CO7-1 Establishment of QA/QC for BNCT Neutron Irradiation Field

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INTRODUCTION: In May 2010, the operation of Kyoto University Reactor (KUR) restarted, which had been suspended during four years for the fuel-low-enrichment. Concurrently with the KUR restart, clinical irradiation of boron neutron capture therapy (BNCT) at the Heavy Water Neutron Irradiation Facility (HWNIF) also restarted [1]. After the restart, 170 BNCT irradiations have already been carried out as of May 2013. In the while, Cyclotron-based BNCT Epi-thermal Neutron Source (C-BENS) was installed in this institute in the end of 2008 [2]. In November 2012, the BNCT clinical trial using C-BENS started. Thus, this institute became a special institute in the world, in where BNCT is performed at the two-type neutron sources such as reactor-based one and accelerator-based one. It is one of the important subjects that the consistent dose-estimation is performed between the both neutron sources, and then the equivalence and homogeneity for the deposited dose during the clinical irradiation are assured. The aim of this research is the establishment of quality assurance and quality control (QA/QC) for BNCT neutron irradiation field. In 2012, one of the important tools for QA/QC, "Multi Ionization Chamber System (MICS)", was prepared by way of trial [3], and its characteristics were estimated and its efficacy was confirmed.

METHODS: The prototype of MICS consists of (i) a chamber of silicon-nitride wall and nitrogen gas for the thermal neutron component ($Si_3N_4(N_2)$), (ii) a chamber of boron-evaporation-coated polyethylene wall and nitrogen gas, covered with ^{6}LiF shield, for the epi-thermal neutron component ($Poly_B(N_2)$), (iii) a chamber of polyethylene wall and methane gas for the fast neutron component ($Poly(CH_4)$), and (iv) a chamber of graphite wall and argon gas for the gamma-ray component (G(Ar)). These chambers were placed on the bismuth-layer side of the collimator on the remote patient carrier, as shown in photo 1. The experiment for the characteristic estimation was performed for the epi-thermal neutron irradiation mode.

RESULTS AND DISCUSSIONS: Figure 1 shows the changes of the separate-estimated values with time for the four components, such as thermal neutron, epi-thermal neutron, fast neutron, and gamma ray, obtained using MICS. These values are for the KUR power of 1 MW. The vertical axis is in flux. The solid lines are for the experimentally-estimated values and the broken lines are for the estimated values by simulation. In the epi-thermal neutron irradiation mode, the thermal neutron flux was practically zero, because it was lower than the limit for the separate-estimation. For the epi-thermal and fast neutrons, those experimental values were in good agreement with the simulation values, in the uncertainty of 4% and 12%, respectively. For the gamma ray, the experimental value overestimated the simulation value at 63%. The flux dispersion in the experiment was 2%, 26% and 27%, respectively for epi-thermal neutron, fast neutron and gamma ray. It was confirmed that the accuracy in the separate-estimation was better and the response was more stable for the component with the larger response.

CONCLUSION: The efficacy of the prototype MICS was confirmed. The possibility of the separate-estimation for the thermal neutron component is expected, as the KUR power is 5 MW, during the actual BNCT.

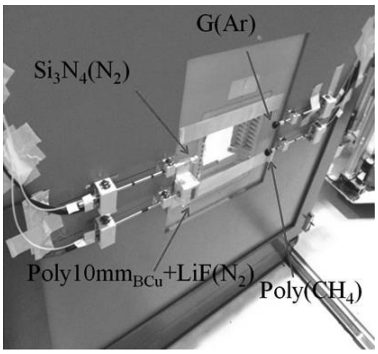

Photo 1. Placement of MICS on the collimator.

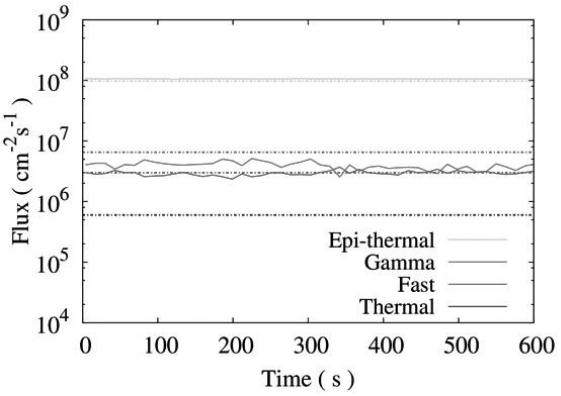

Fig. 1. Changes of the separate-estimated values with time, obtained using MICS.

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採択課題番号 24009　　ＢＮＣＴ用照射場におけるＱＡ／ＱＣの確立　　共同通常
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CO7-2 To Conquer the Clinically Relevant Radioresistant Cell Tumors Targeting Tumor Endohtelial Cells

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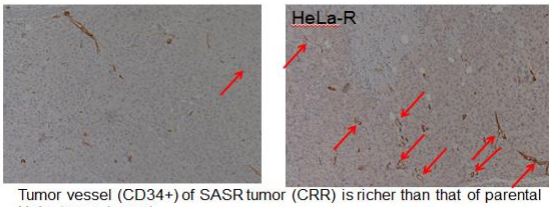
INTRODUCTION: Radiotherapy is one of the major therapeutic modalities for eradicating malignant tumors. However, the existence of radioresistant cells remains one of the most critical obstacles in radiotherapy. To understand the characteristics of radioresistant cells and to develop more effective radiotherapy, we have established clinically relevant radioresistant (CRR) cell lines. Because tumor tissues of CRR cells transplanted into nude mice were richer in tumor blood vessels compared with their radiosensitive parental cell lines. So, we performed boron neutron capture (BNC) method targeting tumor endothelial cells using PEG-10B. Growth rate of HeLa-R (CRR of HeLa) tumors were not significantly different from that of non-irradiated control in 4 weeks after irradiation. Tumors of parental cells treated with BNC using PEG-10B were significantly smaller than those without radiation. Seven days after BNC CD31 positive blood vessels were destroyed in BNCT treated HeLa tumors compared to HeLa tumor without BNCT but were not in HeLa-R tumors irrespective of BNCT. Moreover, to our surprise, 4 weeks after BNC the density of CD34, positive blood vessels were almost the same irrespective of BNCT in both HeLa-R and HeLa tumors.

We need further studies to confirm how the density of blood vessels contributes to tumor radiotherapy.

EXPERIMENTS: Three days before experiments, $1x10^6$ cells of HeLa and HeLa-R were injected subcutaneously into hind legs of male Balb/c nude mice (4 weeks old). The day of the exposure experiment tumor diameter was approximately 3-4 mm. For irradiation of tumor endothelial cells by α-particles, PEG-^{10}B were administered. The compound was suspended in physiological saline at a concentration of 2500 ppm and was injected via the tail vein. Three hours after the administration, mice were exposed to neutron radiation at the Research Reactor Institute, Kyoto University (KURRI).

Tumor size was determined by caliper measurements every three days. Endothelial cells of blood vessels in tumor tissues were immunohistochemically stained for CD34 and CD31. Type Ⅳ collagen for pericytes and functional blood vessels for injected tomatolectin from tails. We counted the number of vessels in 10 high power field (x 400) and calculated the average (n = 3).

Figure 1



Tumor vessel (CD34+) of SASR tumor (CRR) is richer than that of parental HeLa tumor (arrow).

Figure 2

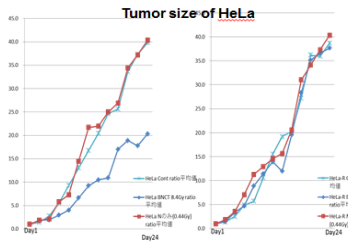


Figure 3

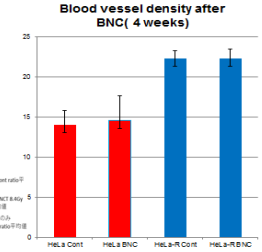


Fig.3 Tumor size of HeLa was significantly decreased in Day 24.
Fig.4 Blood vessel density in CRR tumors was higher than in parental cell tumors after BNC using PEG-10B (10 fields of view).

Figure 4 red: TypeIV Collagen blue: CD31 green: tomatolectin

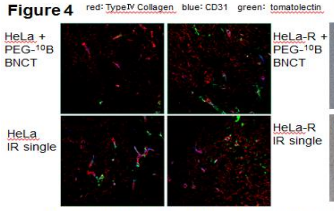


Figure 5

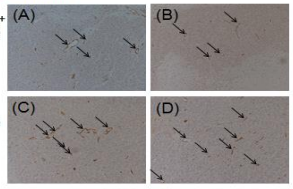


Fig.5 Histological analysis of tumor endothelial cells 7 days after BNCT (8 GyE of α-particles). The intensity of blood vessels of CRR tumors are richer than that of parental cell tumors. And after BNCT using PEG-^{10}B, the number of blood vessels of CRR tumors was recovered more rapidly than that of parental cells.
Fig.6 Histological analysis of tumor endothelial cells 30 days after BNCT. Blood vessels were visualized by CD34 staining. (A) HeLa tumor without BNCT. (B) HeLa tumor after BNCT. (C) HeLa-R tumor without BNCT. (D) HeLa-R tumor after BNCT. Arrow; blood vessels.

RESULTS: We first examined the size of HeLa tumors and HeLa-R daily after BNCT. Within 24 days after BNCT, the size of HeLa-R tumors was not different irrespective BNCT. But the size of HeLa tumors was significantly decreased compare to control tumors.
Mice were sacrificed on day 30 after BNCT. Histological examination showed that HeLa-R tumors were richer in tumor blood vessels compared with their radiosensitive parental cell tumors. CD34 positive blood vessels were also more abundant in SAS-R tumors than in SAS-tumors (Data not shown).

After BNC using PEG-10B, the vessels of HeLa-R tumors were recovered more rapidly in 7 days. On the other hand, those of parental tumors were destroyed.

However, 4 weeks after BNC, the density of CD34 positive blood vessels was almost the same irrespective of BNCT in both HeLa-R tumors and HeLa tumors.

DISCUSSION: In this research, we tried to target tumor endothelial cells of radioresistant HeLa-R tumors, because our preliminary experiment showed that the density of blood vessels in SAS-R tumors was higher than that in SAS tumors. We selectively exposed endothelial cells to α-particles, using BNC with PEG-^{10}B. The tumor volume of HeLa-R was not significantly different after exposure to 8 Gy of α-particles for the examination period. But that of HeLa was significantly decreased compare to control tumors.

7 days after BNC the density of CD31 positive blood vessels were destroyed in HeLa tumor exposed to α-particles compared to control HeLa tumor but did not in HeLa-R tumor irrespective of α-particles exposure. Moreover, to our surprise, 4 weeks after BNC the density of CD34, positive blood vessels were almost same irrespective of α-particles exposure in both HeLa-R tumor and HeLa.

Therefore, further studies are needed to confirm how blood vessel density contributes to tumor radiotherapy.

