I-1. PROJECT RESEARCHES

Project 5

PR5 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.

P5-1: Investigation of cell killing effect by auger electrons emitted during gadolinium neutron capture therapy (Gd-NCT)

We investigated the cell killing effect by auger electrons in Gd-NCT with micronucleus assay in which cytochalasin B was used to inhibit cell division to yield binucleated cells. Although the cause is unclear, CB did not work in the experiment.

We will retry the experiment next year.

P5-2: Development of Nano Carriers Installed with Gd (III)-Thiacalixarene Complex for Gd-NCT

In this study, Gd in a form of 1),2), 3) free Gd3TCAS2, 4) Gd3TCAS2bound to Alb, and 5) Gd-DTPA complex were tested in this study. The cell killing effect was assayed with colony formation assay. 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. The difference in CUF among 1–5) was too subtle to evaluate the effectiveness in the cell killing ability.

P5-3: Gadolinium neutron capture therapy as new treatment for head and neck cancer.

In this study.the authors have developed gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs). This nanoparticulate device is composed of Gd-iethylenetriaminepentaacetic acid (Gd-DTPA) which is a Gd-based MRI contrast agent and chitosan which is a naturally abounded polysaccharide material having bio-degradable, biocompatible and bioadhesive characteristics.

The Gd-NCT using Gd-nanoCPs yielded remarkable tumor (SCCVII) growth suppression compared with irradiation control group. The Gd-NCT using Gd-nanoCPs could be a promising therapeutic option for head and neck cancer.

P5-5: Preparation of functional molecules with Hoechst unit.

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 nucleus,1,2 it was expected that this molecule act as a courier molecule to deliver drugs into the nucleus.

Experiments using F-Hoechst shows that the unit take functional molecules into cell nucleus.

P5-6: Development of Gadolinium-loaded mesoporous silica-based nanoparticles and application to cancer radiotherapy.

We carried out preliminary evaluation of Gd-MSN or Gd-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.

P5-7: Evaluation of Antitumor effectivity by Gd-neutron capture therapy using Gd_2O_3 incorporated nanomicelle.

A Gd_2O_3incorporated nanomicellewas synthesized and the effect of NCT was evaluated with Human Pancreas Adenocarcinoma cell line (AsPC-1) bearing mice.

From the results in this time, we confirmed the anti-tumor effect of Gd_2O_3 incorporated nanomicelle.

P5-8: Development of 10B-enriched GdBO3nanoparticles for neutron capture therapy of cancer.

^{10}B-enriched $GdBO_3$ nanoparticles (NPs) was prepared from Gd_2O_3 and $^{10}B(OH)_3$. After the $Gd^{10}BO_3$ NP was coated by silica (SiO_2), the resulting $Gd^{10}BO_3@SiO_2$ was functionalized with polyglycerol (PG) to give $Gd^{10}BO_3@SiO_2$-PG. The tumor size of BNCT increased at the similar rate to that of the hot control. This indicates the $Gd^{10}BO_3@SiO_2$-PG works as neither BNCT nor Gd-NCT agents.

P5-9: Study about neutron capture therapy using polymeric drug delivery systems chelating Gd.

We recently synthesized functional polymers for chelating Gd, and their size can be easily controlled by fine-tuning the composition. For this purpose, in this fiscal year, we tried to set up the experimental condition for neutron irradiation to subcutaneous tumor models using a prototype of the polymeric drug.

The low-molecular drug and polymeric drug exhibited considerable anti-tumor efficacy, suggesting the successful neutron capture therapy and the appropriate irradiation condition.

P5-10: In vivo dose-dependent administration study in mice of Gd-EDTMP: gadolinium neutron capture therapy formulation for bone metastasis.

We conducted an in vivo administration study in mice to investigate the distribution and safety of Gd-EDTMP, and conducted a basic study focusing on the dose-dependency. The distribution of ^{157}Gd in the tissue was imaged by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The results of semi-quantitative analysis by LA-ICP-MS show that Gd was distributed in the part of femur bone in the order of sub per-cent.

PR5-1 Investigation of cell killing effect by auger electrons emitted during gadolinium neutron capture therapy (Gd-NCT)

M. Suzuki, H. Tanaka
Institute for Integrated Radiation and Nuclear Science Kyoto University

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 in March 2020. Since BNCT will be carried out at the medical institute, the accessibility of BNCT will improve dramatically.

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. 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 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.

In 2020, feasibility of the irradiation system was confirmed. We investigated the cell killing effect by auger electrons in Gd-NCT using this irradiation system. The cell killing effect was assayed by micronucleus assay.

