I-1. PROJECT RESEARCHES

Project 2

PR2 Preclinical studies on gadolinium neutron capture therapy

M. Suzuki

Institute for Integrated Radiation and Nuclear Science, Kyoto University

In this research project, nine research projects were included. Details of each project is referred to the following contents.

P2-1: Investigation of cell killing effect by auger electrons emitted during gadolinium neutron capture therapy (Gd-NCT)

In this year, we developed a new irradiation method to investigate the cell killing effect by auger electrons in Gd-NCT. Using the positive-charged Gd-nanoparticles, the cell killing effect of Gd-NCT was assayed by colony formation assay. In this study, no additive cell killing effect by Gd-NCT was yielded. The Gd concentration of thirty ppm was supposed to be too low. Further study is needed to elucidate the cell killing effect of auger electrons.

P2-2: Development of Nano Carriers Installed with Gd (III)-Thiacalixarene Complex for Gd-NCT

In this year, the cell killing effect of Gd-NCT with Gd_3TCAS_2-installed albumin nanoparticles (NP) was investigated by comparison with those of Gd_3TCAS_2, Gd-DTPA, and PBS control. The results of this study showed that even though the delivery efficiency of Gd_3TCAS_2-installed albumin NP to the cells was lower than that of free Gd_3TCAS_2, Gd_3TCAS_2-installed albumin NP showed higher NCT effect.

P2-3: Gadolinium neutron capture therapy as new treatment for head and neck cancer.

In this study, using masseter muscle invasion model, in vivo tumor-killing effects of Gd-NCT with Gd-loaded chitosan nanoparticles (Gd-nanoCPs) was investigated. The tumor-loaded model was developed by injection of SCC-VII (1x106 cell/mouse) into the left masseter muscle. The result showed that decrease in tumor volume in Gd-NCT group was observed without damage to the normal surrounding mucosa.

P2-4: Preparation of drugs targeting cell nucleus with Hoechst unit

In this study, the synthesized BPA-Hoechst collecting in the nucleus was subjected to cellular experiments.

Experiments using F-Hoechst shows that the unit take functional molecules into cell nucleus. The cytotoxic effect of thermal neutron beam irradiation was significantly enhanced when the cells were irradiated in the presence of BPA-Hoechst. BPA-Hoechst conjugate is a potent candidate agent for BNCT.

P2-5: Development of Gadolinium-loaded mesoporous silica-based nanoparticles and application to cancer radiotherapy.

In this study, preliminary evaluation of Gd-biodegradable periodic mesoporous organosilica （BMPO） using the chicken chorioallantoic membrane (CAM) model that was established by transplanting human ovarian cancer cells.

Preliminary investigation of tumor growth inhibition efficacy Gd-BPMO in the CAM model showed that the tumor growth was significantly inhibited when Gd-BPMO was injected compared to no injection or empty BPMO.

P2-6: Pathological findings of tumor growth suppression of GdNCT with intra-tumoral injection of gadolinium-polyplex in pancreatic cancer model *in vivo*.

In this study, the gadolinium/ hyaluronic acid/ protamine-mixed with cationic liposome (^{157}Gd-plex) was prepared and its efficacy as the compound of GD-NCT was estimated with Human Pancreas Adenocarcinoma cell line (AsPC-1) bearing mice. HE dyeing & apoptotic assay was performed for evaluating the mechanism for cancer cell cytotoxicity by GdNCT. Cancer cell hyalinization and degeneration and apoptotic changes were observed in the GdNCT treated group with ^{157}Gd-plex.

P2-7: Development of gadolinium-coating inorganic nanoparticles with polyglycerol coating for gadolinium neutron capture therapy of cancer.

In this study, Gd-containing inorganic nanoparticles grafted with polyglycerol (Gd-NP-PG) was evaluated as the agent of Gd-NCT using CT26-bearing mice. Gd-NP-PG nanoparticles-mediated Gd-NCT significantly suppressed the growth of CT26 tumors

P2-8: Study about neutron capture therapy using polymeric drug delivery systems chelating Gd.

In this study, new class of Gd-DOTA introduced polymers (polymer-drug) were used for evaluating its efficacy of the Gd-related therapeutic performance thorough neutron irradiation using CT26 bearing Balb/c mice. The result showed that the low-molecular drug and polymer drug exhibited antitumor efficacy with no significant difference. In the next year, modification of experimental designs and polymer structure will be planned.

P2-9: Neutron irradiation after administration of Gd-EDTMP to a mouse mode of mammary tumor bone metastasis: Effects and distribution of Gd formulation as a novel neutron capture therapy agent.

In this year, after neutron irradiation of the tumor-bearing mice with Gd tetra (methylene phosphonic acid) chelate (Gd-EDTMP), the distribution of ^{157}Gd in and around the bone was imaged using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The weight of the right leg in the Gd-NCT group tended to be lower than the control groups. LA-ICP-MS Gd imaging of the legs in the Gd-EDTMP administration groups showed extremely high Gd signal intensity in bone.

