I-1. PROJECT RESEARCHES

Project 11

PR11 Preclinical Studies on gadolinium neutron capture therapy

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The isotope of gadolinium-157 (^{157}Gd) has the highest thermal neutron capture cross-section about 254,000 barn which is about 66 times higher compared with that of boron-10 (^{10}B). The gadolinium neutron capture reaction is as follows, ^{157}Gd+n = ^{158}Gd+gannma-ray+internal conver-sion elections +Auger electrons. Among the byproducts, Auger electrons is categorized as a high linear energy transfer radiation which can induce double strand brake of DNA. Since the range of the Auger electron is the order of a few nanometers, cell killing effect by the electron is evoked at when ^{157}Gd is incorporated into the target such as DNA. Combination therapy with BNCT and Gd-NCT may help to raise the radiation dose for the deep portion of the tumors.

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

P11-1: In the case of irradiation to the cell incorporating ^{157}Gd compound which are suspended liquid, the cells are irradiated with gamma-ray (range is >100 μm) which are emitted from surrounding the cells. To eliminate the irradiation by gamma-ray as much as possible, the irradiation system as follows is effective; the cells adhered to the well of the microplate which stands upright are irradiated with thermal neutrons. A preliminary experiment study was carried out to confirm the feasibility of the irradiation system. Since the point to notice is occurrence of contamination, the microplate should be sealed by sterilized seat to keep the medium in the well of microplate. During the observation time for one week, no contamination was experienced. With respect to the cell killing effect, the BPA-treated cells were morphologically destructed and decreased in number compared with no treatment and irradiation alone control groups.

P11-2: In this study, the effectiveness of new Gd-loaded nanoparticle was tested in vivo study. Since this study was carried out as preliminary study, only one mouse was used. The GdXX-NCT showed a little growth inhibition compared with that of thermal neutron irradiation.

P11-3: In this study, we investigate the in vivo antitumor effects after NCT with intra-tumoral injected nanopartic-ulate formulations. Gd-nanoCPs with different par-ticle sizes were prepared by using chitosan with molecular weights of 10 k (Gd-nanoCP 200) or 950 k (Gd-nanoCP 400) and Gd-DTPA through the previously developed w/o emulsion-droplet coalescence technique. The Gd-nanoCP 200 exhibited a stronger tumor-killing effect than the Gd-nanoCP 400. This significance in tumor-killing effect would be ascribed from a higher Gd reten-tion in the tumor tissue and improved distribution of Gd with intratumorally administered Gd-nanoCP 200.

P11-4: Tetra (methylene phosphonic acid) chelate of Gd (Gd-EDTMP) were evaluated with reference to tissue distribution and effects of thermal neutron irradiation using tumor animal models. Malignant melanoma B16 cells administered through the caudal artery infiltrated the bone marrow in all of the mice. In addition, ^{157}Gd was distributed in the bone matrix around the bony edge line and malignant melanoma. When the distribution of malignant melanoma was compared according to the distribution of melanin in the thermal neutron irradiated and non-thermal neutron irradiated groups, no significant difference was found in the present study.

P11-5: In this study, to construct the molecular system to take drugs into cell nucleus was attempted by using Hoechst molecules. Since the Hoechst group has high DNA-binding function and accumulates in the cell nu- cleus, it was expected that this molecule act as a courier molecule to deliver drugs into the nucleus. Herein, we designed the reaction protocols to couple the drugs with Hoechst unit by Huisgen cycloaddition reaction.

P11-6: The results showed that the chicken egg CAM model can be used as a model to examine the efficacy of the GNCT therapy. The results of gadolinium-loaded nanoparticles compared with that of free gadolinium compound show that the nanoparticle formulation increases efficacy of GNCT.

P11-7: In this study, a series of nanocarriers in sub-50 nm scale and researched its antitumor effect though the change of tumor size was investigated as a novel Gd-compound. The growth rate of the C26 tumors was significantly inhibited after injection of PEG272 and PEG454 with irradiation compared with non-irradiation group.

P11-8: In this study, a silica nano-particle (SiNP, 61.6 ± 3.8 nm in diameter) containing 55 ng/mg Gd3TCAS2, which is promising as a carrier for Gd-NCT was investigated as a new Gd-compound.

PR11-1 Investigation of cell killing effect by auger electrons emitted during gadolinium neutron capture therapy (Gd-NCT)

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INTRODUCTION: An accelerator-based boron neutron capture therapy (BNCT) system and boronophenylalanine (BPA)-based new drug were approved by the Ministry of Health, Labour and Welfare of Japan for the treatment of locally unresectable recurrent or unresectable advanced head and neck cancer on March 2020. Since BNCT will be carried out at the medical institute, the accessibility of BNCT will improve dramatically.

One of the drawbacks of BNCT is that thermal neutrons necessary for tumor control cannot be delivered to the deep portion of the tumor which is located at > 6 cm in depth from the skin surface. To overcome the drawback, in the clinical study on BNCT for malignant glioma, X-ray irra-diation to the deep portion of the tumor was followed by BNCT.