EXPERIMENTS: The V79 cells were maintained in E-MEM supplemented with L-glutamine and 10% fetal bovine serum (FBS). The 1,000 cells per well were seeded in the 96-well microplates. The ^{157}Gd containing medium was adjusted using a contrast media for magnetic resonance imaging (OMNISCAN®). The ^{157}Gd containing medium at a concentration of 100, 1,000 and 5,000 ppm were replaced at 24h before irradiation with thermal neutron. The microplate was sealed with sterilized seat and kept standing upright. The microplate was irradiated with thermal neutron beam at the flux of $1x10^9$ n/cm^2/s^{-1} for 30 minutes. After the irradiation, the each well was rinsed twice with phosphate buffered saline and replaced with cytochalasin B (CB) containing medium (2μg/ml). Since CB inhibits cell division, micronuclei (MN) derived from chromosome damage are detected in binucleated cells. After 48 h incubation, the cells were fixed with Carnoy's solution and their nucleus and micronucleus were stained with propinium iodine. To estimate the cell killing effect by Gd-NCT, function of normal nuclear division was calculated by dividing binucleated cells without MN by binucleated cells.

RESULTS: The figure 1 shows the binucleated cells with micronucleus. However, almost all the cells were mononuclear cells (Fig.2). Although the cause is unclear, CB did not work in this experiment.

Fig.1

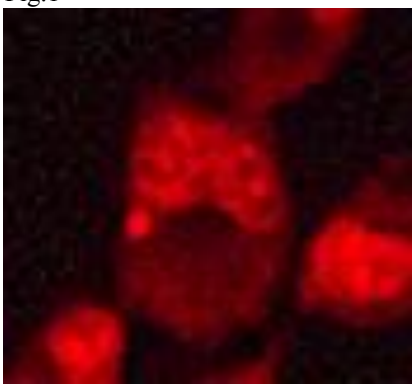
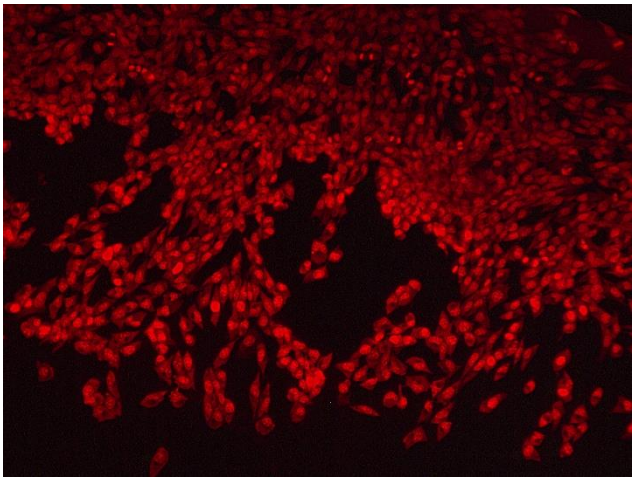


Fig.2



We will conduct this experiment again.

PR5-2 Development of Nano Carriers Installed with Gd(III)-Thiacalixarene Complex for Gd-NCT

N. Iki[1], M. Komiya[1], T. Yamatoya[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]*KURNS*

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. This year, we shifted from a silica nano-particle (SiNP, 61.6 ± 3.8 nm in diameter) containing Gd_3TCAS_2 [5] to albumin NP (AlbNP) as a carrier for Gd-NCT. Here we report the ability to kill cancer cells 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 AlbNP installed with Gd.* The trinuclear complex Gd_3TCAS_2 was prepared as reported elsewhere [2]. The AlbNP was prepared by a method reported [6]. Briefly, BSA solution was added MeOH or 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 1) Gd_3TCAS_2-AlbNP(MeOH) and 2) Gd_3TCAS_2-AlbNP(EtOH), respectively).

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, RPMI medium and solution containing Gd in a form of 1), 2), 3) free Gd_3TCAS_2, 4) Gd_3TCAS_2 bound to Alb, and 5) Gd-DTPA complex were added to each well and incubated for 24 hr. After washing with PBS, the cells were detached from the well and transferred to tubes to be irradiated with thermal neutron (8.6×10^{12} n/cm^2) for 40 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:

The concentration of Gd in the medium to incubate MCF-7 was set to be 24.3 μM for 1, 3–5) and 15.4 for 2). The colony formation units (CFU) normalized with one for no irradiation is shown in Fig. 2. As can be seen, there seems appreciable difference between CFU for 6) control and samples 1–5) containing Gd, suggesting that the gamma ray, internal conversion electron, and/or Auger electron emitted from Gd killed fraction of MCF-7 cells. However, the difference in CUF among 1–5) was too subtle to evaluate the effectiveness in the cell killing ability. Elucidation of the efficiency in the Gd-delivery and the region in the cell where the Gd-agent is delivered are now underway.