PR2-1 Investigation of cell killing effect by auger electrons emitted during gadolinium neutron capture therapy (Gd-NCT)

M. Suzuki and H. Tanaka

Institute for Integrated Radiation and Nuclear Science Kyoto University

INTRODUCTION:

We reported the feasibility of the irradiation system to investigate the cell killing effect by auger electrons in Gd-NCT by micronucleus assay.

In this year, we developed another irradiation system which can irradiate the cells attached to the flasks with horizontal thermal neutron beam derived from the Kyoto University Research Reactor.

Using a new system, we evaluated the cell killing effect of the Gd-containing nanoparticels.

EXPERIMENTS:

Cell line: The V79 cells were maintained in E-MEM supplemented with L-glutamine and 10% fetal bovine serum (FBS).

Gd-nanoparticles: The Gd-nanoparticles which were used in this study were provided from Dr. T. Ando. The surface of Gd-nanoparticles has positive charge which makes the particles adhere to the tumor cells.

Irradiation system: To irradiate the cells attached to the flasks with horizontal thermal neutron beam, the flasks which are processed for transporting cells were used in this study. The flasks are separated with a partition to reduce the volume of medium for filling the space including the bottom.

As shown in Fig. 1, two flasks filled with 30 ml medium were set vertically to be irradiated with horizontal thermal neutron beam.

RESUTLS

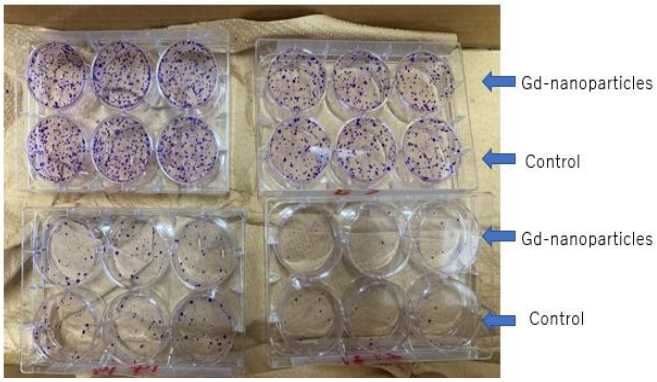
Dosimetry: The flasks were irradiated with thermal neutron fluence of 1.9E+12, 3.7E+12 and 5.8E+12 n/cm^2 which was measured by analysis of activation of gold foil attached to the surface of the bottom of flasks.

Fig. 1 Picture of setting of two flasks for irradiation

Colony formation assay

Figure 2 shows the picture of stained colonies dispersed into the six wells on the microplates.

Fig. 2 Picture of colony formation assay



No apparent difference of numbers of colonies between the cells irradiated with Gd-nanoparticles and those of control was observed. This experiment was repeated three times. In all the experiments, Gd-nanoparticles did not yield the additive cell killing effect.

DISCUSSION: The feasibility of new irradiation system was confirmed in this study. In this system, no leakage of medium during irradiation occurred since the flasks was sealed tightly with caps. Gd-neutron capture reaction using the positive charged Gd-particles which adhere to the cells during irradiation are supposed to emit auger electrons on the cell membrane. The damage of the cell membrane leads to the apoptosis of the cell.

In this study, no cell killing effect by irradiation derived from Gd-neutron capture reaction was observed. The possibility of this result is that the concentration of Gd (30 ppm) was too low to cause the cell killing effect. Further study is needed to investigate the cell killing effect of auger electrons using this system.

PR2-2 Development of Nano Carriers Installed with Gd(III)-Thiacalixarene Complex for Gd-NCT

N. Iki[1], M. Komiya[1], T. Nagasaki[2], and M. Suzuki[3]

[1]*Graduate School of Environmental Studies, Tohoku University*

[2] *Graduate School of Engineering, Osaka City University*

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

INTRODUCTION: Owing to a large thermal neutron capture cross section and total kinetic energy of $^{157}Gd(n,g)^{158}Gd$ larger than that of $^{10}B(n,a)^{7}Li$, 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. We have so far studied nano-carriers for Gd_3TCAS_2 such as silica nanoparticle [5] and albumin NP (AlbNP) [6] aiming at Gd-NCT. Here we report the ability of Gd_3TCAS_2-installed AlbNP to kill cancer cells upon neutron irradiation by comparison with the cases of Gd_3TCAS_2, Gd-DTPA, and PBS control.

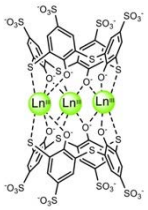


Fig. 1 Structure of Ln_3TCAS_2 complex.

EXPERIMENTS: *Preparation of AlbNP installed with Gd.* The trinuclear complex Gd_3TCAS_2 was prepared as reported elsewhere [2]. The AlbNP was prepared by a method reported [7]. Briefly, BSA solution was added EtOH, followed by addition of glutaraldehyde to obtain the particle containing solution. This was mixed with Gd_3TCAS_2 to afford Gd_3TCAS_2-installed AlbNP (denoted as Gd_3TCAS_2-AlbNP(EtOH)).