CO7-3 Study on Advanced Neutron Measurements Using a Small Size Neutron Scintillator

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INTRODUCTION: The Boron Neutron Capture Therapy (BNCT) has been developed as one of the promising radiotherapies. The neutron dose evaluation for the BNCT is quite important. Optical fiber type detectors as one of the on-line and small neutron flux monitors have been developed. The conventional optical fiber neutron detectors, however, show a continuous distribution without a characteristic shape, such as the full energy peak corresponding to the neutron induced reaction, in a pulse height spectrum due to large fluctuation of collected scintillation photons based on their poor light collection efficiency[1-3]. The sensitivity of these detectors depends on the detector signal gain. We, therefore, develop the advanced optical fiber type neutron detector using a small piece of Eu doped $LiCaAlF_6$ scintillator. This detector can show an obvious neutron absorption peak and suppress the gamma-ray sensitivity. In this report, we characterize the developed neutron detector at the Heavy Water Thermal Neutron Irradiation Facility (HWTNIF) of Kyoto University Research Reactor (KUR).

DEVELOPED DETECTOR: We fabricated the optical fiber type neutron detector using a small Eu:$LiCaAlF_6$ scintillator. The fabricated detector consists of a small piece of Eu doped $LiCaAlF_6$ scintillator, a plastic optical fiber, a photomultiplier tube (PMT) and signal processing circuits. A bulk Eu:$LiCaAlF_6$ scintillator suffers from influence of gamma rays because of its relatively low α/b ratio. Figure 1 shows the pulse height spectra obtained from a bulk Eu:$LiCaAlF_6$ scintillator. Gamma-ray signals are confirmed to interfere the neutron absorption peak.

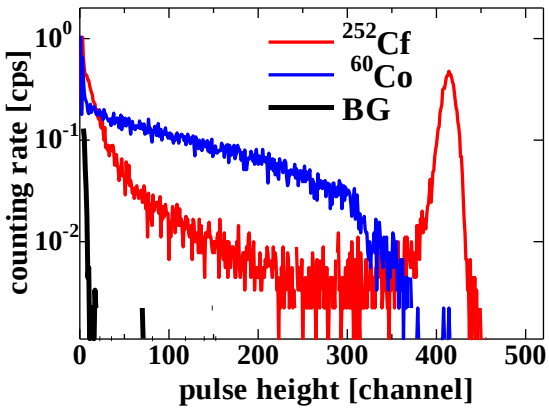

Fig. 1. Pulse height spectra obtained from a bulk Eu:$LiCaAlF_6$ scintillator.

Figure 2 shows the pulse height spectra obtained from an optical fiber detector with a small $LiCaAlF_6$ scintillator. The pulse height of signals induced only by gamma rays can be suppressed compared with neutron induced signals. This is because of differences in ranges of charged particles produced by neutrons and gamma rays.

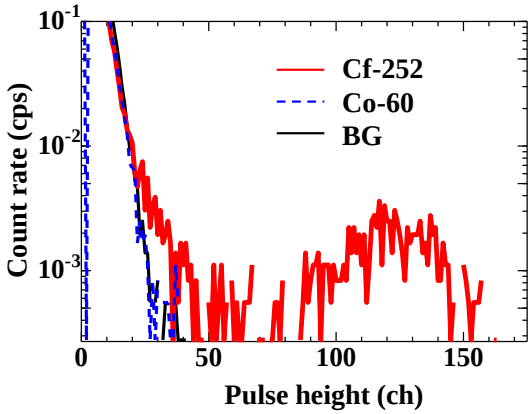

Fig. 2. Pulse height spectra obtained from an optical fiber detector with a small Eu:$LiCaAlF_6$ scintillator.

EXPERIMENTS AT HWTNIF OF KUR: We characterize the fabricated detector at the HWTNIF of KUR. The detector head was placed at various distances from the bismuth filter surface. An example of the pulse height spectrum obtained from the fabricated detector in experiments at the HWTMIF of KUR is shown in Fig. 3, where the distance from the bismuth filter surface was 120 cm. A clear neutron absorption peak was obviously observed.

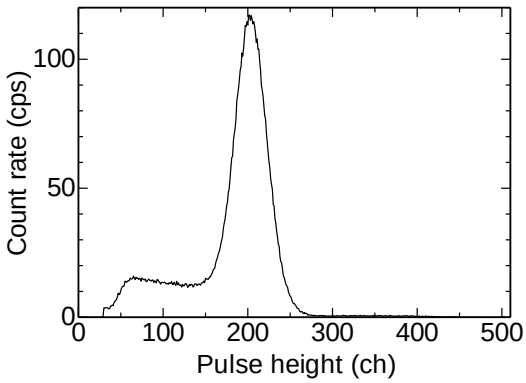

Fig. 3. Example of the pulse height spectrum obtained from the fabricated detector in experiments at the HWTNIF of KUR.

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採択課題番号 24014　極少検出器を用いた中性子計測手法の高度化に関する研究　共同通常
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（京大・原子炉）櫻井良憲、田中浩基

CO7-4 High Boron Content Liposomes and Their Promising Antitumor Effect for BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) functions as a double targeting therapy for cancer. Its therapeutic effect is realized by neutron beam irradiation and a boron delivery system (BDS). BNCT uses the nuclear reaction of two species, boron-10 (^{10}B) and thermal neutrons. Although the low-energy thermal neutrons (0.025 eV) are employed, the resulting α -particle and Li nuclei are high linear energy transfer (LET) particles that travel a short distance (approximately 5−9 μm) to destroy cells containing ^{10}B. If ^{10}B atoms were selectively delivered to intracellular regions of tumor tissue, it would be possible to kill tumor cells selectively without seriously damaging adjacent healthy tissues.

In this study, we focused on lipophilic boron compounds embedded in a liposome bilayer. This strategy is an attractive means to increase the overall incorporation efficiency of boron containing species, as well as to raise the gross boron content of liposomes [1]. We developed high boron content liposomes by incorporating boron into both the interior aqueous core and the membrane of liposomes. Indeed, this strategy yielded significant antitumor effect on tumor-bearing mice after neutron irradiation, as well as a reduction of the total liposome dose, revealing that the current boronated liposome is one of the most promising candidates for practical use in BDSs for BNCT.

EXPERIMENTS: DSPC (MC-8080) and DSPE-PEG (Sunbright DSPE-020CN) were purchased from Nippon Oil and Fats (Tokyo, Japan). Cholesterol (Chol) was purchased from Kanto Chemical (Tokyo, Japan). ^{10}B-enriched BSH and S–cyanoethyl protected ^{10}B -enriched BSH were purchased from Stella Pharma Co. (Osaka, Japan). Boron lipid (DSBL) was synthesized according to the previously described procedures with modification [2].

BSH-encapsulated DSBL-10% liposomes, which were prepared from ^{10}B-enriched DSBL, DSPC, Chol, and DSPE-PEG (0.1:0.9:1:0.11, molar ratio) and 125 mM BSH aqueous solution according to the REV method previously described [3], were injected into colon 26 tumor bearing mice (female, 6−7 weeks old, 16−20 g, 5 mice in each group) via the tail vein at doses of 15 and 30 mg ^{10}B /kg (1500 and 3000 ppm of ^{10}B concentration; 200 μL of boronated liposome solution). The mice were placed in an acrylic mouse holder 36 h after *i.v.* injection. The mice were irradiated in KUR. The antitumor effects of BNCT were evaluated on the basis of the changes in tumor volume of the mice. Mortality was monitored daily and tumor volume was measured at intervals of a few days.

RESULTS: The tumor growth curves are shown in Figure 5. At doses of 15, 30, and 50 mg ^{10}B/kg, tumor growth in mice treated with BSH-encapsulating 10% DSBL liposomes was significantly inhibited after thermal neutron irradiation, and the tumor disappeared within three weeks even when the dose of 15 mg ^{10}B/kg was injected. However, the inhibition was not observed in mice injected with BSH-encapsulating 10% DSBL liposomes (30 mg ^{10}B/kg) without thermal neutron irradiation, or in hot and cold control mice [4].

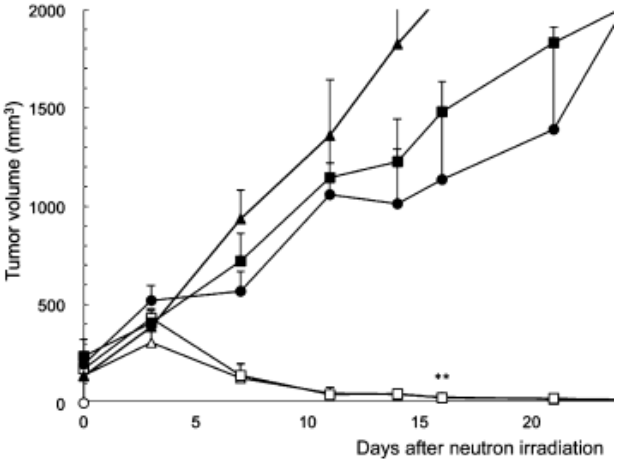

Fig. 1. Tumor volumes in mice (Balb/c, female, 6 weeks old, 14−20 g) bearing colon 26 solid tumor after *i.v.* injection of BSH-encapsulating 10% DSBL liposomes (15 (-Δ-), 30 (-□-), 50 (-○-) mg ^{10}B/kg) and thermal neutron irradiation for 50 min (1 MW) at a rate of (1.5−1.8) x 10^{12} neutrons/cm^2 36 h after injection. Tumor volumes of mice without injection of liposomes with thermal neutron irradiation (-●-), without thermal neutron irradiation (-▲-), or with injection the liposomes (30 mg ^{10}B/kg) without thermal neutron irradiation (-■-)".

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採択課題番号 24023　　中性子捕捉治療のための新規ホウ素薬剤の開発　　共同通常
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CO7-5 Long-Term Result of BNCT for Different Types of Human Clear Cell Sarcoma in Mouse Model

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INTRODUCTION: Clear cell sarcoma (CCS) of tendons and aponeuroses is a rare neoplasm with no effective treatment other than surgery. Furthermore, this malignant tumor has a predilection for young adults and its prognosis is poor [1]. Clearly, therefore, new therapeutic strategies are required. In a previous study, we have shown that the high accumulation of ^{10}B both in cultured human CCS cell lines and in CCS-bearing animal models, is potentially propitious in boron neutron capture therapy (BNCT) with the use of *p*-boronophenylalanine (BPA) [2,3]. We have recently also demonstrated the effectiveness of short-term BNCT in the human CCS-bearing nude mouse model [4]. Thus BNCT could become a potential, new therapeutic option for the treatment of human CCS. Consequently, in this study, we evaluated the long-term efficacy of BNCT for CCS, with the use of nude mice intramuscularly transplanted with human CCS cell lines.