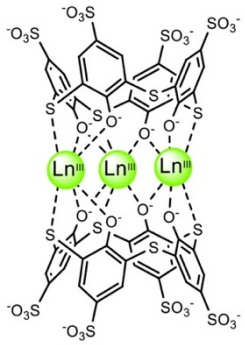


Fig. 1 Structure of Ln_3TCAS_2 complex.

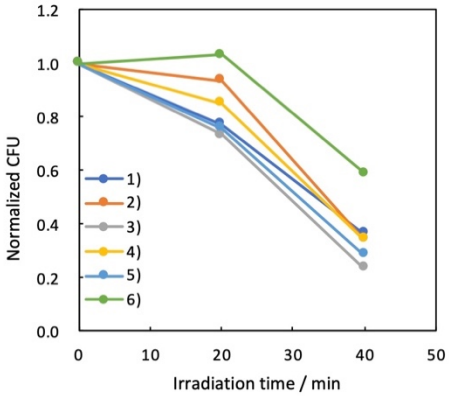


Fig. 2 Dependence of time of neutron irradiation on the colony formation rate for Gd-agents 1–5) and control 6) (n = 3).

REFERENCES:

[1] M. Takagaki *et al*., Future Application of Boron and Gadolinium Neutron Capture Thrapy, in *Boron Science*, Ed N. S. Hosmane (CRC Press) (2012) 243.

[2] N. Iki *et al.*, *Eur. J. Inorg. Chem.,* **2016** (2016) 5020-5027.

[3] R. Karashimada *et al.*, *Chem. Commun*. **52** (2016) 3139-3142.

[4] N. Iki et al., *Inorg. Chem.* **55** (2016) 4000-4005.

[5] T. Yamatoya et al, 31P11-8, p. 105, *KURNS Progress Report 2019*.

[6] C. Weber, C. Coester, J. Kreuter, K. Langer, *Int. J. Pharm*., 194 (2000) 91–102.

PR5-3 Gadolinium neutron capture therapy as new treatment for head and neck cancer

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 for cancer. Unlike boron neutron capture therapy (BNCT), which uses ^{10}B, ^{157}Gd has several potential advantages. γ-rays have long range (. 100 μm) emitted by ^{157}Gd (n, γ) ^{158}Gd reaction, so that considerable tumor-killing effects can be expected to a bulky tumor i.e, head and neck cancer. As a nanoparticulate device for the controlled delivery of Gd in NCT, the authors have developed gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs). This nanoparticulate device is composed of Gd-iethylenetriaminepentaacetic acid (Gd-DTPA) which is a Gd-based MRI contrast agent and chitosan which is a naturally abounded polysaccharide material having biodegradable, biocompatible and bioadhesive characteristics. In the present study, we investigate the *in vivo* antitumor effects after NCT with intratumoral injected nanoparticulate formulations.

EXPERIMENTS: Gd-nanoCPs was prepared by using chitosan with molecular weights of 10 k and Gd-DTPA through the previously developed w/o emulsion-droplet coalescence technique [1]. 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 SCC-VII bearing C3H/HeN mice were used. The mice were divided into NCT group and HOT control group. Gd-nanoCPs incorporating 1.2 mg of natural Gd were injected intratumorally twice (2.4 mg Gd/kg) 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: The mean particle diameter, Gd content and zeta potential of Gd-nanoCPs were 165 nm, 22% and +23 mV, respectively. Before injection, the Gd-nanoCPs was condensed to 6000 μg Gd/mL with a centrifuge. In the NCT trial, eight hours after the last administration, thermal neutron was irradiated to tumor region of the mice. Remarkable tumor-growth was observed in HOT control groups, while the NCT groups showed a suppression of tumor growth (Fig. 1). The decrease in tumor volume was similar to that observed in our previous study using a subcutaneously transplanted melanoma-bearing mouse model [2]. These results have relations that Gd-nanoCPs exhibited a good cell affinity to SCC-VII cells which seems to our previous studies using melanoma cells [3]. Therefore, a Gd-NCT using Gd-nanoCPs could be a promising therapeutic option for head and neck cancer.

