need for new boron drugs that exhibit a cancer-selective uptake mechanism different from that of L-BPA. In this study, we focused on serum albumin as a boron carrier. The serum albumin is an abundant protein that has an extraordinary ligand-binding capacity to carry various endogenous and exogenous compounds in plasma. It accumulates in tumor due to the combination of leaky and abnormal blood vessels with the absence of the lymphatic drainage system known as the enhanced permeability and retention (EPR) effect.
We have developed maleimide-conjugated *closo*-dodecaborate (MID). MID-conjugated albumins accumulated in tumor and exhibited significant tumor suppression in tumor-bearing mice after neutron irradiation [1]. In order to improve further accumulation in tumor, we designed cyclic RGD (cRGD) peptide-conjugated boronated albumin. It is known that cRGD peptide strongly binds to αvβ3 integrin, which overexpress in many cancer cells. BNCT studies were performed at Kyoto University Research Reactor Institute (KURRI).

EXPERIMENTS: U87MG 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 (7.5 mg [^{10}B]/kg) or cRGD-MID-BSA (7.5 mg [^{10}B]/kg). The whole bodies of mice were placed in an acrylic mouse holder and fixed on a 5-mm-thick thermo-plastic plate. At 12 h after administration, the right thighs of mice were irradiated with neutrons in the KUR nuclear reactor. BNCT effects were evaluated on the basis of the changes in tumor volume of the mice.

RESULTS: Anti-tumor effects of cRGD-MID-BSA and MID-BSA were compared in the U87MG xenograft tumor model at the same dose. cRGD-MID-BSA and MID-BSA were injected into U87MG xenograft tumor model mice via the tail vein, and thermal neutron irradiation was performed 12h after injection. Tumor growth curves are shown in Fig. 1 [2].

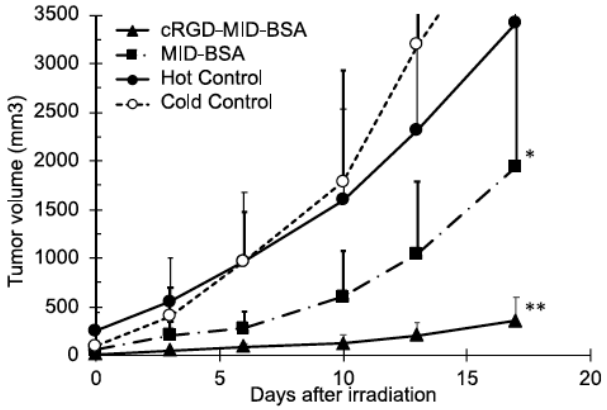

Fig. 1. Tumor volume in U87MG xenograft tumor model mice irradiated with thermal neutron (hot) for 50 min (1.8 x 10^{12} neutrons/cm^2) or without irradiation (cold). The irradiation was performed at 12 h after i.v. injection of cRGD-MID-BSA and MID-BSA (7.5 mg [^{10}B]/kg). Data are expressed as means ± SD (n = 5). Statistical significance: *P< 0.05 and **P < 0.001 compared with hot controls. Reproduced from ref 2. Copyright 2020 American Chemical Society.

CONCLUSION: We succeeded in the preparation of cRGD-MID-BSA. The conjugation of the cRGD peptide ligand at Cys 34 in BSA was confirmed by MALDI-TOF-MS analysis after trypsin digestion. In vivo fluorescence live imaging of near infrared dye (NID)-conjugated cRGD-MID-BSA and MID-BSA revealed that both cRGD-MID-BSA and MID-BSA similarly reached the maximum accumulation during 8−12 h after injection. However, NID-cRGD-MID-BSA was more selectively accumulated and retained in tumor than NID-MID-BSA after 24 h. An *in vivo* BNCT study emerged with the cRGD peptide ligand enhanced accumulation of MID-BSA in tumor cells through αvβ3 integrin, attributed to significant tumor growth suppression after neutron irradiation. Therefore, the MID-albumin conjugate is a potential platform not only for tumor targeted by introducing tumor-affinity ligands but also for *in vivo* live imaging by introducing visualization functional groups, providing a possible theranostic module for BNCT.

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CO7-4 Development of *closo*-dodecaborate-containing pteroyl derivatives targeting folate receptor-positive tumors for boron neutron capture therapy

Hiroyuki Nakamura[1,2], Kai Nishimura[2], Fumiko Nakagawa[2], Satoshi Okada[1,2], Taiki Morita[1,2], Shinji Kawabata[3], Takushi Tanaka[4], Minoru Suzuki[4]

[1]*Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan*
[2]*School of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan*
[3]*Department of Neurosurgery, Osaka Medical College, Osaka 569-8686, Japan*
[4]*Institute for Integrated Radiation and Nuclear Science, Kyoto University, Osaka 590-0494, Japan*

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) has been attracting attention as a noninvasive radiotherapy in cancer treatment. In 2020, accelerator-based BNCT for head and neck cancer using L-BPA was approved by the Pharmaceuticals and Medical Devices Agency in Japan. L-BPA is known to accumulate into tumor cells thorough L-type amino acid transporter 1 (LAT-1). However, there are many cancers with low L-BPA accumulation, therefore there is a need for new boron drugs that exhibit a cancer-selective uptake mechanism different from that of L-BPA. We focused on folate receptor α (FRα). Folate is one of the B vitamins and known to be taken into cells via FRs, which are overexpressed on the surface of many cancer cells including HeLa and U-87 MG. Therefore, FRs have attracted attention as targets for cancer treatment. In this study, we synthesized pteroyl-*closo*-dodecaborate conjugate (PBC) and examined their cell uptake using folate receptor (FRα) positive and negative cells. It is known that the pteroyl group of folate is essential for the interaction with folate receptors, which overexpress in many cancer cells.

EXPERIMENTS: (1) WTT Assay: Cells were seeded in 96-well plates in medium at the density of 5×10^3 cells/well. After 24 h of cell attachment, the cells were exposed to PBC and L-BPA-Fructose complex at final concentration ranging from 0 to 3 mM (L-BPA : 0 to 10 mM), for 72 h at 37°C. At the end of the incubation period, the mitochondrial function was verified with 0.5 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 2 h at 37°C and quantified spectrophotometrically at 595 nm by Biorad microplate reader.
(2) *In vitro* BNCT Effects toward U87MG Cells: Cells were plated on 96-well plates (500 cells per well) 12 h before irradiation. They were incubated with PBCs or L-BPA-fructose (25 or 50 ppm [^{10}B]) for 3 h at 37 °C and irradiated with thermal neutron for 100 min (2.03×10^{12} – 1.05×10^{13} neutrons/cm^2) in the KUR nuclear reactor. After replaced by a flesh medium, the cells were further incubated for 72 h. A PBS solution of MTT (2.5 mg/mL) was added to each well (10 μ L per well), and the plate was incubated for 2 h at 37 °C. After the medium was removed, dimethyl sulfoxide (DMSO) was added to each well (100 μ L per well). Absorbance at 570 nm was measured with a plate reader (TECAN, infinite F200). The irradiated cell viability was calculated by comparison of the absorbance of a nonirradiated cell (cold control).

RESULTS: A high dose is necessary to achieve the required boron concentration in the tumor for BNCT. Thus, low cytotoxicity is essential for BNCT boron agents. We first examined the cytotoxicity of synthesized PBCs toward three human cancer cell lines using MTT assay: HeLa (human cervical carcinoma) and U87MG (human glioblastoma) cells are FRα positive and A549 (human alveolar adenocarcinoma) cells are FRα negative. L-BPA was used as a positive control. PBC exhibited IC_{50} values (the concentrations required for 50% inhibition) in a range of 1–3 mM toward these human cancer cells, indicating that PBC has adequately low cytotoxity, enough to use as BNCT boron agents. We next compared BNCT anti-tumor effect between PBC and L-BPA toward U87MG human glioblastoma cells (FRα positive cells). Irradiation dose-dependent BNCT effects toward U87MG cells as shown in Fig. 1 indicated that PBC exhibited more potent than L-BPA at 25 ppm B concentration and that the cell-killing effect of PBC at 25 ppm B concentration was higher than that at 50 ppm B concentration.

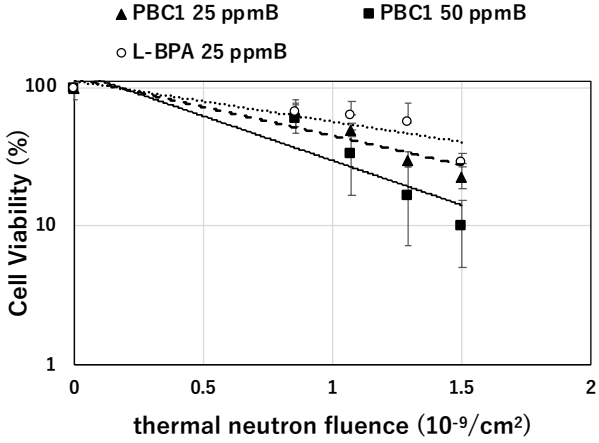

Fig. 1. BNCT effect of PBC toward U87MG after thermal neutron irradiation for 100 min (2.03×10^{12} – 1.05×10^{13} neutrons/cm^2) in the KUR nuclear reactor. L-BPA was used as a positive control.

CONCLUSION: We succeeded in the development of PBC targeting folate receptor positive tumor cells. PBC showed higher cell-killing effects than L-BPA against U87MG FRα positive cells after thermal neutron irradiation. Therefore, PBC is considered to be a promising boron agent for the treatment of L-BPA negative patients in BNCT.

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CO7-5

Influence of the abscopal effect to the survival rate following head neutron-irradiation between the different inbred mice

Y. Kinashi[1], T. Takata[1], Y. Sakurai[1], H. Tanaka[1]

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

INTRODUCTION: It is reported that immune response is activated by partial radiation [1]. The influence on immune organization of the mouse at the time of the head irradiation is not well known. The purpose of this study is to evaluate the relative biological effectiveness in the severe combined immunodeficiency (SCID), so-called SCID mice, those are having well-known high radiation sensitivity following thermal neutron irradiation for mice cranial.

EXPERIMENTS: CB17/Icr-*Prkdc*^{scid}/CrICrIj (SCID mice) were obtained from Charles River Inc. As a comparison experiment for the SCID mice, Balb/c and C3H/He mice were obtained from Japan Animal Inc.
Neutron irradiation and Gamma-ray irradiation was performed as follows. The Heavy Water Facility of the Kyoto University Research Reactor (KUR) was used. Mice were restrained in a plastic box on a radiation board. Neutron fluence was measured by radio-activation of gold foil and gamma-ray doses by TLD. Gamma rays were delivered with a ^{60}Co gamma ray machine. Mice were restrained in a plastic box on a radiation shelf. For the apoptotic assay of the splenic cells, the cell suspension was adjusted and incubated for 1weeks. At 2 days after irradiation, apoptotic induction of the cells was examined by Cell Death Detection ELISA (Roche).

RESULTS: As shown in Table 1, the RBE values estimated by the apoptotic changes of the SCID, Balb/c and C3H mice splenic cells following the partial neutron irradiation.

Table1. RBE (Relative Biological Effectiveness) calculated from apoptosis of splenic cells following neutron radiation

	SCID	Balb/c	C3H
RBE*	1.57	2.09	2.28

*RBE was calculated the Enrichment factor at 3Gy neutron radiation dose / the Enrichment factor at 3Gy gamma-ray radiation dose.

The apoptotic induction of the splenocytes of SCID mice was larger than that of Balb/c and C3H mice at 2 days after irradiation.