EXPERIMENTS: (1) *Tumor cell line*: Human CCS cell lines, MP-CCS-SY [5], SU-CCS-1 [6] and HS-MM [7] were grown in RPMI 1640 and DMEM (for HS-MM) with fetal bovine serum in a 5% CO_2 humidified incubator at 37˚C.
(2) *BNCT for CCS-bearing animal*: All animal experiments were carried out according to the regulations of the Animal Care and Use Committee. Cells of CCS cell lines were transplanted into the left femoral region of BALB/c nude mice. When CCS tumors grew to about 10-20mm in diameter, the animals were divided into 3 BNCT groups and 3 control groups for each cell line. The animals, under anesthesia and through the femoral vein, were intravenously administered BPA-Fr (24mg ^{10}B/kg; the BNCT groups) or saline (the control groups), and then immediately placed in a chamber for thermal neutron irradiation experiments. Thermal neutrons (1MW) were delivered from the dorsum of the mouse, in the heavy water facility at KURRI. LiF tiles were used to shield parts of the body other than the left leg. After the irradiation, the size of the tumor was measured as the maximum elliptical area of the tumor mass as follows: Maximum tumor area=(minor axis)×(major axis), and the "Area ratio" was defined as the area of each divided by the area of day 0. Tissue samples of the tumor mass resected under anesthesia from the control groups on day 45, and from the BNCT groups on day 90 were fixed in 10% buffered-formalin solution and embedded in paraffin according to standard protocols. Sections were then stained with hematoxylin-eosin (HE) for histological examination.

RESULTS: The doses absorbed (Gy) by the mice intramuscularly transplanted with the CCS cells were 7.2 (HS-MM), 6.8 (MP-CCS-SY) and 7.1 (SU-CCS-1). After irradiation, the size of the decreased time-dependently in the BNCT groups until around day 20, and then regrew time-dependently. In the control groups, the growth was not suppressed by thermal neutron irradiation; the tumor mass simply increased with time [Fig.1]. Histological examination of the BNCT groups on day 90 revealed regrowth of the CCS tumor of each cell line, with no damage to normal surrounding tissue.

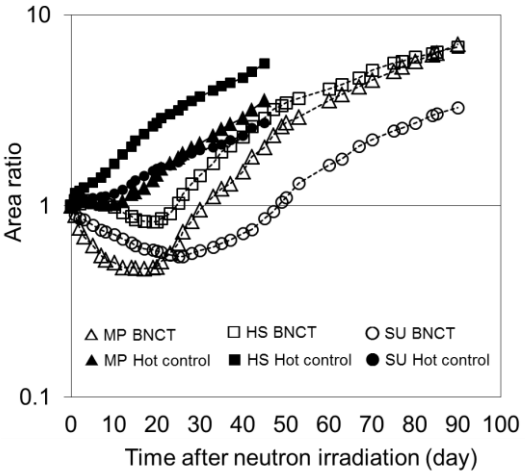

Fig.1. After the neutron irradiation, the size of the tumor was measured as the maximum elliptical area of the tumor mass. BNCT groups (ΔMP BNCT; MP-CCS-SY, □HS BNCT; HS-MM, ○SU BNCT; SU-CCS-1) and control groups (▲MP Hot control; MP-CCS-SY, ■HS Hot control; HS-MM, ●SU Hot control; SU-CCS-1).

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採択課題番号 24029　　明細胞肉腫に対するホウ素中性子捕捉療法の検討　　共同通常
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CO7-6 Application of B_4C Nanoparticles for Boron Neutron Capture Therapy

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INTRODUCTION:

In carcinoma in situ (CIS) in the bladder, the cancer cells are still only in the mucosa of the bladder, but are in flat sheets that look a bit like moss. CIS bladder cancer is called a high risk, early bladder cancer because if it is not treated it is very likely to spread into the deeper layers of the bladder.

Nonradioactive isotope ^{10}B atoms that absorb low-energy (<0.5 eV) neutrons (thermal neutrons) disintegrate into an alpha (^4He) particle and a recoiled lithium nucleus (^7Li). These particles deposit high energy along their very short path (<10 μm). Thus, only malignant cells with ^{10}B are destroyed following thermal neutron irradiation. Theoretically, any normal cells abutting the cancer cells are spared from high linear energy transfer irradiation by ^4He and ^7Li particles.

We have been working on a nanoparticle fabrication technique by irradiating laser light onto a plate placed or powder dispersed in liquid media. This technique has several advantages over conventional nanoparticle preparation in liquid phase, highly pure nanoparticles with less use of surfactant molecules mostly toxic in biological systems, and crystallized nanoparticle formation due to the transient high temperature process induced by pulsed laser irradiation. Thus, these features are expected to be suitable for B_4C nanoparticle preparation by pulsed laser irradiation of B_4C particles dispersed in ethyl acetate under atmospheric pressure and room temperature [1].

In the treatment of BNCT for CIS bladder cancer, intravesical administration of B_4C nanoparticles may be appropriate drug delivery system as Bacille de Calmette et Guérin (BCG) vaccine treatment since carcinoma cells in CIS bladder cancer is exposed directly by B_4C nanoparticles applied to bladder.

We carried out a preliminary experiment investigating availability of B_4C nanoparticles in the treatment of BNCT for CIS bladder cancer.

EXPERIMENTS:

HeLa cells were used in this study. The cells were dispersed in the 96-well microplates at the concentration of 10,000 cells per well the day before neutron irradiation. In this experiment, B-10 rich nanoparticles ($^{10}B_4C$ nanoparticles) were used in the BNCT treatment groups. The treatment conditions were sorted into 5 groups as follows.

1. No treatment
2. $^{10}B_4C$ exposure without neutron irradiation
3. Neutron irradiation without $^{10}B_4C$ exposure.
4. BNCT with $^{10}B_4C$ nanoparticles (no rinse).
5. BNCT following 1 h-exposure with $^{10}B_4C$ and rinse the cells with PBS.

In the treatment groups 4 and 5, $^{10}B_4C$ nanoparticles were exposed for 1hour at the concentration of 10 μg/well (0.31 cm^2). Thermal neutron beam (Mixed mode) irradiated the cells for 20 minutes. Cell viability was assayed by a colorimetric assay (WST-1assay).

RESULTS:

Thermal neutrons delivered to the cells were estimated as 1.0E+12 n/cm^2. Results on viability in 5 treatment groups were shown in Fig. 1.

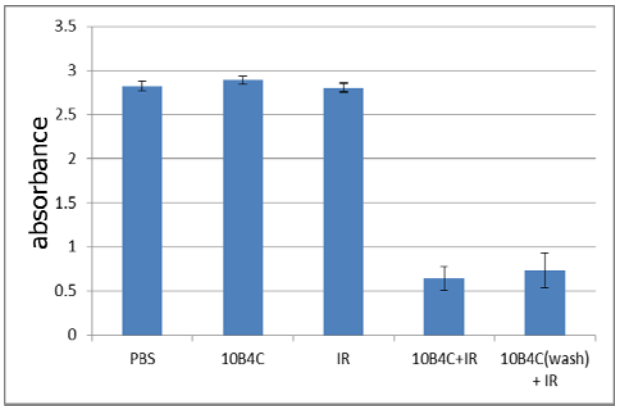

Fig. 1 In two control groups (groups 2 and 3, 10B4C and IR), cell proliferation was not inhibited compared with no treatment group (PBS). In two BNCT groups (groups 4 and 5, 10B4C+IR and 10B4C (wash)+IR), decrease in cell viability was equally observed.

In the group 5, following rinse of the cells with PBS, $^{10}B_4C$ nanoparticles attached to the cells was observed. The observed nanoparticles were much lesser compared with those in the group 4, which suggested that BNCT with much less $^{10}B_4C$ nanoparticles may be enough for sufficient cell toxicity.

DISCUSSION:

BNCT with intravesical administration of $^{10}B_4C$ nanoparticles for CIS bladder cancer has three advantages from a clinical viewpoint. One is that occurrence of systemic toxicity derived from the newly-developed boron compound is not necessary to be considered. Second is that the irradiated doses to rectum or small intestine around the bladder is very low since no boron compound distributed in these normal organs. Third is that $^{10}B_4C$ nanoparticles are more economically prepared compared with other boron compounds, boronophenylalanine (BPA) and borocaptate sodium. We have planned to carry out in vivo BNCT experiments with $^{10}B_4C$ nanoparticles using orthotropic bladder cancer model mice.

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CO7-7 Development of Novel Boron Compounds for BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) is an attractive technique for cancer treatment. Although many kinds of boron compounds such as amino acid, nucleic acid and peptides have been reported as boron carrier for BNCT [1], only two compounds, *p*-borono-L-phenylalanine (BPA) and mercapto-*closo*-undecahydrododecaborate (BSH) are clinically used in cure of cancer with BNCT. As part of our developing studies on new boron delivery agents for BNCT, we have designed and synthesized thiododecaborate ($[B_{12}H_{11}]^{2-}$-S-) unit containing L-amino acids (**1a–c**), which constitute a new class of tumor-seeking and water soluble amino acids (Fig. 1). *In vitro* evaluation studies on the cytotoxicity, killing effects by neutron irradiation, and micro distribution analysis performed previously by our group suggested that **1a–c** might be potential delivery agents for BNCT [2]. On the other hand, recently, radio-labeled with ^{18}F α,α-cycloalkyl amino acids such as 1-aminocyclobutane-1-carboxylic acid (ACBC) are highly noted as useful PET (positron emission tomography) tracers for diagnosis of cancer, since unusual amino acids having α,α- alkyl ring are incorporated selectively and temporarily retained by cancer cells.
To develop practical materials utilizing ^{10}B carrier, we have newly synthesized dodecdaboratethio-unit containing α, α-cycloalkyl amino acids such as *cis/trans*-[1-amino-3-(thiododecaboranyl)methyl]cyclobutane-1-carboxylic acid (ACBC-BSH, **2a** and **2b**) bearing no asymmetric carbon atom, by extension of our reported method. Here, we report the tumor cell killing effects of **2** against cultivated cancer cells.

MATERIAL and METHOD: Cultures were inoculated with 1.0 x 10^6 cells/dish, and cells were grown for 24 h in DMEM. The medium was replaced with the each medium containing each boron amino acid (final concentration was 1.0 mM in each case). The cells were cultured for 24 h, and the medium was removed by aspiration. The cells were washed with PBS, harvested by trypsinization, and then cell numbers were counted. After centrifugation, the tripsin was removed by aspiration, and to the residual cells was added DMEM. The suspension of the cell in DMEM (5.0 x 10^3 cells/mL, 1mL) was irradiated with thermal neutron for 0 - 90 min in column-shape tube. The thermal neutron fluence was determined by

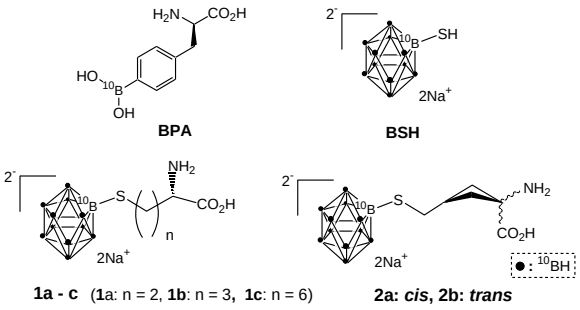

Fig. 1. Boron compounds.

averaging two gold foils symmetrically attached to the surface of the column-shape tube along the direction of incidence of thermal neutrons. After thermal neutron exposure, 600 cells were placed in three Corning 60 mm tissue culture dishes containing 3 mL DMEM to examine colony formation. Seven days later, the colonies were fixed with ethanol and stained with 0.1% crystal violet for quantitative visualization by the naked eye.