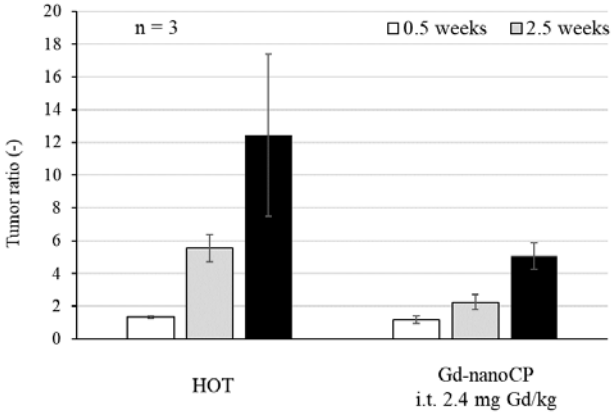


Fig. 1. Tumor volumes after thermal neutron beam irradiation of NCT and HOT control groups.

REFERENCES:

[1] H. Tokumitsu et al., Pharm. Res., 16, 1830–1835 (1999).
[2] H. Ichikawa et al., Appl. Radiat. Isot., 88, 109-113 (2014).
[3] T. Andoh et al., Appl. Radiat. Isot., 164, 109270 (2020).

PR5-4 Preparation of functional molecules 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]*Integrated Radiation and Nuclear Science, Kyoto University*

INTRODUCTION:

Neutron capture therapy (NCT) is an effective medical cure for treatment of tumor tissue. This therapy is based on the accumulation of neutron capture agents at the target tumor to which thermal neutrons are irradiated. NCT is valuable because the thermal neutrons with low energy showed negligible damage to normal tissue in which neutron-capturing agents were not accumulated. This technique thus provides a solution to the major problem of radiation therapy; the radiation led to a damage of normal tissue. Thus, the techniques for the accumulation of NCT agents in the diseased tissues and cells is important to prevent side-effect of radiation.

Gadolinium (^{157}Gd)-based NCT (Gd-NCT) has recognized as a useful cancer treatment approach. To obtain success with ^{157}Gd-NCT, we designed a novel Gd complex which had neutron capturing properties in cell nucleus, because target DNA was accommodated in the cell nucleus. 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 nucleus,[1,2] it was expected that this molecule act as a courier molecule to deliver drugs into the nucleus. Herein, we estimated the modification procedure of Hoechst molecules by Huisgen cycloaddition reaction and conducted cellular experiments to evaluate their properties in living cells.

EXPERIMENTS:

Preparation of modified Hoechst unit for Huisgen reaction. The Hoechst skeleton was synthesized as follows. Diaminobenzene derivative 1 was coupled with benzaldehyde 2 to give desired A-Hoechst (Figure 1A).

Huisgen cycloaddition reaction between A-Hoechst and fluorophore. A-Hoechst (1.0 mg, 2.2 μmol) was added to the solution of fluorescein 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 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 fluorescein derivative with Hoechst unit (F-Hoechst).

Cellular experiments using fluorophore-labeled Hoechst derivative (F-Hoechst). A549 cells were seeded in 96 well plates at a density of 5000 cells/well in DMEM and allowed to grow at 37 ˚C for 24 hours. The cells were incubated with 30 μM F-Hoechst in DMEM (DMSO 1%) for 1 h. After the cells were washed with phosphate buffered salts (PBS), the cells were subjected to laser scanning confocal microscope to investigate the cellular uptake of F-Hoechst.

RESULTS:

Initially, we conducted the Huisgen reaction of A-Hoechst. In this experiment, we employed fluorescein as a phantom drug for easy tracking in the cells, and conducted the cycloaddition reaction. The reaction was proceeded at ambient temperature in the presence of catalytic $CuSO_4$ and ascorbic acid. Efficiently, the triazole ring formation occurred, and fluorophore with Hoechst unit (F-Hoechst) was formed.

We next conducted cellular experiments using F-Hoechst. A549 cells were incubated with F-Hoechst for 1 h, and then subjected microscopy. As shown in Figure 1B, we observed strong fluorescence of F-Hoechst from cell nucleus. Thus, Hoechst unit efficiently take functional molecules into cell nucleus.

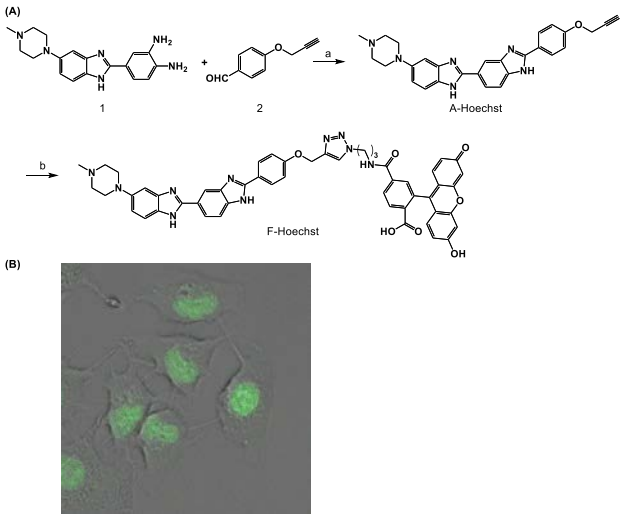


Figure 1. (A) Synthesis of A-Hoechst and F-Hoecsht. (a) $Na_2S_2O_5$, EtOH, H_2O, 55% (b) $CuSO_4$, TPTA, Sodium ascorbate, DMF, H_2O, 86%. (B) Confocal fluorescence microscopy imaging of living A549 cells incubated with F-Hoechst (Ex. 488 nm).

