CO1-1 Analysis of Heavy Metal Elements in Chinese Medicine and Herbs

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INTRODUCTION: Chinese medicines and herbs were analyzed in a previous experiment at KUR. At that results, toxic elements of As and Hg were contained with high concentrations in 2 Chinese medicine; *Niuhuang Jiedu Pian* and *Liushen Wan*. However, when they are sulphide, they were not absorbed into the body. So, the chemical structures are very important. Furthermore, low concentrations of Hg were contained in many Chinese medicines and herbs. However, Chinese medicines and herbs produced in Japan contained these toxic elements rarely. The previous experiment raised mainly 3 queries; 1. Do the same named medicines contain same toxic elements with same level concentrations? 2. What chemical structures were the As and Hg? 3. Do not Chinese medicines and herbs produced in Japan contain toxic elements? The purpose of this study was to clear these issues.

EXPERIMENTS: Additional samples used in this study were 30. (The total sample number was 149.) They were grinded into powders with an agate mortar and enclosed each in a double polyethylene bag by measuring their weight. The standard samples used were JA-2 and JR-2 of rock standard, and 50 ppm of As, Hg, Sb, Co and Cr of atomic absorption reagents (Wako) were used applying to 5 filter papers (Advantec 5A). The samples and the standards were irradiated 1MW × 30 min at KUR. After cooling among 5 to 10 days, the gamma-rays of medium and long half life radionuclides were measured. Also, a XRD was used to study chemical structure of the 2 Chinese medicines of total 7 samples, which were produced in China and Japan.

RESULTS and DISCUSSION: All of *Niuhuang Jiedu Pian*, 3 samples produced in China, were contained from 2.2 to 8.6% of As and from 1.2 to 74 ppm of Hg. The main mineral was realgar by XRD, and the chemical structure of As was sulphide; As_4S_4. We could not find the products except for Chinese one. On the other hand, among 4 samples, 2 of *Liushen Wan* were produced in China, and contained approx. 7% of As and 7.5% of Hg. Also, among 2 Japanese *Liushen Wan,* one for child was contained approx. 1% of As and 30ppm of Hg, and one for adult was contained approx. 550 ppm of As and 1.2 ppm of Hg. The main minerals of these 2 Chinese *Liushen Wan* were realgar and cinnabar by XRD; As_4S_4 and HgS. On the other hand, the main minerals of these 2 Japanese *Liushen Wan* were gold and calcite by XRD; Au and $CaCO_3$. So, the same named medicines never contained same toxic elements with same level concentrations. Because the chemical structures were sulphide, they are not absorbed into a body, but just given a shock for the body. So, toxicity of the elements was no problems. Additionally, some minerals in Chinese medicines tended to contain other toxic elements like Co, Cr and Sb by INAA. Furthermore, almost all herbs contained low level concentration of Hg. It was considered the sources of Hg of low concentrations were not minerals but ground contamination or dust contamination in the air.

Conclusion: It was clarified that some Chinese medicine contained high concentration of toxic elements and many herbs contained low concentration of Hg; however, it was considered their chemical structures were not dangerous. At the same time, the safety images of Chinese medicines and herbs were not correct.

採択課題番号　26001　　漢方薬、薬草に含まれる重金属元素の分析　　通常採択
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CO1-2 Status Report 2014 for B–3 Beam Port of KUR

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INTRODUCTION: The B–3 beam port of Kyoto University Research Reactor (KUR) had long been used as a four-circle single-crystal neutron diffractometer (4CND). For the last decade, however, the 4CND was so old that its research activity on neutron science was quite low. Therefore, the 4CND needed to be replaced and a new neutron diffractometer has been required at the B–3 beam port. Also, the new neutron diffractometer (Compact multipurpose neutron diffractometer) is critical for the structural investigations of energy storage materials such as hydrogen absorbing alloys and rechargeable lithium-ion batteries. The neutron (powder) diffraction is a powerful tool to determine the positions of light elements (e.g., hydrogen and lithium) in solids. Here, we report the current status of the B-3 beam port of KUR and the preliminary neutron diffraction experiments using a hydrogen absorbing alloy.

INSTRUMENT: The compact multipurpose neutron diffractometer is now being installed on the B–3 beam port. The neutron wavelength (λ), which is monochromatized by the (220) plane of a Cu single crystal, is 1 Å. To cover the detector area (6 º ≤ 2θ ≤ 150 º), 24 ^{3}He tube detectors (1/2 inch in diameter) have been prepared. The distances from the monochromator to the sample and from the sample to the detector will be 1.9 m and 1.2 m, respectively. Fig. 1(a) shows the goniometer for the compact multipurpose neutron diffractometer; we performed an operation check. A detector bank including the 24 ^{3}He tube detectors will be placed on an arm of the goniometer. In addition, the new beam shutter was installed at the B-3 beam port, as shown in Fig. 1(b).

PRELIMINARY EXPERIMENTS: The preliminary neutron diffraction experiments were performed using the hydrogen absorbing alloy, $(Ti_{0.31}Cr_{0.33}V_{0.36})D_{1.7}$, where D is the deuterium. It is worth noting that $Ti_{0.31}Cr_{0.33}V_{0.36}$ is a null alloy for neutron scattering due to its atomic compositions (i.e., $b_c[Ti_{0.31}Cr_{0.33}V_{0.36}] = 0$; b_c[Ti] = –3.370 fm, b_c[Cr] = 3.635 fm, and b_c[V] = –0.443 fm), where b_c is the coherent scattering length. The crystal structure has been refined on the basis of a cubic CaF_2-type structure with a = 4.288(1) Å, using X-ray diffraction with Cu $K\alpha$ radiation (see Fig. 2(b)). As shown in Fig. 2(a), we succeeded to observe several Bragg reflections for the $(Ti_{0.31}Cr_{0.33}V_{0.36})D_{1.7}$ on the B–3 beam port; which could be indexed on the basis of λ = 1 Å. Note that the Bragg reflections correspond to the D–D correlations because of the null alloy.