The isotope of gadolinium-157 (^{157}Gd) has the highest thermal neutron capture cross-section about 254,000 barn which is about 66 times higher compared with that of bo-ron-10 (^{10}B). The gadolinium neutron capture reaction is as follows, ^{157}Gd+n = ^{158}Gd+gannma-ray+internal conver-sion elections +Auger electrons. Among the byproducts, Auger electrons is categorized as a high linear energy transfer radiation which can induce double strand brake of DNA. Since the range of the Auger electron is the order of a few nanometers, cell killing effect by the electron is evoked at when ^{157}Gd is incorporated into the target such as DNA. Combination therapy with BNCT and Gd-NCT may help to raise the radiation dose for the deep portion of the tumors.

To investigate the cell killing effect by Auger electron, irradiation by other byproducts yielded by ^{157}Gd neutron capture reaction should be eliminated as much as possible from the site of the reaction. In the case of irradiation to the cell incorporating ^{157}Gd compound which are sus-pended liquid, the cells are irradiated with gamma-ray (range is >100 μm) which are emitted from surrounding the cells. To eliminate the irradiation by gamma-ray as much as possible, the irradiation system as follows is ef-fective; the cells adhered to the well of the microplate which stands upright are irradiated with thermal neutrons.

A preliminary experiment study was carried out to con-firm the feasibility of the irradiation system. Since the point to notice is occurrence of contamination, the micro-plate should be sealed by sterilized seat to keep the me-dium in the well of microplate.

EXPERIMENTS: The colon-26 cells were maintained in RPMI-1640 supplemented with L-glutamine and 10% fetal bovine serum (FBS). The 1,000 cells per well were seeded in the 96-well microplates. After overnight incu-bation, the medium of each well was replaced by BPA-containing medium at the ^{10}B concentration of 100 ppm for 2 hours before irradiation with thermal neutron. The microplate was sealed with sterilized seat and kept stand-ing upright. The microplate was irradiated with thermal neutron beam at the flux of 1x109 n/cm^{2}/s^{-1} for 30 minutes. After the irradiation, the medium in each well was re-placed with fresh medium. To confirm the feasibility of this experiment, the microplate was kept in the incubator for one week. The occurrence of contamination was visu-ally checked and the cell killing effect by BPA-treated cells was inspected thorough a microscope.

RESULTS: During the observation time for one week, no contamination was experienced. With respect to the cell killing effect, the BPA-treated cells were morphologi-cally destructed and decreased in number compared with no treatment and irradiation alone control groups.

DISCUSSION: To irradiate the cells attached to the cul-ture device vertically against the thermal neutron beam, the irradiation system in which microplate was standing upright was used in this experiment. Although some ad-ditional procedure such as sealing the plate with sterilized seat is needed, the analysis of the cell-toxic effects such as WST-1 assay is easily carried out by using microplate reader. Since the feasibility of this irradiation system was confirmed, we will investigate the cell killing effect of the positive-charged ^{157}Gd-compound which was at-tached to the cell membrane.

31P11-1

PR11-2 **Investigation of gadolinium neutron capture therapy (Gd-NCT) using gadolinium-loaded nano-particle**

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INTRODUCTION: An accelerator-based boron neutron capture therapy (BNCT) system and boronophenylalanine (BPA)-based new drug were approved by the Ministry of Health, Labour and Welfare of Japan for the treatment of locally unresectable recurrent or unresectable advanced head and neck cancer on March 2020. Since BNCT will be carried out at the medical institute, the accessibility of BNCT will improve dramatically.

One of the drawbacks of BNCT is that thermal neutrons necessary for tumor control cannot be delivered to the deep portion of the tumor which is located at > 6 cm in depth from the skin surface. To overcome the drawback, in the clinical study on BNCT for malignant glioma, X-ray irradiation to the deep portion of the tumor was followed by BNCT.

The isotope of gadolinium-157 (^{157}Gd) has the highest thermal neutron capture cross-section about 254,000 barn which is about 66 times higher compared with that of boron-10 (^{10}B). The gadolinium neutron capture reaction is as follows, ^{157}Gd+n = ^{158}Gd+gannma-ray+internal conversion elections +Auger electrons. Among the byproducts, Auger electrons is categorized as a high linear energy transfer radiation which can induce double strand brake of DNA. Since the range of the Auger electron is the order of a few nanometers, cell killing effect by the electron is evoked at when ^{157}Gd is incorporated into the target such as DNA. Combination therapy with BNCT and Gd-NCT may help to raise the radiation dose for the deep portion of the tumors.