REFERENCES:

[1] K. Tanabe *et al.*, *Chem.Bio.Chem.* **19** (2018), 956–962.

R2P5-5

PR5-5 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). 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]. We have also developed mesoporous silica-based nanoparticles loaded with gadolinium instead of boron. We assumed this Gd-MSN or Gd-BPMO could be applied to gadolinium neutron capture therapy (GNCT). These nanoparticles have a large surface area where Gd can be attached for GNCT application. We carried out preliminary evaluation of these gadolinium nanoparticles using the chicken chorioallantoic membrane (CAM) model that was established by transplanting human ovarian cancer cells.

EXPERIMENTS:
Mesoporous silica nanoparticles (MSN) were synthesized by sol-gel synthesis of TEOS. BPMO nanoparticles were 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. These nanoparticles were surface modified with an amino group. Gadolinium diethylenetriamine penta-acetic acid (DTPA) was then mixed with amine-modified BPMO to couple Gd on the nanoparticles 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 eggs. After intravenously injection of Gd-loaded nanoparticles 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: Nanoparticles synthesized had approximately 80-100 nm of diameter and homogenous shapes examined by SEM and TEM microscopy. After coupling of Gd, we detected that the amount of Gd coupled with the nanoparticles by ICP. Gd occupied 2.6% of the weight of Gd-BPMO nanoparticle.

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. Tumor weight after the injection of Gd-BPMO followed by neutron irradiation and incubation was 27% of that compared with no injection of empty nanoparticle injection. Free Gd was 75% of the no injection control.

Further experiments on the biodistribution of Gd-BPMO are planned.

REFERENCES:
[1] Tamanoi, F. *et al*., Int. J. Mol. Sci. **22**, 2251 (2021).

PR5-6

Evaluation of Antitumor effectivity by Gd-neutron capture therapy using Gd_2O_3 incorporated nanomicelle

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

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

INTRODUCTION

In the previous research, we developed a series of polymers containing gadolinium for neutron capture therapy (Gd-NCT) [1,2,3,4]. Gd-NCT is a noninvasive therapy for treating tumors with secondary particle (Auger electron and photon) released after neutron capture reaction. Compared with boron, which has been research many times, gadolinium-157 has higher thermal neutron cross section (66 times more than boron). Therefore, less amount of gadolinium can receive same effect with boron. Besides, because the compound of gadolinium is a kind of contrast agent used for MRI, a suitable Gd compound can be expected to achieve diagnostic treatment.

However, due to the strong toxicity, low molecular weight and poor selectivity of Gd compound, a carrier, for example, nanomicelle should be introduced to solve these problems. And gadolinium oxide (Gd_2O_3) is more stable than gadolinium ion and higher tumor intention time can be expected.

In this time, a Gd_2O_3 incorporated nanomicelle was synthesized and the effect of NCT was evaluated with Human Pancreas Adenocarcinoma cell line (AsPC-1) bearing mice.

EXPERIMENTS

Female Balb/c nude mice were used to prepare AsPC-1 bearing model. Tumor cells were subcutaneously injected into the right leg of the mouse. After two weeks for tumor growth, the mouse injected Gd_2O_3 incorporated nanomicelle and Gd-DTPA (a Gd chelate without nanomicelle) received neutron irradiation (2.0×10^{12} n/cm^2) for 1h. The tumor size was measured by a caliper and the tumor volume was calculated using the equation, $V = (a \times b^2) / 2$, where a and b are the major and minor axes of the tumor.

RESULTS

Anti-tumor ability was evaluated by tumor growth speed. The slower tumor increased, the better anti-tumor effect was confirmed. From the Fig. 1, the mice injected Gd_2O_3 incorporated nanomicelle and received neutron irradiation showed the best anti-tumor effect. Therefore, the better anti-tumor effect of Gd_2O_3-nanomicelle than other samples was improved. Among irradiation group, the mice injected Gd-DTPA showed higher tumor growth speed than mice without injection. It can be considered as the introduction of extraneous materials like Gd-DTPA. Another point should be noticed is that the mice only received neutron irradiation also showed the anti-tumor effect. The reason was most likely considered as the gamma ray mixed with neutron beam. Because gamma has the ability to kill tumor cells, all irradiation group showed anti-tumor effect. Besides, no acute toxicities were recognized among all groups injected samples.