Cell experiment 1. MCF-7 cells were seeded in a 6-well plate at a cell concentration of 1.0×10^5 cells/mL and incubated for 24 h. After supernatant was removed, RPMI medium and solution containing Gd in a form of 1) Gd_3TCAS_2-AlbNP(EtOH), 2) free Gd_3TCAS_2, 3) Gd-DTPA, and 4) PBS (as control) were added to each well and incubated for 24 hr. The concentration of Gd in the medium to incubate MCF-7 was set to be 25 μM for 1–3). After washing with PBS, the cells were detached from the well and transferred to tubes to be irradiated with thermal neutron for 20 min.

Cell experiment 2. MCF-7 cells were mixed with RPMI medium containing Gd_3TCAS_2-AlbNP(EtOH), the absolute amount of which was adjusted to the same value of one delivered into the cells in the experiment 1. Afterward, the cell was irradiated with thermal neutron for 20 min.

Assay. To the wells containing 2 mL of RPMI medium in 6-well plates, irradiated cells were seeded at the concentration of 1,000 cells/well. After incubation for 14 days, the colony was stained with crystalviolet.

RESULTS:

In Experiment 1, the ansolute amount of Gd into 2.0×10^4 cells was estimated to be 1) 9.8 , 2) 73.6, and 3) 2.6 pmol by using ICP-AES. The colony formation units (CFU) normalized with one for no irradiation is shown in Fig. 2a. As can be seen, there seems appreciable difference between CFU for 1) and others 2–4). Thus, despite the fact that the delivery efficiency of Gd_3TCAS_2-installed AlbNP to the cells was lower than that of free Gd_3TCAS_2, Gd_3TCAS_2-installed AlbNP showed higher NCT effect. In Experiment 2, neutron was irradiated to a sample containing the same amount of Gd in the medium but outside the cells. The CFU showed that the NCT effect is lower than that attained by Experiment 1 (Fig. 2b). This unambiguously shows that internalization of Gd_3TCAS_2-installed AlbNP in the cells is essential to show the NCT effect.

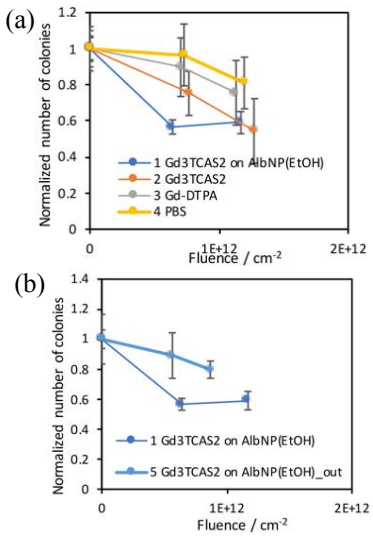


Fig. 2 Dependence of neutron fluence on the colony formation rate (n = 3). a) Comparison among the Gd-agents 1–3) internalized in MCF-7 cells. b) Comparison between internalized and outside Gd_3TCAS_2-installed AlbNP.

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PR2-3 Gadolinium neutron capture therapy as a new treatment for head and neck cancer: tumor-killing effects on a masseter muscle invasion model

T. Andoh[1], T. Fujimoto[2], M. Suzuki[3], T. Takata[4], Y. Sakurai[4] and H. Ichikawa[1].

1Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Japan.
2Department of Orthopaedic Surgery, Hyogo Cancer Center, Japan.
3Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University, Japan.
4 Division of Radiation Life Science, Institute for Integrated Radiation and Nuclear Science, Kyoto University, Japan

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

EXPERIMENTS: Gd-nanoCP was prepared by using chitosan with molecular weights of 10 k and Gd-DTPA through the previously developed w/o emulsion-droplet coalescence technique [2]. Mean particle size and zeta potential of the resultant Gd-nanoCPs were measured by Zetasizer® (Malvern Instruments Ltd, UK) in water at 25ºC. Gd content in Gd-nanoCPs was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, SPS3100, Hitachi High-Tech Science Corporation, Japan) followed by incineration of each sample. In the NCT trial, male 5-week-old C3H/HeN mice were used. SCC-VII (1×10^6 cells/mouse) were injected into the left masseter muscle [3]. The mice were divided into NCT group and HOT control group. Before injection, Gd-nanoCPs were concentrated to 6000 μg Gd/mL by centrifugation. Gd-nanoCPs incorporating 1.2 mg of natural Gd were injected intratumorally twice (2.4 mg Gd/kg) to the mice. The tumors were exposed to thermal neutron irradiation at the Institute for Integrated Radiation and Nuclear Science, Kyoto University. For determining the tumor volume, two bisecting diameters of the tumor were measured with a slide caliper and calculated with the longest and shortest length of the tumor in millimeters (mm). The tumor-growth suppressing effect was assessed by the ratio of tumor volume before and after neutron irradiation.

RESULTS: Mean particle diameter, Gd content, and zeta potential of the Gd-nanoCP were 178 nm, 19%, and +24 mV, respectively. In the NCT trial, thermal neutron irradiation was applied to mouse tumors 8 h after the last administration. Remarkable tumor growth was observed in the HOT control group, whereas the NCT group showed tumor growth suppression (Fig. 1). Decreases in tumor volume were similar to those observed in our previous study using a transplanted tumor-bearing mouse model [4]. The tumor mass was selectively suppressed without damage to the normal surrounding mucosa because Gd-nanoCPs could not penetrate the surrounding tissue. These results indicate that Gd-nanoCPs displayed potent tumor tissue affinity after intratumoral injection, reduced tumor tissue volume, and did not damage the mucosa. GdNCT using Gd-nanoCPs could thus be a promising therapeutic option to shrink tumors occurring close to the mucosa.

