CO7-1 Establishment of protocol for neutron capture therapy for head and neck cancer

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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 can not 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 2 patients and undertook BNCT during this period as follows:

Patient #1: 66 y.o. male
Recurrence of oropharyngeal carcinoma
Histology: squamous cell carcinoma
Effect: SD
SAE: none; oral mucositis, Grade 2

Patient #2: 61 y.o. male
Recurrence of unknown head and neck carcinoma
Histology: squamous cell carcinoma
effect: PR
As an Severe Adverse Event (SAE) on Patient #2, on the second day after BNCT treatment, a cardiopulmonary arrest was detected at midnight, and cardiopulmonary resuscitation was started immediately. Fortunately, cardiopulmonary function recovered, but post-resuscitation encephalopathy was noted, and treatment is currently underway. The direct involvement of BNCT is unknown.

CONCLUSION:
We will continue to accumulate the cases carefully to establish a safe and stable treatment of BNCT.

CO7-2 Adjuvant therapy with BNCT for advanced or recurrent Head and Neck Cancer

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INTRODUCTION

BNCT has been undergone to advanced head and neck (AHNC) cancer and recurrent head and neck cancer (RHNC). Most patients developed, lymph or distant metastasis within 6 months after BNCT [1]. BNCT has achieved a good local control, however patient's survival rate has never been good. That's why these cancer cells have already spread to another organ at the first of BNCT [2]. We report the therapeutic effect and safety of BNCT combined with systemic therapy.

METHODS AND MATERIALS

11patients were treated by BNCT with BPA [3] at KURNS (Institute for Integrated Radiation and Nuclear Science, Kyoto University) between Sep. 2017 and Feb. 2019. All patients were taken F-BPA-PET to estimate the tumor/blood boron ratio (T/B ratio), and these values ranged from 2.5 to 6 (median 3.2). In eight out of 11 patients, systemic therapy was initiated as neoadjuvant therapy prior BNCT. Systemic therapy was administered for 0.7-17.2(median 10.2) months. Most patients, six out of 11, were administered nivolmab as adjuvant therapy. Cetuximab, TS-1 and Immunotherapy were used to three, one and one patient respectively.

RESULT

Follow-up time was 1.8-16 (median 5.2) months. Local recurrence appeared in three patients out of 11 patients in 2.8-12.5 months (median 4.5) after BNCT. Local relapse developed in BNCT field without findings of any lymph node or distant metastasis. Eight out of 11 patients have achieved good local control and good QOL. Two of 4 relapse patients were taking cetuximab and other two patients were taking nivolmab. The relapse patients using nivolmab had had already resistance to nivolmab, though, they were kept taking nivolmab because had no other medical therapy to be chosen. Five patients, the rest of patients who took nivolmab, were controlled.

Adverse effect was not enhanced by systemic therapy. Severe adverse effect which is more than grade 3 on CTCAE v4.0 had never seen after BNCT. No patients died while the follow-up period.

CONCLUSION

BNCT combined with adjuvant anti-cancer therapy, such as chemotherapy, molecular-targeted drug or immunotherapy, was safe and has possibility of improving the therapeutic effect and survival rate. Especially, BNCT combined with nivolmab seems to be good combination for the control of cancer, when tumor has good response to nivolmab.

Patients characteristic	
Number of patients	11
Age (median)	48.5 y.o. (34-75 y.o.)
Gender	
Male	5
Female	6
Pathlogical type	
SCC	10
Mucoepidermoid carcinoma	1
Prior treatment	
Operation	6
Chemotherapy	10
Radiation therapy	11
Disease Presentation	
Local recurrence	8
Cervical lymph node metastasis	3
Systemic therapy	
Nivolmab	6
Other	5
Result	
Controlled	8
Relapse	3
Metastasis	0

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CO7-3 Synthesis and Biological Evaluation of *closo*-Dodecaborate Ibuprofen Conjugate (DIC) as a New Boron Agent for Neutron Capture Therapy

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INTRODUCTION: The icosahedral symmetrical cluster, *closo*-dodecaborate, contains twelve boron and twelve hydrogen atoms. The sodium form of *closo*-dodecaborate is water-soluble and has low toxicity. Indeed, mercaptoundecahydrododecaborate (BSH; $[B_{12}H_{11}SH]^{2-}$) was developed as a boron agent and used to treat brain tumors via boron neutron capture therapy (BNCT) for many years. We previously developed maleimide-containing *closo*-dodecaborate (MID) that was conjugated not only with cysteine residue–SH but also with lysine residue–NH_2 in proteins.[1,2] We focused on serum albumins, which are essential transporter proteins for many drugs and endogenous compounds, using these as boron carriers for BNCT. As expected, the MID albumin conjugate (MID-AC) accumulated selectively in mouse tumors. It is known that human serum albumin has two primary drug binding sites (i.e., sites 1 and 2) located in subdomains IIA and IIIA. Ibuprofen exhibits high affinity to human serum albumin (HSA) via binding to site 2, and we believe that the ibuprofen *closo*-dodecaborate conjugate will be a novel boron agent, binding to human serum albumin at site 2 for delivery to tumors. In this study, we report the design and synthesis of *closo*-dodecaborate ibuprofen conjugate (DIC) **1** (Figure 1).

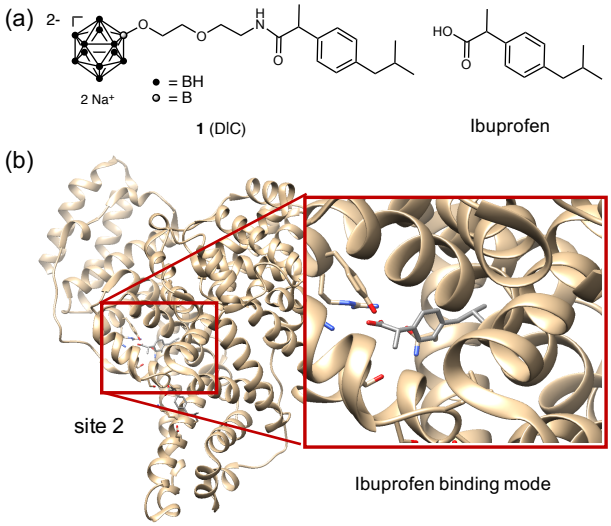

Fig. 1. (a) Chemical structures of ibuprofen and *closo*-dodecaborate ibuprofen conjugate (DIC) **1**. (b) Ibuprofen-human serum albumin (HSA) cocrystal structure (PDB: 2BXG). Ibuprofen binds to site 2 of HSA.

EXPERIMENTS:
Human cervical cancer HeLa cells were seeded at a density of 8×10^5 cells/ml with media 1 mL in the 6 well plate dishes, and incubated for 12 h. Each sample solution was added to a final 300 ppm boron concentration, and the cells were incubated for 1, 3, and 6 hours. Then the medium was removed and cells were washed three times in the dish with 1 mL of PBS buffer. A mixed solution of $HClO_4$ and H_2O_2 ($HClO_4$: H_2O_2 = 1: 2, 2 mL) were added to dishes and the cells were collected. The resulting solution was heated at 70 °C for 1 h. and filtered through 500 nm hydrophobic filter. The boron concentration of the obtained solution was measured by ICP-AES.

RESULTS: The cell uptake of DIC by HeLa cells was examined. The cells were incubated with boron agents for 1-6 h at a 300 ppm boron concentration with and without fetal bovine serum (FBS) to investigate the effect of BSA in medium on the cell uptake. The results are shown in Figure 2. The boron uptake reached a maximum 3 h after administration and became a plateau in the case of BSH. In contrast, the time-dependent boron uptake in the cells was observed in the case of DIC. The boron concentration of DIC in HeLa cells reached at 0.68 μg/10^6 cells 6 h after administration. The uptake of DIC by HeLa cells was similar in the presence or absence of FBS, suggesting that FBS does not affect the uptake of DIC by the cells.