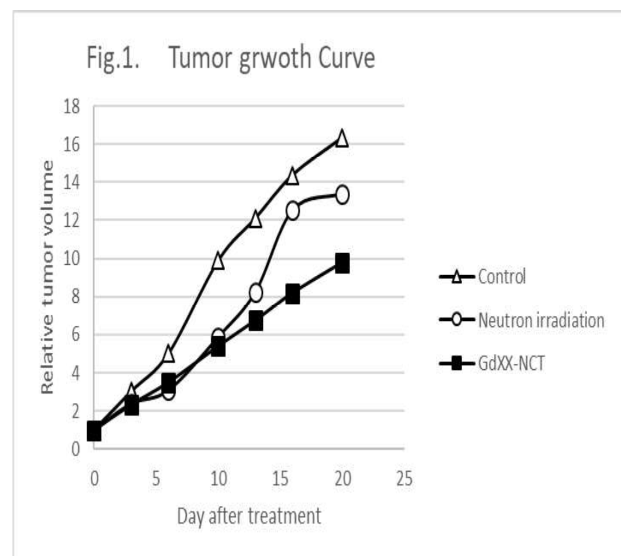
In this study, the effectiveness of new Gd-loaded nanoparticle was tested in vivo study. Since this study was carried out as preliminary study, only one mouse was used.

EXPERIMENTS: The colon-26 cells were maintained in RPMI-1640 supplemented with L-glutamine and 10% fetal bovine serum (FBS) in cell culture flasks (T-75). The cells were processed into single cells by exposure of 0.05% trypsin. The single cell suspended medium was prepared at the concentration of four million cells per 100 μm. At 10 days before the irradiation, four million colon-26 cells in 100 μm FBS-free medium were implanted to the left hind leg of 8-week old female Balb/c mice. At the irradiation, size of the tumors ranged from 10 cm to 15 cm. The new Gd-loaded nanoparticle (GdXX) was injected to one mouse intravenously via tail vain at 24 hours before the irradiation. The mouse was irradiated at the heavy water facility of Kyoto University Research Reactor (KUR) at the thermal neutron flux of 5.1E10+8 (n/cm^2/s) for 60 minutes. The long and short diameter of the tumor and the weight of the mouse was measured twice a week. The tumor volume was calculated by the following equation:

The tumor volume = (long diameter x short diameter x short diameter) / 2.

Since this preliminary experiment was carried out in accompanied with other experiment using 50-60 mice, the tumor growth curve for GdXX was compared with those of no-treatment control and thermal neutron irradiation control in the other experiment.

RESULTS: Decrease of the weight of the GdXX-NCT treated mouse 10% greater than that of the treatment day was not observed. Fig.1 shows the tumor growth curves for no-treatment control, thermal neutron irradiation, and GdXX -NCT treated mice. The GdXX-NCT showed a little growth inhibition compared with that of thermal neutron irradiation.



DISCUSSION: No suggestion was drawn from this preliminary experiment in which only one mouse was treated with GdXX-NCT. In 2020, the pharmacokinetic and GdXX-NCT in vivo studies were planned.

PR 11-3

Nanoparticulate formulations for neutron capture therapy: Evaluation of anti-tumor effect after intratumor injection

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INTRODUCTION: The successful treatment of cancer by neutron capture therapy (NCT) requires selective delivery of large amounts of ^{10}B or ^{157}Gd isotope to tumor cells. In the previous study, we developed two types nanoparticulate formulations for NCT. One is formulated nanosuspension (NS) composed of *p*-borono-L-phenylalanine (L-BPA) itself. In this BPA-NS formulation, solid particles of L-BPA are dispersed in water with a surface-active stabilizer. The other one is gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs). Gd in Gd-nanoCP-treated tumors is based primarily on the bioadhesive (cationic), biocompatible (nontoxic), and biodegradable (bioerodible) properties of chitosan nanoparticles. In the present study, we investigate the *in vivo* antitumor effects after NCT with intratumoral injected nanoparticulate formulations.