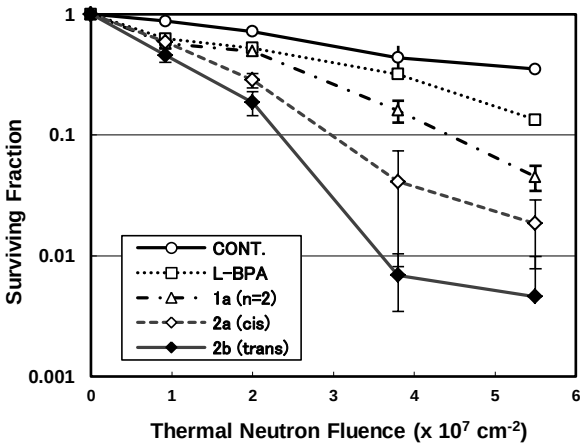

Fig. 2. Tumor cell killing effects of boron amino acids against C6 Cell.

RESULTS: To confirm the usefulness of ACBC-BSH **2a** and **2b** for BNCT, we examined the tumor cell killing effects of L-BPA, **1a, 2a,** and **2b** against C6 (rat glioma), cell. As shown in Fig. 2, ACBC-BSH **2a** and **2b** showed higher killing effects than that of BPA and **1a**. This result suggests that ACBC-BSH **2** is useful for ^{10}B carrier on BNCT for glioma.

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採択課題番号 24036　ホウ素中性子捕捉療法の新規ホウ素薬剤の開発研究　共同通常
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CO7-8 BNCT for Malignant Melanoma and Head and Neck Cancer

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INTRODUCTION: Advanced head and neck carcinoma (AHNC) and recurrent head and neck cancer (RHNC) are often radio-/chemo-resistant and show extensive growth, requiring a wide resection including surrounding normal tissues. To avoid severe impairment of head and neck structures, it is necessary to explore new treatment for AHNC. Mishima first proposed employing BNCT for malignant melanomas utilizing the specific melanin synthesis activity of melanoma cells [1]. Kato et al. [2] began BNCT using both BSH and BPA for recurrent parotid gland carcinoma for the first time and reported excellent preliminary results. On the basis of the encouraging results of their pioneering clinical trial, our many years' experience with melanoma BNCT and the trend toward emphasizing the quality of life after treatment, we also began treating our patients with BNCT using BPA alone [3-4]. This report is a summary of treatment by BNCT using BPA alone in 2012.

16 cases are consulted to Kawasaki Medical School Hospital as referral visit for BNCT in 2012. 10 patients were treated, consisting 4 men, 6 women; a median age of 60 years old (range 37-86 years). The number of head and neck recurrent carcinomas was 7, and that of cutaneous malignant melanomas was 3. 6 in head and neck recurrent cancer were squamous-cell carcinoma (SCC) recurrence, and one was the neck lymph node metastasis of the malignant melanoma. The local recurrence was five, and the number of neck lymph node metastasis was one among SCC. All cutaneous malignant melanomas treated BNCT as first choice. Patients of head and neck recurrent carcinoma have undergone the operation in the past except the one of malignant melanoma. The local recurrence was five, and the number of neck lymph node metastasis was one among SCC. All cutaneous malignant melanomas treated BNCT as first choice. Patients of head and neck recurrent carcinoma have undergone the operation in the past except the one of malignant melanoma.

RESULT: Although the malignant melanoma faded, it still remains. Neither a recurrence nor progression is accepted. Any adverse event is not occurred. One of the head and neck recurrent carcinomas which is neck lymph node metastasis of the malignant melanoma died in a month after BNCT cause of rapid progression of cancer. In two patients of head and neck cancer (SCC), the local recurrence occurred within two months after BNCT. The shortage of doses was able to be considered as acause. And two of four patients which carried out partial control accepted the recurrence outside the exposure field. Although the partial control by BNCT is good, it does not contribute to overall survival.

Conclusion: We are considering combined use with systemic therapy may also be useful to find the better result.

Details:

Age (median)	60 y.o. (37-86 y.o.)
male	4
Female	6
Head and neck carcinoma	7
SCC	6
nasopharynx	1
hypopharynx	1
buccal mucosa	1
maxillary sinus	1
external auditory canal	2
malignant melanoma	1
local recurrence (SCC)	5
cervical lymph node metastasis	2
external auditory canal	1
malignant melanoma	1
cutaneous malignant melanomas	3
heel	2
upper lip	1

Result:

local recurrence	2
metastasis	2

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採択課題番号 24037　　中性子補足療法適応癌腫のプロトコル確率　　共同通常

（川崎医大）平塚純一、原田保、粟飯原輝人、宇野雅子、森田倫正、牧野英一、笹岡俊輔、小西圭、神谷伸彦（京大・原子炉）小野公二、丸橋晃

CO7-9 Study on the Semiconductor Device Error Irradiated with Thermal Neutrons

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INTRODUCTION: Recently, the irradiation experiments of fast neutrons for semiconductor devices have been performed using cyclotron facility of Research Center for Nuclear Physics, Osaka University [1]. However, irradiation test using thermal neutron for semiconductor device has not been systematically performed. If the nuclide with large capture cross section of thermal neutrons is contained in semiconductor devices, charged particles are produced in semiconductor devises. Charged particles create electron-hole pair toward those tracks that become the current of noise in semiconductor devises. There is fear that the current in semiconductor devices triggers incorrect operation. Furthermore, the miniaturization of semiconductor device is proceeding and the current of signal is becoming small. Thus, the sensitivity for electronic noise becomes large. The aim of this study is the investigation of the influence for semiconductor device of thermal neutron irradiation.

EXPERIMENTS: The sample of semiconductor device was irradiated at Heavy Water Neutron Irradiation Facility (HWNIF) of KUR [2]. To investigate the influence of thermal neutrons, the difference of the number of error (bit flip of memory) for epithermal mode (CO-0000F) and mix mode (OO-0000F) was recorded. Fig. 1 shows the neutron spectrum of two irradiation mode. Epithermal mode can produce the epithermal and fast neutrons without thermal neutrons using a cadmium filter.

A number of semiconductor devices were irradiated simultaneously. The irradiated thermal neutron distribution was not uniform because of its large irradiation area. In order to measure thermal neutron flux, gold wire was set at the surface of each semiconductor device. Gold wire was taken from the surface of a semiconductor device to determine thermal neutron flux. The number of error of each semiconductor device was recorded during the irradiation of thermal neutron. Next, the number of error was also recorded for epithermal and fast neutron irradiation.

RESULTS: Table 1 shows the number of error of each semiconductor for each irradiation mode and thermal neutron flux. The deviation of thermal neutron fluxes for each semiconductor device was shown in this table. The importance of the measurement of thermal neutron fluxes was revealed according to these results. Fig. 2 shows the comparison of the number of error, that was removed the influence of epithermal and fast neutrons, per thermal neutron fluence. As shown in Fig. 2, the number of error for sample D, E, F was larger than other samples. We established the thermal neutron irradiation field for the investigation of error of semiconductor devices.

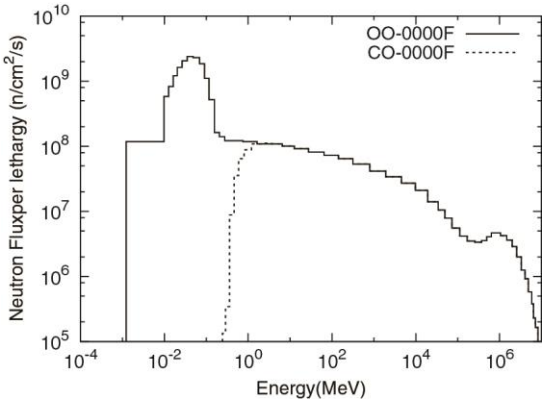

Fig.1. Neutron spectrum of CO-0000F and OO-0000F mode at the surface of gamma ray shield of HWNIF.

Table 1. the number of error of each semiconductor for each irradiation mode and thermal neutron fluxes

	Thermal neutron Flux [n/cm/s]	The number of error	
		OO-0000F	CO-0000F
Sample A	1.0E+8	53	21
Sample B	1.0E+8	39	17
Sample C	9.1E+7	110	54
Smaple D	2.0E+8	7378	293
Sample E	2.1E+8	3959	110
Sample F	2.1E+8	3537	92
Sample G	1.2E+8	39	12

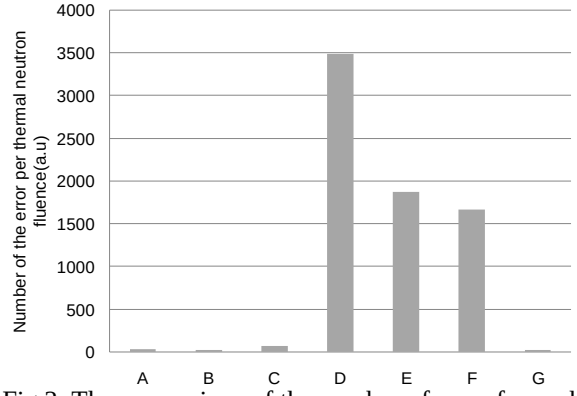

Fig.2. The comparison of the number of error for each sample.

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採択課題番号　24043　　中性子照射による半導体デバイスエラーに関する研究　　一般通常

（京大・原子炉）田中浩基、櫻井良憲、堀順一

CO7-10 Tissue Changes by BNCT of the Oral Cancer Tissue at Having Used Hyaluronan Conjugated PEG Liposome

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INTRODUCTION: In Boron neutron capture therapy (BNCT), selective and highly concentrated boron accumulation in tumor cells is important. A study was carried out in our division using various liposomes [1, 2].
Hyaluronan is a ligand of CD44 and RHAMM, which is excessively expressed in tumor cells, and Hyaluronan-conjugated PEG Liposome (HA PEG Liposome) is an active targeting candidate for tumor cells.

In this study, the effect of neutron irradiation was investigated by histological observation, in order to consider BSH transport using HA PEG Liposomes in mice with oral squamous cancer cells.

EXPERIMENTS: Tumor bearing mice were prepared by injecting cultured SAS cells into the right thigh of BALB/c mice. When tumor size reached approximately 10 mm, HA-PEG-Liposomal BSH was intravenously administered from the tail vein. This injection was carried out at the point in time when boron concentration in the tumor tissue could be maintained at a comparatively higher condition than the surrounding tissue than that of previous experiments, in other words, two days before of neutron irradiation. For reference, a control group which used liposomes without boron was also set. After neutron irradiation, the mice were allowed to grow for 8 weeks. At the 8 weeks, all mice were euthanized, and tissue samples were taken. Formalin fixation was carried out on the samples, and the samples were set as a formalin fixed paraffin sections according to the standard method, HE dyed, and observed by a microscope.