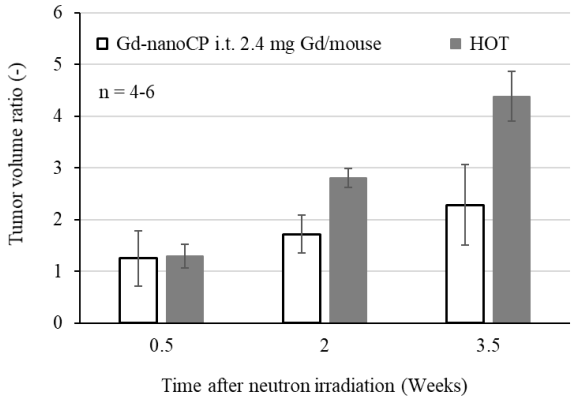

Fig. 1. Tumor volumes after thermal neutron beam irradiation of NCT and HOT control groups.

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PR2-4 Preparation of drugs targeting cell nucleus with Hoechst unit

K. Tanabe[1], M. Suzuki[2], T. Nishihara[1] and M. Mizutani[1]

[1]*Department of Chemistry and Biological Science, College of Science and Engineering, Aoyama Gakuin University*

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

INTRODUCTION:

In recent years, Boron Neutron Capture Therapy (BNCT) has been attracting attention as the fifth cancer treatment method next to surgery, chemotherapy, radiation, and immunotherapy. BNCT is a treatment that selectively destroys cancer cells by accumulating ^{10}B compounds, which cause nuclear reaction with neutrons, on the cancer cells and irradiating them with neutrons. However, at present, there are only two types of boron drugs available for BNCT: BPA and BSH. Therefore, it is an urgent issue to develop drugs that accumulate in the tumor tissue with high selectivity and show effective therapeutic effects. In this study, we attempted to construct the molecular system to take the complex into cell nucleus by using Hoechst molecules. Since the Hoechst group has high DNA −binding function and accumulates in the cell nu-cleus,[1] it was expected that this molecule act as a courier molecule to deliver drugs into the nucleus. We expected that by collecting the drug in the nucleus, we could achieve an effective DNA-targeted attack in tumor cells. We attempted to modify the conventional drug, BPA, for BNCT and the gadolinium complexes. The synthesized BPA-Hoechst was subjected to cellular experiments, and we confirmed that BPA-Hoechst exhibited effective cytotoxic effects under irradiation conditions of thermal neutrons.

EXPERIMENTS:

Huisgen cycloaddition reaction between A-Hoechst and BPA to form BPA-Hoechst. A-Hoechst (1.0 mg, 2.2 μmol) was added to the solution of BPA with azide unit (0.5 mg, 2.1 μmol) in DMF-H_2O. Then, $CuSO_4$, TBTA and sodium ascorbate were added to the solution. The resulting mixture was stirred for 20 h at ambient temper-ature. After the reaction, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography to give BPA derivative with Hoechst unit (BPA-Hoechst).

Cellular experiments using BPA-Hoechst. A549 cells were seeded in 96 well plates at a density of 4.5×10^3 cells per well in DMEM and allowed to grow at 37 ˚C for 24 hours. The cells were incubated with 0, 10, 100 μM BPA-Hoechst in DMEM (DMSO 1%) for 3 h. After incubation, the cells were irradiated with neutron for 45 min at KUR (1 MW). Then, the cell was cultured for 2 days in the fresh DMEM at 37 ˚C. Then, cell viability assay was performed Cell Counting Kit-8 (Dojindo) and microplate reader (Absorbance at 450 nm).

RESULTS:

Preparation of BPA-Hoechst was achieved as shown in Figure 1A. Initially, we conducted the Huisgen reaction of A-Hoechst and BPA derivative bearing azide group. The reaction was proceeded at ambient temperature in the presence of catalytic $CuSO_4$ and ascorbic acid. Efficiently, the triazole ring formation occurred, and BPA with Hoechst unit (BPA-Hoechst) was formed. On the other hand, the modification of gadolinium complexes is still ongoing. Using cyclic amine 3 as starting material, two amino groups were protected with BOC groups and the remaining amino groups were alkylated. The removal of the protecting groups and the Huisgen reaction are currently under investigation.

For understanding of the biological activity of BPA-Hoechst, we measured its cytotoxic effect against A549 cells, which were exposed to neutron (1 MW) in the presence of aqueous solution of BPA-Hoechst and then incubated at 37 ˚C. Cell survival was determined by WST assay. Figure 1C compares cell survivals in the presence and absence of BPA-Hoechst after irradiation. The cytotoxic effect of radiation was significantly enhanced when the cells were irradiated in the presence of BPA-uridine. It is likely that BPA is effectively activated in the cell nucleus and thereby exhibits high cytotoxicity against tumor cells upon irradiation. In a separate experiment, we evaluated the cytotoxic effect of BPA in the presence of fluctose that complexed with BPA to dissolve into water. The cytotoxic assay revealed that BPA-fluctose conjugate showed similar cyototoxic effect upon irradiation. Thus, it is reasonable to conclude that BPA-Hoechst conjugate is a potent candidate agents for BNCT. In vivo experiments to evaluate the function of BPA-uridine is in progress.