EXPERIMENTS: Macrogol 15 hydroxystearate (Solutol® HS 15, SO) and soybean lecithin (SL) were used as stabilizers. BPA-NS using SO and SL was prepared by a wet-milling method with the use of a Pulverisette-7 planetary ball mill (Fritsch). The obtained BPA-NS was sonicated using a 2510J-DTH water-bath sonicator (Branson Ultrasonics Co.) for 5 min at room temperature. Gd-nanoCPs with different particle sizes were prepared by using chitosan with molecular weights of 10 k (Gd-nanoCP 200) or 950 k (Gd-nanoCP 400) and Gd-DTPA through the previously developed w/o emulsion-droplet coalescence technique [1]. In the NCT trial, male B16F10 melanoma bearing C57BL/6J mice were used. The mice were divided into NCT group and HOT control group. BPA-Fructose complex (BPA-Fr), BPA-NS (500 mg BPA/kg), Gd-nanoCP 200 and 400 (2.4 mg Gd/kg) were administered intratumoral (i.t.) injection to the mice. The tumors in the left hind legs 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 calculation 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: BPA-NS displayed a mass median diameter of 176 nm. After i.t. administration, BPA-NS gave rise to the remarkably prolonged retention of ^{10}B in tumor tissue possibly due to the slow diffusion and/or dissolution of solid BPA-nanoparticles in a tumor. Gd-nanoCPs prepared using chitosan with a higher MW (950 kDa) had a mean particle size of 468 nm; Gd-nanoCPs prepared using chitosan with a lower MW (10 kDa) had a mean particle size of 185 nm. After i.t. administration, Gd-nanoCP 200 showed significantly higher Gd concentration in tumor tissue in comparison to Gd-nanoCP 400. In the NCT trial, growth of tumor masses was observed in the control group, while the NCT groups showed an equivalent suppression of tumor growth (Fig. 1). In BNCT, two BPA formulations had been similar tumor-killing effect. These results suggested that boron accumulates specifically in the tumor after i.t. administration of BPA formulations and that BNCT after i.t. dosing of BPA-NS is equally efficacious in the treatment of BNCT after i.t. administration of BPA-Fr. In GdNCT, the Gd-nanoCP 200 exhibited a stronger tumor-killing effect than the Gd-nanoCP 400. This significance in tumor-killing effect would be ascribed from a higher Gd retention in the tumor tissue and improved distribution of Gd with intratumorally administered Gd-nanoCP 200.

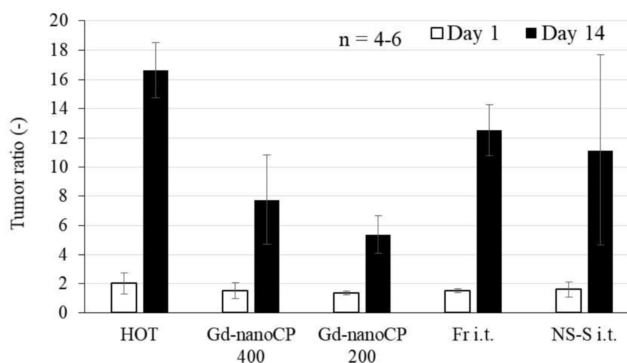

Fig. 1. Tumor volumes after thermal neutron beam irradiation of NCT and HOT control groups.

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[1] H. Tokumitsu *et al.*, Pharm. Res., 16, 1830–1835 (1999).

PR11-4 Examination on gadolinium neutron capture therapy in mouse malignant melanoma bone metastasis model

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INTRODUCTION: To develop the next generation of cancer radiotherapy, we evaluated tissue distribution and effects of thermal neutron irradiation on tumor animal models of tetra (methylene phosphonic acid) chelate of Gd (Gd-EDTMP), a neutron capture therapy formulation containing gadolinium. The distribution of ^{157}Gd in the tissue was imaged by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

EXPERIMENTS: Gd-EDTMP solutions were prepared from gadolinium chloride and EDTMP; C57BL/6JJcl female mice (6 weeks old) were obtained from CLEA Japan, Inc. (Tokyo, Japan). Mouse malignant melanoma B16 cells (JCRB0202) were purchased from JCRB Cell Bank (Ibaraki, Japan). Cultured B16 cells were injected from the tail artery of C57BL mice anesthetized with isoflurane and transplanted into the lower limbs (1×10^6 cells per mouse). On day 7 after transplantation, a saline dilution solution of Gd-EDTMP was administered intraperitoneally at a concentration of 20.0 mg-Gd/kg. After 24 h of injection, mice were randomly divided into two groups (n = 3) and one group was irradiated with thermal neutrons at the Kyoto University reactor at 6.0×10^8 cm^{-2} for 60 min. The remaining one group was not irradiated. After 7 days, mice were sacrificed and femur and tibia were sampled. For each mouse, the left femur was demineralized for 24 h, paraffin-embedded, and 5 µm sections were made in thin sections parallel to the long axis of the bone and stained with hematoxylin and eosin (H.E.). The right femur was cut into thin sections every 5 µm using the Kawamoto method, a non-demineralized frozen section preparation method, and the ^{157}Gd distribution was determined by LA-ICP-MS[1]. The invasion of the tumor into the bone was assessed by micrographs of H.E. stained sections of the femur and tibia.

RESULTS: Malignant melanoma B16 cells administered through the caudal artery infiltrated the bone marrow in all of the mice studied here. In addition, ^{157}Gd was distributed in the bone matrix around the bony edge line and malignant melanoma. When the distribution of malignant melanoma was compared according to the distribution of melanin in the thermal neutron irradiated and non-thermal neutron irradiated groups, no significant difference was found in the present study.
Although bone is hard tissue, it is actually undergoing constant remodeling by osteoblasts and osteoclasts. Gd-EDTMP may have been included in this remodeling. In bone metastatic tumors, tumor cells that flow into the bloodstream create a foothold in the bone marrow, disrupting the balance between osteoclasts and osteoblasts and proliferating. It is known that there are many cases of osteoclastic enhancement due to tumor growth, and it is thought that ^{157}Gd is enriched by the incorporation of Gd-EDTMP into the bone tissue generated by osteoblasts that work for repair in the vicinity of these osteoclasts. In particular, the acidic environment caused by osteolysis is known to be one of the causes of pain, so this formulation, which can capture neutrons in the vicinity, may have an effect on the pain of bone metastases. In the present study, there was no significant difference in the distribution of melanin in malignant melanoma caused by thermal neutron irradiation, which may be due to the small number of animal models produced and the fact that we could not compare the malignant melanoma cells themselves because we used pigment as an indicator. In the future, it is necessary to study the experimental animal model system that can follow the therapeutic effect of neutron capture more strictly, especially the model focusing on the pain of bone metastasis.