RESULTS: Regarding tumor size observation after irradiation, among the group administered BSH, significant tumor shrinkage effect was observed in some specimens and no effect was observed in other specimens. No tumor shrinkage effect was observed in the control group. Variation in the results in the administered group is assumed to be due to technical error in intravenous injection, etc.

In specimens where tumor shrinkage effect was observed, the site of the tumor was macroscopically ulcer-like in appearance and no tumor was observed. Histologically, the ulcer sites were observed on the epithelial surface, and tumor residue was observed in the connective tissue layer under the ulcer. In the control group, images of tumor tissue proliferation were observed.

In the future, methods to increase the effectiveness of killing tumor cells should be considered.

Fig. 1. Macroscopic photo of a mouse before neutron irradiation

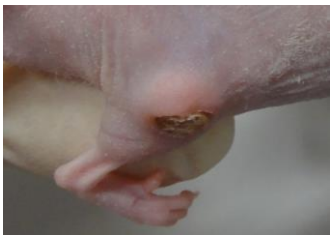

Fig. 2. Macroscopic photo at the 8 weeks after neutron irradiation, boron injected group

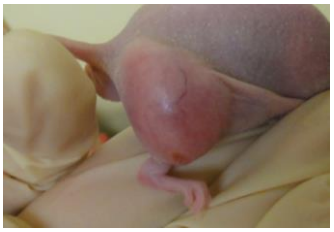

Fig. 3. Macroscopic photo at the 8th week after neutron irradiation, boron non-injected group

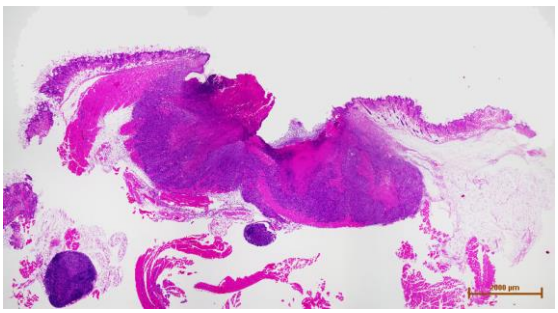

Fig. 4. HE tissue image, ulcer-bearing section in the epithelium, and tumor cells are observed in the connective tissue layer under the ulcer. On the ulcer side of the tumor, necrotic tissue and fibrin deposition was observed. (bar=2mm)

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採択番号　24046　　口腔癌に対する硼素中性子補足療法の基礎的研究　　共同通常

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CO7-11 Development of Water-Soluble Gd Metallofullerenes/PEG-b-Polyamine Complexed Nanoparticles for Neutron Capture Therapy

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INTRODUCTION: Gadolinium element is one of promising candidates for neutron capture therapy (NCT) because of high neutron capture cross section. The essential toxicity of Gd ion is one of the problems to solve. Gadolinium chelates with specific ligand such as diethylenetriaminepentaacetic acid (DTPA), and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), have been proposed to reduce toxicity. However, these Gd chelates still show toxicity and are not enough for NCT, because high dose is required for NCT treatment. Gadolinium endohedral metallofullerene ($Gd@C_{82}$) are emerging compounds and promising as new biomaterials to suppress the toxicity of Gd by fullerene cage. In order to use $Gd@C_{82}$ compounds in biological fluid, delivatization of $Gd@C_{82}$ has been done in most cases, which causes unwanted bioactivity. Thus, we have been used fullerenes without delivatization. To solubilize fullerenes in aqueous media, poly(ethylene glycol)-block-poly(2-(N,N-diethylamino)ethyl methacrylate (PEG–*b*–PAMA) were employed in our previous work[1]. The result of the NCT experiment in vitro showed that $Gd@_{82}$/PEG–*b*–PAMA complexed nanoparicle was effective NCT agent. To develop the more safety water-soluble $Gd@C_{82}$ nanoparticles as NCT agent, radical containing amphiphilic block copolymer (PEG–*b*–PMNT) was used as dispersion agent [2]. PEG–*b*–PMNT is low toxic polymer (LD_{50} > 600 mg/Kg), and it acts as reactive oxygen species (ROS) scavenger to suppress the unwanted immune response induced by ROS after neutron irradiation. In this report, we investigate the NCT by $Gd@C_{82}$/PEG–*b*–PMNT complexed nanoparti-cles (GdNPs). To confirm the tumor treatment, tumor sizes of the mice were measured after neutron irradiation.

EXPERIMENTS:
Preparation of $Gd@C_{82}$ nanoparticles: 10 mg of $Gd@C_{82}$ was dissolved in 50 mL of DMF and sonicated for 1 h. 50 mg of PEG–*b*–PMNT was added to the mixture and the sonication continued for 3 h. The mixture was transferred to a dialysis tube and dialyzed against 2 L of water. The external water was changed 5 times at t = 3, 15, 39, 63 and 87 h. The solution was condensed by ultrafiltration and dispersed in PBS for in vivo experiment.
Preparation of tumor bearing mice (BLAB/c, male, 5weeks): Tumors were prepared in mice legs by hypodermic injection of colon-26 cells (1,000,000 cells per mouse). This procedure was carried out a week before the neutron irradiation.
Neutron irradiation: The administration of the GdNPs were carried out via tail vein (1 mg of $Gd@C_{82}$ per mouse). After 2 d, the mice were irradiated thermal neutrons for 120 min at a rate of 9.4 – 9.8 × 10^{12} neutrons/cm^2). After the neutron irradiation, the tumor sizes of mice were measured for 26 d.

RESULTS:
Evaluation of obtained $Gd@C_{82}$ nanoparticles: The size of the GdNP was evaluated by DLS. The result showed that the average size of GdNP was ca. 40 nm and the dispersion property of the particles is quite stable enough to keep the dispersion for over a year.
Neutron Irradiation: The tumor grew up to 6 – 7 cm^3 after 26 d without GdNPs and no thermal neutron irradiation (n = 4). On the other hand, the growth was effectively suppressed in the mice treated with GdNPs with the irradiation of thermal neutron (average size was 2 cm^3, n = 4). The suppression of tumor growth was also observed in the mice with the irradiation of thermal neutron without GdNPs (average size was approx. 4 cm^3, n = 5), the suppression efficacy of tumor growth of the mice with GdNPs with the irradiation was higher than that of the mice without GdNPs with the irradiation (p-value = 0.144). Our results demonstrate that the rational material design of GdNPs holds promise for the future of GdNCT.

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採択課題番号24047　　高い腫瘍選択性を有するボロン含有ナノ粒子の創製と中性子捕捉療法への展開　　共同通常

（筑波大・数理）長崎幸夫、矢口達也、角谷省吾、高振宇、堀口諭吉（名古屋大学）野田祥子、中西勇介、篠原久典（京大・原子炉）鈴木実、小野公二

CO7-12 Pilot Biodistribution Study of Poly-ion Complex Mediated Gadolinium Delivery System to Cancer Model *In vivo*

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INTRODUCTION:

Tumour cell destruction in gadolinium (Gd) neutron-capture therapy (GdNCT) is due to the nuclear reaction between Gd atoms and thermal neutrons. It is necessary for effective neutron capture therapy to accumulate Gd atoms in the tumour cells without affecting adjacent healthy cells.

We have developed novel efficient gene transfection system, comprising the plasmid/polycation complex core and the outer polyanion-coating. We used Polyethyleneimine (PEI) as polycationic polymer, and Polyethylene glycol derivatives having carboxylic acid [1,2]. We had also applied this poly-ion complex as heavy ion delivery systems. In this study, we prepare Gadoteridol entrapped poly-ion complex for selective intravenous or intratumoural injection for mouse colon cancer model applicating to GdNCT, and evaluate the poly-ion complex as selective Gd delivery carrier to cancer tissues.

EXPERIMENTS:

(1) Cell: Mouse colon cancer cell line: Colon 26
(2) Polyethyleneimine: ExGen 500 (Fermentas Ltd.) was used. ExGen 500 is linear shaped 22 kDa PEI. The efficient gene transfer activity of ExGen 500 is related to its capacity for condensing DNA, interacting with anionic proteoglycans of the cell membrane, protecting DNA and inducing endosomal swelling and rupture before DNA degradation.
(3) PEG-C: Polyethylene glycol derivatives having carboxylic acid was synthesized (MW of PEG was 8940, 17.7 carboxylic acid groups were binding per PEG molecule).
(4) JTS-1: pH dependent fusogenic peptide was kindly gifted by Professor Leaf Huang, Department of Pharmacy, University of Pittsburgh, PA, USA.
(5) Gadoteridol: (±) -10 - (2-hydroxypropyl) -1,4,7, 10- tetraazacyclo-dodecane -1,4,7- triacetatogadolinium ［III］($C_{17}H_{29}GdN_4O_7$) (MW: 558.69, 1396.5 mg/5ml) .
(6) Gadolinium Delivery in the Colon 26 tumours

①Colon 26 cells (2 x 10^6) were injected subcutaneously into the back of female BALB/c mice. At 10–14 days after injection, when the estimated tumour weight reached about 500 mg, the recipient animals were injected intravenously or intratumorally with either Gd/PEI, Gd/PEI/PEG-C/JTS-1, or Gd solution.
②One, and three days after injections, the Gd concentrations of the tumor nodules, blood, and normal organs were measured. The Gd concentrations of tissues were determinated by ICP- Mass Spectroscopy of Juntendo University.

RESULTS:

The Gd concentration in tumour by intratumoural (IT) delivery with Gd/PEI was 53.7 ppm, 22.5 ppm, after 2 hours and 12 hours, respectively. The Gd concentration in tumour by IT delivery with Gd/PEI/PEG-C/JTS-1 was 21.9 ppm, 12.3 ppm, after 2 hours and 12 hours, respectively. The Gd concentration in tumour by IT delivery with Gd solution was 17.4 ppm, 6.8 ppm, after 2 hours, 12 hours, respectively.

The Gd concentrations in Colon 26 tumour on delivery with poly-ion complex (Gd/PEI) was 3 times superior to simple Gd solution after 2 hours IT injection.

The Gd concentration in tumour by intravenous (IV) delivery with Gd/PEI was 4.0 ppm, 1.2 ppm, after 2 hours, 12 hours, respectively. The Gd concentration in tumour by IV delivery with Gd/PEI/PEG-C/JTS-1 was 9.8 ppm, 2.7 ppm, after 2 hours, 12 hours, respectively. The Gd concentration in tumour by IV delivery with Gd solution was 1.1 ppm, 0.0 ppm, after 2 hours, 12 hours, respectively.

The Gd concentrations in Colon 26 tumour on delivery with poly-ion complex (Gd/PEI) was 4 times superior to simple Gd solution after 2 hours IV injection, and also 9 times superior to control groups in the usage of poly-ion complex (Gd/PEI/PEG-C/JTS-1) .