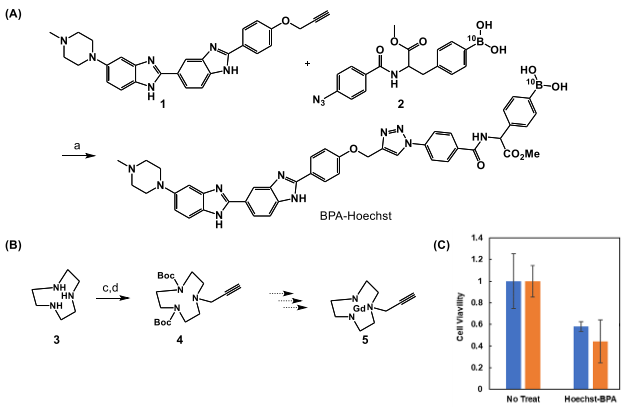

Figure 1. (A,B) Synthesis of BPA-Hoechst (A) and ligand of gadolinium ion (B). (C) Cytotoxic effect of BPA-Hoechst conjugate upon thermal neutron irradiation (1 MW, 45 min).

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PR2-5 Development of Gadolinium-loaded mesoporous silica-based nanoparticles and application to cancer radiotherapy

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

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

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

INTRODUCTION: Boron phenylalanine (BPA) has been developed as useful boron compound which is available for Boron neutron capture therapy (BNCT) therapy. We have developed two types of mesoporous silica-based nanoparticles that are loaded with BPA and found that the BPA-loaded nanoparticles have ability of improving the BNCT efficacy [1]. On the other hand, we have recently developed novel type of biodegradable periodic mesoporous organosilica (BPMO) which was loaded Gadolinium instead of BPA (Gd-BPMO). We assumed this Gd-BPMO could be applied to Gadolinium neutron capture therapy (GdNCT). GdNCT has been devised as a less inversive cancer therapy as well as BNCT. Gd-BPMO has a large surface area where Gd can be attached for GdNCT application. We preliminary evaluated the GdNCT efficacy with Gd-BPMO in chicken chorioalaantoic membrane (CAM) model that was transplanted ovarian cancer cells.

EXPERIMENTS:
BPMO was synthesized by sol-gel synthesis of two precursors, bis[3-(triethoxysilyl) propyl] tetrasulfide and 1, 2-bis(triethoxysilyl) ethane. This resulted in the incorporation of tetrasulfide bonds into the framework of the nanoparticles. BPMO was then modified with amino group. Gadolinium diethylenetriamine penta-acetic acid (DTPA) was then mixed with amine-modified BPMO to couple Gd on BPMO by electrostatic interaction between positively charged $NH3^+$ and negatively charged COO^- of DTPA. The synthesized nanoparticles were characterized by using SEM and EDX-TEM. The amount of Gd attached on the nanoparticles was examined by ICP-AES, and Gd content was determined.

CAM model was established by transplanting human ovarian cancer cells OVAR8 on the CAM in fertilized chicken egg. After intravenously injection of Gd-BPMO, the eggs were placed at the center of emerging neutron beam. Eggs were irradiated with thermal neutron for 1 h at an operating power of 1MW. After the irradiation, eggs were incubated for 3 days at 37℃ with 65% humidity. Tumors were then cut out to evaluate the tumor size.

RESULTS: As seen in Fig. A, Gd-BPMO synthesized had approximately 80-100 nm of diameter and homogenous shapes examined by SEM microscopy (TEM image is not shown). After coupling of Gd to BPMO, we detected the amount of Gd that was coupled with BPMO by ICP. The 2.6% of Gd were coupled with BPMO.

As seen in Fig. B and C, Investigation of tumor growth inhibition effect of Gd-BPMO was preliminary resulted that the tumor growth was significantly inhibited when Gd-BPMO was injected compared to no injection or empty BPMO (27% tumor growth inhibition effect compared to No injection). We have been carrying out further experiments about the characterization and biodistribution of Gd-BPMO, and their tumor growth inhibition efficacy by neutron irradiation is currently ongoing.