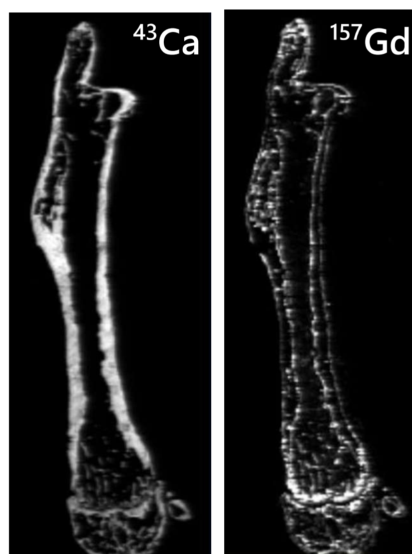

Fig. 1.
Distribution of ^{43}Ca and ^{157}Gd in the tumor bearing mice femur 24h after injection of Gd-EDTMP.

REFERENCES:

[1] A. Kubota *et al.*, Juntendo Medical Journal., **65** (2019) 461-467.

PR11-5 Preparation of drugs bearing Hoechst unit that target cell nucleus

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INTRODUCTION: The cell nucleus has been recognized as an important target in boron neutron capture therapy (BNCT). Therefore, there are increasing demands for the development of the methods to take drugs for BNCT to cell nucleus.

In this study, we attempted to construct the molecular system to take drugs into cell nucleus by using Hoechst molecules. Since the Hoechst group has high DNA −binding function and accumulates in the cell nu-cleus,[1,2] it was expected that this molecule act as a courier molecule to deliver drugs into the nucleus. Herein, we designed the reaction protocols to couple the drugs with Hoechst unit by Huisgen cycloaddition reaction.

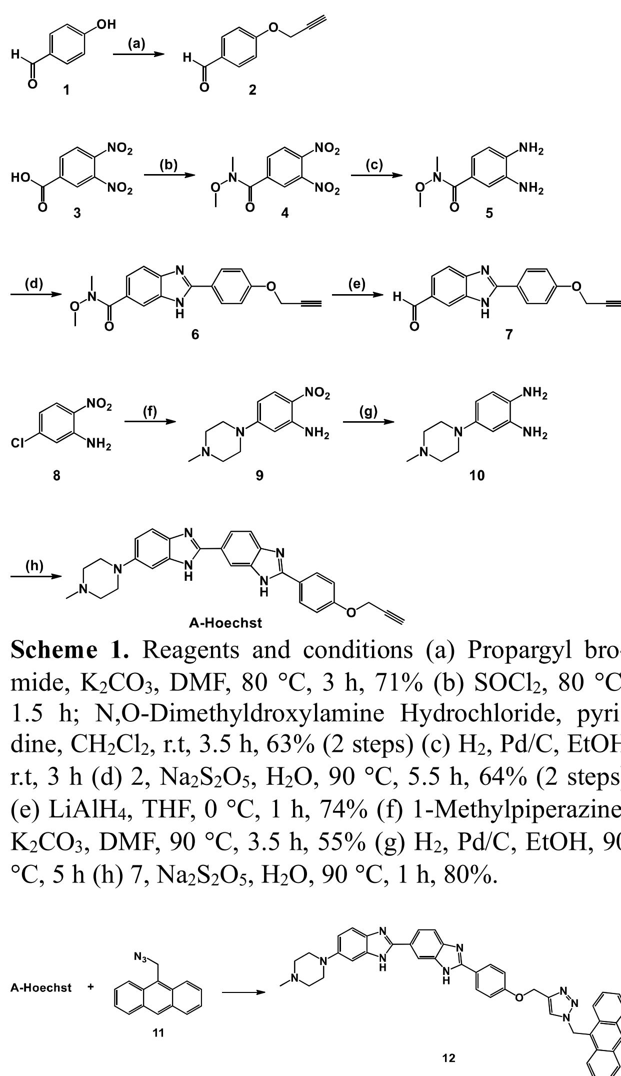
EXPERIMENTS:

Huisgen cycloaddition reaction between A-Hoechst and 9-azidemethylanthracene 11. A-Hoechst (1.0 mg, 2.2 μmol) was added to the solution of 9-azidemethylanthracene (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 temperature. 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 anthracene derivative with Hoechst unit 12.