CONCLUSION:

Poly-ion complex (Gd/PEI/PEG-C/JTS-1) can be applied to the Gd delivery systems with the retention activity and dispersion activity. We are ongoing to evaluate the suppressive activity with these complexes by thermal neutron irradiation.

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採択課題番号 24050　中性子捕捉療法の一般外科領域癌への展開に向けた基礎的研究　共同通常（東大・原子力国際専攻）高橋浩之、柳衛宏宣、Novriana Dewi（東大・獣医）柳川将志、飯塚智也（東大・心臓外科）櫻井由里子、毛利きくえ（大阪市大・バイオ）長崎　健、李　家暐、朱　宇翔、河崎　陸（京大・原子炉）小野公二、増永慎一郎、鈴木　実、櫻井良憲

CO7-13 Pilot Clinical Study of Boron Neutron Capture Therapy for Recurreced Hepatic Cancer

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INTRODUCTION:

Applications of boron neutron-capture therapy (BNCT) has been increased clinically in patients with a lot of cancers in hole body. The main two ^{10}Boron compounds(sodium mercaptoundecahydro dodeca- borate : ^{10}BSH, ^{10}B-p borono- phenylalanine (^{10}BPA) and its fructose complex) are used to clinical trials. Tumour cell destruction in BNCT is due to the nuclear reaction between ^{10}Boron and thermal neutrons. For effective BNCT therapy, it is necessary to accumulate ^{10}B atoms in the tumour cells without affecting adjacent healthy cells.

Most of hepatocellular carcinomas (HCC) are thought to be incurable, and limited surgical operation, chemotherapy, or radiation therapies are available for a prolonged survival. Suzuki et al. had reported that the intra-arterial administration of a boron compound with IPSO is technically an application of chemoembolization, which has been widely used for the treatment of liver tumours. They also reported the clinical results of the first patient with multiple hepatocellular carcinomas (HCCs) treated with BNCT. Higashi et al. prepared a long term inseparable, water-in-oil-water emulsion (WOW) containing 8-60 mg of epirubicin for use in arterial injection therapy to treat patients with HCC. The WOW was prepared by membrane emulsification technique using a controlled pore glass.

We started the pilot clinical studies of BNCT to recurrence breast cancer, hepatic cancer, and gastrointestinal cancers. In this paper, we present pilot clinical study in patients of hepatic cancer.

RESULT [Case 1]

In accordance with the clinical results of Higashi and colleagues, water-in- oil-in-water (WOW) emulsion has been used as the carrier of anti-cancer agents on intra-arterial injections in clinical trials. We would like to apply BNCT for the treatment of HCC in order to increase the selection of therapies available for HCC patients. We developed a ^{10}BSH containing WOW emulsion using a double emulsification technique.

A 63-year-old man with multiple HCCs was enrolled as the first patient in a pilot study for treating BNCT with ^{10}BSH containing WOW emulsion. The patient had been performed right hepatectomy in 6 years ago. Hepatic arterial chemotherapies with epirubicin containing WOW emulsion were performed in the recurrence stages. The multiple tumours in the left liver lobe were treated with BNCT by selective intra-arterial infusion of ^{10}BSH containing WOW emulsion. The pre-BNCT dosimetry was performed using SERA(mean tumour fluence is 12Gy-Eq on 56 minutes BNCT (Maximum 19Gy-Eq on tumour), and maximum fluence of normal mucosa is 5.0 Gy-Eq).

The tumour size was remained stable during 3 months after BNCT. No adverse effect as a result of BNCT was observed during the treatment and follow-up period. The BNCT-treated tumours showed regrowth 3 months after BNCT, so the patient has continued the repeated hepatic arterial chemotherapy of epirubicin containing WOW emulsion.

The present results showed that 10B-containing WOW emulsion can be applied as a novel intra-arterial boron carrier for BNCT for HCC.

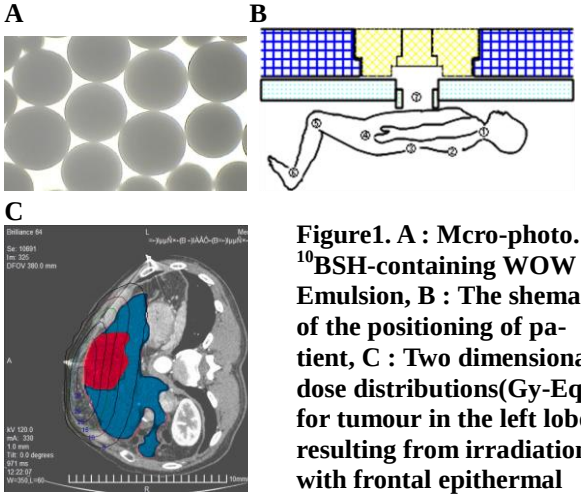

Figure1. A : Mcro-photo. ^{10}BSH-containing WOW Emulsion, B : The shema of the positioning of patient, C : Two dimensional dose distributions(Gy-Eq) for tumour in the left lobe resulting from irradiation with frontal epithermal neutron beams (surface is 30Gy-Eq)

採択課題番号 24051　中性子捕捉療法の一般外科領域癌への展開に向けた臨床的研究　共同通常
（東大・原子力国際専攻）高橋浩之、柳衛宏宣（宏仁会メディカルシティ東部病院）東　秀史、瀬口浩司（結核予防会　新山手病院）江里口正純、丸山正二、小山和行（啓愛会　宝陽病院）野中泰政（京大・原子炉）小野公二、増永慎一郎、鈴木　実、櫻井良憲

CO7-14 Development of Boronated Liposome for Boron Neutron Capture Therapy

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) has cell selective radiation therapy theoretically. Therapeutic effect of the boron compound is based on alpha particles produced by the ^{10}B (n, α) ^{7}Li reaction and tumor selectivity [1]. We developed novel boronated liposome for BNCT, analyzed the neutron capture effect for the cancer cell line and tumor bearing mouse model [2].

EXPERIMENTS: Synthesized the new boronated lipid compound (PBL). Boronated lipid and other lipids (DSPC: cholesterol: PBL = 1:1:0.12) were dissolved in organic solvent and assembled as a liposome of 100 μm diameter using lipid film methods and extruder.
i) *In Vitro* Colony forming assay using V79 and 379A was performed with three groups of samples; (1) Boronated liposome **without** medium change, (2) Boronated liposome **with** medium change, (3) **no boron** argents groups. Each cell suspesions were irradiated at KUR. Irradiation time of each groups were 15min, 30min, and 45min. After 1week incubation, the colonies were counted.
ii) ***In Vivo*** tumor growth inhibitory test with animal experimental tumor bearing model. 10^7 of CT26 colon cancer cells were injected subcutaneously to right thigh of BALB/c mice. 2week after the injection, 5% PBL liposome was administrated via tail vein. The Boron dose was 10mg/kg, volume of 100μl. Concentration of the boron was 2000ppm.
Mice were devided four groups; (1) 5% PBL lipsome group, (2) BSH water solutions, (3) Neutron only and (4) no treatment groups.

RESULTS:
i) Boronated liposome and neutron capture effect *In Vitro*. (2) Medium wash group and (3) no boron agent (neutron only) groups had same dose response. It means that the 5% PBL liposome was not accumulating into cells from the surrounding medium. (1) Without wash group had cell killing effect compared with (2) and (3). It means surrounding medium containing 5% PBL liposome could effect to cell. (**Fig.1**)
ii)5%PBL lipsome group revealed tumor growth inhibition. There are significant difference after 10 days from the irradiation compared with other groups. (student T test P<0.05) (**Fig.2**) In 5% PBL liposome group, there was one complete remission.

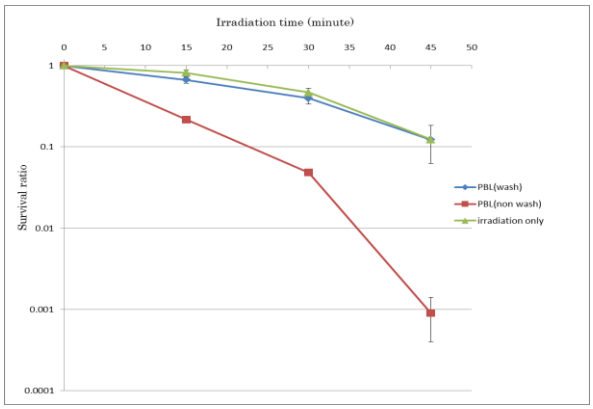

Fig. 1. Cytotoxicity reaction by thermal neutron irradiation with PBL modified liposome. The V79 379A cells were irradiated with 5.6Gy for 15 minute, 12.1Gy for 30minute and 18.4Gy for 45minute. Data are represented as ratio of control. (mean± S.D.).

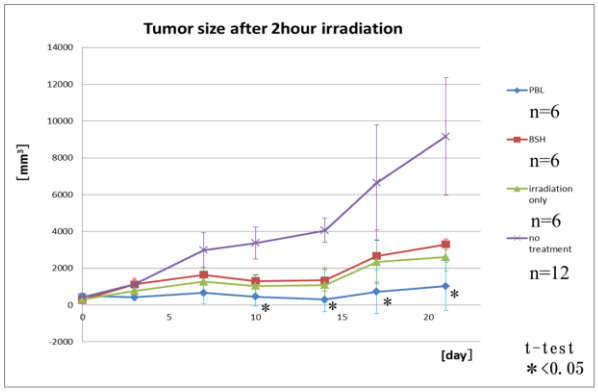

Fig. 2. Tumor growth inhibitory effect by thermal neutron irradiation with PBL modified liposome. The tumor-bearing mice were irradiated with 14.2 Gy for 2 hours. (mean± S.D.)

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採択課題番号 24052　新規化合物および細胞内濃度変調による中性子捕捉反応による　共同通常細胞生存試験および腫瘍増殖抑制効果

（筑波大学・医学医療系）中井　啓、山本哲哉、吉田文代、白川　真、山本陽平、上前洋二、佐藤英介（京大・原子炉）増永慎一郎、櫻井良憲、田中浩基

CO7-15 The Benefit of High LET-Radiation to Glioma Stem-Like Cells

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PURPOSE:
Radiation therapy with surgery and chemotherapy is the standard treatment for glioblastoma multiforme (GBM) [1]. However, almost half of GBM patients cannot survive one year after diagnosis, and the prognosis of patients with GBM has not been improved over the past decades. Recently, there have been some reports showing the presence of glioma stem cells (GSCs) in malignant gliomas which are regarded as highly radio-resistant to low linear energy transfer (LET) photons[1, 2]. On the other hand, we have applied boron neutron capture therapy (BNCT) for GBM. This is a unique tumor-selective particle radiotherapy using neutron irradiation, especially thermal neutron irradiation. Boron-10 (^{10}B) releases alpha (^{4}He) and ^{7}Li particles by ^{10}B(n,α)^{7}Li reaction. The key players of anti-tumor effects in BNCT are these high linear energy transfer (LET) particles. With BNCT, good results have been achieved for patients with GBM and recurrent malignant glioma[3, 4]. Here we analyzed the benefit of high LET particles to GSCs.