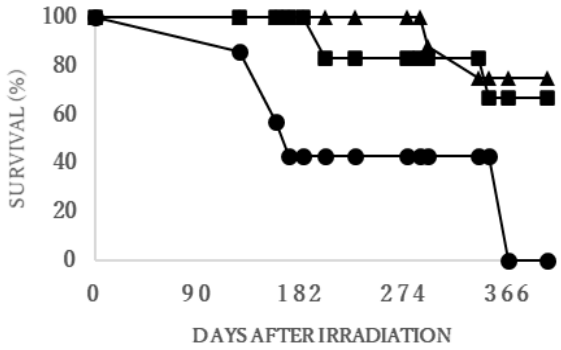

Fig.1 The survival rate after the partial head-irradiation of the neutron by KUR. Each marker shows the survival rate of the three kinds of mice. (●: SCID, ■: Balb/c, ▲: C3H)

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

DISSCUSSION

In the radiation sensitive mice study, the RBE values of SCID mice was 1.57, comparing the neutron and the gamma studies. The RBE values of Balb/c and C3H/He mice was 2.09 and 2.28, respectively. SCID mice show extreme sensitivity to ionizing radiation, because cells lack functional DNA-dependent protein kinase. Our results suggest that the difference of RBEs for radiation sensitive mice were smaller than the wild type mice, that is to say, the hyper radiation sensitivity does not have a disadvantage in BNCT. On the other hand, 60% of the SCID mice died by partial neutron head-irradiation after 100 days and died all on the 356th day. Because neutron sensitivity becomes higher, as for the SCID mouse inferior to a wildtype mouse in immunoreaction, BNCT works in the survival rate disadvantageously.

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CO7-6 Optimization of chemical structures of polymer-BPA complexes for a nonclinical study

T. Nomoto[1], K. Konarita[1], K. Uehara[2], M. Ishimura[2], Y. Ishino[2], A. Sudani[2], Y. Sakurai[3], M. Suzuki[3], N. Nishiyama[1]

[1]*Institute of Innovative Research, Tokyo Institute of Technology*
[2]*Stella Pharma Corporation*
[3] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION: *p*-Boronophenylalanine (BPA) has been most widely investigated in clinical studies of boron neutron capture therapy (BNCT) because of its selective accumulation within tumor cells through LAT1 amino acid transporter overexpressed on many tumor cells [1]. However, the antiport mechanism of LAT1 sometimes causes unfavorable efflux of intracellular BPA by exchanging it with extracellular amino acids including tyrosine [2], resulting in short retention time within a target tumor and compromised therapeutic efficiency.

In this regard, we recently reported that poly(vinyl alcohol) (PVA) can form a complex with multiple BPA molecules through boronate ester formation and that PVA-BPA complex can be internalized into the cells through LAT1-mediated endocytosis and entrapped in endo-/lysosomes, thereby preventing the unfavorable efflux by LAT1 antiport mechanism [3]. Even in *in vivo* studies, PVA-BPA exhibited prolonged retention within the target tumor and significantly augmented antitumor efficiency with thermal neutron irradiation.

For the clinical application of PVA-BPA, it is important to optimize the composition of PVA-BPA especially from the viewpoint of molecular weight because it usually affects biodistribution. Herein, to optimize the chemical structure of PVA-BPA, we prepared a series of PVA-BPA complexes with various physicochemical properties and examined their antitumor efficacy in BNCT.

EXPERIMENTS: BALB/c mice bearing subcutaneous CT26 tumors were prepared by subcutaneous injection of the cell suspension. PVA-BPA was intravenously injected to the mouse at a dose of 10 mg BPA/mouse, and the thermal neutrons were irradiated to the tumor using KUR 3 h after injection. The tumor volume (V) was calculated using the following equation:

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

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

RESULTS: As shown in Fig. 1, PVA-BPA with different physicochemical properties exhibited the strong antitumor effect with thermal neutron irradiation, indicating that the physicochemical properties of PVA did not critically affect the potency of PVA-BPA. This tendency may be advantageous in manufacturing PVA for PVA-BPA complexes in practical applications.

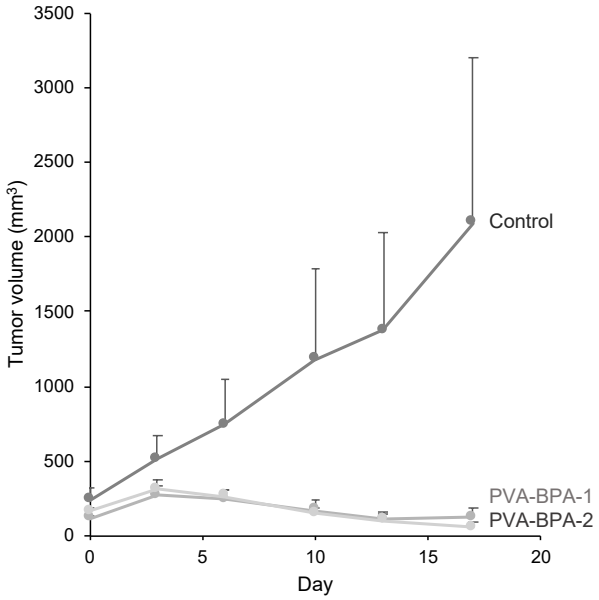

Fig. 1. Antitumor efficacy of representative PVA-BPA complexes (PVA-BPA-1 and PVA-BPA-2) to subcutaneous CT26 tumor models.

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CO7-7 OKD-001-based BNCT successfully prolongs the overall survival of orthotopic xenograft mouse model of a patient-derived glioblastoma stem-like cell line

A. Fujimura[1,2], .K.Igawa[2], H. Michiue[2], A. Ueda[2], N. Kondo[3], Y. Sakurai[3] and M. Suzuki[3]

[1]*Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Department of Physiology, Okayama University*

[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 fine-tuned intracellular atomic reaction of ^{10}B boron. Because BNCT utilizes a combination of ^{10}B pre-treatment and neutron irradiation, and thus induces a consequent ^{10}B nuclear reaction inside cancer cells, a highly cancer-specific ^{10}B delivery system is required for safety and efficacy. In 2020, a promising BNCT system with BPA (4-borono-L-phenylalanine) has been approved as a treatment against head and neck (H&N) cancer in Japan. BPA is a ^{10}B-added derivative of tyrosine/phenylalanine and therefore is mainly transported into cells via amino acid transporters, such as LAT1[(1)]. In H&N cancers, the expression levels of LAT1 are upregulated in almost all cases. However, in glioblastoma patients, there are many cases with low expression levels of LAT1. To expand the possibility of BNCT against these patients, the other types of boron agents are necessary. In this study, we showed an efficacy of BNCT with a novel boron agent, OKD-001[(2)], against orthotopic xenograft mouse models of a patient-derived glioblastoma stem-like cell line.

EXPERIMENTS:

NOD-SCID mice were orthotopically transplanted with 100,000 cells of a patient-derived glioblastoma stem-like cell line, MGG8[(3)] 15 days before BNCT with OKD-001. On the day before neutron irradiation, the mice were injected with 40 mg/kg of mercaptoundecahydrododecaborate (BSH) or OKD-001 (a mixture of BSH and A6K self-assembling peptide). On the next day, these mice were irradiated with neutron for 1 hour at KUR under anesthesia. Note that the neutron irradiation was done only on head part, not on whole body of mice. After BNCT, these mice were checked twice a week to obtain the overall survival time.

RESULTS:

As shown in Table 1, the medians of overall survival time of non-irradiated group (Group A: cold control, n=6), BSH-pretreated group (Group B), and OKD-001-pretreated group (Group C) are 36±5.8, 41±5.7, 51.5±3.9 days, respectively. OKD-001-BNCT, but not BSH-BNCT, significantly improved the prognosis of the orthotopic xenograft models.

	Group A	Group B	Group C
	cold control	BSH (40 mg/kg)	OKD-001 (40 mg/kg)
Median	36	41	51.5
stdev	5.8	5.7	3.9

Table 1. OKD-001, but not BSH, successfully prolonged the overall survival time (days) in the orthotopic xenograft mouse model of patient-derived glioblastoma stem-like cell line. MGG8.

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CO7-8 Enhancement of the cancer cell-killing effects of boron neutron capture therapy by overexpression of *LAT1* in human cancer cells

K. Ohnishi[1], M. Misawa[2], N. Sikano[3] and M. Suzuki[4]
Departments of [1]Biology, [3]Radiological Science, Ibaraki Prefectural University of Health Sciences
[2]National Institute of Advanced Industrial Science and Technology
[4]Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Outcome from BNCT largely depends on amount of intracellular accumulation of boron compound. L-type amino-acid transporter 1 (LAT1) [1], through which boronophenylalanine (BPA) is transported into cells, is frequently expressed in various types of tumor cells including glioblastoma but not in normal cells [2]. We transfected *pCMV/LAT1-GFP* plasmids into a glioblastoma cell line, T98G, and selected several clones and confirmed that those clones stably overexpress LAT1 in cell membrane with confocal laser microscopic observation and western blot analysis. In this study, we measured the sensitivity to the neutron and gamma-ray fluences generated by KUR in T98G/K1 and T98G/K4 clones that uptake ^{14}C-BPA 2.5 and 5.0 times, respectively, larger than T98G/KC2 control clone.

EXPERIMENTS: We repeated the previous experiments performed in 2019. The experiments are as follows. T98G/K1, T98G/K4, T98G/KC2 and transiently *pCMV/LAT1-GFP*-lipofected T98G/KC2 cells (treated with Lipofectamine 2000 overnight) were treated with medium containing ^{10}BPA (0, 5 or 20 ppm) for 3 hours. The cells were trypsinized and the cell suspensions in 1.5-ml cryo-tubes were irradiated with the fluences (0.4 or 0.8 Gy in total doses of neutrons and γ-rays) from KUR. The irradiated cells were plated on three replicate dishes for colony formation assay. The cells were fixed with ethanol and stained with crystal violet after cell culture for 10-14 days.

RESULTS: We confirmed the previous results obtained from 2019 experiments. The results are as follows. T98G/K4 cells showed slightly enhanced sensitivity to the fluences compared with T98G/K1, T98G/KC2 and the lipofected T98G/KC2 cells in the case of 5 ppm ^{10}BSA treatment. There is no significant difference in the sensitivity between T98G/K1 and T98G/KC2 cells as well as transiently lipofected T98G/KC2 cells. In the case of 20 ppm ^{10}BSA treatment, T98G/K4 and the lipofected T98G/KC2 cells showed largely enhanced sensitivity to the fluences compared with T98G/KC2 cells (ER=1.5). T98G/K1 cells showed slightly enhanced sensitivity (ER=1.2). The sensitivity of the cells to the fluences was correlated with the expression level of LAT1 of the cells. Figures of results are now in press for publication in Radiation Research [3].

CONCLUSION: This study confirmed the conclusion of 2019 report that overexpression of LAT1 in cancer cells causes enhanced anticancer effects of BNCT and BNCT combined with gene therapy is beneficial for tumors bearing low LAT1 expression.

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CO7-9 Mechanism of Glioma Stem Cells' Survival Conferred by Glioma Niche after BNCT

N. Kondo[1], E. Hirata[2], M. Natsumeda[3], M. Nakada[4], Y. Sakurai[1], T. Takata[1], T. Kinouchi[1] and M. Suzuki[1]

[1] Institute for Integrated Radiation and Nuclear Science, Kyoto University (KURNS)
[2]Division of Tumor Cell Biology and Bioimaging Cancer Research Institute of Kanazawa University
[3]Department of Neurosurgery, Brain Research Institute, Niigata University
[4]Department of Neurosurgery, Kanazawa University Graduate School of Medical Science

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.

Glioma stem cells is known to be resistant to chemo-radiation therapy. This study is aimed to investigate whether glioma stem like cells (GSLCs), which is resistant to chemo-radiation therapy, take up a boron compound, p-boronophenylalanine (BPA) or not.

EXPERIMENTS:

Cell culture: We used human GBM cell lines that were established from tumor samples from two patients and named the cell lines no. 1 and no. 2. Cells were cultured as non-adherent spheroids in serum-free DMEM/F12 containing GlutaMax (Thermo Fisher Scientific), B27 without vitamin A (Thermo Fisher Scientific), penicillin and streptomycin (Nacalai Tesque, Kyoto, Japan), hEGF (20 ng/mL) and hFGF (20 ng/mL, Peprotech, TX, USA). To induce cell differentiation, we exposed the cells to media containing 10% FBS for 24 h. We dissociated cells to single cells using accutase (Nacalai Tesque) and rinsed them with PBS before mass cytometry analysis at 37 °C in CO_2 incubator.