RESULTS: We designed the synthetic protocols of the Hoechst-tethered molecules by using Huisgen cycloaddition. It is well-known that azide group is coupled with acetylene unit in the presence of Cu(I) catalyst via a formation of triazole ring. Thus, we designed Hoechst unit with acetylene group (A-Hoechst in Scheme 1) and drugs with azide substituent.

The synthesis of A-Hoechst is outlined in Scheme 1. Phenol derivative 1 was alkylated by propargyl bromide under basic conditions to form 2. On the other hand, dinitro benzoic acid 3 was converted to amide 4, and then 4 was reduced to give diamine 5. The diamine 5 was treated with aldehyde 2 to form benzimidazole derivative 6, which was reduced to give formyl benzimidazole 7. The Hoechst skeleton was synthesized as follows. Nitroaniline 8 was coupled with 1-methylpiperazine under basic conditions and following reduction gave diaminobenzene 10. The coupling of 7 and 10 furnished the desired A-Hoechst.

We next evaluated the Huisgen reaction of A-Hoechst. As a phantom drug, we employed 9-azidemethylanthracene 11 and conducted the cycloaddition reaction. The reaction of A-Hoechst and anthracene 11 was conducted at ambient temperature in the presence of catalytic $CuSO_4$ and ascorbic acid. Efficiently, the triazole ring formation occurred, and anthracene derivative with Hoechst unit 12 was formed. These results strongly indicate that A-Hoechst will be a promising molecule to prepare Hoechst-tethered molecules. At present, introduction of Gadolinium complexes or boron compounds into A-Hoechst is in progress.



Scheme 1. Reagents and conditions (a) Propargyl bromide, K_2CO_3, DMF, 80 °C, 3 h, 71% (b) $SOCl_2$, 80 °C, 1.5 h; N,O-Dimethyldroxylamine Hydrochloride, pyridine, CH_2Cl_2, r.t, 3.5 h, 63% (2 steps) (c) H_2, Pd/C, EtOH, r.t, 3 h (d) 2, $Na_2S_2O_5$, H_2O, 90 °C, 5.5 h, 64% (2 steps) (e) $LiAlH_4$, THF, 0 °C, 1 h, 74% (f) 1-Methylpiperazine, K_2CO_3, DMF, 90 °C, 3.5 h, 55% (g) H_2, Pd/C, EtOH, 90 °C, 5 h (h) 7, $Na_2S_2O_5$, H_2O, 90 °C, 1 h, 80%.

Figure 1. Huisgen reaction between A-Hoechst and 11.

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[1] Tsukiji, S. *et al., Chem. Comm.* **50** (2014) 6149–6152.

[2] Tanabe, K. *et al., ChemBioChem*, **19** (2018) 956-962.

PR11-6 Establishment of the chicken egg tumor model for GNCT

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INTRODUCTION:

Gadolinium Neutron Capture Therapy (GNCT) provides a promising alternative to BNCT. Because gadolinium is used as an MRI enhancing reagent, various compounds containing gadolinium have been developed. Our aim is to establish a capability to test various gadolinium containing reagents for GNCT at the Kyoto University Research Nuclear Reactor. In this study, we have evaluated the possibility to use the chicken egg tumor model that has been shown to be effective for the study of BNCT.

Nanoparticles can be tuned to accumulate in the tumor. This feature is attractive for GNCT, as tumor accumulation of gadolinium may dramatically enhance GNCT efficacy. We chose mesoporous silica-based nanoparticles as a carrier for gadolinium. This material is a homogeneous preparation of nanoparticles with a diameter of 40-400 nm. Approximately 1400 pores are present in one particle. Because the wall of the pore can be considered as a part of the surface area, they have a large surface area; it is reported that 1g of the nanoparticle contains 400 square meter of surface area.

EXPERIMENTS:

(Exp.1) The CAM assay was established by using fertilized chicken eggs. After incubation for ten days, a window was made on the egg shell and ovarian cancer cells were transplanted onto the CAM membrane of fertilized chicken eggs. Tumor formation was examined.

(Exp.2) Mesoporous silica-based nanoparticles were synthesized by incubating TEOS in an alkaline solution which promotes the formation of the Si-O-Si framework as a base structure of the nanoparticle. CTAB was added as a templating agent. Surface modification was carried out by the grafting method that enabled attachment of gadolinium to the surface of the nanoparticles. Stability of the gadolinium attachment was examined.

(Exp.3) The CAM model established as described in Exp. 1 was used to intravenously inject gadolinium-loaded nanoparticles and the eggs were exposed to neutron at the nuclear reactor for 1 hour. Effect on tumor growth was examined by observing tumor size as well as by examining tumor weight three days after the exposure.

RESULTS:

Exp.1:
Three days after transplanting human ovarian cancer cells onto the CAM membrane, tumor formation was confirmed. The tumor was examined by green fluorescence of ovarian cancer cells that express GFP. In addition, H&E staining of the tumor was carried out.