MATERIALS AND METHODS:
Glioma stem-like cells (GSLCs) were induced from GBM cell line A172 in stem cell-culture medium [5]. The phenotype of these GSLCs and wild type cell lines were confirmed by western blot analysis and fluorescence-activated cell sorting (FACS) using stem cell markers. These cells were irradiated with ^{60}Co gamma rays or neutron beams. Radio-sensitivity was assessed by a colony-forming assay[6] and the number of DNA double strand breaks (DSBs) using histone gamma-H2AX foci detection assay[7, 8].

RESULTS:
In stem cell-cultured medium, GSLCs could form neurosphere-like spheroid cells. And GSLCs expressed neural stem cell markers more frequently in western blot analysis and the ratio of CD133 positive cells increased day by day. GSLCs were radio-resistant to gamma-rays in comparison with parental cultured cell lines, but neutron beams could overcome the resistance. Twenty-four hours after irradiation with gamma-rays, the number of gamma-H2AX foci in GSLCs was significantly less than that of parental cells, while there was no apparent difference in the number of these foci between GSLCs and parental cultured cell lines following neutron beam irradiation. In addition, neutron beam irradiation induced larger gamma-H2AX foci than those observed after gamma-ray irradiation in both types of A172 cells.

CONCLUSION:
Neutron beams can induce elastic scattering and nitrogen neutron capture reaction, and produce proton particle (H^{+}). This particle is high LET radiation and it could overcome radioresistance of GSLCs with unrepairable DSBs. So we could demonstrate that high LET radiation may be able to overcome GSCs that are resistant to low LET radiation. It is necessary to further investigate the usefulness of high LET radiation to control GSCs, and high LET radiation therapy such as BNCT has a very important role in further treatment for therapy-resistant GBM.

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採択課題番号 24054　　新規硼素化合物の有用性と腫瘍幹細胞の克服　　共同通常
（大阪医大）宮武伸一、弘田祐己、川端信司、平松亮、頼経英倫那
（岡山大）道上宏之、森亜希子、王飛霏
（熊本大）富澤一仁
（京大原子炉）小野公二

CO7-16 **A Biodistribution Study of Carboran Sugar**

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Carboran sugar (carboranyl-thio-d-glucose: TDG) has been chemically modified via novel approach and reduction of its IC_{50} has been achieved to be very low $5.3x10^{-2}$ M that is almost half of that of BSH ($2.75x10^{-2}$ M) [1].

In this study, biodistribution of TDG derivative has been preliminary investigated via α-auto-radiography. C6 tumor cells were implanted via stereotactic maneuvers into Wister's rat brain. 2 weeks after the implantation, 100mg/kg body weight of the compound was injected into peritoneal cavity. 3hs after injection, whole brain was removed and rapidly frozen in liquid nitrogen. Frozen sections were mounted onto the solid-state track detectors: Kodak LR 115 and were exposed by thermal neutrons. The detector were then etched in 10% NaOH solution at 60℃ to emerge α- and/or recoil ^{7}Li particles tracks of $^{10}B(n,\alpha)^{7}Li$ that could be numerically evaluated via an ordinary light microscope.

Figure 1 showed the α-track autoradiography of the compound in a rat C6 brain tumor.

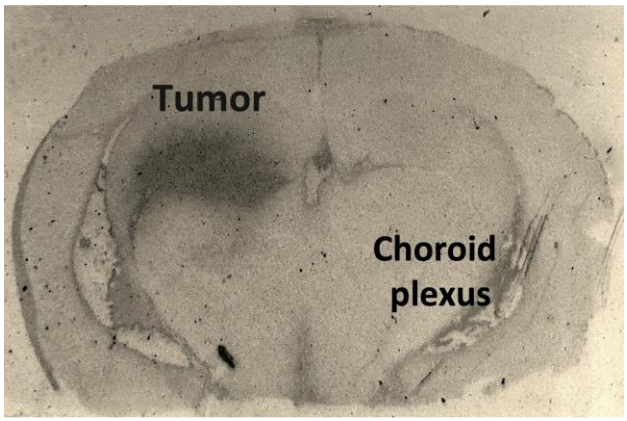

Fig. 1. α-track autoradiography of boron biodistribution in C6 rat brain tumor.

The biodistribution of the compound was very similar with that of BSH. Perhaps it distributes in the brain via non-permeable fashion of blood brain barrier, but blood tumor barrier.

Although our preliminary *in-vitro*-BNCT studies of the compound showed that the surviving fraction was smaller than that of BPA, its tumor cell killing effect is still low for clinical purpose as BPA. Further modification of its ligand has been addressed in this study. Perhaps the compound combines the best of BSH and BPA.

REFERENCE

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CO7-17 Clinical Studies on BNCT for 5 Cases of Head and Neck Cancer

I. Kato, N. Yamamoto[1], Y. Fujita[2], M. Ohmae[3], M. Suzuki[7], S. Masunaga[7], M. Nakazawa, T. Sumi, A. Maruhashi[7], Y. Imahori[5], M. Kirihata[6] and K. Ono[7]

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INTRODUCTION: We had first reported that six patients with head and neck cancer (HNC) had been treated with BNCT [1]. We also report long term (more than 5-year) clinical outcomes of our 26 patients with recurrent HNC treated with BNCT [2]. We summarized 5 patients with HNM who had treated with BNCT at KUR in last year in Table 1.

PURPOSES: The purpose of this study was to estimate safety and effectiveness of BNCT for patients with advanced/ recurrent HNC for which there were no other treatment options.

RESULTS: We also report here latest clinical outcomes of 35 patients with recurrent HNC

All cases are advanced such as 17 (53%) out of 35 patients had developed regional lymph node metastases. Distant metastases were developed in 10 cases (29%) during treatment. (1)^{10}B concentration of tumor/normal tissue ratios (T/N ratio) of FBPA-PET studies were SCC: 1.8-6.0, sarcoma: 2.5-4.0, parotid tumor: 2.5-3.7. (1) Regression rates were CR: 13cases (51%), PR: 13cases (37%), PD: 3cases (9%), NE (not evaluated):1case. Response rate was 88%. (2) Mean Survival time was 24.2months. 2-year overall survival rate (OS) and 6-year OS were 42% and 36%, respectively. (3)BNCT improved QOL, PS and survival periods. (4)Survival periods after BNCT were 1-84 months. (5) Adverse events were brain necrosis, osteomyelitis and transient mucositis and alopecia and so on.

Case 1: A 40 year-old female with recurrent adenoid cystic carcinoma of nasal cancer (rT0N3M0, ACC), who had got a skull-base operation and irradiated RT 60Gy post operatively in 2004. About 5-year later, she had got another surgical operation because of intra-orbital recurrence. In 2011 she had developed another recurrence just after she had got endoscopic surgery for a solitary lung metastasis. FBPA-PET study revealed ^{10}B concentration of T/B ratio was 2.0. Then she has been treated with BPA (500mg/kg) mediated BNCT at KUR in June 2012 and she has been disease free survival for so far 11-month.

Case 5: A 65-year old woman with SCC at Lt-WK (T4N0M0) had got surgery with microscopic forearm reconstruction in March 2011 and she had got surgery of Rt-RND and postoperative radiotherapy (54Gy) in June, Lt-RND in December. She had got bilateral selective intra-artery chemotherapy (CDDP+TXT) after having developed recurrence in February 2012. Again she had developed cervical LN metastases (L-Level II:4cm, R-Level V:1.5cm) so she had weekly treated with [Cetuximub (400mg/m^2,250mg/m^2)+Paclitaxel:60mg/m^2]x7. FBPA-PET study resulted that T/B ratio=4.0. Just before BNCT, the left of level II LN had grown to 5cm with undefined margin which was infiltrated into para-pharyngeal area. The LN was necrotic and skin was ruptured and discharged cancer milk. She treated BNCT in February, 2013. After BNCT the LN had completely disappeared and had covered with normal skin. Then she has been disease free survival for so far 5-month.

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Table 1. Treatment Summary of 5 Cases (May , 2013)

Case No.	Pt's Initial (Age)	Clinical Diag. (Histopathol. Diag.)	10B-conc. Blood(ppm)	T/B ratio	T-max of thermal neutron (D)		Total-RBE-Dose Eq (Gy-Eq)			Irradiation time(min.)	% Reduction (Period) Prognosis (Survival)
					Fluence (E+11n/cm^{-2})	History of RT: (Gy)	T-Peak Gy-Eq	T-deepest Gy-E	Skin/Mucosa		
1	Y・M (40)	Rt-op.OKK, op. lt-Lung meta (ACC)	25.7	2.0	18.0	50	26(1.6 ㎝)	24(4cm)	6.9/18.0	55	CR(11M)・Alive(11M)
2	A・H(51)	Rt-Op. ZK, Rt-LN meta.(SCC)	28.0	2.2	23.0	63	23(4.0 ㎝)	22(4cm)	6.4/10.0	90	CR(10M)・Alive(10M)
3	K・M (71)	Rt-Op.OKK (SCC)	34.0	6.0	13.0	50	44(1.5 ㎝)	28(5cm)	6.8/15.0	56	CR(4M) Alive((4M)
4	K・Y(83)	Rt-0p.OGK (SCC)	28.0	7.0	15.0	40	60(2.5cm)	44(5 ㎝)	2.8/11	33	CR(3M) Alive((3M)
5	A・K(60)	Lt-Op-WK、Lt-LN meta	23.0	4.0		45	44(1.5 ㎝)	28(5 ㎝)	6.8/12	56	CR(3M) Alive((3M)

採択課題番号 24088　頭頸部悪性腫瘍におけるホウ素中性子捕捉療法の臨床的研究　共同通常（阪大・2 口外）加藤逸郎、岩井聡一、墨　哲郎、中澤光博、由良義明（阪大・工）村田　勲（慶大）岡本正人（長崎大）梅田博昭、柳本惣市（りんくう医療セ）大前政利（東大阪総合）千足浩久（市立池田）大西徹郎（田中クリニック）田中　善（京大・原子炉）田中浩基、鈴木　実、櫻井良憲、増永慎一郎、丸橋晃、小野公二

CO7-18 B4C Particle as a Boron Compound for BNCT

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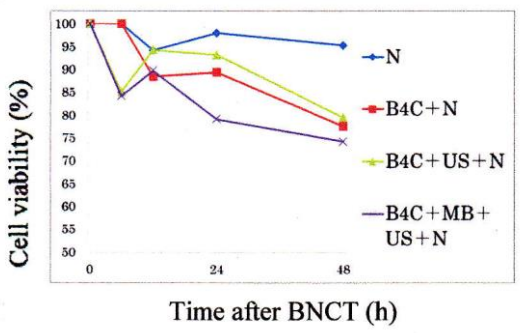
INTRODUCTION: B4C nano particles are produced by liquid phase laser irradiation method [1]. The diameter of the particle is 200 nm. Sonoporation is a low ultrasound which makes small transient holes in the cell membrane and introduces external materials such as drug and gene into the cell [2]. In this study, we investigated whether sonoporation could be used to introduce B4C particles into the oral squamous cell carcinoma (SCC) cells.