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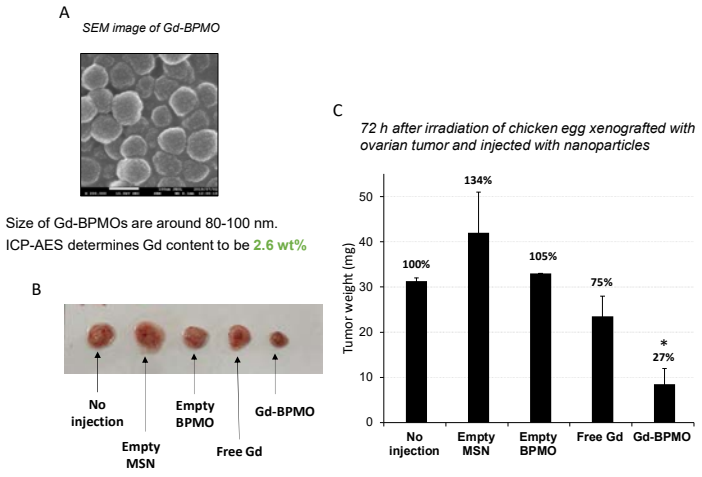

PR2-6 Pathological Findings of Tumor Growth Suppression of GdNCT with Intra-Tumoral Injec-tion of Gadolinium-Polyplex in Pancreatic Cancer Model *in vivo*

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

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

INTRODUCTION:

Gadolinium atoms react thermal neutron and offers cytotoxic effect by 1μm-range high LET Auger electron, and long-range gamma rays on Gadolinium-neutron capture therapy(GdNCT) [1, 2, 3]. For effective GdNCT, it is necessary to accumulate Gadolinium atoms into the tumor tissues selectively, so we prepared the gadolinium / hyaluronic acid / protamine-mixed with cationic liposome (^{157}Gd-plex) as neutron capture agent. In this study, we evaluated the mechanism for cancer cell cytotoxicity by GdNCT performing with HE dyeing & Apoptotic analysis using tumor tissues after GdNCT in vivo.

EXPERIMENTS:

We prepared nanoparticles mixed with 1.5mL of Gadolinium compound "Gadovist" (MW: 604.71), 0.2mL of a solution of 10mg/mL-hyaluronic acid sodium, and 0.1mL of 20mg/mL of protamine incubating at room temperature for 30min. Then, these mixing solutions were poured into Coatsome EL-C. Human pancreatic cancer AsPC-1 cell was used for the *in vivo* anti-tumor effect evaluation. We prepared AsPC-1($5x10^5$) model by transplanting to right lower leg. Twelve hours after intra-tumoral injection of 0.1mL of ^{157}Gd-plex, we performed thermal neutron irradiation at Institute for Integrated Radiation and Nuclear Science, Kyoto University (average neutron fluence of 3.0×10^{12} n/cm^2). The change in tumor growth and survival rate of the mice reflected the anti-tumor effect of ^{157}Gd-plex. We obtained a tumor and various organs (heart, the lungs, liver, kidney, spleen) one month after irradiation, and performed a formalin fixation and the freeze fixation using the OCT compound and examined the pathological cell cytotoxic effects. We performed apoptotic analysis using the TUNEL dyeing to analyze the mechanism of the antitumor effect (Apoptosis in situ detection Kit Wako, 293-71501, Osaka, Japan) [4].

RESULTS:

The nuclear dysplasia of the cancer cell was strong in the non-irradiation group, but the degeneration was not seen. The degeneration was not seen in the groups with only irradiation, but the cancer cell hyalinization and degeneration were seen in the ^{157}Gd solution and ^{157}Gd-plex treated groups(Figure 1). The apoptotic changes of the cancer cells were not seen in the non-irradiation group. There were few apoptotic changes in the control group with irradiation, but the apoptotic changes were dominant in the GdNCT treated group with ^{157}Gd-plex (Figure 2).

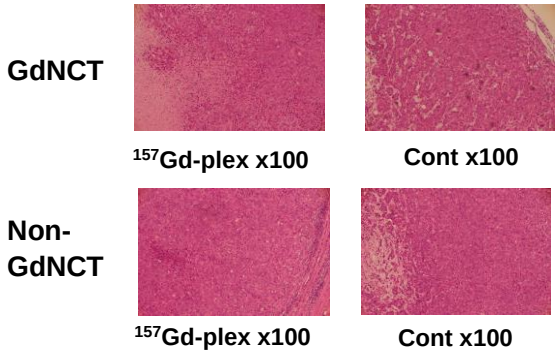

Figure 1. HE staining of tumor growth suppression by ^{157}Gd-plex on GdNCT

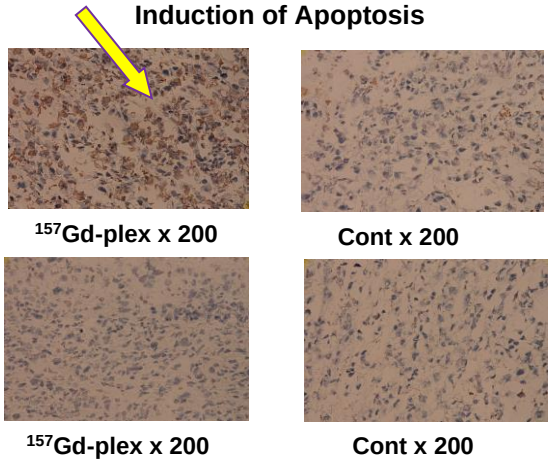

Figure 2. TUNEL staining of tumor growth suppression by ^{157}Gd-plex on GdNCT

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PR2-7 Development of gadolinium-containing inorganic nanoparticles with polyglycerol coating for gadolinium neutron capture therapy of cancer

Li Zhao[1], Heon Gyu Kang[2], Masahiro Nishikawa[2], Minoru Suzuki[3] and Komatsu Naoki[2]