Exp.2:
Successful synthesis of gadolinium-loaded silica-based nanoparticles was confirmed by SEM, TEM and elemental mapping using TEM (EDX-TEM). The size of the nanoparticle was 120 nm. Loading of gadolinium was 4% of the weight of the nanoparticle, as determined by ICP.

Exp.3:
We carried out a GNCT experiment using the nuclear reactor. Gadolinium-loaded nanoparticles were intravenously injected into the chicken egg and irradiated with a neutron beam for one hour two days after the injection. Three days after the irradiation, the size and weight of the tumor was examined. The results showed that the weight of the tumor after injection of gadolinium-loaded nanoparticles was 27% of the control tumor (no injection). This contrasts with the injection of free gadolinium compound in which case the weight of the tumor was 75% of the control. Nanoparticles without gadolinium gave results that were similar to that of the control.

CONCLUSION AND FUTURE PROSPECTS:

Our results show that the chicken egg CAM model can be used as a model to examine the efficacy of the GNCT therapy. The results of gadolinium-loaded nanoparticles compared with that of free gadolinium compound show that the nanoparticle formulation increases efficacy of GNCT. However, this should be taken as a preliminary result, as the experiment was carried out only once. Further experiments are planned using different conditions such as varying irradiation time.

31P11-6

PR 11 - 7 Evaluation of Antitumor effectivity on Gd-neutron capture therapy with polymeric nanocarries

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INTRODUCTION:

Gadolinium neutron capture therapy (GdNCT) is a promising cancer treatment. Compared with the conven-tional boron NCT, because Gd compound can be used as MRI contrast agent [1], it allowed for MRI-guided GdNCT. However, clinical Gd-chelates lack high and selective accumulation in tumors for electing safe and potent GdNCT [2].

Nanoscale drug carriers are a promising way to pro-mote the accumulation of Gd agents in tumors through the enhanced permeability and retention (EPR) effect, which is based on the leaky vasculature of tumor tissues and their impaired lymphatic drainage. For developing successful nanocarriers for GdNCT, it is necessary to design systems with high Gd loading capacity that avoid leakage of free Gd. Thus, we developed a series of nanocarriers in sub-50 nm scale and researched its anti-tumor effect though the change of tumor size.

EXPERIMENTS:

We developed nanocarriers based on poly(aspartic ac-id) (P(Asp)) and were modified with poly(ethylene gly-col) (PEG) chains having different molecular weight (Mw). After chelating Gd-DOTA, the final products, which are PEG_{272}(PEG_{272}-P(Asp-Gd-DOTA)) and PEG_{454}(PEG_{454}-P(Asp-Gd-DOTA)), were made.

Colon 26 cancer cell was used for the *in vivo* an-ti-tumor effect evaluation. After 24h administration, the tumor-bearing mice received neutron irradiation 60 minutes at Nuclear Reactor Facility of Kyoto Univ Insti-tute for Integrated Radiation & Nuclear Science with average neutron fluence of 2.0×10^{12} n/cm^2. the change in tumor growth and survival rate of the mice reflected the anti-tumor effect of nanocarriers. Considering the influence of irradiation, we also set the mice injected same samples but without receiving irradiation as con-trols groups. Besides, while measuring the size of tumor, the weight change was also recorded for evaluation of the toxicity of these samples.

RESULTS:

The result of tumor growth showed at Figure 1. From the results of antitumor effect after GdNCT (Fig. 1), the growth rate of the C26 tumors was significantly inhibited after injection of PEG_{272} and PEG_{454} with irradiation compared with non-irradiation group. However, PEG_{272} showed more potent antitumor ability. From day 9, the relative tumor volume became smaller than before irradi-ation, and until day 24, the relative tumor volume was 0.27 of the initial size. Despite PEG_{454} showed higher tumor accumulation, the enhancement of the antitumor activity of PEG_{272} could be attributed to the higher cellu-lar uptake, as intracellular delivery of Gd has been indi-cated to be critical for effective cell killing [3].

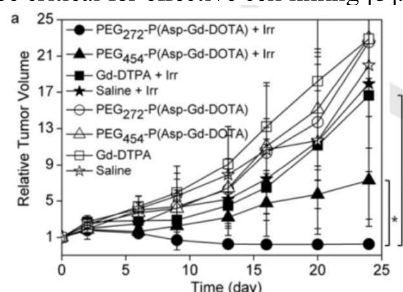

Fig. 1. Tumor growth suppression result.

Moreover, the body weight of the mice did not de-crease after the treatments, which indicate the safety of these polymers and the GdNCT (Fig. 2).