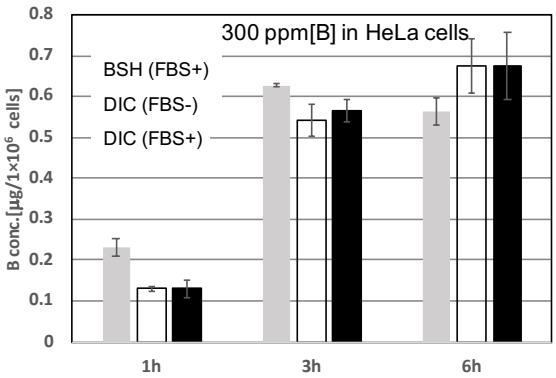

Fig. 2. Cell uptake of DIC by HeLa cells.

In conclusion, DIC was accumulated into HeLa cells in a time-dependent manner. The boron concentration of DIC in the cells 6 h after administration was higher than that of BSH whereas the boron concentration of BSH in the cells was higher than that of DIC 1 h after administration, suggesting that, once accumulated, DIC retains in HeLa cells longer than BSH.

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CO7-4 The Effect of Boron Neutron Capture Therapy to Normal Bones in Mice

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INTRODUCTION: Primary malignant bone tumors have been mainly treated with preoperative chemotherapy followed by surgery. Wide or radical margins including limb amputation are required for local control. Although surgical techniques named limb-salvage therapy become a mainstay of treatment to avoid the limb amputation, complications such as postoperative infection, fracture, or local recurrence often occurred.

Although primary bone tumors have been generally considered as radio-resistant tumors, radiation therapy has been used for the purpose of the functional and cosmetic status of patients. When a large single dose of photon radiation therapy is delivered to achieve the effective tumor control, clinically relevant late effects in the surrounding normal tissues include skin ulceration, neuropathy, and fracture.

Boron neutron capture therapy (BNCT), a tumor cell -selective particle radiation therapy, is considered to be effective for the tumors without any late effects to the normal bone. However, an appropriate BNCT dose irradiated safely to the normal bone, that is evaluated using experiment animals, is not determined.

In this study, we performed BNCT to normal bone in mice, and evaluated the influence on their bone strengths.

EXPERIMENTS: Eight to ten-week-old C3H/He mice were used for the study. As boron compound, p-boronophenylalanine (BPA) was prepared at a dose of 25 mg/ml. Irradiation was carried out using X-ray and thermal neutron at Gifu University and Kyoto University Reactor, respectively.

Boron concentration measurement After subcutaneously injected into mice at doses of 125, 250, 500 mg/kg of BPA, the boron concentrations at each time point (30, 60, 90, 120 min after administration) in the blood and bone were measured by prompt gamma ray spectroscopy. Five mice were used for each group.

X-ray irradiation Mice were irradiated to their right hind limb at single doses of 12, 24, 36, 48 Gy. Five mice were used for each X-ray dose.

Neutron irradiation On the next day after X-ray irradiation of 24 Gy, which was the dose that did not affect bone strength evaluated by X-ray irradiation experiments, mice were irradiated with a reactor neutron beam at a power of 1 MW. Following types of irradiation were carried out; neutron beam only, neutron beams with 125, 250, and 500 mg/kg of BPA administration. Based on a preliminary study of the biodistribution of BPA, irradiation was performed between 30 and 120 min after the injection. Five mice were used for each group.

Bone strength analyses Tibias were collected at 12 weeks post-irradiation. Subsequently, they were mechanically tested in three-point bending to determine the bone strength. Tests were performed at HAMRI CO., LTD.

RESULTS: As shown in Fig. 1, both boron concentrations in blood and bone increased dose dependently. While blood concentrations decreased over time, bone concentrations maintained until 120 min after BPA administration.

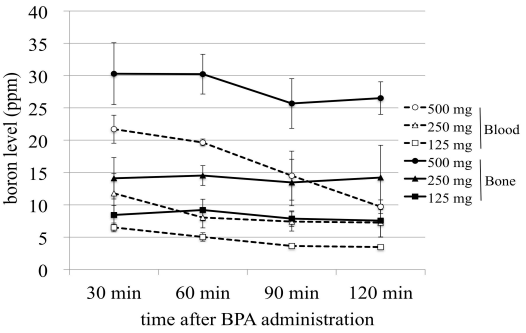

Fig. 1. Boron concentration levels in blood and bone

Tibial bending strengths at 12 weeks after X-ray irradiation decreased at doses of 36 Gy and 48 Gy compared to that at doses less than 24 Gy (Fig. 2).

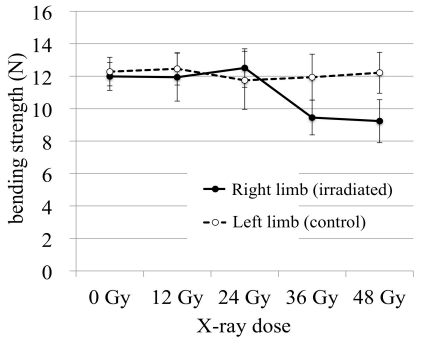

Fig. 2. Bone strengths at 12 weeks after X-ray irradiation.

Tibial bending strengths at 12 weeks after X-ray irradiation (24 Gy) followed by neutron irradiation did not have significant differences between any groups (Fig. 3).

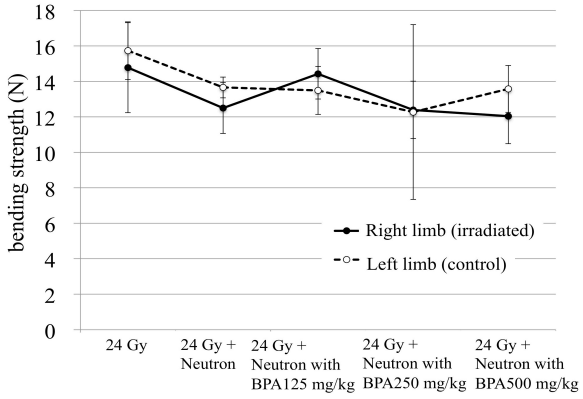

Fig. 3. Bone strengths at 12 weeks after X-ray irradiation followed by neutron irradiation.

CO7-5 Establichment of a novel mutation breeding using Boron Neutron Capture Reaction (BNCR)

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INTRODUCTION: Mutation breeding has provided significant benefits to breed improvement. The type of mutagenic treatment for plant breeding have been used chemical mutagens, such as EMS and MNU, or physical mutagens, such as gamma rays, X-rays and ion beams[1, 2, 3, 4]. In this present study, we focused on Boron Neutron Capture Reaction (BNCR) which based on the nuclear reaction of ^{10}B atom with thermal/epithermal neutron already applied to cancer treatment (BNCT)[5, 6].

The purpose of this study is to establish a novel mutation breeding using BNCR (Fig. 1). 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.

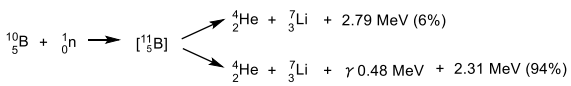

Fig 1. BNCR Reaction.

In other words, the mutagenic effect depends on chemical and physical factors, such as ^{10}B concentration, thermal neutron intensity, and irradiation time. This method is expected as a new approach of mutagenesis.

EXPERIMENTS: The experimental material used *Oryza sativa* L. cv. Nipponbare. The dry seeds were immersed into different concentrations (0, 10, 100, 200ppm) of ^{10}B-enriched *p*-boronophenylalanine (BPA)[7, 8] (Fig. 2) for 16hours. The samples were washed with water and re-dried at room temperature. The seeds in 2-mL tubes were irradiated with thermal neutron for 90 minutes in the Kyoto University Research Reactor (KUR). To provide four different levels of neutron fluence, the tubes were set to four columns microtube rack at the time of irradiation. Irradiation experiments were carried out three times.

As preliminary experiment, the immersed seeds into BPA were germinated on petri-dishes with continual moistening of filter paper at 25℃, it was investigate the germination rate.

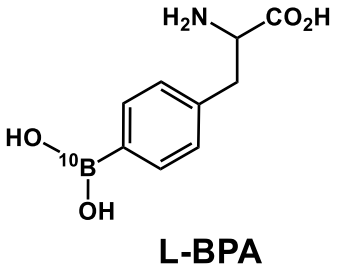

Fig. 2. Boron Carrier.