[1] School for Radiological and Interdisciplinary Sciences (RAD-X) and Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Soochow University, P. R. China
[2] Graduate School of Human and Environmental Studies, Kyoto University
[3] Institute for Integrated Radiation and Nuclear Science, Kyoto University

Neutron capture therapy (NCT) that employs boron or gadolinium isotopes as sensitizers holds great promise for cancer treatment. ^{157}Gd is the most effective isotope in terms of thermal neutron capture, holding a large cross-section of 254000 barn. The Gd neutron capture reaction, ^{157}Gd(n, γ)^{158}Gd, is capable of generating high linear energy transfer (LET) Auger-Coster-Kronig (ACK) electrons as well as low LET γ photons to damage cancer cells. Another advantage of GdNCT is that Gd(III) is an efficient contrast agent for magnetic resonance imaging (MRI), which enables noninvasive detection of Gd content in the tumor and can guide GdNCT.[1] Unfortunately, because of the lack of appropriate tumor-selective Gd agents, the GdNCT concept has thus far not been clinically tested.

Gd-containing inorganic nanoparticles (Gd-NP) carrying a large number of Gd atoms in a single particle are considered as a potential Gd agent for GdNCT. We have recently prepared a kind of Gd-NP, which were further grafted with polyglycerol (PG) to increase the colloidal stability and dispersibility in physiological media.[2] The resulting Gd-NP-PG nanoparticles were characterized and investigated as a Gd agent for GdNCT of murine CT26 colon cancer. The results showed that Gd-NP-PG nanoparticles can disperse in phosphate buffer saline (PBS) at a fairly high concentration of > 10 mg Gd/mL, showing no aggregation and precipitation over 3 months. Upon intravenous admin-istration, Gd-NP-PG nanoparticles circulated in the mouse blood for a relatively long time, and accumulated in CT26 tumor through enhanced permeability and re-tention (EPR) effect, reaching a high Gd concentration of about 150 μg/g at 24 h postinjection. As expected, Gd-NP-PG nanoparticles-mediated GdNCT (thermal neutrons: 1 MW for 50 min) significantly suppressed the growth of CT26 tumor as compared to the control groups, suggesting that the nanoparticles can be a po-tential Gd agent.

We are going to study the MRI of tumor using Gd-NP-PG nanoparticles as a contrast agent, with an aim to realize MRI-guided GdNCT (Figure 1), which can not only help optimize the GdNCT plan, but also enhance the accuracy and efficacy of GdNCT. In addition, the therapeutic mechanism of Gd-NP-PG nanoparticles-mediated GdNCT will also be explored.

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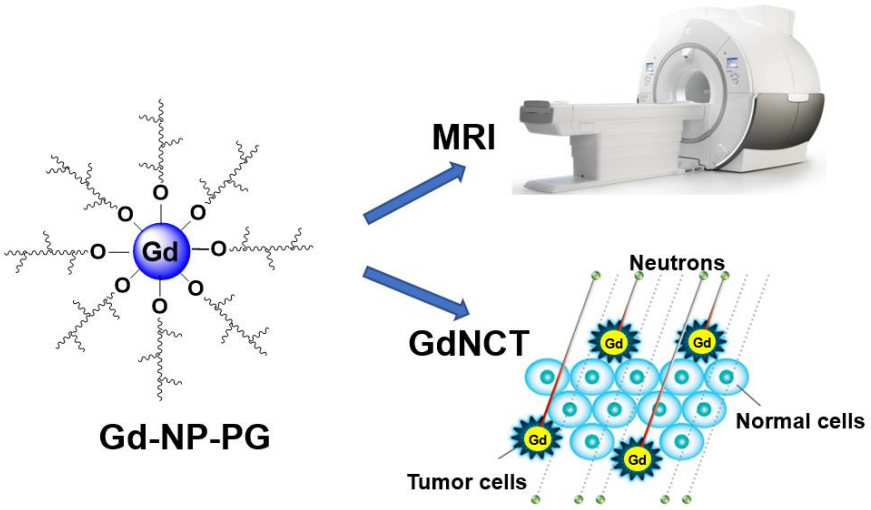

Figure 1. MRI-guided GdNCT using Gd-NP-PG as a Gd agent.

PR2-8 Study about neutron capture therapy using polymeric drug delivery systems chelating Gd

Y. Miura[1], T. Nomoto[1], K. Konarita[1], D. Tokura[1], Y. Sakurai[2], M. Suzuki[2], N. Nishiyama[1]

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

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

INTRODUCTION: ^{157}Gd has the high neutron capture cross section and can generate Auger electrons and γ-rays upon thermal neutron irradiation. Thus, Gd has been expected to be a promising atom in neutron capture therapy. We previously synthesized the inorganic-organic hybrid nanoparticle encapsulating Gd and demonstrated strong antitumor efficiency in subcutaneous tumor models [1]. However, the tumor accumulation level of Gd and ultimate antitumor efficiency was not always well correlated [2]. To improve the tumor accumulation and penetration performance of drug carriers, we designed and synthesized new class of Gd-DOTA introduced polymers (polymer-drug) by using controlled polymerization techniques and selective polymer modifications. According to *in vivo* biodistribution study, obtained polymer-drug enable selective Gd delivery against targeted tumors. Thus, in this year, we tried to confirm the Gd-related therapeutic performance of the polymer-drug through neutron irradiation.