Boronophenylalanine (BPA) Treatment and Thermal Neutron Irradiation: We treated cells with medium containing BPA at the concentration of 25 ppm for 24 h. The BPA was formulated and its concentration was measured as previously described [1].

Multiparameter Mass Cytometry: Cells were dissociated into single cells, labelled with heavy metal conjugated antibodies and analyzed by mass cytometer [2].

RESULTS: The percentage of the total BPA-positive cells decreased in differentiated cells compared with GSLCs in both no. one and no. two cells (stem vs. differentiated cells: no. one, 56.0% vs. 25.7% and no. two, 35.8% vs. 21.5%) (Table 1). In differentiated no. one cells, only 9.3% were BPA+/Oct3/4+ cells compared with 29.4% of no. one GSLCs. Similarly, lower percentages of no. one differentiated cells expressed both BPA and a stem cell marker than detected in no. one GSLCs. In contrast, 18.4% and 24.9% of differentiated cells positive for BPA were positive for GFAP and Tuj1, respectively, compared with 20.5% and 21.2% in GSLCs positive for BPA (Table 1). In differentiated no. two cells, only 2.9% were BPA+/SOX2+ cells compared with 15.5% of no. two GSLCs. Similarly, lower percentages of no. two differentiated cells expressed both BPA and a stem cell marker than were seen among the no. two GSLCs. In no. two cells, 8.6% and 9.4% of differentiated cells positive for BPA were positive for GFAP and Tuj1, respectively, compared with 10.5% and 11.1% GSLCs positive for BPA (Table 1).

BPA$^+$	No. 1 Stem	No. 1 DC	No. 2 Stem	No. 2 DC
Total	56.0	25.7	35.8	21.5
Differentiation$^+$				
GFAP	20.5	18.4	10.5	8.6
Tuj1	21.2	24.9	11.1	9.4
Stem $^+$				
Oct3/4	29.4	9.3	11.4	4.9
CD15	19.2	10.6	11.0	6.1
CD171	21.3	13.9	8.0	4.2
IL6Ra	15.1	6.4	8.1	4.1
SOX2	51.9	15.2	15.5	2.9
Nestin	35.0	8.3	12.8	3.6
CD144	11.6	5.3	6.8	3.9
Musashi-1	28.1	14.5	19.0	8.2
CD133	18.8	9.0	11.6	5.8
PDGFRα	44.9	15.6	12.0	4.6
Notch2	18.9	12.1	11.8	4.6
CD44	19.1	9.9	11.6	5.9
Nanog	20.6	6.9	11.5	5.0
STAT3	44.9	17.2	16.2	1.1
CXCR4	18.7	12.2	13.5	5.7
c-Myc	27.1	11.6	13.2	5.7
CD49f	29.8	18.7	15.0	2.0

Table 1. Percentages of total *p*-boronophenylalanine (BPA$^+$) cells, BPA$^+$/differentiation marker$^+$ cells and BPA$^+$/stem marker$^+$ cells among glioma stem-like cells (Stem) and differentiated cells (DC) from no. 1 and no. 2.

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CO7-10 Establishment of protocol for neutron capture therapy for head and neck cancer

I. Ota[1], H. Uemura[1], A. Nishimura[1], T. Kimura[2], S. Mikami[1], T. Yamanaka[1], M. Suzuki[3], Y. Sakurai[3], H. Tanaka[3], N. Kondo[3], T. Tamamoto[2], M. Hasegawa[2] and T. Kitahara[1]

[1]Department of Otolaryngology-Head & Neck Surgery,
[2]Department of Radiation Oncology, Nara Medical University
[3]Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Neutron capture therapy (BNCT) for head and neck tumors has been clinically studied since 2001, with the BNCT research group at Kyoto University Reactor Laboratory, which is a co-investigator, highly effective, with high safety. It is being established. Since November 2012, we implemented the therapy as a joint research with Kyoto University Reactor Laboratory, with the consent of the patients in 4 cases of refractory recurrent head and neck cancer. As a result, the response rate was a very high 100%. The tumor reduction effect in recurrent cases after radical irradiation, which could not be achieved by conventional treatment methods, strongly suggests the possibility of expanding the indications for BNCT for refractory carcinomas as well as for head and neck cancer cases. Here, we will perform BNCT for refractory and recurrent head and neck tumors and examine their efficacy and optimal protocol.

EXPERIMENTS: We will treat BNCT for refractory and recurrent head and neck tumors that meet the following criteria and examine their efficacy and optimal protocol.
Inclusion Criteria
(1) The patient with local recurrence of head and neck cancer who cannot perform the standard therapy any more after radiotherapy.
(2) The patient with local recurrence of head and neck cancer by the imaging diagnosis, such as CT, MRI and PET.
(3) The patient with previous radiotherapy (total 40-75 Gy, 2Gy/fq) for the recurrent region.
(4) The patient with the period of more than one month since the previous treatment.
(5) The patient with recurrence lesion in the less than 6cm of depth from skin as GTV for BNCT.
(6) The Patients who have PS less than 2 and are expected to survive more than 6 months after BNCT.
(7) The patient with good condition of renal function: creatinine <1.2 mg/dl for male and
<1.0 mg/dl for female.
(8) The patient with the age between 20 and 80.
(9) Written informed consent with one own will.

Exclusion Criteria
(1) The patient with active multiple primary cancers; synchronous or metachronous (within 5 years) double cancers .
(2) The patient with metastatic lesion.
(3) The patients with severe complications.
(4)The patients with infection requiring systemic treatment.
(5) The patient with severe adverse event
(>Grade3, CTCAE v4.0) in the BNCT region.
(6) The patient with cardiac pacemaker.
(7) The patient judged to have difficulty in maintain posture during the protocol treatment.
(8) The patient with WBC; < 3000/mm3, PLT; < 100000/mm3
(9) The patient with recurrence lesion invasive to carotid artery and toskin.
(10) Patients with phenylketonuria.

RESULTS: We enrolled no patient and did not undertake BNCT during this period. However, the patient enrolled last year was followed up safely.

Patient #1: 46 y.o. female
Recurrence of cancer of the external ear
Histology: squamous cell carcinoma
Effect: SD
SAE: none; dermatitis, Grade 2

CONCLUSION:
We have accumulated the cases carefully to establish a safe and stable treatment of BNCT. Finally we will evaluate to the efficacy of BNCT for refractory and recurrent head and neck tumors.

CO7-11 Identification of host immunostimulatory effects induced by boron neutron capture therapy

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

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

INTRODUCTION: Boron neutron capture therapy is a type of radiation therapy that utilizes the physical phenomenon of boron atoms (^{10}B) fissioning into alpha particles and lithium nuclei when they capture neutrons. The particles generated are heavy particle beams with a high cell-killing effect, and each particle has a short range of less than 10 μm (less than the diameter of a cell). Therefore, by selectively delivering boron atoms to tumor cells and then irradiating them with neutrons from outside the body, it is theoretically possible to selectively treat only tumor cells. Radiation has classically been thought to weaken the immunity of the irradiated organism, but this is only the case for the whole body or large areas of bone marrow irradiation. However, this is only the case for irradiation of the whole body or a large area of bone marrow. As technology has advanced, it has recently become clear that radiotherapy can have an anti-tumor immunostimulatory effect on the host, especially when the irradiation is focused on the tumor. In this research project, we investigated the effects of boron neutron capture therapy on host immune cells and evaluate whether boron neutron capture therapy has the same immune-stimulating effects that are known to occur with reference radiation such as X-rays/gamma-rays. In addition, we explored the possibility of combining boron neutron capture therapy and immunotherapy using a mouse model to provide a basis for combination therapy in actual clinical practice.

EXPERIMENTS: Mouse-derived malignant melanoma cell B16 and mouse-derived squamous cell carcinoma SCCVII were implanted subcutaneously in the lower leg of C57BL/6 and C3H mice, respectively. Tumors of a specific size were irradiated with BNCT or the same dose of reference radiation (γ-ray), and the effect of host immune response was evaluated.

RESULTS: When CD8 positive T cells in the mouse body are removed simultaneously with reference radiation using removal antibodies, a similar tumor volume reduction effect is usually observed up to about 7 days after irradiation, but after that, the anti-tumor effect of radiation is weakened and the tumor volume becomes significantly larger. Using model mice, the BNCT group and the CD8-positive T cell removal antibody simultaneously with BNCT showed a different course from the standard reference radiation. In the case of the reference radiation, the initial effect of the radiation was not only a direct anti-tumor effect but also an immune contribution, whereas the direct anti-tumor effect of BNCT was intense, suggesting that the initial effect was not significantly affected by the immune status of the host. On the other hand, the use of antibodies to remove CD8-positive T cells resulted in statistically significant tumor regrowth compared to the BNCT group after about 2-3 weeks, reflecting the importance of host immune function in regrowth after BNCT. Since the results obtained were unexpected, we next examined whether BNCT and immunotherapy (anti-PD-1 antibody) could be used to prevent tumor repopulation and recurrence, reflecting the results. When anti-PD-1 antibody was administered at the same time as BNCT, tumor regrowth was usually observed 3 weeks after treatment in this mouse model, but when anti-PD-1 antibody was used in combination with BNCT, regrowth was suppressed and the survival rate was improved. In addition, the immune function of the host is involved in repopulation and recurrence after BNCT, suggesting that combined immunotherapy may be effective in reducing the risk of recurrence after BNCT. The detailed mechanism remains to be elucidated, and as a future prospect, we would like to analyze the mechanism that inhibits recurrence using RNA-seq and flow cytometry.

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CO7-12 Preliminary study of antitumor effectivity by Gd-neutron capture therapy using RGD binding Gd-DTPA-incorporated calcium phosphate nanoparticles to canine hemangiosarcoma model

M. Yanagawa [1], H Xuan [2,3], H. Yanagie[4,5,6], Y. Sakurai[5,6], K. Mouri[6], N. Dewi[6], H. Cabral[3], T. Nagasaki[7], Y. Sakurai[8], H. Tanaka[8], M. Suzuki[8], S. Masunaga[8], and H. Takahashi[2,3,4,5]

[1]Obihiro Univ of Agriculture and Veterinary Medicine, [2]Dept of Nuclear Engineering & Management, School of Engineering, Univ of Tokyo, [3]Dept of Bioengineering, School of Engineering, Univ of Tokyo, [4]Institute of Engineering Innovation, School of Engineering, Univ of Tokyo, [5]Cooperative Unit of Medicine & Engineering, Univ of Tokyo Hospital, [6]Niigata Univ of Pharmacy & Applied Life Sciences, [7]Osaka City University Graduate School of Engineering, [8]Kyoto Univ Institute for Integrated Radiation & Nuclear Science, JAPAN

INTRODUCTION: Hemangiosarcoma is a malignant tumor arising from vascular endothelial cells, and common tumor of the canine spleen [1]. Hemangiosarcoma of the spleen has early distant metastases and peritoneal dissemination and has a poor prognosis despite surgery and chemotherapy [2].

Gadolinium neutron capture therapy (Gd-NCT) is the unique radiation therapy that uses ^{157}Gd and has a large thermal neutron cross section (255,000 barns). The range of induced high LET Auger electrons is few micron, so increase of ^{157}Gd concentration in the cancer cells is important for increasing the therapeutic effect. We had reported that the gadolinium neutron capture reaction (GdNCR) showed the tumour growth suppression, and could be applied to the intensive cancer treatmens in near future [3,4,5].

Recently, Arg-Gly-Asp(RGD) sequence is very impressive in the targeting fields of pharmaceutical sciences, because, RGD sequence can bind to the Integrin receptor of cancer cell surface. So, it can be used for cancer targeting by endocytosis mechanism of RGD sequence [6].

In this work, we prepared the RGD motief binded Gd-DTPA/CaP nanoparticles for selective cancer targeting, and applied the GdNCR to the canine hemangiosarcoma model by intraveneous injection.

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

RGD sequense binding nanomicelle and Gd-DTPA / CaP nanomicelle were injected tumor-bearing mice via the tail vein 24 hours prior to neutron irradiation.