RESULTS: The immersed seeds were germinated normally at four BPA concentrations at the range of from 70% to 100%. Thermal neutron fluence were 1.1~1.2x10^{13} cm^2, 7.7~8.3x10^{12} cm^2, 5.3~6.3x10^{12} cm^2, 3.7~4.2x10^{12} cm^2, at the time of irradiation. We are set to confirm effects of irradiation. To verify the mutagenic effects of different mutagens. Because of that, the efficiency, mutation rate spectrum, and optimum processing conditions need to be examined. Also, as basic research, ^{10}B selectively accumulate to meristematic cells. If not accumulate, we try the search of suitable ^{10}B compound for plant.

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CO7-6 A Fundamental Investigation on Using Known Samples as a Standard to Evaluate Various Constrction Materials

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INTRODUCTION: Concrete is widely used as radiation shield in nuclear reactors and irradiation facilities, because of its flexibility and sufficient supply. However, there are few discussions on the content of the shielding concrete composition, and very old data (which have uncertain information regarding material properties beside of elemental data) are still used in the shielding calculation. For the above situation, Radiation Shielding Material Standardization Working Group under Atomic Energy Society of Japan (AESJ) was established in order to proceed the standardization of shielding concrete[1]. On the other hand, once those facilities start to operation, the concrete for the shield are affected by the radiation ray from the operating radiation source in the facilities and activated. For the above situation, low activation concrete[2]-[4] is the one of the ways to solve the problem. Especially, boron neutron capture therapy (BNCT) should be effective facilities to apply the low activation concrete.

For the above two points (standardization of concrete composition and low activation), elemental analyses for the materials in concrete for radiation shielding are necessary and important by neutron activation analysis in KUR. As a fundamental study, investigation of variation of three kinds of known sample were conducted in order to discuss proper standard material for the above purpose.

METHODS: More than 3000 of raw material for low activation concrete and ordinary concrete were gathered from all over the Japan and oversea, including hundreds of raw materials newly corrected during the period of KUR shut down. Several tenth of the materials were chosen among the material stock library. These materials were crushed to certain size (typically under 0.7mm or less), and were packed for 0.1 to 0.3 g with special treatment for the irradiation in KUR. After the irradiation with 10 to 60 minutes and with the certain cooling period, these samples were measured by Ge detector one by one. The quantity of the target elements, which were selected by former investigations as Co, Cs, Sc, Fe and Eu[2]-[4], in each sample were estimated by the comparison of the known standard material in the same package for the irradiation. Three kinds of known materials were prepared, such as JSAC0411[5], JSAC0522[6] and fused alumina with Eu and Co (FAwEC). Table 1 shows the content of target elements for the samples, and Table 2 shows the relative standard variation of estimating elements for focused gamma ray peaks. These tables were introduced that the samples for FAwEC had less variation compared to those for JSAC0411 and JSAC0522.

CONCLUSION: Investigation on estimation of the variation for standard samples was conducted in order to estimate the quantity of target elements in concrete raw materials. Fused alumina samples with Europium and Cobolt have less variation compared to those for JSAC0411 and JSAC0522.

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Table 1 Content of target elements in samples

	K	Fe	Co	Cs	Eu
	g/kg		mg/kg		
JSAC0411	2.4	33	6.5	3.97	1.4
JSAC0522	9.3	29.2	47.4	–	–
FAwEC	–	–	15.1	–	75.9

Table 2 Relative standard variation (%) of estimating elements for focused gamma ray peaks

Sample name	Eu-152		Co-60		Cs-134		Irradiation		Number
	344keV	1408keV	1173keV	1332keV	605keV	796keV	Month	time(min)	of sample
JSAC0411	5.55	2.99	5.21	3.74	4.71	3.52	2018/8	30	4
JSAC0411	4.44	4.60	1.69	2.02	ND	7.28	2018/8	10	6
JSAC0411	6.75	2.64	4.90	6.85	8.25	9.73	2018/11	30	4
JSAC0411	16.28	10.84	10.34	10.45	ND	18.35	2018/11	10	6
JSAC0522	12.92	2.61	2.32	1.43	24.24	6.92	2018/11	10	3
FAwEC	1.64	1.88	1.65	1.16	ND	ND	2018/11	10	6

CO7-7

Pathological findings after GdNCT using Gd-DTPA-incorporated calcium phosphate nanoparticles

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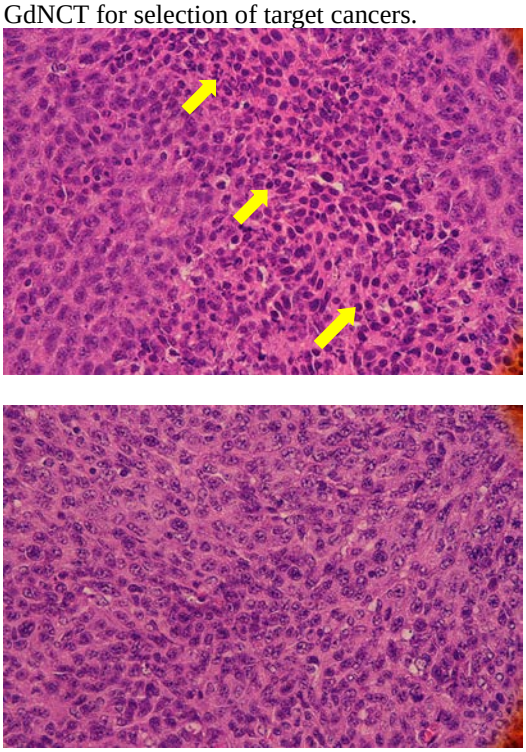
INTRODUCTION: Gadolinium-157 has been paid the most attention as a novel atom for neutron capture therapy (NCT) agent because of its high thermal neutron cross section (255 000 barns). The ^{157}Gd atoms can induce Auger electrons and gamma rays on gadolinium neutron capture reaction (Gd-NCR). The range of high LET Auger electorn is few micron, so it is necessary to accumulate the ^{157}Gd atoms in the cancer cells, especially in the nucleus for effective GdNCT [1,2,3].

In this work, we performed checking the pathological findings in the evaluation of tumor growth suppression on Colon26 tumour bearing mice by GdNCT with the intraveneous injection of Gd-DTPA/CaP nanoparticles as continued work from previous evaluation on single/multiple-injected group [2, 3].

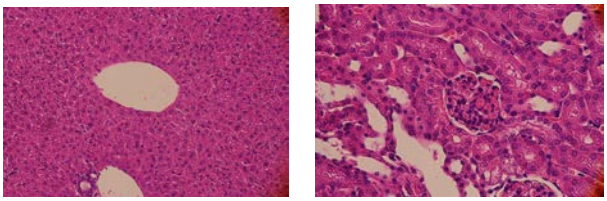
EXPERIMENTS: *In vivo* evaluation was performed on colon-26 tumor-bearing mice irradiated for 60 minutes at nuclear reactor facility of Kyoto Univ Institute for Integrated Radiation & Nuclear Science with average neutron fluence of 2.0×10^{12} n/cm^2. Antitumor effect was evaluated on the basis of the change in tumor growth and survival rate of the mice. The pathologicl findings of each organ were checked with H.E. stainings.

RESULTS: No acute toxicities were recognized in the treated mice after GdNCT using intraveneous injection of Gd-DTPA/CaP nanomicelle. Tumor growth was suppressed until four times of the non-treated group in the same manner of previous experiments (data not shown). The tumour volume was decreased after GdNCT, and the infiltration of mononuclear cells were seen in the tumour. Non-treated group shows normal histology with clear cytoplasm and nucleus(Fig.1). The abnormal change in the liver, the kidney, the heart, and the lung were not found in the histologic examination one month after Gd NCT (Figure1). Thease results indicate that Gd-DTPA /CaP nanomicelle has the promising possibility as novel GdNCT agent.