EXPERIMENTS: BALB/c mice bearing subcutaneous CT26 tumors were prepared by subcutaneous injection of the cell suspension. The polymer drug or the low-molecular drug (Gd-DOTA) as a control were intravenously injected to the mouse, and the thermal neutrons were irradiated to the tumor using KUR at 5 MW for 10 min. The tumor volume (V) was calculated using the following equation:

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

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

RESULTS: As shown in Fig. 1, the low-molecular drug and polymer drug exhibited antitumor efficacy, suggesting the successful neutron capture therapy and the appropriate irradiation condition. However, the two samples did not exhibit a significant difference for tumor growth inhibitory effect, suggesting that the dosr of polymeric drug and/or administration schedule were insufficient. In the next fiscal year, we will improve the therapeutic efficacy by modification of experimental designs and polymer structure.

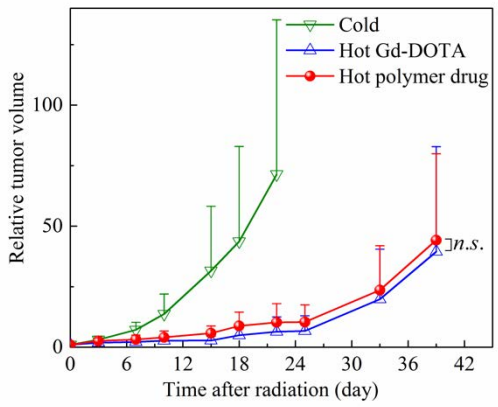

Fig. 1. Antitumor efficacy to subcutaneous CT26 tumor models.

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PR2-9 Neutron irradiation after administration of Gd-EDTMP to a mouse model of mammary tumor bone metastasis: Effects and distribution of Gd formulation as a novel neutron capture therapy agent

T. Matsukawa[1], M. Suzuki[2], A. Kubota[1], A. Shinohara[1,3], K. Yokoyama[1,4]

[1] *Juntendo University Faculty of Medicine*
[2] *Institute for Integrated Radiation and Nuclear Science, Kyoto University*
[3] *Research Institute for Cultural Studies, Seisen University*
[4] *International University of Health and Welfare*

INTRODUCTION: To develop the next generation of cancer radiation therapy, we evaluated the tissue distribution of Gd tetra (methylene phosphonic acid) chelate (Gd-EDTMP), a gadolinium-containing neutron capture therapy preparation, and the effects of thermal neutron irradiation on animal tumor models. Previous our experiments with a single intraperitoneal administration of Gd-EDTMP in young mice suggested that Gd-EDTMP has a high dose distribution in parts of the femur, especially in bones with epiphyseal line. In this study, mice model of carcinogenesis was created in elderly mice with a closed epiphyseal line to investigate the basis for neutron capture therapy. After neutron irradiation of the mice, the distribution of ^{157}Gd in and around the bone was imaged using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

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

RESULTS and DISCUSSION: syu
Comparing the weight of the right legs, including the tumor (Fig. 1), the Gd+/Nu+ group (822 ± 27 mg) tended to be lower than the Gd+/Nu- (1255 ± 390 mg) or Gd-/Nu+ (1147 ± 173 mg) groups. This may suggest that tumor growth in the Gd+/Nu+ mice was suppressed. LA-ICP-MS Gd imaging of the legs of Gd +/Nu + and Gd +/Nu - showed extremely high Gd signal intensity in bone. In 4T1 tumor areas, Gd signal intensity was higher than in surrounding muscle tissue (Fig. 1). It was suggested that 4T1 cells may uptake Gd-EDTMP.

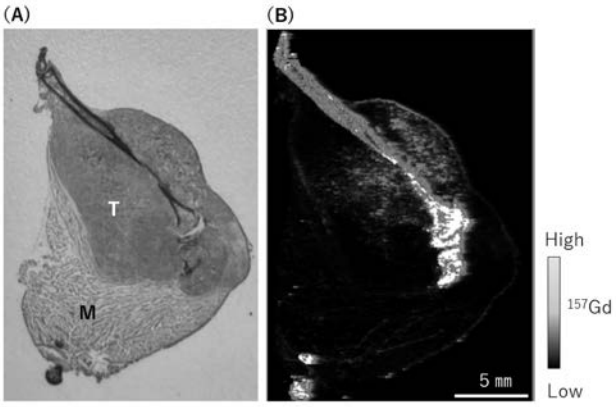

Fig. 1. Leg section of a 4T1 mammary tumor cell transplanted mouse 14 days after treatment with Gd tetra (methylene phosphonic acid) chelate (Gd-EDTMP) (20 mg/kg). (A) Hematoxylin Eosin stained microscopic image: T indicating tumor area and M indicating muscle area. (B) Imaging of ^{157}Gd distribution by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). In the figure (B), white areas indicate areas of high ^{157}Gd signal intensity.

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