From the results of histologic analysis, no abnormal change in the liver, the kidney, the heart, and the lung were found one month after Gd-NCT treatment.

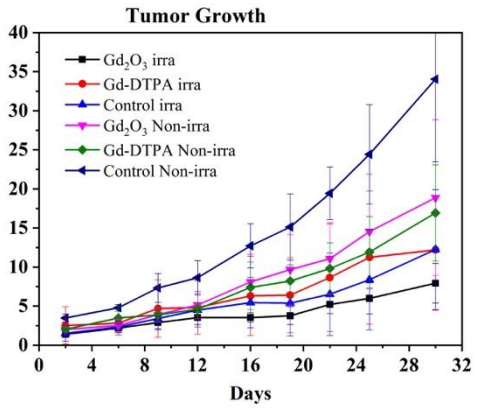


Fig 1. Tumor growth suppression in the group of Gd_2O_3 incorporated nanomicelle and bare Gd-DTPA

In the next works, due to the hollow structure of nanomicelle, we are considering the introduction of anti-tumor drugs to improve the tumor killing ability. What's more, we will check the expression of integrin receptors in many cancer cells lines and we hope to check that if the cell absorbs Gd_2O_3-nanomicelle through endocytosis. The mechanism of cytotoxicity on Gd-NCT is also very important consider the clinical application in the future. Therefore, it will be evaluated with apoptosis, autophagy, senescence etc.

In a word, from the results in this time, we confirmed the anti-tumor effect of Gd_2O_3 incorporated nanomicelle. But there are still some points should be solved and improved.

REFERENCES:

[1] Dewi N et al., Biomed & Pharmacother (2013) **67**:451-7.

[2] Dewi N et al., J Can.Res.Clin.Oncol. (2016) **142**(4):767-75.

[3] Mi P, et al.: J Cont. Release (2014) **174**:63-71.

[4] Qin C, et al., Chem Nano Mat. (2020) **6**:1-9.

PR5-7 Development of ^{10}B-enriched $GdBO_3$ nanoparticles for 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

INTRODUCTION: Gadolinium neutron capture therapy (GdNCT) has been attracting increasing interest together with boron neutron capture therapy (BNCT). Herein, we combined the two NCTs by synthesizing nanoparticle including both Gd and B.

EXPERIMENTS: ^{10}B-enriched $GdBO_3$ nanoparticles (NPs) was prepared from Gd_2O_3 and $^{10}B(OH)_3$. After the $Gd^{10}BO_3$ NP was coated by silica (SiO_2), the resulting $Gd^{10}BO_3@SiO_2$ was functionalized with polyglycerol (PG) to give $Gd^{10}BO_3@SiO_2$-PG. The product was characterized by X ray diffraction, FTIR spectroscopy and transmission electron microscopy (TEM). The size and thickness of the core ($Gd^{10}BO_3$ NP) and the SiO_2 layer were determined to be 120 and 40 nm, respectively, by TEM. After PG functionalization, $Gd^{10}BO_3@SiO_2$-PG showed very good dispersibility (>2 mg/mL) both in water and saline. The hydrodynamic size and the zeta potential of $Gd^{10}BO_3@SiO_2$-PG were 242 nm and –16.8 mV.

For in vivo experiments using tumor mice, we injected $Gd^{10}BO_3@SiO_2$-PG in phosphate buffer saline (17 mg mL^{-1}, 200 μL) from the tail vein. After 24 h, anesthesia was injected and the mice were fixed in holding plastic tool. Neutron was irradiated to the mice tumor (1 MW for 50 min). Then, tumor size was monitored and compared with the results of the control experiments.

RESULTS: As shown in Figure 1, the tumor size of BNCT increased at the similar rate to that of the hot control. This indicates the $Gd^{10}BO_3@SiO_2$-PG works as neither BNCT nor GdNCT agents. We need to do pharmacokinetics to confirm if the NP accumulates in tumor. We also have to optimize the experimental procedure for neutron irradiation, especially, the timing when we irradiate neutron after the injection of the $Gd^{10}BO_3@SiO_2$-PG.