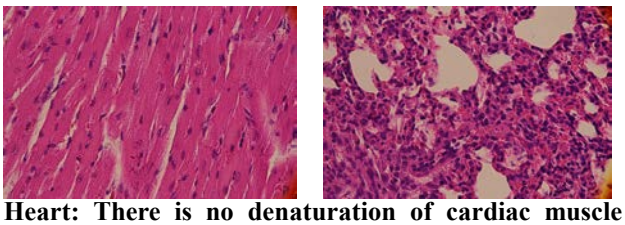
In the next experiments, evaluation of the mechanism of cytotoxicity on GdNCT is necessary. We must check the mechanisms, for examples, apoptosis, autophagy, senescence, etc. We hope to increase a dose in quantity of Gd-DTPA /CaP nanomicelle. We hope to refer these results of toxicity examinations to the clinical studies of GdNCT for selection of target cancers.



Tumour: The tumour volume was decreased after GdNCT, and the infiltration of mononuclear cells were seen in the tumour(Upper:GdNCT, Lower: Non treated, x400).



Liver: Hepatocellular denaturation and the destruction are absent(Lt:x400). Kidney: There is no glomerulus or tubular denaturation(t:x400).



Heart: There is no denaturation of cardiac muscle cells (Lt:x400). Lung: There is no denaturation of alveolar cells (Rt:x400).

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CO7-8 The feasiblity study of Eu:LiCAF neutron detector for an accelerator-based BNCT

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INTRODUCTION: The stability of neutron flux at an accelerator-based BNCT facility is relatively worse than that at a reactor-based one. Therefore, it is necessary to measure the neutron flux precisely in real-time to optimize the patient's exposure dose for the accelerator-based BNCT. However, the neutron flux is so intense (about 10^9(n/cm^2/s)) that the real-time measurement has not been realized yet. Hence we tried to measure the neutron flux with a small detector using a Eu:LiCAF scintillator [1] on the tip of optical fibers, as shown Fig.1. For this detector, the linearity to the neutron flux higher than 10^8(n/cm^2/s) has not been measured and we have measured the linearity of the detector to the neutron flux of 10^8 to 10^9(n/cm^2/s) with the correlation coefficient of 0.99 last experiment done at KUR [2]. In this experiment, we have tried to check the linearity of the detector to the neutron flux higher than 10^9(n/cm^2/s).

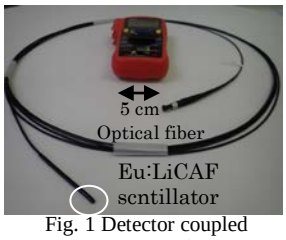

Fig. 1 Detector coupled with the optical fiber.

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EXPERIMENTS: The experiments were performed at the KUR-SLY where the maximum neutron flux of about 10^{12}(n/cm^2/s) is available at the bottom when the reactor power is 1MW [3]. Figure 2 shows the experimental set-up for the measurement, where the photon counting unit C8855 and 871 is able to count up to 1 Mcps. The optical output from the Eu:LiCAF scintillator through the optical fibers was properly converted to an electric signal and counted with the counting units. Prior to the measurement, the detector was put into the plastic bottle, where Figure 3 shows the situation, and loaded to 20 cm from the bottom of the KUR-SLY. At this position, the maximum neutron flux is about 10^{11}(n/cm^2/s). Figure 4 shows the loading of the detector into the KUR-SLY, which is same method done in 2017. The measurement was carried out from the starting-up to 1 MW-arrival of the reactor.

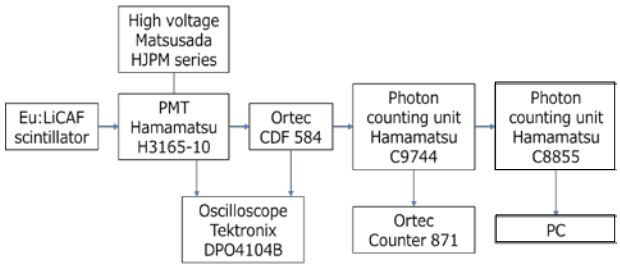

Fig. 2 Experimental setup.

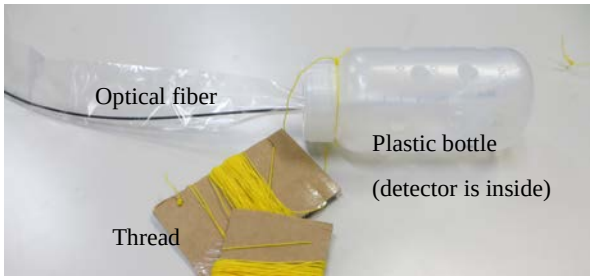

Fig. 3 Situation about the putting into the plastic bottle. (Same method done in 2017)

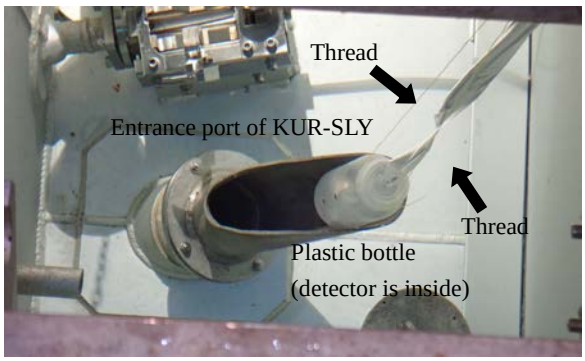

Fig. 4 Loading of the detector into the KUR-SLY. (Same method done in 2017)

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RESULTS: In this experiment, the count-rate measured by C8855 and Ortec counter 871 has saturated before the reactor reached 1 MW and the linearity to the neutron flux could not be identified. I think that the effect to the count-rate from Cherenkov radiation in optic fiber was high and the setting of the high voltage was high.

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CONCLUSIONS: A small detector using a Eu:LiCAF scintillator has been tested at the KUR-SLY experimental port. Unfortunately, the linearity to the neutron flux could not be identified.

To overcome the results, we will review two points. First, we will investigate the influence of Cherenkov radiation of which wavelength spreads from 400 nm to 750 nm. Since the scintillation peak of the Eu:LiCAF is about 370 nm, using the optical filters, the influence of the Cherenkov radiation should be estimated.

For the setting of high voltage, we will experiment at the iBNCT in Tsukuba University and we will decide the suitable setting of high voltage.

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CO7-9 Evaluation of Relative Biological Effectiveness of the splenic cells in SCID mice following Thermal Neutron Irradiation

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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, 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. After irradiation, the splenic cell suspension were adjusted and incubated for 1weeks. At 1 hour, 24 hours and 7 days after irradiation, apoptotic induction of the cells was examined by Cell Death Detection ELISA (Roche).

RESULTS: As shown in Fig. 1, the apoptotic changes of the SCID mice splenic cells increased with a radiation dose.

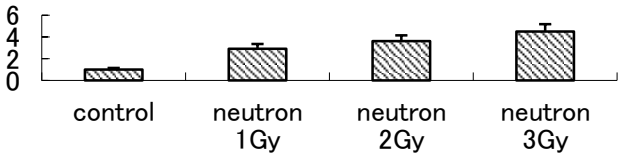

Fig.1. Apoptotic induction of the splenocytes of SCID Mice at 24 hour after neutron irradiation. The vertical axis shows an enrichment factor level.

The apoptotic induction of the splenocytes of SCID mice was larger than that of C3H mice at 1 hour, 24 hours and 7 days after irradiation. The difference of the apoptosis of the splenic cells between SCID mice and C3H mice was the biggest at the 24 hours after the neutron radiation.

The values of relative biological effectiveness were biological effectiveness were shown in below (Table 1).

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

	SCID	C3H
RBE*	1.47	2.08

*RBE was calculated the Enrichment factor at 2Gy neutron radiation dose / the Enrichment factor at 2Gy gamma-ray radiation dose.

DISSCUSSION
Previously we reported the RBE values for SCID mice of the LD50 by radiation oral death assay and gamma H2AX foci number of the lymphocytes. The RBE values calculated from radiation oral death assay and gamma H2AX foci number of the lymphocytes were 1.6 for SCID mice and 2.0 for C3H mice and 1.5 for SCID mice and 1.9 for C3H mice, respectively. In the radiation sensitive mice study, the RBE values of SCID mice was 1.5-1.6, comparing the neutron and the gamma studies. The RBE values of C3H/He mice was 1.8-2.0. 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.