EXPERIMENTS: SAS cells derived from oral SCC were used. Cells were exposed to thermal neutron at Kyoto University Reactor (KUR) [3]. An ultrasound machine, Sonitron 2000V, and a microbubble, SV-25, were used [4]. The cell viability was examined by MTT assay. The cell surface was observed using a scanning electron microscope.

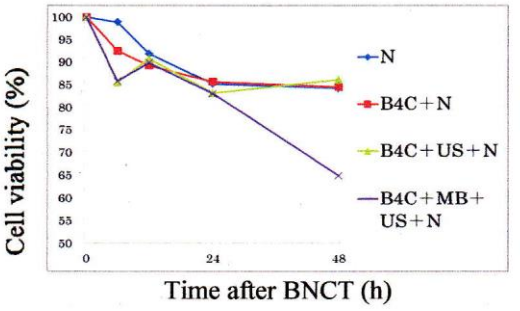
RESULTS: After sonoporation in the presence of microbubbles, small holes (1-2μm) were observed on the cell surface by a scanning electron microscope. The cell viability in the groups treated with B4C-mediated BNCT was lower than that in the group treated with neutron alone (Fig.1). B4C-mediated BNCT in combination with sonoporation (ultrasound in the presence of microbubble) was most effective. When cells were mixed with B4C, washed in PBS and exposed to thermal neutron, cell killing effect of B4C was lost, but it remained in combination with sonoporation (Fig.2).

CONCLUSION: B4C particle can be used as a boron compound for BNCT. Sonoporation may introduce B4C particle into oral SCC cells.



N:neutron, US:ultrasound, MB:microbubble

Fig. 1. Cell viability after neutron irradiation with B4C in the medium



N:neutron, US:ultrasound, MB:microbubble

Fig. 2. Cell viability after neutron irradiation without B4C in the medium

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採択課題番号 24089　口腔悪性腫瘍におけるホウ素中性子捕捉療法に関する基礎的研究　共同通常（阪大・歯）岩上隆紀、加藤逸郎、由良義明（済生会千里病院）山本直典（京大・原子炉）田中浩基、櫻井良憲、増永慎一郎、鈴木実、丸橋晃、小野公二

CO7-19 Microdosimetry of Neutron Field with Low-Enriched Uranium at Kyoto University Reactor

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INTRODUCTION: Kyoto University Reactor was shut down in 2006. After that, the fuel has been changed from high-enriched uranium of 93% to low-enriched uranium of 20%. The reactor has been restarted from 2010. The neutron energy spectrum has not hopefully unchanged by this change. However, there are lacking of measurements. Our group had accumulated data of microdosimetric studies at BNCT beam therapeutic beam before the changes of the fuel enrichment [1,2]. In this study, microdosimetric measurements are carried out to estimate BNCT neutron beam for the low-enriched fuel operation and compared with previous spectra.

EXPERIMENTS: Microdosimetric single event spectrum for the BNCT clinical irradiation filed (epithermal neutron mode: CO0000 and mixed neutron mode: OO0000) [3] has been measured with tissue equivalent proportional counter (TEPC) with a condition of a 1 μm site size. In order to take wide lineal energy range, signals from TEPC were divided into two types of amplifier-gain (high-gain and low-gain). Pulse heights were analyzed by two USB-MCAs (Kromek Co LTD, Kspec). Pulse height distribution of two types of gain have been connected and microdosimetric single event spectrum have been obtained.

RESULTS: The microdosimetric single event spectrum of $yf(y)$ and $yd(y)$ for epithermal neutron mode: CO0000 and mixed neutron mode: OO0000 are shown in Fig. 1 (a) and (b), respectively. The broad structure below 20 keV/μm of OO0000 mode is due to the (n,γ) reaction by thermal neutrons. Similarly, peak structure around 200 keV/μm of mixed mode is due to the $^{17}O(n,\alpha)$ reaction by thermal neutrons. These structure relatively small contribution for epithermal neutron mode. These results are consistent with each other.

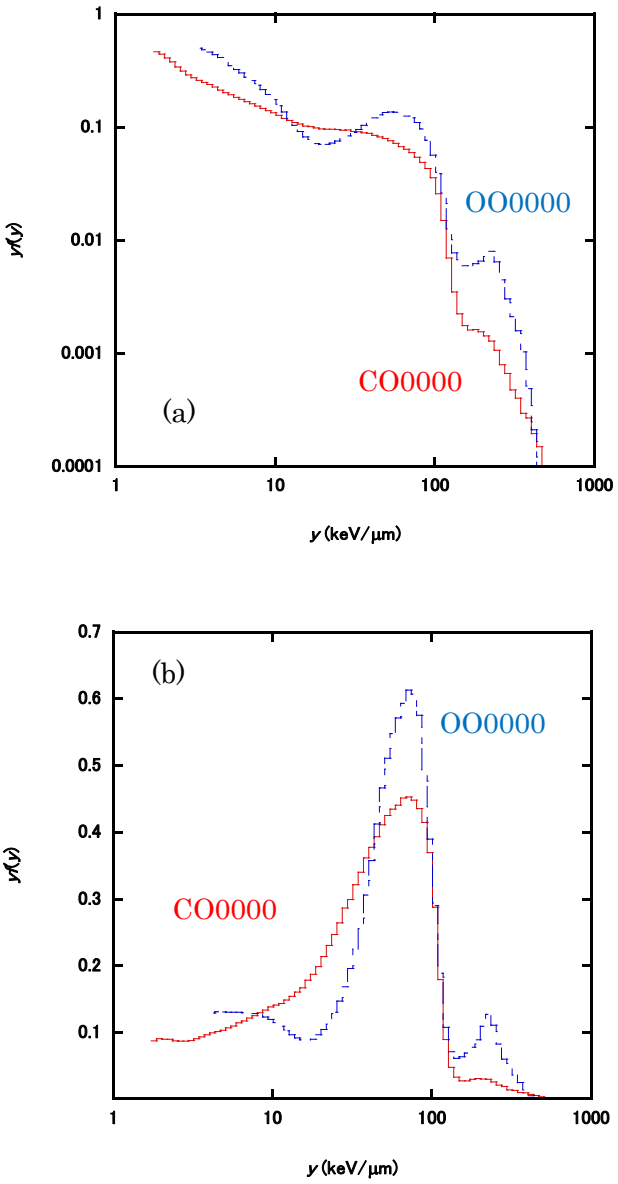

Fig. 1 (a) $yf(y)$ and (b) $yd(y)$ single event spectrum

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採択課題番号 24098　低濃縮燃料移行後の BNCT 医療照射場のマイクロドシメトリ　　共同通常
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（京大・原子炉）田中浩基、櫻井良憲

CO7-20 Combination therapy of C6 Glial Tumor Cell by BNCT and PDT Sensitized by a Boron Lich Porphyrin Derivative

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INTRODUCTION: The distribution of BNCT as one of clinical cancer treatments will be spread by the progress of the sensitizers and the compact instruments in future. We had developed a sensitizer with lich of F(16) and B(44) elements in one molecules by collaboration research of Russian groups [1]. The molecule has both effects of photosensitizing and the other capture of neutron, which is easy water-soluble (>10mM) and the photosensitizing effect is very high even at ppm order of the concentration. We had been studied photodynamic therapy (PDT) of cancer for 30 years using many porphyrin derivatives. There had be not seen the reports about the combination studies yet using one sensitizer.

EXPERIMENTS: **(1)** Uptake Conditions: $2x10^6$ C6 rat glial tumor cells/ ml were incubated in the 10μM Compound-B (Figure-1: MW=1564.63) of the medium solution for 14.5hr at CO_2 incubator. The Boron-10 incorporated in the cells was 3.15 ppm. **(2)** Irradiation Dose and the Sequence: The neutron beam dose was 0.575Gy for 10 min. The laser dose at 665 nm was 115.2 J/cm^2 for 10 min. The photodynamic irradiation was done within 2 hr after the BNCT treatment. **(3)** Damaged Cell Analysis: The cell number at the 36 hr incubation after the combination treatments were measured by the flow cytometory analysis (FACS Canto-II type, BD Ltd. Co.) using double fluorescent staining (Apoptosis Kit: annexine-V/FITC and PI, MBL Co.) 15 min before the analysis.

RESULTS: The Compound-B in Fig. 1 was used for the combination therapy of BNCT and PDT at low concentration of 3.15 ppm.

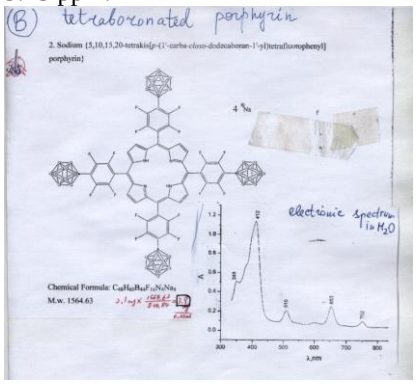

Fig.1. Chemical structure and the absorption spectrum of the capture molecule.
Molecular weight **=1564.63,$C_{48}H_{60}B_{44}F_{16}N_4Na_4$=** {5,10,15,20-terakis[4-(1-caba-*closo-do*decabrane-1-yl)tetrafluorophenyl]17,18-dihydrophyrin}} tetrasodium.
Two observations of the cell damages, 100% - survival fraction = damaged fraction (%) and the necrotic fraction were obtained from the flow cytometory analysis as shown in Figure 2.

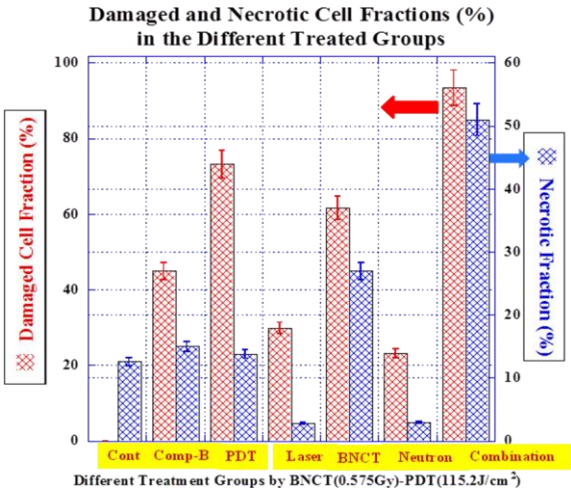

Fig.2. Cell damaged and Necrotic Cell Fractions (%) in the Different Treated Groups.
The cell damaged fraction was correlated with the necrotic fraction, especially BNCT and the combination with PDT. It was considered that both treatments of BNCT and combination was more effective treatment of cancer cell comparing PDT because the necrotic fraction of combination of 51% was the highest value comparing with the total (41%) value with PDT (21%) and BNCT (27%), respectively. It is expected that there will be presented a synergic effect between BNCT and PDT treatments.

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採択課題番号 24103　　BNCT と光線力学治療の併用療法に両用の増感剤を使用した癌培養細胞共同実験　　共同通常
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