In vivo evaluation was performed on Ju-A1 tumor-bearing mice irradiated for 60 minutes at nuclear reactor facility of Kyoto University Institute for Integrated Radiation & Nuclear Science with average neutron fluence of 3.0×10^{12} n/cm^2. Antitumor effect was evaluated on the basis of the change in tumor growth.

RESULTS:
Tumor growth was suppressed in the groups of RGD sequence binding Gd-DTPA/CaP nanomicelle and bare Gd-DTPA/CaP nanomicelle compared with the non-irradiated groups with the injection of same DDS.

The tumour volume was decreased after GdNCT. In this preliminary experiment, the tumour decresence by RGD sequence binding Gd-DTPA/CaP nanomicelle was superior than the group of bare Gd-DTPA/CaP nanomicelle.

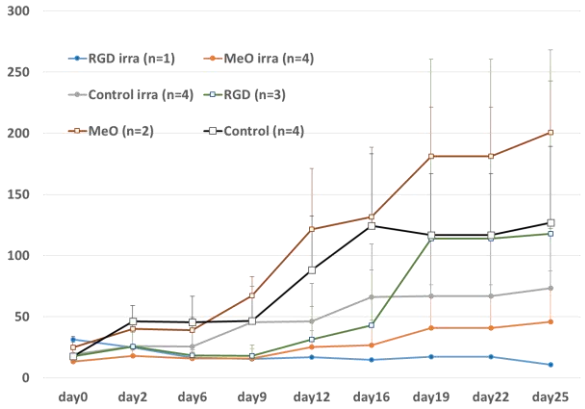

Figure 1. Tumor growth suppression in the group of RGD sequence binding Gd-DTPA/CaP nanomicelle and bare Gd-DTPA/CaP nanomicelle on canine hemangiosarcoma model

In the next experiments, we will check the expression of integrin receptors on the surface of canine hemangiosarcoma cells on liver metastasis, and also hope to check the uptake of ^{157}Gd atoms in the cancer cells by endocytosis. We hope to apply RGD Gd-DTPA/CaP nanomicelle to liver metastatic tumors by intra-arterial injection for GdNCT, and evaluate the mechanism of cytotoxicity on GdNCT, for examples, apoptosis, autophagy, etc.

We hope to apply this delivery systems to the clinical studies of GdNCT for canine patients in the future.

Thease preliminary results indicate that RGD sequence binding Gd-DTPA /CaP nanomicelle has the possibility as novel active targeting ^{157}Gd carrier to canine hemangiosarcoma for GdNCT.

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CO7-13 **Screening of boron compounds for BNCT on 2020 International collaboration studies**

M. Takagaki[1], Kazuko Uno[2], and M. Suzuki[3]

[1]*RCNP, Osaka University*
[2]*Louis Pasteur Center for Medical Research*
[3]*IIRNS, Kyoto University*

We have been investigating boron/gadolinium compounds for screening them as a candidate for B/Gd-NCT and also to promote boron chemistry for educational purpose in worldwide.
BNCT effect for malignant brain tumors has been still controversial, and investigation for further effective boron/gadolinium compounds (B-com) has been expected national wide for accelerator based BNCT of Cancer. To promote this investigation, we will academically provide quick bioassay of new boron compounds for BNCT with easy free access. The project is supported by motivation and enthusiasm for BNCT of Kyoto University researchers for neutron irradiation experiments as well as Pasteur researchers. We hope our project might improve BNCT research for cancer patients. Our areas of research concerns are not only for BNCT, but also boron science. This project is supported by a joint use research program of Institute for Integrated Radiation and Nuclear Science, Kyoto University.

In the joint use experiment in 2020, screening experiments of 6 samples of boron and gadolinium compounds in 3 countries were investigated for B/Gd-NCT. The compound concept for brain tumors and experimental methods are described at the following URL:

Screening Protocol:
Samples are first look screening of experiments 1, 2 and 3, and they are investigated more along 4 and 5.
1. Solubility in a physiological condition
2. Cell toxicity
3. Cellular BNCT (in-vitro BNCT)
4. Bio-distribution study (neutron induced boron autoradiography)
5. Animal BNCT (in-vivo BNCT)
6. Pre-clinical study

Concept:
https://1458ab30-7501-42df-8c2e-ff59d20cecb7.filesusr.com/ugd/ddd07a_6d26937e29ee4177bfa37ea6d33de022.pdf

Method:
https://1458ab30-7501-42df-8c2e-ff59d20cecb7.filesusr.com/ugd/ddd07a_cbe194d92fd14397a5db1690d68a185c.pdf

Samples and Results:
The samples were a carborane-containing small protein docking to epidermal growth factor receptor (EGFR), gadolinium Nano inclusion bodies, and a carborane-containing glioma cells infiltration inhibitor. In each case, a higher BNCT effect than the existing BPA was confirmed, and it is planned to continue to be investigated for this fiscal year's experimental plan. The experimental results are being prepared for quick submissions and publications.

CO7-14 The evaluation of boron neutron capture therapy (BNCT) to the novel mouse model of pelvic recurrence of colorectal cancer

J. Arima[1], M. Yamamoto[1], K. Taniguchi[1,2], H. Hamamoto[1], Y. Inomata[1], A. Miyamoto[1], H. Kashiwagi[3], S. Kawabata[3], S. Miyatake[4], M. Suzuki[5], K. Tanaka[1], J. Okuda[1] and K. Uchiyama[1]

[1] *Department of General and Gastroenterological Surgery, Osaka Medical College, Osaka, Japan*
[2] *Translational Research Program, Osaka Medical College, Osaka, Japan*
[3] *Department of Neurosurgery, Osaka Medical College, Osaka, Japan*
[4] *Section for Advanced Medical Development, Cancer Center, Osaka Medical College, Osaka, Japan*
[5] *Department of Particle Radiation Oncology, Research Reactor Institute, Kyoto University, Kumatori, Osaka, 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 to pelvic colorectal cancer using the mouse model of pelvic recurrence of colorectal cancer.
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EXPERIMENTS: We used Boronophenylalanine (BPA) as a boron compound. Colon26-Luc cells were concentrated to 1.0×10^6/150μL in 0.15ml of PBS and injected into the pelvic cavity of each mouse. Animals were divided into three groups (8 animals per group); BNCT with BPA (i.p.) without tumor, only neutron irradiation, BNCT with BPA (i.p.).
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RESULTS: We performed the histopathological assessments of tumor, rectum and vagina of three groups.
In the both groups of only neutron irradiation and BNCT with BPA, tumors infiltrated to rectum and vagina. In the control group of BNCT with BPA (i.p.) without tumor, all mice did not have symptoms such as diarrhea and survived until the endpoint. It suggested that BNCT induced no side effect. (Fig.1)
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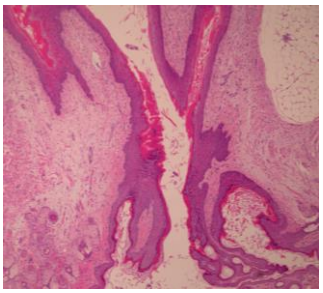
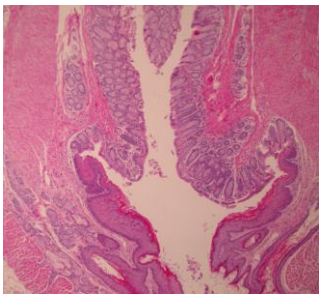
Figure.1


vagina


rectum

In the histopathological assessments of the control group, there was not structural change.

Ongoing study: We changed BALB/c mice to nude mice at the present because we would like to use a human colorectal cancer. We implanted DLD-1 clone#1-LUC to the pelvis of nude mice and observed the growth of tumors. The average overall survival was about a month. We are planning to investigate the usefulness of BNCT comparing with control. Moreover, we developed BSH-transferrin-PEG-liposome as a novel boron drug. We are planning to use this boron drug in our mouse model.
We will continue this study and the results will be published in the future.

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CO7-15 Self-assembling A6K peptide nanotubes for BSH delivery

Hiroyuki Michiue [1]*, Mizuki Kitamatsu[2], Asami Fukunaga[3], Nobushige Tsuboi[4], Atsushi Fujimura[3], Hiroaki Matsushita[3], Kazuyo Igawa [1], Tomonari Kasai [1], Natsuko Kondo[5], Hideki Matsui [1, 3], Shuichi Furuya [1]

[1]Neutron Therapy Research Center, Okayama University,
[2]Department of Applied Chemistry, Kindai University,
[3]Department of Physiology, Okayama University,
[4]Department of Neurological Surgery, Okayama University
[5]Institute for Integrated Radiation and Nuclear Science, Kyoto University,

INTRODUCTION:
Drug delivery systems (DDS) for boron agents, including polymeric macromolecule DDS such as liposomes and emulsions, which combine BSH and several boron compounds, are interesting and adaptable tools in preclinical BNCT experiments. In this work, we focused on a peptide DDS using A6K peptide. A6K comprises six alanine residues and one lysine (AAAAAAK) and has been reported as an siRNA (small interfering RNA) delivery tool. In this work we aimed to demonstrate a new boron delivery system based on A6K peptide and BSH, and open up a novel direction for boron agents in the next generation of BNCT.

EXPERIMENTS: 24 h before neutron irradiation, the U87 delta EGFR cell line was treated with 200 µM A6K/2 mM BSH or 100 µM A6K/1 mM BSH. Just before neutron irradiation, the cell samples were transferred to collecting tubes and subjected to 1 MW neutron irradiation (thermal neutron flux 1.4×109 neutron/cm2/s) for 5 min, 15 min or 30 min (Fig 1-A, C, D). In addition, U87 delta EGFR were treated with 2 mM BSH; 200 µM A6K/2 mM BSH; or 100 µM A6K/1 mM BSH (final concentrations) and subjected to 1 MW neutron irradiation at the KURNS facility for 15 min and 30 min. After irradiation, all glioma cells were re-cultured in 96 well plates (9×103 cells/well) for 24 h and 48 h, and cell proliferation was measured with Cell Proliferation Reagent WST-1 using a microplate reader (Fig.1-B). A colony formation assay was carried out after 2 weeks of culture with U87 delta EGFR in 60 mm culture dishes (n=4) and all culture cells were stained with 0.5% Crystal Violet (CV) in 20% methanol. The colonies of CV stained samples were counted automatically with an aCOLyte 3 automatic colony counter machine, and all data were statistically analyzed.

RESULTS: The U87 delta EGFR cell line was treated with 100 µM A6K/1 mM BSH or 200 µM A6K/2 mM BSH 24 h before neutron irradiation. Following 1 MW neutron irradiation, we carried out a cell proliferation assay (WST-1 assay) over 48 h and colony formation assay over 14 days (Fig. 1-A, C, D). Figure 1-B shows the WST-1 assay results for U87 delta EGFR after 24 h and 48 h, following 1 MW neutron irradiation at KURNS