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CO7-10 Effect of BNCT on dissemination and invasion of brain tumor cells

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INTRODUCTION: The prognosis of patients with high grade gliomas remains poor despite surgical resection followed by radiation therapy and concomitant adjuvant chemotherapy with temozolomide (TMZ). Tumor recurrence is almost inevitable and the median survival time (MST) of patients with newly diagnosed glioblastomas (GBMs) is only 14.6 months [1]. We have utilized boron neutron capture therapy (BNCT) for the treatment of patients with either recurrent or newly diagnosed high grade gliomas. A significant survival benefit was seen in those patients with newly diagnosed GBMs who received BNCT, followed by photon boost, with a MST of 23.5 months [2,3]. Furthermore, for those patients with recurrent gliomas, especially those with poor prognosis who were classified as 3+7 by recursive partitioning analysis (RPA) [4], had a MST of 9.1 months compared to 4.4 months for those in the same RPA class [3] who had been treated by current standard therapy. In our experience with all clinical trials of BNCT, the most frequent cause of death following treatment has been leptomeningeal dissemination (LMD) [2,3]. Assuming that more than 85% of tumor recurrence following conventional treatment is local, that is within 2 cm of the original margin of the contrast-enhancing lesion [5,6], local control by BNCT is significantly better than that obtained by current conventional radiotherapy [5,6]. The aim of this research is to investigate whether BNCT influences invasion or dissemination of brain tumor stem like cells. Emerging evidence suggests that exosomes (extracellular vesicles) serve an important role in intercellular communications. Therefore, we focus on exosome from brain tumor stem cells after BNCT in this study.

EXPERIMENTS: Cells: U87MG and U87 delta *EGFR* cells were cultured in DMEM with 10 % fetal bovine serum at 37° C in a humidified atmosphere of 95% air and 5% CO_2. Two days before irradiation, we changed the medium without serum. In case of neutron irradiation, we added boronophenylalanine (BPA) at 25 ppm in medium for 24 hrs.
Irradiation: we irradiated the cells in T25 flasks using X-ray or neutron.
Preparation of exosomal fraction: 48hrs after irradiation, the culture medium was collected and centrifuged at 2000 *g* for 20 min at 4° C and supernatant was filtered with 0.22 μm PVDF filter (Millipore). Then the supernatant was centrifuged at 100000 *g* for 70 min. The pellet was rinsed with PBS and centrifuged at 100000 *g* for 70 min.
Sample preparation for western blot: Irradiated cells were collected 48 hrs after irradiation and lysed in RIPA lysis buffer containing protease inhibitors.

RESULTS: We obtained enough amount of exosomes to analyze for non-irradiated control. However, we did not obtain enough exosomes for irradiated groups.
We are performing western blot analysis for irradiated cells.

CONCLUSION: We will change the method for exosome isolation using qEV kit etc next year.

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CO7-11 Response Assessment of Meningioma Treated by BNCT

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INTRODUCTION: High-grade meningioma (HGM) is a good candidate for boron neutron capture therapy (BNCT). BNCT shows good local response to this entity. However the response assessment is still under developing. Generally speaking, one dimensional RECIST is widely accepted for response assessment for solid cancers. However meningioma has dural attachment and this may make difficult for the one dimensional assessment. Therefore we evaluate the response assessment with two dimensional Macdonald assessment and three dimensional volumetric method.

MATERIALS and METHODS: From August 2017 to February 2019, we treated 14 cases of HGM in KUR. Among them, we applied above 3 methods for the response assessments, RECIST, Macdonald and volumetric assessments for best response, for 7 selected cases.

RESULTS: Here we demonstrated 3 typical cases of HGM treated by BNCT. Patient 6582368 was grade 2 HGM (Fig. 1). She was operated 6 times and applied IMRT and SRT, prior to BNCT. Five months after BNCT, MRI showed transient enlargement of enhanced volume. One dimensional RECIST assessment was 16.8% increase, two dimensional Macdonald assessment was 28.4% increase. The lesion became shrunk after this study. Therefore this transient increase in size seemed to be pseudoprogression. Patient 7081295 showed 1.3% decrease, 2.1% increase and 62.1% decrease in RECIST, Macdonald and volumetric assay, respectively 5 months after BNCT (Fig. 2). Patient 7103553 showed 1.2% increase, 26.5% decrease, 40.8% decrease in RECIST, Macdonald and volumetric assay, respectively 5 months after BNCT (Fig. 3). Table 1 showed the response assessment of BNCT in cumulative 7 cases of HGM.

DISCUSSION: Response assessment for meningioma has not yet been established [1]. RECIST is most popular for response assessment for solid cancer, however, meningioma usually has dural attachment which **is** not affected by any treatments. Therefore, MacDonald may be better than RECIST for this purpose. However, even by Macdonald it may be difficult to exclude treatment-related necrosis for the assessment. Thus volumetric analysis may be adequate for response assessment of HGM treated by BNCT. Case 7081295 and 7212464 demonstrated this theory in Table 1.

Fig. 1
Before BNCT **5M after BNCT**

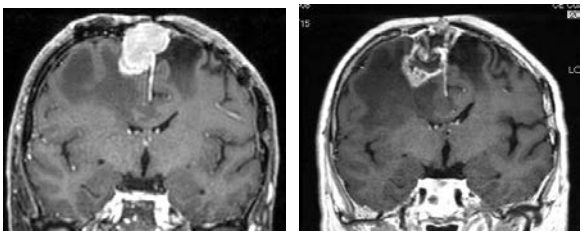

Fig. 2
Before BNCT **5 M after BNCT**

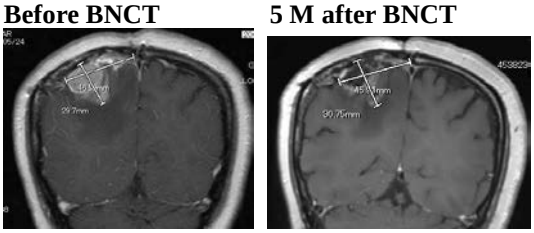

Fig. 3
Before BNCT **2M after BNCT**

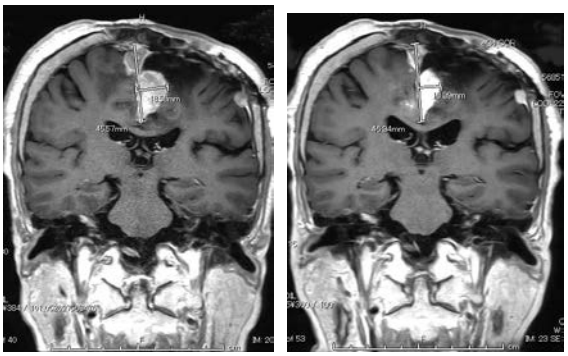

Table 1

ID	6562368#	7081295	7212464	7103553	7277315*	5919134	6795335
RECIST	+16.8%	-1.3%	+4.0%	+1.2%	-11.8%	-10.4%	-14.8%
Macdonald	+28.4%	+2.1%	+6.4%	-26.5%	-67.0%	-14.0%	-10.6%
Volumetry Excluding necrosis	+1.0%	-62.1%	-52%	-40.8%	-41.9%	-35.2%	-46.1%

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CO7-12 Development of a Silica Nano Particle Installed with Gd(III)-Thiacalixarene Complex as a Gadolinium Carrieres to Tumor for Gd-NCT

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INTRODUCTION: Owing to a large thermal neutron capture cross section and total kinetic energy of $^{157}Gd(n,\gamma)^{158}Gd$ larger than that of $^{10}B(n,\alpha)^{7}Li$, gadolinium attracts growing attention as an alternative to boron in neutron capture therapy [1]. Because free gadolinium ($Gd(OH_2)_9$) has toxicity, a safe carrier of Gd to tumor not to release free Gd is required. We recently found that thiacalix[4]arene-*p*-tetrasulfonate (TCAS) self-assembled three lanthanide (Ln) cores including Gd to form a sandwich-type complex, Ln_3TCAS_2 (Fig. 1) [2], the characteristic features of which are high kinetic stability, luminescence signal [3], and ^1H relaxation arising from the Ln center [4]. Nano-sized particles are frequently used as a drug carrier toward tumor by enhanced permeability and retention (EPR) effect. Here we attempted to prepare a silica nano-particle (SiNP) containing Gd_3TCAS_2 and evaluate its ability as the NCT agent by cell viability.