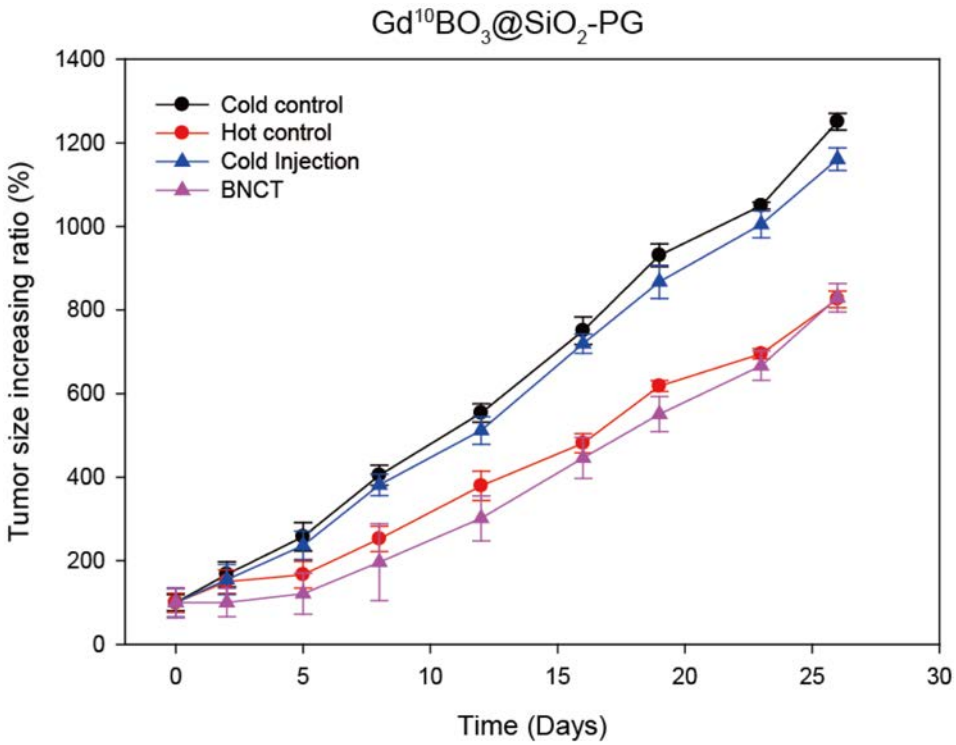


Figure 1 Time coarse of tumor size (%) as compared with the size on day 0 (100%). Cold control: no injection and no neutron irradiation. Hot control: no injection with neutron irradiation. Cold injection: no neutron irradiation with injection. BNCT: injection of $Gd^{10}BO_3@SiO_2$-PG with neutron irradiation.

PR5-8 Study about neutron capture therapy using polymeric drug delivery systems chelating Gd

Y. Miura[1], T. Nomoto[1], K. Konarita[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]. Thus, it is important to quantitatively investigate the intratumoral distribution and tumor accumulation level of Gd and study the correlation between such pharmacokinetic parameters and therapeutic effect.

We recently synthesized functional polymers for chelating Gd, and their size can be easily controlled by fine-tuning the composition. Using these polymers, we will comprehensively investigate the biodistribution and therapeutic efficacy in neutron capture therapy. For this purpose, in this fiscal year, we tried to set up the experimental condition for neutron irradiation to subcutaneous tumor models using a prototype of the polymeric drug.

EXPERIMENTS: BALB/c mice bearing subcutaneous CT26 tumors were prepared by subcutaneous injection of the cell suspension. The polymeric drug or the low-molecular drug 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 polymeric drug exhibited considerable antitumor efficacy, suggesting the successful neutron capture therapy and the appropriate irradiation condition. In the next fiscal year, we will correlate the therapeutic efficacy with the tumor accumulation level of Gd.

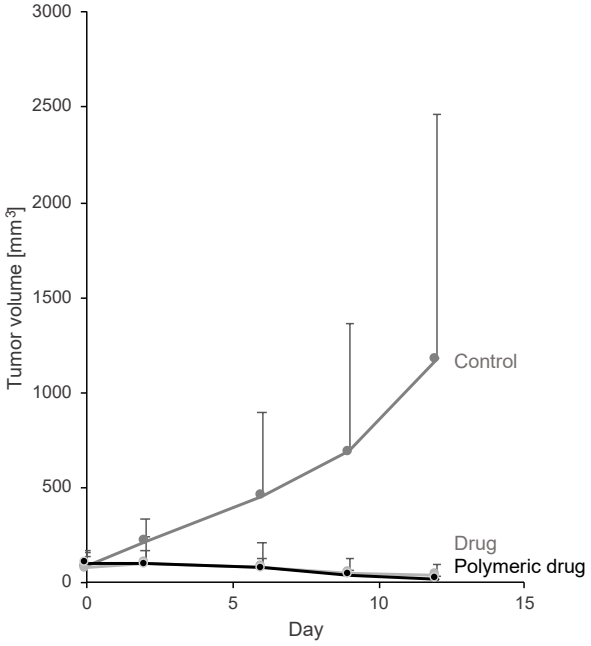


Fig. 1. Antitumor efficacy to subcutaneous CT26 tumor models.