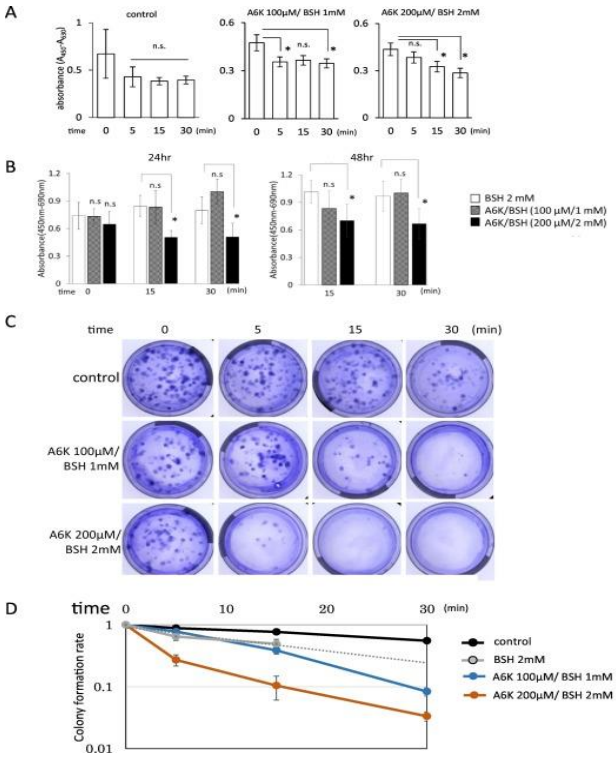

Fig. 1. **The boron neutron reaction *in vitro* with low or high dose A6K/BSH complex in U87 delta EGFR cells analyzed at KURNS: (A)(B) Cell proliferation by WST-1 assay after 24 and 48 h incubation for different neutron irradiation times (C)(D) U87 delta EGFR cell colony formation assay with CV staining after 14 days for different neutron irradiation times (black line: control group, gray line: 2 mM BSH, blue line: low dose 100 µM A6K/1 mM BSH, and orange line high dose 200 µM A6K/ 2 mM BSH, in each case n=4).**

for 15 min, or 30 min. No significant differences were observed for the control group for any radiation time (Fig. 1-A). In contrast, the 100 µM A6K/1 mM BSH and 200 µM A6K/2 mM BSH groups showed inhibition of cell proliferation dependent on neutron radiation dose and irradiation time (Fig.1-A). In addition, the colony formation assay carried out 14 days after the boron neutron reaction showed late and slow cell reaction (Fig. 1-C, D). The colony formation assay for the A6K/BSH complex groups showed survival ratios of 1, 0.78, 0.39, and 0.08 and 1, 0.27, 0.10, and 0.03, for 0, 5, 15, and 30 min neutron irradiation periods, respectively (Figure 1-C, D). The group treated with BSH only showed ratios of 1, 0.65, and 0.5 for 0, 5, and 15 min neutron irradiation periods. In the colony formation assay, the suppressive effect was clear for both A6K/BSH complex groups with thermal neutron irradiation dose escalation (Figure 1-D). Compared with the 2 mM BSH treated group, the A6K/BSH complex groups showed inhibition of cell proliferation following 15 min and 30 min neutron irradiation in both 24 h and 48 h WST-1 assays (Figure 1-B).

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R2136

CO7-16 Experiment on BNCR Effect of a Novel BPA Formulation using Ionic Liquid by Thermal Neutron Irradiation

M. Shirakawa[1,2], Y. Sato[1], N. Kamegawa[3], R. Takeuchi[3], K. Nakai[2], A. Zaboronok[2], F. Yoshida[2], T. Tsurubuchi[2], T. Takata[4], M. Suzuki[4], A. Matsumura[2] and H. Hori[3,5]

[1]*Department of Pharmaceutical Sciences, University of Fukuyama*
[2]*Department of Neurosurgery, Faculty of Medicine, University of Tsukuba*
[3] *Morita Pharmaceutical Ind., Ltd.*
[4] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[5] *Niigata University of Pharmacy and Applied Life Sciences*

INTRODUCTION: Owing to its outstanding anti-tumor effect by thermal neutron irradiation, *p*-boronophenylalanine (BPA) have been used in clinical on BNCT. However, BPA formulation dissolved in a fructose complex is known that a large dose is required for clinical use due to its poor solubility. In other words, a fructose-based BPA formulation imposes a large physical burden on the patient as well as management and transportation costs.

Therefore, we have studied the use of Ionic Liquids (ILs) as a novel solvent for BPA. ILs are composed of organic cations and anions, and have been attracting more attention in pharmaceutical fields because of their high solubility and stability [1].

We have already studied various combinations of compounds for cations and anions, some of which have resulted in high solubility exceeding that of fructose complex [2]. This paper presents the experimental results obtained on anti-tumor effect *in vivo* using BPA dissolved in ILs (BPA-ILs).

EXPERIMENTS:

1. Synthesis of ILs and Preparation of BPA-ILs

ILs using experiment of this paper were synthesized in meglumine as the cations and L-serine as the anion. Equimolar meglumine and L-serine solutions were stirred at room temperature for 24 hours, after that the water content contained within ILs was removed to 50 wt% by rotary evaporator at 100℃.

BPA-ILs was prepared by dissolving BPA in its ILs and mixing it with buffer solution (PBS), Tween 80, ethanol and citric acid.

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

The tumor-bearing mice were prepared by grafting 2 x 10^6 of mouse colon carcinoma cells (CT26) to the right thigh of female BALB/cA mice (4 weeks old, weighing 16-20 g) to have a tumor diameter of 6-8 mm. These mice were purchased at the age of 3 weeks from CLEA Japan Inc. (Tokyo, Japan) and tamed in Institute for Integrated Radiation and Nuclear Science, Kyoto University.

About 10 days after, 200μL of BPA-ILs were administrated by intraperitoneal injection before irradiation. The dosage was 24mg^{10}B/kg and similarly, BPA fructose complex (BPA-Fru) was administered. At the interval was 2hours, the irradiation was performed with thermal neutrons with a flux of 2.0-5.6 x 10^9 neutrons/cm^2/s over 12 min. The tumor size was measured over time after the irradiation until Day 29 and calculated using the general formula [3].

Also, a significant difference in tumor size on the last measurement day of each group was calculated by independent t-test. The value of the significant difference and the number of asterisks are as follows.
(* : p<0.05, ** : p<0.01, *** : p<0.005, **** : p<0.001, ns: No significant difference)

RESULTS: As shown in Fig. 1 and Fig. 2, BPA-ILs significantly suppressed the tumor growth as compared to other control groups without remarkable side effect (e.g. weight loss) similarly to BPA-Fru.

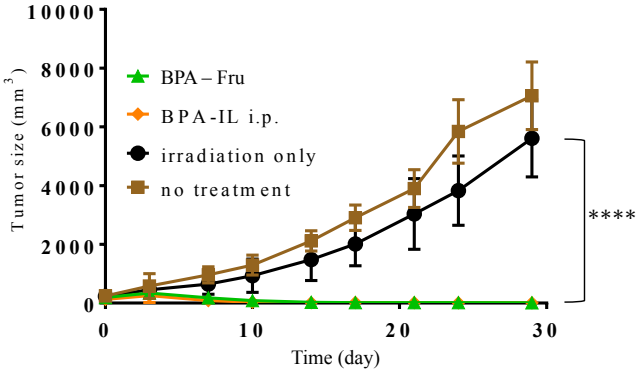

Fig.1) Anti-tumor effect of BNCT by BPA-ILs.
(BPA-ILs vs. BPA-Fru : ns, vs. irradiation only : ****, vs. no treatment : ****)

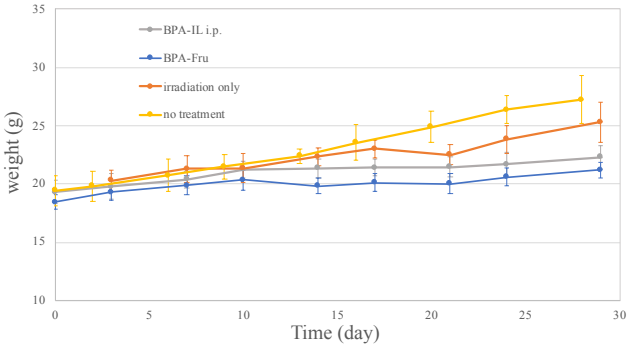

Fig.2) Body weight of mice after irradiation with the injection of BPA-ILs

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CO7-17 Application of Intra-Tumoral Injection of Gadolinium-Polyplex for Gadolinium-Neutron Capture Therapy to Pancreatic Cancer Model *in vivo*

Hironobu Yanagie[1,2,3], Yoshiteru Yanagie[4], Xuan Hou[5], Masashi Yanagawa[6], Tatsuya Ishikawa[3], Yuriko Sakurai[1,2,3], Kikue Mouri[3], Novriana Dewi[3], Yasuyuki Morishita[7], Changyuan Qin[5], Takehisa Matsukawa[8], Ayano Kubota[8], Minoru Suzuki[9], Shin-ichiro Masunaga[9], Yoshinori Sakurai[9], Hiroki Tanaka[9], Takeshi Nagasaki[10], Kazuhito Yokoyama[8], Takefumi Hirata[11], Masayuki Nashimoto[3], Jun Nakajima[2,12], Minoru Ono[2,13], Horacio Cabral[5], Takumichi Sugihara[3], and Hiroyuki Takahashi[1,2,5]

[1]Institute of Engineering Innovation, School of Engineering, Univ of Tokyo, [2]Cooperative Unit of Medicine & Engineering, Univ of Tokyo Hospital, [3]Niigata Univ of Pharmacy & Applied Life Sciences, [4]Faculty of Medicine, Teikyo University, [5]Dept of Bioengineering, School of Engineering, Univ of Tokyo, [6]Obihiro Univ of Agriculture and Veterinary Medicine, [7]Dept of Human & Molecular Pathology, Graduate School of Medicine, The University of Tokyo, [8]Department of Hygiene, Faculty of Medicine, Juntendo University, [9]Kyoto Univ Institute for Integrated Radiation & Nuclear Science, [10]Osaka City University Graduate School of Engineering, [11]Geochemical Research Center, School of Science, The University of Tokyo, [12]Dept. of Pulmonary Surgery, The University of Tokyo Hospital, [13]Dept. of Cardiac Surgery, The University of Tokyo Hospital, JAPAN

INTRODUCTION:

Gadolinium-neutron capture therapy(GdNCT) is a particle beam therapy using gadolinium and thermal neutron [1, 2, 3]. Gadolinium reacts thermal neutron and offers cytotoxic effect by 1μm-range high LET Auger electron, and long-range gamma rays. Therefore, for effective GdNCT, it is necessary to accumulate Gadolinium atoms into the tumor tissues selectively. Compared with the conventional boron NCT, because Gd compound can be used as MRI contrast agent, it allowed for MRI-guided GdNCT. In this study, we evaluated gadolinium / hyaluronic acid / protamine-mixed with cationic liposome (^{157}Gd-plex) as neutron capture therapy agent by in vivo experiment on AsPC-1 human pancreatic tumor-bearing mice.

EXPERIMENTS:

We prepared nanoparticles mixed with 1.5mL of Gadolinium compound "Gadovist" (MW: 604.71), 0.2mL of a solution of 10mg/mL-hyaluronic acid sodium, and 0.1mL of 20mg/mL of protamine incubating at room temperature for 30min. Then, these mixing solutions were poured into Coatsome EL-C. Human pancreatic cancer AsPC-1 cell was used for the *in vivo* anti-tumor effect evaluation. We prepared AsPC-1($5x10^5$) model by transplanting to right lower leg. Twelve hours after intra-tumoral injection of 0.2mL of ^{157}Gd -plex, we performed thermal neutron irradiation at Institute for Integrated Radiation and Nuclear Science, Kyoto University (average neutron fluence of 2.0×10^{12} n/cm^2). The change in tumor growth and survival rate of the mice reflected the anti-tumor effect of ^{157}Gd-plex. While measuring the size of tumor, the weight change was also recorded for evaluation of the toxicity of these samples.

RESULTS:

The ^{157}Gd concentration in the ^{157}Gd -plex was 13700ppm by measured ICP-AES, and the diameter was 200nm. Thirty percent of tumor growth suppression was achieved in the ^{157}Gd -plex injected NCT group compared with non-irradiated group. The tumor growth suppression of the ^{157}Gd -plex injected group was superior than the only Gdovist injected group by NCT.

We attempted enhancement of retention of gadolinium atoms by mixing Gd-polyplex. The experimental results showed that the tumor growth suppression of ^{157}Gd -plex-injected irradiated group was revealed superiority compared to the group with Gd solution injection, or non-treated control group after NCT, and no significant weight loss were observed after treatment suggesting low systemic toxicity of this system. We would like to consider the best irradiation time and dose of administration. The ^{157}Gd -plex will become one of the candidates for Gd delivery system on NCT. Moreover, the body weight of the mice did not decrease after the treatments, which indicate the safety of ^{157}Gd-plex and the GdNCT.