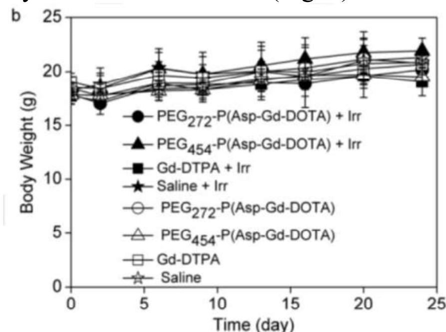

Fig. 2. The weight loss.

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PR 11-8 Development of Nano Carriers Installed with Gd(III)-Thiacalixarene Complex for Gd-NCT

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INTRODUCTION: Owing to a large thermal neutron capture cross section and total kinetic energy of $^{157}Gd(n,\gamma)^{158}Gd$ larger than that of $^{10}B(n,\alpha)^{7}Li$, gadolinium attracts growing attention as an alternative to boron in neutron capture therapy [1]. Because free gadolinium ($Gd(OH_2)_9$) has toxicity, a safe carrier of Gd to tumor not to release free Gd is required. We recently found that thiacalix[4]arene-*p*-tetrasulfonate (TCAS) self-assembled three lanthanide (Ln) cores including Gd to form a sandwich-type complex, Ln_3TCAS_2 (Fig. 1) [2], the characteristic features of which are high kinetic stability, luminescence signal [3], and ^1H relaxation arising from the Ln center [4]. Nano-sized particles are frequently used as a drug carrier toward tumor by enhanced permeability and retention (EPR) effect. Previously, we obtained a silica nano-particle (SiNP, 61.6 ± 3.8 nm in diameter) containing 55 ng/mg Gd_3TCAS_2, which is promising as a carrier for Gd-NCT. Here we attempted to evaluate the ability to kill cancer cell upon neutron irradiation by comparison with the cases of Gd_3TCAS_2, BSA-Gd_3TCAS_2 complex, Gd-DTPA, and PBS control.

EXPERIMENTS: *Preparation of SiNP installed with Ln.* The trinuclear complexes Ln_3TCAS_2 (Ln = Gd, Tb) were prepared as reported elsewhere [2]. The Ln_3TCAS_2-installed SiNP was prepared by a Stöber's method [5], which was modified with using 3-aminopropyltrimethoxysilane (APTES) as an anchor of negatively charged Ln_3TCAS_2. Furthermore, the surface was modified with poly(ethylene glycol) (PEG) by PEG-NHS (MW 2000) to retain water-dispersibility and biocompatibility.
Cell experiments. MCF-7 cells were seeded in a 6-well plate at a cell concentration of 1.0×10^5 cells/mL and incubated for 24 h. After supernatant was removed, DMEM and solution of SiNP loaded with Gd_3TCAS_2 (5.0×10^5 M as Gd) were added to each well and incubated for 24 hr. After washing with PBS, the cells were de- tached from the well and transferred to tubes to be irradi- ated with thermal neutron (8.6×10^{12} n/cm^2) for 90 min. Assay: To the wells containing 2 mL of RPMI medium in 6-well plates, irradiated cells were seeded at the concen- tration of 1,000 cells/well. After incubation for 14 days, the colony was stained with crystal violet.

RESULTS:
The images of 6-well plates for samples without irradiation showed formation of colonies (Fig. 2). This implies that Gd_3TCAS_2, BSA-Gd_3TCAS_2, and Gd_3TCAS_2-loaded SiNP are not toxic. By contrast, no colony was found for those samples with irradiation of thermal neutron, suggesting that the experimental conditions such as irradiation time were too harsh for the cell. Optimization of the irradiation conditions is necessary for further survey of the potential of the Gd-carrier.

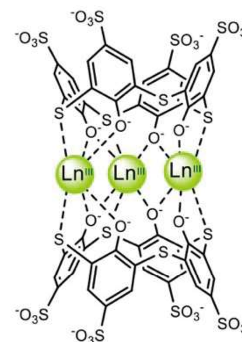

Fig. 1 Structure of Ln_3 $TCAS_2$ complex.

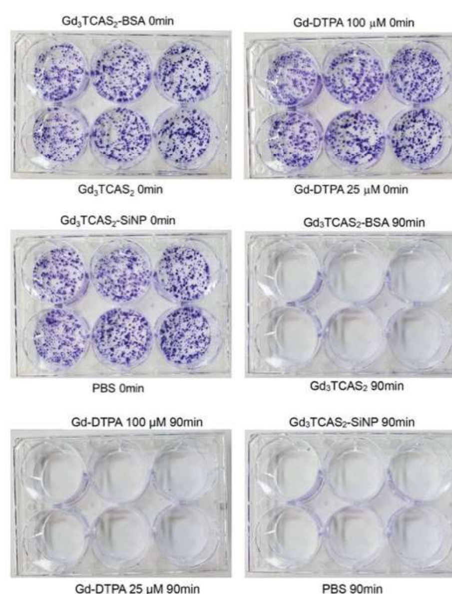

Fig. 2 Images of plates for the colony assay of MCF-7 cells with and without irradiation of thermal neutron.

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