EXPERIMENTS: *Preparation of SiNP installed with Ln.* The trinuclear complexes Ln_3TCAS_2 (Ln = Gd, Tb) were prepared as reported elsewhere [2]. The Ln_3TCAS_2-installed SiNP was prepared by a Stöber's method [5], which was modified with using 3-aminopropyltrimethoxysilane (APTES) as an anchor of negatively charged Ln_3TCAS_2. Furthermore, the surface was modified with poly(ethylene glycol) (PEG) by PEG-NHS (MW 2000) to retain water-dispersibility and biocompatibility.

Cell experiments. MCF-7 cells were seeded in a 6-well plate at a cell concentration of 7.5×10^5 cells/mL and incubated for 24 h. After supernatant was removed, DMEM and solution of SiNP loaded with Gd_3TCAS_2 were added to each well and incubated for 24 hr. After washing with PBS, the cells were detached from the well and transferred to tubes to be irradiated with thermal neutron.

RESULTS: The TEM images of SiNPs revealed the size of the particles were 61.6 ± 3.8 nm in diameter, which is compatible to the one (10-100 nm) for the EPR effect. The amount of Gd loaded in the SiNPs were determined by ICP-AES to be 55 ng/mg, which was 1.8-fold larger than one obtained without using APTES, suggesting that Gd_3TCAS_2 was successfully anchored to ammonium group of APTES in the SiNP.

Optical images of MCF-7 cells incubated in the presence of SiNP loaded with Tb_3TCAS_2 were obtained with bright field and fluorescence microscopes (Fig. 2). As can be seen, emission of green light from Tb(III) inside the cell was observed to show successful delivery of the SiNPs to the cells. This implies that the SiNP loaded with Gd_3TCAS_2 could be used as the carrier of Gd to tumor in NCT.

We then attempted cell viability study after irradiation of neutron for 0, 30, and 60 min to MCF-7 cells incubated with 0, 50, and 100 μM of Gd in the medium added as Gd_3TCAS_2 in the SiNP. The cell viability for samples incubated in the presence of 100 μM of Gd showed decrease from 117 to 65% as time elapsed from 0 to 60 min. But similar trend was observed for control without containing Gd. Thus, it was still unclear whether the Gd in the SiNP sufficiently emit γ ray or internal conversion electron and Auger electron. SiO_2 shell of SiNP may have hindered the emission of these electrons from the material. Hence, we will seek other carriers of Gd_3TCAS_2 which allows emission of those electrons.

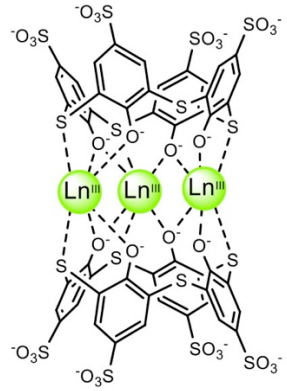

Fig. 1 Structure of Ln_3TCAS_2 complex.

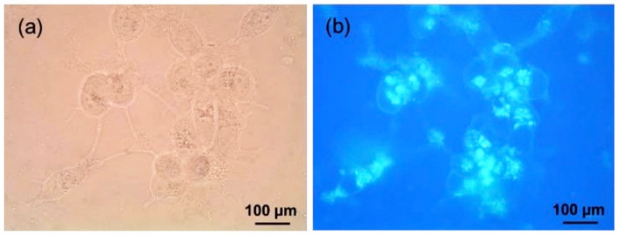

Fig. 2 Images of MCF-7 cells obtained with (a) bright field and (b) fluorescent microscopes.

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CO7-13 Development of New Gadrinium Neutron Capture Therepy Agent for Bone Cancer

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INTRODUCTION

For developing the next generation cancer radiation therapy, we established a new method to evaluate tissue distribution of neutron capture therapy formulation containing gadolinium-157. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used for elemental imaging in the field of earth and planetary sciences. We applied this method to biological samples, enabled imaging intra-tissue distribution of gadolinium-157[1]. In this study, by using LA-ICP-MS, we revealed that EDTMP [ethylenediamine tetra (methylene phosphonic acid)] chelate of gadolinium (Gd-EDTMP) .

EXPERIMENTS

Female C3H/HeNJcl aged 7 weeks ware obtained from CLEA Japan, Inc (Tokyo, Japan). LM8 (RBRC-RCB1450) cells were purchased from Riken BRC Cell Bank (Ibaraki, Japan). Cultured LM8 cells were prepared for intratibial injection. The cells were injected (2×10^6 cells per mouse) into the right hind tibia of CH3 mice anesthetized with isoflurane, and mice were randomized into four groups (n=4 per group). At 4 days subsequent to the injection of cells, mice were injected Gd-EDTMP or saline.

Gd-EDTMP chelate solution was prepared from gadolinium chloride and EDTMP [1]. Gd-EDTMP was diluted with saline (1.0 mg-Gd/mL) and intraperitonealy administered to two groups of mice to 10.0 mg-Gd/kg, other eight mice were injected same volume of PBS as control. Twenty-four hours after injection, each one groups of mice which were injected Gd-EDTMP or saline were irradiated for 120 min with 8.0×10^{12} cm^{-2} thermal neutrons at the Kyoto University Reactor. Seven days after thermal neutron irradiation tibia samples were excised and analysed laser ablration inductively coupled plasma mass spectrometry (LA-ICP-MS). The size of the tumors are going to measure by photomicrograph of a hematoxylin-eosin stained section of tibia.

RESULTS

The distribution of ^{157}Gd in the tibia determined by LA-ICP-MS is shown in Fig. 1. Gd-EDTMP was concentrated to a bone at high concentration, that was 1500 times of muscle tissue. By limiting the application to bone tumors, Gd-EDTMP might be used sufficiently for neutron capture therapy by overwhelming tumor concentration effect. As for effect of neutron capture therapy, we would like to give it more consideration moving forward.

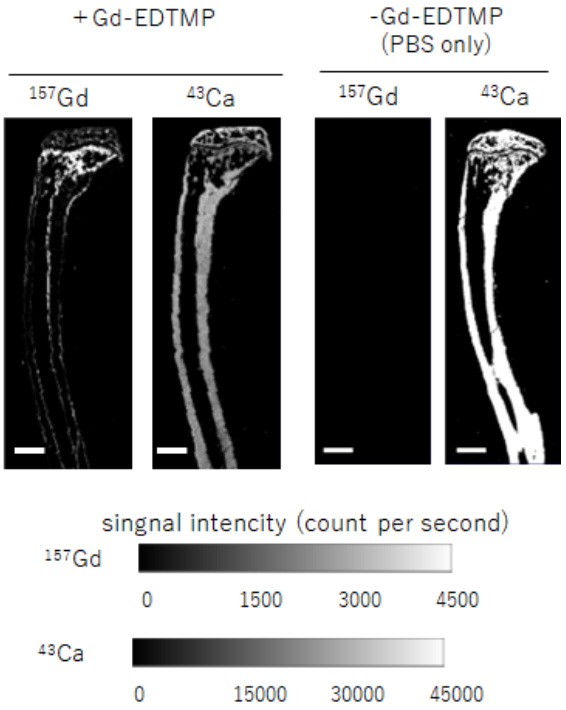

Fig. 1. Distribution of ^{157}Gd and ^{43}Ca in the mice tibia. Tibia section from Gd-EDTMP treated mice (left) and PBS treated mice as control (right) were analysed by LA-ICP-MS. Scale bar is 1mm.