Table1. Tumor growth suppression of subcutaneous AsPC-1 model by intra-tumoral injection of ^{157}Gd-plex with thermal neutron irradiation *in vivo*

	Tumor growth rate		
	Day6	Day10	Day13
GdNCT			
^{157}Gd sol.	**1.55±0.38**	**2.29±0.67**	**2.50±0.42**
^{157}Gd-plex	**1.58±0.71**	**1.82±0.81**	**2.14±0.93**
Non-treat.	**1.62±0.06**	**2.30±0.27**	**2.64±0.07**
Non NCT			
^{157}Gd sol.	**1.79±0.34**	**3.92±1.33**	**4.64±1.64**
^{157}Gd-plex	**1.41±0.17**	**2.26±0.18**	**2.62±0.03**
Non-treat.	**1.62**	**2.02**	**3.21**

Tumor growth suppression in ^{157}Gd-plex group by NCT was 30 % superior compared with non-irradiated group. Tumor growth suppression in ^{157}Gd-plex injected group was 25 % superior compared with ^{157}Gd solution injected group in NCT groups.

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CO7-18 Functionalization of Boron-Containing Nanoparticle and its Application to Boron Neutron Capture Therapy

Yuquan Wang[1], Heon Gyu Kang[1], Masahiro Nishikawa[1], Minoru Suzuki[2] and Komatsu Naoki[1]

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

INTRODUCTION: Boron neutron capture therapy (BNCT) is one of the promising cancer therapy with minimized side effect, because ^{10}B atoms located in the cancer tissue generate alpha particles locally upon neutron irradiation. Herein, we will report that boron-containing nanoparticle (B-NP) is functionalized with polyglycerol (B-NP-PG) and the resulting B-NP-PG was employed for the pharmacokinetic experiments to make clear the biodistribution of B-NP-PG.

EXPERIMENTS: B-NP was functionalized with PG according to the method previously reported by us [1-3]. The resulting B-NP-PG was fully characterized by nuclear magnetic resonance (NMR) spectroscopy, Fourier transfer infrared (FTIR) spectroscopy and thermogravimetric analysis (TGA).

PBS dispersion (200 μL) of B-NP-PG was intravenously injected to the BALB/c tumor-bearing mice from the tail vein. After the mice were euthanized, the ^{10}B contents were assessed in blood, tumor and organs (liver, spleen and kidney) by the prompt γ-ray microanalysis system.

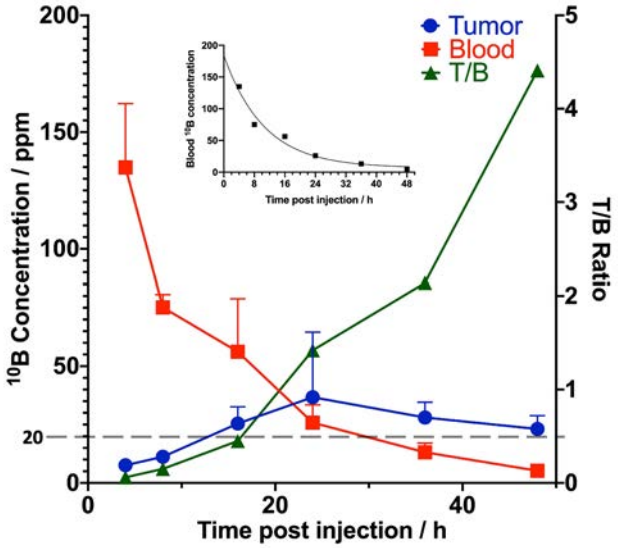

Figure 1. ^{10}B concentrations in tumor (T) and blood (B), and the T/B ratio. Data are presented as mean ± SD. Half-life (7.3 h) is calculated by one phase decay fitting of blood ^{10}B concentration shown in the inset.

RESULTS: B-NP-PG is dispersed in a phosphate buffer saline (PBS) at very high concentration and the resulting dispersion is very stable for more than one month.

While the enough ^{10}B concentration level for BNCT is kept in tumor for 30 h from 18 h to 48 h, the ^{10}B concentration in blood decreases gradually with the half-life of 7.3 h as shown in Figure 1. Accordingly, the T/B ratio increases with time and reaches 4.4 at 48 h which fulfills the requirement 3) (T/B ratio ≥ 3). After evaluation of these pharmacokinetic data based on the requirements, we decided the timing of neutron irradiation to be at 24 and 48 h after B-NP-PG administration exhibiting the highest ^{10}B tumor concentration (36.7 ppm) and T/B ratio (4.4), respectively [3].

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CO7-19 Basic research to expand the application of BNCT to companion animals.

Y. Wada[1,2] and M. Suzuki[2].

[1] Veterinary Medical Center, Osaka Prefecture University
[2]*Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University*

Introduction

In veterinary medicine, radiation therapy is often used to treat head and neck tumors in recent years. However, in most cases, re-growth is observed. Therefore, it is desirable to expand the indications for BNCT in the veterinary field. Squamous cell carcinoma of the canine oral cavity is one of the diseases that can be treated with radiation therapy. Squamous cell carcinoma in the oral cavity has a different behavior between tonsillar and non-tonsillar origin.

Tonsillar squamous cell carcinoma (TSCC) has been demonstrated as not only locally invasive, with extension into surrounding tissues commonly seen at the time of diagnosis。Although surgery, chemotherapy and radiotherapy seem to increase the median survival time of dogs diagnosed with tonsillar squamous cell carcinoma, there is no highly effective treatment for canine TSCC. Non-tonsillar oral squamous cell carcinoma (NTSCC) shows good results when combined with surgery and radiotherapy, but there are many cases where surgery cannot be performed and only radiotherapy can be performed. However, in many cases with radiotherapy alone results in recurrent tumor. The purpose of this study is to investigate whether canine TSCC and NTSCC are eligible for BNCT.

Experiments

TSCC (TSCCLN-5) cell line and NTSCC (oSCC-4) was used for this experiment. First, a chlorogenic assay was performed to confirm the radiation response to X-rays in vitro. In addition, the cells were incubated under 50 ppm BPA for 2 hours and then irradiated with neutrons for clonogenic assay to check the response of the cells to BNCT.

Result

As shown in Fig. 1, the survival rate was significantly lower in oSCC at 2, 4, 8, and 12 Gy of X-ray irradiation**.** As shown in Fig. 2, BNCT-induced cell death was higher in oSCC than in TSCCLN.

In the future, we plan to investigate the uptake of BPA into cells and the expression of LAT-1, and to study the response in vivo.

Fig.1

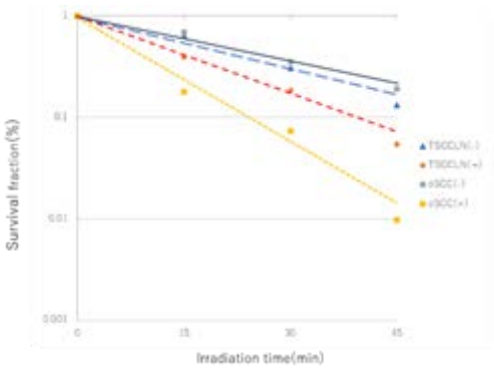
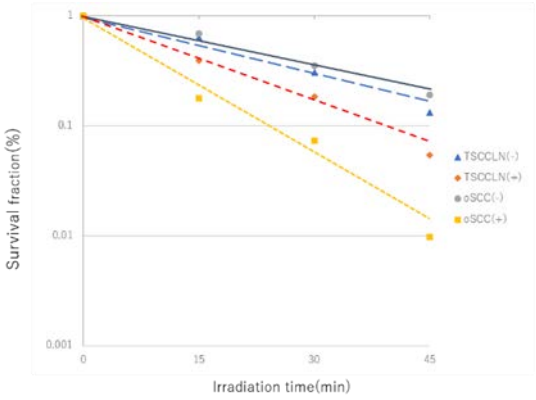


Fig. 2



R2151

CO7-20 Development of real-time boron-concentration estimation system for whole-organ-irradiation BNCT

Y. Sakurai, H. Matsunaga[1], T. Takata, H. Tanaka and M. Suzuki

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

INTRODUCTION: Whole organ irradiation in radiotherapy is effective on multiple tumors and minimal residual tumors, such as whole brain irradiation for brain metastasis, whole conserved-breast irradiation in conservative therapy for breast tumor, etc.. However, it cannot be realized for liver and lung tumors. It is because the tolerant dose of these organs is 20 to 40 Gy, which is smaller than the tumor control dose of 50 to 80 Gy. In BNCT, the ranges of the heavy charged particles due to the $^{10}B(n,\alpha\gamma)^{7}Li$ reaction in the boron compound, which selectively accumulates in tumor cells, are too short to reach the adjacent normal cells. Therefore, BNCTs with the whole organ irradiation for liver and lung tumors can be realized as radiotherapy.

In BNCT clinical studies for liver and lung tumors at KUR, the equivalent doses for normal liver and/or lung have been estimated on the basis of boron concentration in blood, which is measurable by prompt gamma-ray analysis. But actually, the variation for the boron concentration in normal liver and/or lung is assumed to be larger among the patients with basal disease, such as cirrhosis, pulmonary fibrosis, pulmonary emphysema, etc..

The purpose of this research is the development of the real-time boron-concentration estimation system in liver and/or lung during BNCT, in order to improve the dose estimation accuracy for BNCTs with the whole-liver and/or whole-lung irradiation. In this system, the technique of prompt gamma-ray telescope is applied. A gamma-ray telescope system has been in use for liver tumor BNCT at Heavy Water Neutron Irradiation Facility of KUR (KUR-HWNIF) from 2005 [1]. The collimation system of this telescope was improved in 2017 [2].

In 2020, verification experiment for the discrimination-ability between tumor and normal parts using this telescope system were performed.

MATERIALS AND METHODS: A liquid rectangular phantom of 20 cm in width, 20 cm in length and 40 cm in depth was used in the experiment. An acrylic hollow sphere of 5 cm in outer diameter, which was filled with boric acid of 193 ppm for B-10 concentration, was placed as a tumor part in the phantom. The phantom liquid was pure water or boric-acid water of 23 ppm for B-10 concentration. The epi-thermal neutron irradiation was performed in the irradiation field of 12 cm in diameter. The initial position of the tumor-sphere center was settled to be the center for the telescope-view-field on the beam axis, and it was moved from 0 to 6 cm in the right direction for the view from the beam-aperture side.

The positions of two telescope collimators were fixed to be the lowest. At these positions, the effective telescope-view-field on the beam axis was within 3 cm in width in the right direction for the view from the beam-aperture side. The prompt gamma rays due to the neutron reactions with B-10 and hydrogen (H-1) from the tumor part and its surroundings during the epi-thermal neutron irradiation were counted.

RESULTS AND DISCUSSION: Figure 1 shows the relationship between the position of the tumor-sphere center and the count ratio for B-10 gamma rays to H-1 gamma rays (B/H count ratio). It was confirmed that the B/H count ratio was larger as the position of the tumor-sphere center was closer to the beam axis, namely as it was closer to the center of the effective telescope-view-field. It was also confirmed that the B/H count ratio was larger for the boron-acid water phantom than for the pure water phantom. It is because that the B-10 gamma rays from the boric-acid water of 23 ppm for B-10 concentration in the telescope view-field additionally contribute to the B-10 gamma-ray count.

CONCLUSION: The B-10 concentration ratio for the tumor sphere to the boron-acid water phantom was 8.4 in this experiment. For such a level of the concentration ratio, the discrimination between tumor and normal parts can be expected by comparing the count ratio between the cases with and without the tumor part in the telescope view-field. In the actual BNCT clinical study, the B-10 concentration of the normal part is 10 to 25 ppm, and that of the tumor part is more than three times larger. In future, the more precise estimation will be performed for the B-10 concentration, size and position of the tumor sphere, and for the position of two telescope collimators. Moreover, the effective range for the discrimination between tumor and normal parts will be clarified.

ACKNOWLEDGEMENTS: This study was supported by The Kyoto University Research Fund (Core Stage Back-up, in 2017).

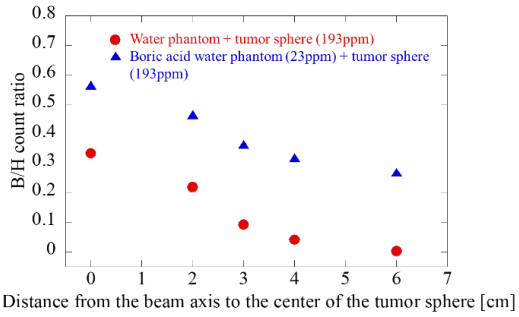


Fig. 1. Relationship between the center position of the tumor sphere and the B/H count ratio.