REFERENCES:

[1] P. Mi *et al.*, ACS Nano, **9** (2015) 5913-5921.
[2] N. Dewi *et al.*, J. Cancer Res. Clin. Oncol., **142** (2016) 767-775.

PR5-9 In vivo dose-dependent administration study in mice of Gd-EDTMP: gadolinium neutron capture therapy formulation for bone metastasis

T. Matsukawa[1], M. Suzuki[2], A. Shinohara[1,3], K. Kajino[1], 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 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. On the other hand, based on findings from previous MRI contrast media studies, Gd formulation are known to cause skin fibrosis called nephrogenic systemic fibrosis (NSF) and calcification of rhabdomyosarcoma and tendons in rare cases. Therefore, in the present study, we conducted an in vivo administration study in mice to investigate the distribution and safety of Gd-EDTMP, and conducted a basic study focusing on the dose-dependency. 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. BALB/cAJcl female mice (5 weeks old) were acclimated for 1 week and then intraperitoneally administered a single dose of phosphate-buffered saline solution of Gd-EDTMP (1 mg-Gd/ml) at 5, 10, and 20 mg/kg body weight (n=2 or 3). The mice were fed and watered ad libitum until 21 days post-dose, and their general condition was monitored. Body weight was measured every 2 days until 6 days post-dose, and then every 3-5 days. Cardiac blood sampling was performed under pentobarbital anesthesia on the 21st day of administration. After euthanasia, bones and muscles of the lower limbs were collected to observe Gd distribution. The ^{157}Gd concentrations in blood and left femur were measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 8800) after acid digestion by microwave. The right frmur 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 and DISCUSSION: No clear toxicity was observed in the dose range of the present study, although a slight weight loss was observed immediately after administration. There were no behavioral changes suggestive of NSF. These results suggest that the Gd-EDTMP formulation is applicable to neutron capture therapy for bone tumors.

Although the blood Gd concentrations were higher in the 20 mg/kg group, no clear dose-dependence was observed in blood. The blood Gd concentration was lower than 50 μg/L (50 ppb), suggesting that most of the administered Gd-EDTMP was distributed promptly in the bone and the Gd amount circulating in the blood was small.

From the result of Gd concentration by conventional ICP-MS analysis, a linear dose dependence of ^{157}Gd distribution to bone was observed. The ^{157}Gd concentration in the whole femur was enriched, about 80 mg/kg in the 20 mg/kg dose group. The results of semi-quantitative analysis by LA-ICP-MS show that Gd was distributed in the part of femur bone in the order of sub per-cent. It was found that a single intraperitoneal administration of Gd-EDTMP resulted in high dose distribution to the bone as the target of this formulation. In general, neutron capture therapy is expected to be effective if the concentration of the neutron capture element in the target tumor is on the order of parts per million (ppm). By limiting the application to bone tumors, Gd-EDTMP might be used sufficiently for neutron capture therapy.

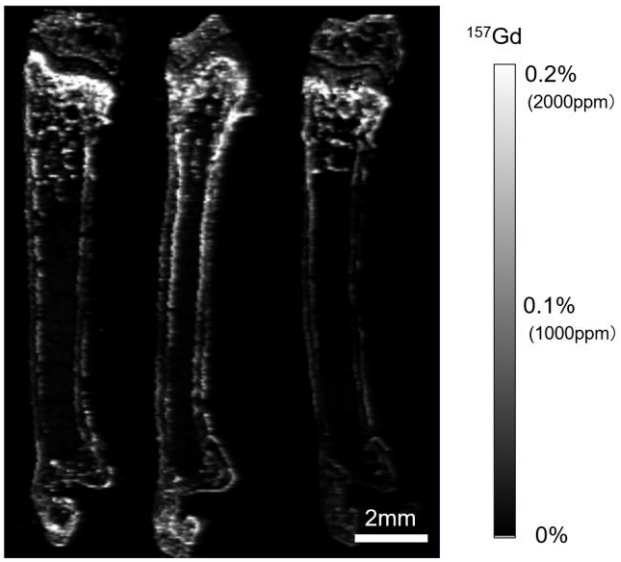


Fig. 1. Semi-quantitative ^{157}Gd imaging of mice femurs 21days after injection of Gd-EDTMP (20mg/kg).

REFERENCES:

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

R2P5-10