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CO7-14 Enhanced neutron sensitivity by overexpression of *LAT1* in human cancer cells

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INTRODUCTION: Outcome from BNCT largely depends on amount of intracellular accumulation of boron compound. L-type amino-acid transporter 1 (LAT1) [1], through which boronophenylalanine (BPA) is transported into cells, is predominantly 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. Confocal laser microscopic observation confirmed that those clones stably overexpress LAT1 in cell membrane. Uptake of ^{14}C-BPA was measured by use of a RI tracer method in the LAT1-overexpressing T98G cells. The amount of intracellular ^{14}C-BPA was 1.5-5.0 times larger in several LAT1-overexpressing clones than that in a control clone. Cell growth rate was not affected by the LAT1 overexpression. We intend to examine the sensitivity to neutrons generated by KUR in stably LAT1-overexpressing T98G cells after ^{10}BPA treatment.

EXPERIMENTS: Stably LAT1-overexpressing T98G cells (T98G/K4 clone), control T98G cells (T98G/KC2 clone, transfected *LAT1*-empty plasmids) and transiently *pCMV/LAT1-GFP*-lipofected T98G/KC2 cells were plated on dishes. After overnight culturing, the cells were treated with ^{10}BPA (5 or 20 ppm) and 3 hours later the cells were trypsinized and irradiated with the beams (neutrons and γ-rays, 0.4 or 0.8 Gy in total dose) 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: T98G/K4 cells showed slightly enhanced sensitivity to the beams compared with T98G/KC2 and the lipofected T98G/KC2 cells in the case of 5 ppm ^{10}BSA treatment (Fig. 1a). There is no significant difference in the sensitivity to the beams between T98G/KC2 and the transiently lipofected T98G/KC2 cells. In the case of 20 ppm ^{10}BSA treatment (Fig. 1b), T98G/K4 and the lipofected T98G/KC2 cells showed largely enhanced sensitivity to the beams compared with T98G/KC2 cells (ER=1.5). The sensitivity to the radiation flux depended on dose of ^{10}BSA.

CONCLUSION: Results obtained from this study showed that overexpression of LAT1 results in enhanced sensitivity to neutrons, depending on dose of ^{10}BPA, in human cancer cells.

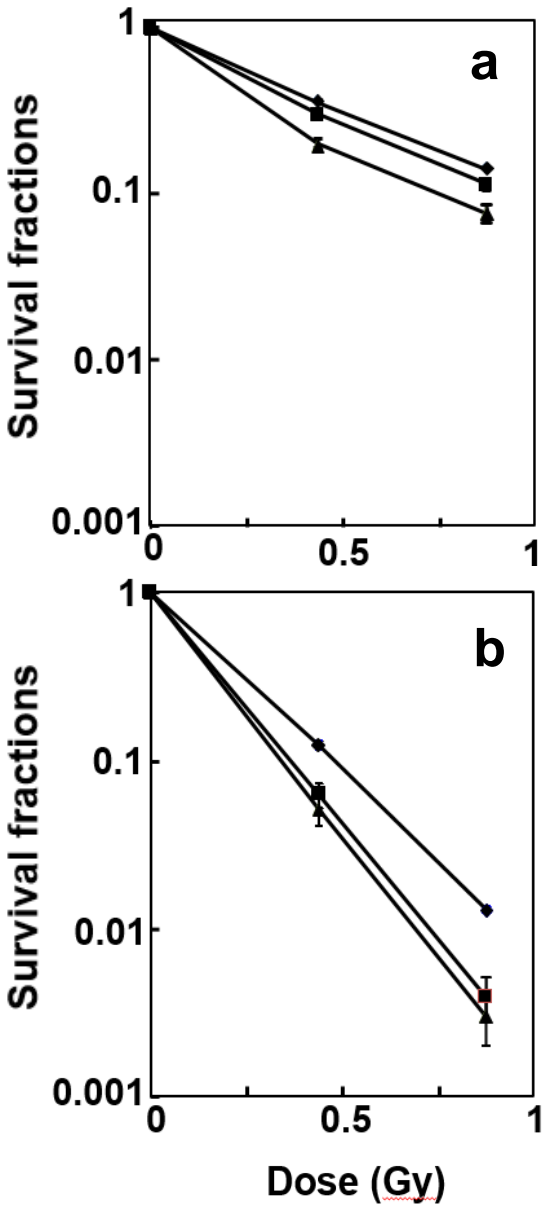

Fig. 1. Sensitivity to neutrons and γ-rays beams from KUR in T98G/K4 (▲), T98G/KC2 (♦) and transiently *pCMV/LAT1-GFP*-lipofected T98G/KC2 cells (■). Cells were treated with 5 (**a**) or 20 ppm ^{10}BPA (**b**) 3 hours before irradiation.

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[2] K. Kaira *et al.*, Br. J. Cancer, **107** (2012) 632-638.

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CO7-15 Clarify the Heterogeneity of Boron Distribution in BNCT

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INTRODUCTION:

Today, boronophenylalanine (BPA) is widely used as a boron compound for boron neutron capture therapy (BNCT). BPA is reported to be uptaken by SLC7A5 (LAT1), SLC7A8 (LAT2) and SLC6A14 (ATB^{0+}) which were identified as the transporters of BPA *in vitro* study [1]. However, relationship between BPA and these transporters *in vivo* has been unrevealed. The aim of this study is to investigate the relationship *in vivo* to clarify the mechanism of heterogeneity of BPA distribution.

EXPERIMENTS:

T3M4 (human pancreatic cancer cell line) was subcutaneously inoculated into the legs of BALC/c-nu/nu mice. BPA at the dose of 500mg/kg was injected subcutaneously and the tumor was removed two hours later. Frozen tumor sections (6-μm thickness) were made by cryostat and put them on CR-39 which are widely used as the solid state nuclear track detector. CR-39 plates were irradiated with thermal neutron using Tc-Pn in Kyoto Univesity Research Reactor (KUR). The thermal neutron fluence was approximately 8×10^{11} (n/cm^2). Two heavy particles (^4He and ^7Li) yielded by $^{10}B(n,\alpha)^7Li$ reaction made dots on the CR39 plate. The position of the dot correlated with that of BPA. Hematoxylin-Eosin (HE) staining of tumor sections were carried out followed by chemical etching process to enlarge the dot detectable by optical microscope. Before chemical etching, the image of HE-stained tumor sections were stored since the tumor sections were lost in the process of chemical etching.

To investigate the relationship between HE-stained tumor sections and dots images on CR 39, these images ware merged [2].

RESULTS:

Figures 1 and 2 show the images of boron distribution depicted as small black circles which were overlaid on the HE-stained image. The relationship between histological structure and BPA distribution was successfully imaged. Feasibility of the method for detecting BPA distribution on the tumor section image was confirmed. As the next experiment, we will investigate the identification of the transporters of BPA using the immunohistochemical staining.

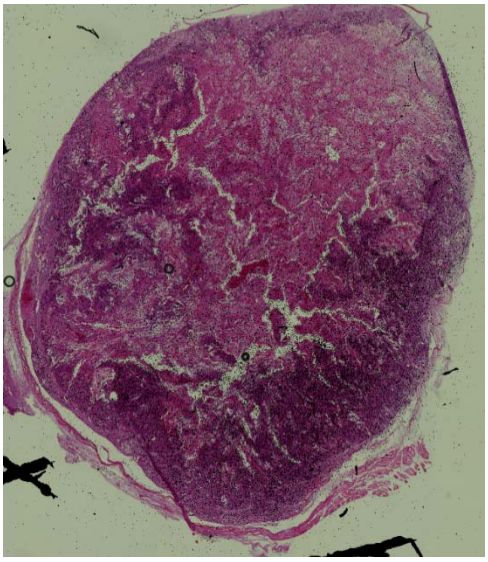

Fig.1 HE staining and boron distribution (x4)

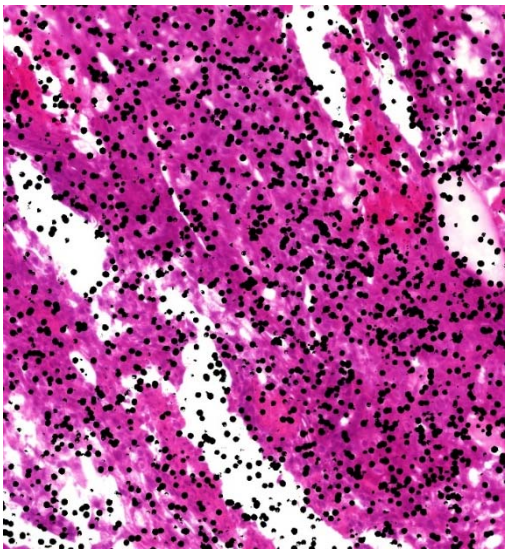

Fig.2 HE staining and boron distribution (x20)

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CO7-16 The Effect of boron neutron capture therapy (BNCT) on normal lung in mice

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Introduction

Boron neutron capture therapy (BNCT) has been applied mainly for the treatment of locally recurrent malignant brain tumors or head and neck cancers in the irradiated region. The outcomes of the clinical BNCT studies on BNCT for both tumors have been reported to be promising in some studies.