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CO7-21 Development of ^{10}Boron-loaded silica nanoparticles for BNCT application

F. Tamanoi[1], K. Matsumoto[1] and M. Suzuki[2]

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

INTRODUCTION: Boron neutron capture therapy (BNCT) was proposed as novel type of radiation therapy in 1936 and has been investigated in a variety clinical studies against brain cancer, head and neck tumor or other type of cancer. Boron phenylalanine (BPA) is a useful boron compound which advanced BNCT therapy yielding promising results. However, new types of boron reagents with increased tumor accumulation and less toxicity are being sought by a number of groups. We have developed biodegradable periodic mesoporous organosilica (BPMO) based reagents which have a large surface area where BPA can be attached for BNCT application.

To investigate the potential of BPA-loaded BPMO (BPA-BPMO) for BNCT, we used chicken chorioallantoic membrane model (CAM model) which is generated by transplanting human cancers onto the CAM membrane. This assay is versatile and convenient. After intravenous injection of BPA-BPMO to the blood stream, chicken eggs were irradiated with neutron beams to evaluate the tumor growth inhibition effect. Dramatic inhibition of tumor growth was observed.

EXPERIMENTS: BPMO was synthesized by sol-gel synthesis of two precursors, bis[3-(triethoxysilyl) propyl] tetrasulfide and 1, 2-bis(triethoxysilyl) ethane. This resulted in the incorporation of tetrasulfide bonds into the framework of the nanoparticles. BPMO was then processed to modify with GOPTS (3-glycidyloxypropyl trimethoxysilane) that contain epoxy groups. After converting epoxy groups to diols, BPA was mixed with diol-BPMO to graft BPA using a method to chelate boron with diol groups.

To investigate how BPA-BPMO behaves in biological systems, we evaluated cellular uptake of BPA-BPMO to cancer cells. Various types of cancer cells such as FaDu human head and neck cancer, OVCAR8 human ovarian cancer and A549 human lung cancer, were used. They were incubated with BPA-BPMO for 24 h at 37℃ humidified CO_2. Cellular uptake of BPA-BPMO was observed by confocal microscopy. We then used the CAM model to gain insight into tumor accumulation of BPA-BPMO. 2.0×10^6 of OVCAR8 cells were transplanted onto CAM of fertilized chicken egg to form tumor. 0.2 mg of BPA-BPMO was intravenously injected into the chicken egg, and tumor accumulation of BPA-BPMO was examined by red fluorescence of Rhodamine B-labeled BPMO by stereomicroscopy and confocal microscopy.

We finally tested whether the growth of the CAM tumor can be inhibited the neutron exposure. After injection of BPA-BPMO to chicken eggs that have OVCAR8 tumor on CAM, the eggs were placed at the center of the emerging neutron beam. Eggs 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℃ with 65% humidity. Tumors were then cut out to evaluate the tumor size and weight.

RESULTS: BPMO synthesized had approximately 200 nm of diameter and homogenous shapes examined by SEM and TEM microscopy. BPMO had 985.96 m^2/g of surface area, 0.557 cm^3/g of pore volume and 2.34 nm of pore diameter as determined by nitrogen adsorption-desorption analysis. FT-IR analysis showed diagnostic peaks of typical Si-O-Si, -C-S-, $-(CH_2)_2-$ and $-CH_2-$ vibrations. After loading of BPA to BPMO, we analyzed surface charge of BPA-BPMO which was negative due to modification with phosphonate. The zeta potential of BPA-BPMO was -42.48 mV.

Cellular uptake of BPA-BPMO to various types of cancer cells was observed. From confocal microscopy and flow cytometry analysis, the red fluorescence of BPA-BPMO could be observed inside these cells and was localized at the perinuclear region. These results are consistent with our previous observation that mesoporous silica-based nanoparticles are efficiently taken up by endocytosis system [1]. We also observed tumor accumulation of BPA-BPMO on CAM model. Significant red fluorescence of BPA-BPMO was detected in the tumor that was formed using GFP-expressed OVCAR8; overlapping of red and green fluorescence was detected. The red fluorescence of BPA-BPMO was also detected in liver and kidney, but the level of fluorescence was less than that seen in the tumor.

We finally investigated tumor growth inhibition effect on BPA-BPMO. After injecting BPA-BPMO, the eggs were subjected to neutron irradiation. As seen in Figure 1, the weight of tumor after neutron irradiation exposure with the BPA-BPMO-administered samples were around 12 mg, while the tumor weight of control (no injection or empty BPMO injection) was around 60 mg. 80% inhibition was observed by BPA-BPMO injection. Thus BPA-BPMO exhibits efficacy in BNCT. These results are published. Mouse experiments to follow up this study are ongoing.

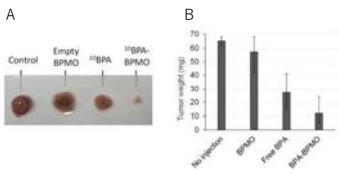

Figure 1. Tumor growth inhibition by neutron irradiation. (A) Pictures of CAM tumors after neutron irradiation; (B) Tumor weight mesurment

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CO7-22 Three dimentional model for pre-clinical investigations in BNCT

K.Igawa, [1]A.Sasaki, [2]K.Izumi, [2] E.Naito, [3]M.Suzuki, [3]N.Kondo, [3] Y.Sakurai

Neutron Therapy Research Center, Okayama University
[1]Department of Oral Maxillofacial Surgery, Okayama University
[2]Graduate School of Medical and Dental Sciences Oral Life, Niigata University
[3]Research Reactor Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: In Boron Neutron Capture Therapy (BNCT), the Boron dose and neutron dose in tumor cells are the key factors. The development of boron agents for BNCT requires the evaluation system bridging the gap between *in vivo* and *in vitro* research. Also, from the viewpoint of the animal welfare and the 3Rs: Replacement, Reduction and Refinement, the humane alternative research methods that do not use animals, are required for BNCT. On the other hand, three-dimensional (3D) models have been widely used in cancer research due to their ability to mimic multiple features of the tumor microenvironment [1]. Therefore, the validity of the 3D cancer model for pre-clinical investigations in BNCT is examined in this study.

EXPERIMENTS: First, we measured the distribution of neutron irradiation dose in an acellular collagen gel on a 6 wells plate, identical to the culture vessel to fabricate the oral cancer 3D model. The neutron fluence on six different locations of a 6 well plate was measured using the gold foils. Based on this mock irradiation, the optimal irradiation time for 3D model is calculated. For the development of oral cancer 3D model, the oral mucosal fibroblast cells （Niigata University）and oral squamous cell lines（SAS, JCRB） were embedded and cultured on type I -A collagen (Niita-gelatin, Japan) in αMEM medium (Wako, Japan) supplemented with 10 % fetal bovine serum (Sigma -Aldrich, USA) and 100 unit/ml penicillin and 100 μg/ml streptomycin (1% p/s) (Thermo Fisher Scientific, USA) [2,3]. Before neutron irradiation, Boron (Steboronine®, Stella pharma, Japan) or Phosphate Buffered Saline（PBS, Sigma -Aldrich, USA）was added to the oral cancer 3D model and incubated for 2 hour. After washing by PBS, the 3 D models were irradiated by neutron and cultured for another 7 days (Fig. 1). The 3D models were fixed with 10 % formalin, embedded in paraffin, cut in 5 μm sections and stained with hematoxylin and eosin.

RESULTS: The neutron fluence at six various points of a 6 wells plate was measured using the gold foil as shown Fig.2. The mean value of neutron fluence on the top and bottom of a 6 wells plate (1.42×10^{12} cm^{-2}) is almost same value in the collagen gel (①, ②). Therefore, the neutron dose in 3D model is decided to measure on the top and bottom of a 6 wells plate to keep sterilization.

The histological examinations of the oral cancer 3D model after boron neutron capture reaction revealed that the epithelial thickness of cancer cell layers on the top of the fibroblasts-embedded collagen gel was thinner, compared with control as shown in Fig.3. However, the thickness of fibroblast-embedded collagen gel did not differ between the two. These results indicate the 3D model would be an alternative tool to animal test for boron neutron capture reaction.

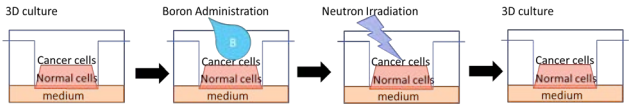
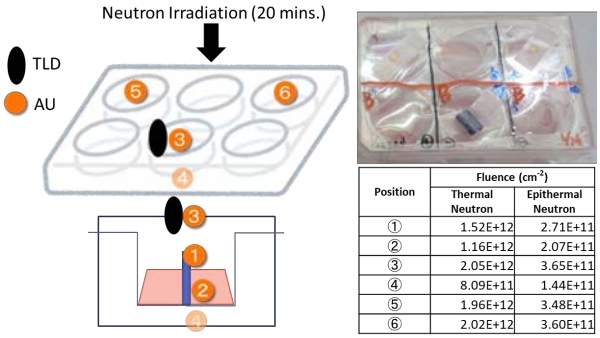


Fig. 1. The 3D model evaluation system for BNCT.



Position	Fluence (cm^{-2})	
	Thermal Neutron	Epithermal Neutron
①	1.52E+12	2.71E+11
②	1.16E+12	2.07E+11
③	2.05E+12	3.65E+11
④	8.09E+11	1.44E+11
⑤	1.96E+12	3.48E+11
⑥	2.02E+12	3.60E+11

Fig. 2. The measurement of dosimetry in 3D model.

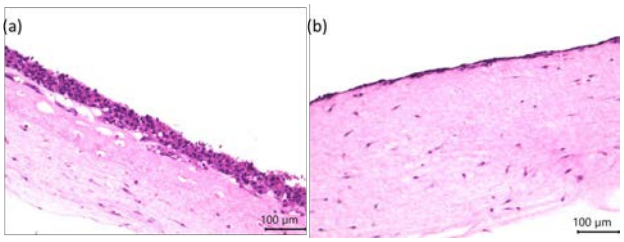


Fig. 3. Histology of the oral cancer 3D model at 7 days after neutron irradiation. Before neutron irradiation, (a) PBS or (b) Boron was injected into the 3D model.

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CO7-23

A novel carbon nanohorns for BNCT *in vitro* study

T. Tsurubuchi[1], F. Yoshida[1], A. Zaboronok[1], K. Nakai[1,2], M. Shirakawa[3], M. Suzuki[4]

Graduate School of Science, Kyoto University
[1] Department of Neurosurgery, Faculty of Medicine, University of Tsukuba
[2] Department of Radiation Oncology, Faculty of Medicine, University of Tsukuba
[3] Department of Pharmaceutical Sciences, University of Fukuyama
[4] Institute for Integrated Radiation and Nuclear science, Kyoto University

INTRODUCTION:

Only p-boronophenylalanine (BPA), sodium borocaptate (BSH) already have been used in Boron Neutron Capture Therapy (BNCT) trials as boron compounds. However, to keep enough tumor to normal concentration ratio of boron for effective BNCT, BPA and BSH usually need to be continuously administrated to the patient just before neutron irradiation. Less toxicity, more accumulation and retention in tumor cells but not in the normal cells, and clear washout from the normal tissue are the important points for newly boron comounds for BNCT.

We developed the BN-CNH was coated with phospholipid polyethylene glycol having folate, BN-CNH/PLPEG-FA (Iizumi 2015) called carbon nanohorns.

The previous experiment data (Tsurubuchi 2019) showed carbon nanohorns effectively suppressed the colony forming units of GL261 mice glioma cells. We considered whether carbon nanohorns accumulated in other types of tumor cells. To assess the therapeutic effects of carbon nanohorns in BNCT, we tried another irradiation study in vitro in the Kyoto University Research Reactor (KUR).

EXPERIMENTS: This time, CT26, mice colon tumor cells were used for colony formation test. The $1*10^6$ cells were plated on wells of 6 well plate 24 hours before experiment. The drug, carbon nanohorns were added to each well 24 hours before irradiation. The BPA were added 2 hours before irradiation. The control group was radiation only group without drug. For counting plating efficiencies (PE), each group had only CT26 tumor cells without treatment. The cells were trypsinized and counted. The irradiation was performed with thermal neutrons with a flux of 1.6×10^{12} neutrons/cm^2 over 15min at the KUR. The 200 cells per dish were plated respectively. PE were checked after counting colonies on day 14. The colony forming units were calculated as mean colony counts devided by PE. This time carbon nanohorns only contains natural boron.

RESULTS: The carbon nanohorns group showed better tumor suppression compared to irradiation only group (Figure 1). Wash treatment did not reduce colony form-ing units. The BPA group showed better tumor suppres-sion effect compared to the carbon nanohorns group. Considering our carbon nanohorn consisting of natural boron, the carbon nanohorns group showed the same tu-mor suppression effect compared to the BPA group. The irradiation experiment using carbon nanohorns showed enough tumor suppression effect independent on the dif-ferent kinds of tumor cells.

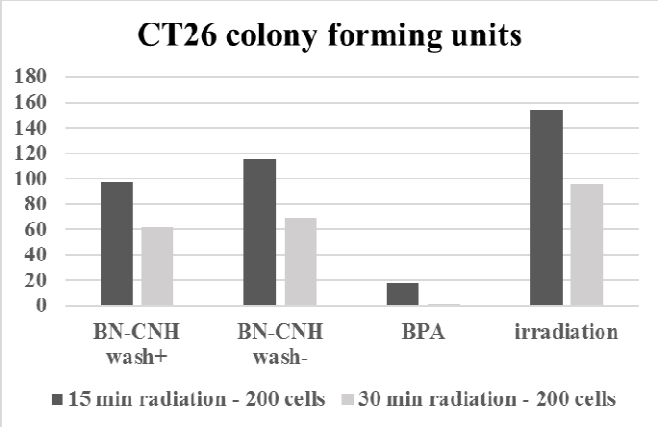

Figure 1 The results of CT26 colony forming units

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CO7-24 Development of ^{10}BPA-loaded mesoporous silica-based nanoparticles and preliminary evaluation in BNCT mouse experiments

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

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

INTRODUCTION: Boron phenylalanine (BPA) has been developed as useful boron compound which is available for Boron neutron capture therapy (BNCT) therapy. We have recently developed two types of mesoporous silica-based nanoparticles that are loaded with BPA. One is mesoporous silica nanoparticles loaded with BPA. Another is biodegradable periodic mesoporous organosilica (BPMO) loaded with BPA. These nanoparticles have a large surface area where BPA can be attached for BNCT application. We have found and reported that BPA-BPMO exhibits efficacy in BNCT experiments which used chicken. chorioallantoic membrane model (CAM model) [1]. In this study, we carried out experiments to prepare for mouse experiments to evaluate their BNCT efficacy.

EXPERIMENTS: MSN was synthesized by sol-gel synthesis employing TEOS as a precursor. 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. MSN and BPMO were then processed to modify with GOPTS (3-glycidyloxypropyl trimethoxysilane) that contain epoxy groups. After converting epoxy groups to diols, BPA was mixed with diol-BPMO to graft BPA using a method to chelate boron with diol groups. The synthesized nanoparticles were characterized by using SEM, TEM, FT-IR, nitrogen adsorption-desorption analysis and zeta potential. The amount of boron attached on the nanoparticles was examined by ICP, and boron content was determined.

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

Irradiation of mice with neutron beams was carried out by placing mice in specially prepared holders. Irradiation was carried out with thermal neutron for 12 min at an operating power of 5MW. After the irradiation, mice were returned to cage, and tumor size was longitudinally and transversely measured every 2 days for up to 25 days.

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

Boron accumulation in the CT26 tumor was investigated with ICP. We were able to achieve delivery of boron in the tumor but the route of delivery needs to be further investigated. We also observed accumulation of BPA-BPMO in the tumor by confocal microscopy. When BPA-BPMO was injected to tumor, the red fluorescence of BPA-BPMO could be observed in the tumor. However, the distribution was not even. We are further investigating the optimum condition for BPA-BPMO accumulation in the tumor.

Investigation of tumor growth inhibition effect of BPA-loaded nanoparticles is currently ongoing. After injecting the nanoparticles, all mice are subjected to neutron irradiation and tumor growth is followed for 25days. BNCT efficacy of BPA-loaded nanoparticles is compared with that of free BPA.

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CO7-25 Effects of overexpression of *LAT1* on suppression of tumor growth by boron neutron capture therapy

K. Ohnishi[1], M. Misawa[2], N. Sikano[3] and M. Suzuki[4]
Departments of [1]Biology, [3]Radiological Science, Ibaraki Prefectural University of Health Sciences
[2]National Institute of Advanced Industrial Science and Technology
[4]Institute for Integrated Radiation and Nuclear Science, Kyoto University

INTRODUCTION: Outcome from BNCT largely depends on amount of intracellular accumulation of boron compound. L-type amino-acid transporter 1 (LAT1) [1], through which boronophenylalanine (BPA) is transported into cells, is frequently expressed in various types of tumor cells including glioblastoma but not in normal cells [2]. We transfected *pCMV/LAT1-GFP* plasmids into a glioblastoma cell line, T98G, and selected several clones and confirmed that those clones stably overexpress LAT1 in cell membrane with confocal laser microscopic observation and western blot analysis. Our *in vitro* neutron irradiation experiments using LAT1 overexpressing clone showed that overexpression of LAT1 in cancer cells causes enhanced anticancer effects of BNCT and BNCT combined with gene therapy is beneficial for tumors bearing low LAT1 expression [3]. In this study, we transplanted LAT1 overexpressing clone (T98G/K4) into nude mice and effects of neutron irradiation on tumor growth were preliminary examined after ^{10}BPA treatment.

EXPERIMENTS: We transplanted minced tumors formed with LAT1 overexpressing clone (T98G/K4) into femoral area of nude mice. Accumulated amounts of ^{10}BPA in blood and tumor were measured using prompt gamma-ray assay (PGA) on 1 h after ^{10}BPA s.c. injection (100 mg/kg, 1 h before irradiation). The transplanted tumors in 4 mice were irradiated with thermal neutron beam at the fluences of 3.3x 10^{12} n/cm^{-2} on 1h after the ^{10}BPA injection.

RESULTS: Accumulated amounts of ^{10}BPA in blood and tumor were 7.2 ppm and 19.4 ppm, respectively. The relative tumor volumes in ^{10}BPA-treated 3 mice and ^{10}BPA-nontreated 1 mouse were 0.049, 0.127, 0.586 and 1.830, respectively on 24th day after the irradiation.

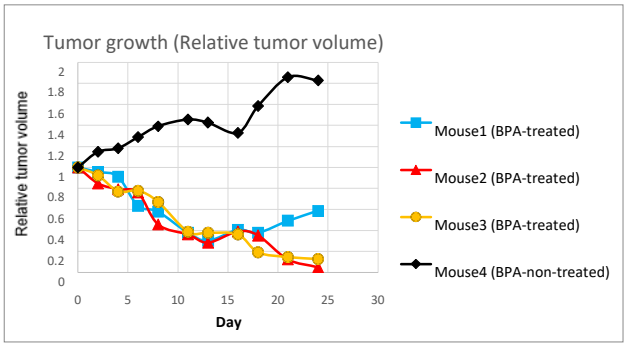

Fig. 1. Tumor growth curves of LAT1 overexpressing tumors (T98G/K4) in ^{10}BPA-treated 3 mice and ^{10}BPA-non-treated 1 mouse.

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

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[3] K. Ohnishi *et al.*, Radiat. Res., in press.

CO7-26 Synthesis of a Novel Boron Compound with Potential Peptide-Related Nuclear Import

M. Shirakawa[1,2], A. Okuda[3], A. Zaboronok[4], Y. Sato[1], A. Shigenaga[1], N. Kamegawa[5], R. Takeuchi[5], H. Hori[5,6] and M. Sugiyama[3]

[1]*Department of Pharmaceutical Sciences, Fukuyama University*
[2]*Department of Radiation Oncology, Faculty of Medicine, University of Tsukuba*
[3] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[4] *Department of Neurosurgery, Faculty of Medicine, University of Tsukuba*
[5] *Morita Pharmaceutical Ind., Ltd.*
[6] *Niigata University of Pharmacy and Applied Life Sciences*

INTRODUCTION:

The therapeutic effect of BNCT is provided by the alpha -rays generated in the ^{10}B 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 ^{10}B is present in or near the nucleus of the tumor cell [1].

Therefore, we developed a peptide-based carrier that can efficiently and selectively transport boron in the nucleus of the tumor cell. This article presents the obtained experimental results of the synthesis of a novel boron peptide combined with the Cell Penetrating Peptide (CPP), Nuclear Localization Signal (NLS), and the membrane fusion peptide.

EXPERIMENTS:

Boron peptides were combined with mercaptododecaborate (BSH) and two functional peptides, and the synthesis was performed on Fmoc-Lys(ivDde)-wang resin by the Fmoc solid-phase method [2]. As a peptide sequence, we selected two of TAT (GRKKRRQRRRPQ), H5 (HHHHH), H16 (HHHHHHHHHHHHHHHH), R8 (RRRRRRRR), c-Myc (PAAKRVKLD), GALA (WEAALAEALAEALAEHLAEALAEALEALAA) and synthesized them by inserting a linker between the two peptides. After that, BSH was conjugated with the peptide C-terminal region.

Each amino acid coupling reaction was carried out for 3 min at 90℃ using a microwave. 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 drying procedure, we obtained the desired compound.

RESULTS: As shown in Fig. 1 and Fig. 2, we succeeded in synthesizing the novel boron peptide (e.g., TAT-GALA-BSH). In the identification of this boron peptide, the Ultra Performance Liquid Chromatography (UPLC) analysis showed almost one peak (retention time at 4.72min), and the substance was confirmed to be of high purity (96%). Also, MALDI-TOFMS (Matrix Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry) showed *m/z* 4978 of the peak as an exact mass of *m/z* 4978.

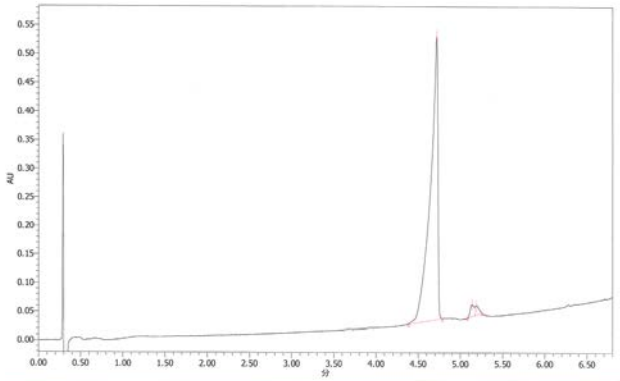

Fig.1) Purity of TAT-GALA-BSH by UPLC

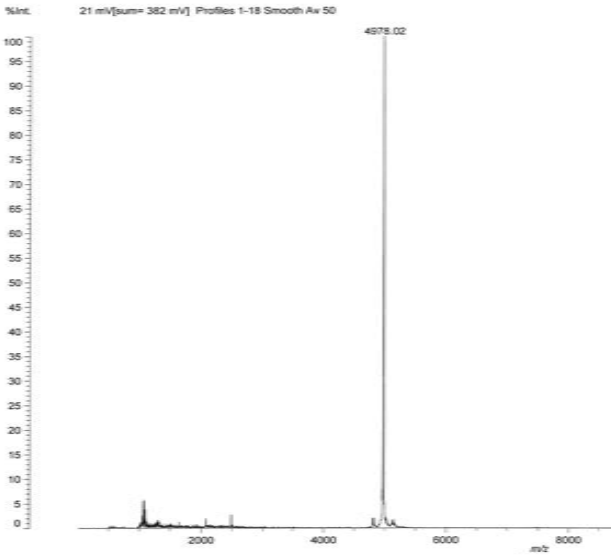

Fig.2) Identification of TAT-GALA-BSH by MALDI-TOFMS.

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