Clinical trials using accelerator-based (AB)-BNCT system are currently in progress. Since the AB-BNCT system is much compact compared with a research reactor, the system can be installed in the existing medical institutes. The AB-BNCT system in the hospital is available to more patients suffering from malignant tumors compared with the BNCT system using research reactor. Lung cancer, breast cancer and hepatic tumors including hepatocellular carcinoma and multiple metastatic tumors are more common than malignant brain tumors and head and neck tumors.

In this study, for BNCT to apply more common cancer such as lung cancer, breast cancer and hepatic tumors, the effect of BNCT irradiation on normal was investigated. These normal lung are irradiated in the treatment of lung cancer, and breast cancer with BNCT. The research on the effect of BNCT on the normal lung is still ongoing. The preliminary results of the study on normal lung were reported.

Materials and methods

Experimental animals

Twelve- to thirteen-week-old female C3H/He mice were used for this study. All procedures for animal experiments were carried out in accordance with the regulations of Kyoto University Research Reactor Institute regarding animal care and handling.

Experimental protocols

In this study, the radiobiological effectiveness of high linear energy transfer (LET) irradiation by $^{10}B(n,\alpha)^{7}Li$ reaction on normal lung tissues is investigated in comparison to that of X-ray. The endpoint is occurrence of fatal radiation injuries.

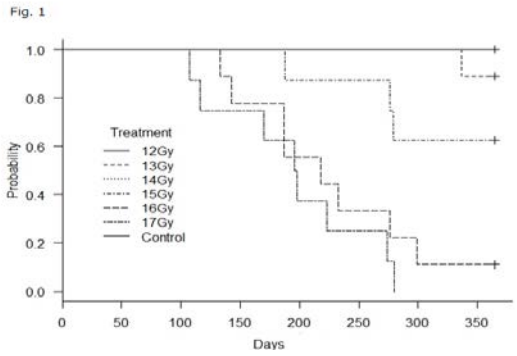
Three types of irradiation carried out in this study ware as follows, X-ray irradiation, thermal neutron beam irradiation and BNCT-irradiation using p-boronophenylalanine –fructose complex (BPA-F). In each irradiation, the whole lung was irradiated with shielding other part of the body. In X-ray-irradiation, anesthetized mice were confined in 1mmPB box with 25x20 mm-sized window. The whole thoracic put within the window was irradiated with 150keV X-ray at 1.16 Gy min^{-1}. In thermal neutron beam irradiation, mice was held within a specially designed cage made with thick paper at the flux of 3.73E+10 cm^{-2}·min^{-1}. LiF tiles (50-mm thick) were used to shield parts of the body other than the chest. Since all the mice treated with BNCT at the dose of 500 mg/kg have been alive in BNCT-irradiation groups, BPA-F was subcutaneously injected in each mouse at the dose of 1,000 mg/kg. Irradiation was started at two hours after the injection of BPA-F. Six- to nine mice were used for each data point.

At two or three days' intervals during one months from the irradiation and weekly intervals after two months, the mice were weighted and carefully observed.

Results

In X-ray treatment, mice were irradiated with a single dose (12 to 17 Gy). Fig.1 shows the survival curve for 12 months after the treatment day. Eight, seven and three mice died in 17 Gy, 16 Gy, and 15 Gy groups, respectively. One mouse in 13 Gy group died at 11 months after the treatment day. All the mice in no-treatment, 12 Gy and 14 Gy groups were alive for 12 months after the treatment day.

Fig. 1



All the mice died in a week after the treatment day in thermal neutron irradiation experiments although two or three irradiation protocols at 5MW were tried. Since the course of death is thought to be acute radiation injury of intestine, optimization of irradiation time and adequate shield of abdomen will be needed.

The mice treated with BPA (1,000 mg/kg)-BNCT were sorted into three groups according to the irradiation time. Since all the mice irradiated with BPA-BNCT for 150 min died in a week, the mice in 60, 90, and 120 min-irradiation groups have been observed. All the mice are alive for 3 to 6 months after the treatment day.

CO7-17 Study of the influence on normal liver tissue by BNCT

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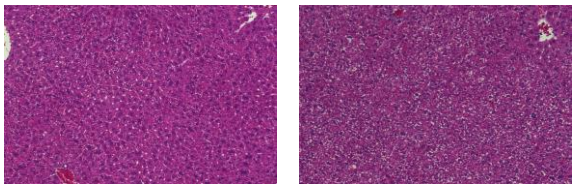
INTRODUCTION: Pioneering clinical research aimed at expanding the application of BNCT is planed to be implemented as a joint use research adoption task at the Kyoto University Institute for Integrated Radiation and Nuclear Science. At the part of the study, a clinical study of BNCT for the hepatocellular carcinoma is planned.

The BNCT for liver tumor, which has been conducted up to the present, has used the compound effectiveness factor (CBE) determined by using genotoxicity for hepatocytes as an indicator, which has been clarified by Suzuki et al. But there is a problem whether it is appropriate as a real clinical endpoint. Fundamental researches of liver fibrosis that are the late effect of radiation therapy are necessary. It is necessary to do basic research that uses liver fibrosis, which is a late radiation injury to the liver, as an evaluation index.

A purpose of this study is to establish systematically and continuously technique that can analyze the harmful phenomenon in the normal liver tissue of BNCT.

EXPERIMENTS: Female C57BL6 mice at 6weeks of age were purchased from CLEA Japan Inc. BPA solution (500mg/kg or 1000mg/kg) was injected Subcutaneously 2 hours before neutron irradiation. The mice were irradiated for 60 minutes at the 1MW output. 1week after irradiation, mice were sacrificed and the livers were analyzed. It has been suggested that radiation of normal liver tissue cause steatosis leading to fiblosis [2]. HE staining and Triglyceride quantification were performed to investigate degree of the steatosis in the mouse normal liver tissue after BNCT.

RESULTS: As shown in Fig. 1, the result of HE staining demonstrated that the steatosis of the BNCT group was increased. Furthermore, quantification of triglyceride was performed to determine the degree of steatosis of normal mouse liver tissue after BNCT. Triglycerides in mouse normal liver tissue after BNCT tended to be increased compared to control (Fig.2). In the future, it is necessary to carry out verification of liver fibrosis and quantification of related proteins.



Control 1000mg/ml BPA + neutron

Fig.1. HE staining of mice liver tissue after BNCT. Left panel is control and right panel is 1000mg/ml BPA plus neutron irradiation.

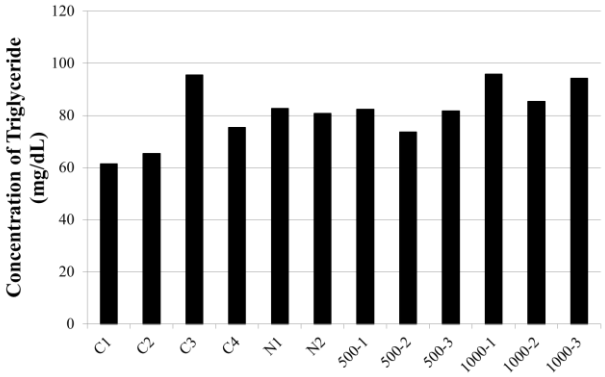


Fig.2. Triglyceride determination of mice normal liver tissue 1 week after BNCT. C: control, N: neutron alone, 500: 500mg/ml BPA + neutron, 1000: 1000mg/ml BPA + neutron.

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