Furthermore, the data acquisition group of the neutron science division of KEK (KEK–KENS DAQ group) has used the B–3 beam port to assess their new ^{6}Li-glass neutron detector system, LiTA12. The LiTA12 system consists of a ^{6}Li-glass neutron detector with a multianode photo multiplier tube (MA–PMT), an amplifier, and an analog-to-digital converter (ADC) board. The B–3 beam port has a wide space around the sample position; therefore we can easily install any other system like the LiTA12 system.

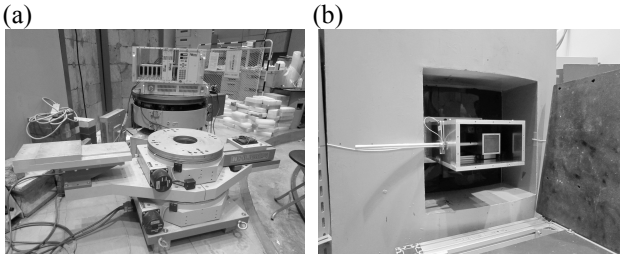

Fig. 1. Current status of the B-3 beam port of KUR: (a) new goniometer for the CMND and (b) new beam shutter installed at the B-3 beam port.

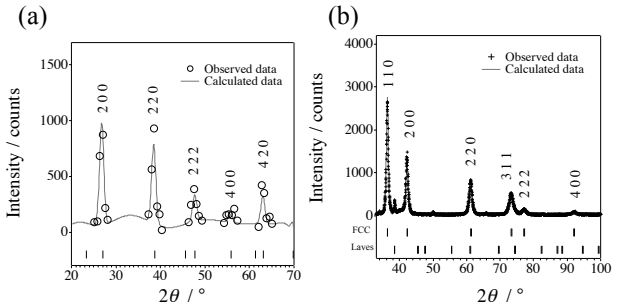

Fig. 2. Neutron and X-ray diffraction data for a hydrogen storage alloy, $(Ti_{0.31}Cr_{0.33}V_{0.36})D_{1.7}$, collected at (a) the B-3 beam port of KUR and (b) a X-ray diffractometer with Cu $K\alpha$ radiation.

採択課題番号 26007　材料研究および中性子検出器開発を目的とした小型多目的中性子回折装置の建設　　通常採択

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CO1-3 Synthesis of Metal Nanoparticles under the Gamma-ray Irradiation Field

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INTRODUCTION: It is known that metallic nanoparticles have some specific properties, which are not appear in bulk materials such as catalytic activities, magnetic properties and so on. Also the character of them depends on its size, shape, structure, chemical composition and so on. For instance, it is known that Au nanoparticles exhibit the surface plasmon resonances (SPRs) in specific wavelength at about 520 nm. They have many possibilities to applied for various industrial fields. Generally, many kinds of metal nanoparticles commercially are synthesized by using chemical reaction method. On the other hands, it has been reported that specific feature of metallic nanoparticles can be synthesized under energetic irradiation fields, such as ultrasonic, electron, gamma-ray, plasma and so on. Using these irradiation reduction methods, it is possible to fabricate the unexpected structured nanoparticles. So far, we have successfully synthesized Cu nanoparticles, which is not easy to reduce in aqueous solution by chemical reaction, by using gamma-ray irradiation reduction method [1]. However, many of them are not only pure Cu but also copper oxides. In this study, we have tried to fabricate pure Cu nanoparticles by adding of ethylene glycol instead of diethylene glycol.

EXPERIMENTS: Aqueous solutions with a given concentration of copper complex ($(CH_3COO)_2Cu{\cdot}H_2O$) with an additive of of sodium dodecyl sulfate (SDS) and ethylene glycol (EG). The solution was argon gas purged and sealed into polystyrene vessels. They were irradiated at about 300 K with 1.17 and 1.33 MeV gamma-rays from ^{60}Co radio active source at gamma irradiation facility in KURRI, Kyoto University. The total dose was fixed to 23.4 kGy with dose rate of 15.6 kGy/h. UV–vis absorption spectra were measured by using Shimadzu UV-2550 spectrophotometer in the wavelength range in 300-800 nm and the shapes and the structures for all colloidal products were observed by conventional TEM (JEOL JEM-2000FX). Samples for TEM observations were made by putting a drop of colloidal solutions on a carbon film with a Mo mesh and dried them in a vacuum.

RESULTS:
Figure 1 shows the UV/vis absorption spectra for aqueous solutions before and after gamma-ray irradiation. It appears only one absorption peak around 570 nm, which shows pure Cu nanopartcles production, after gamma-ray irradiation. This is quite different from the result that of adding of DEG [1]. This result clearly shows that only pure Cu nanoparticles were generated and no oxidation takes place during gamma-ray irradiation. This is because ethylene glycol act as the scavenger of oxidation radicals from H_2O by gamma-ray irradiation radiolysis. TEM observation shows that generated particles are monodispersive spherical shape and its average diameter is about 17 nm (fig. 2).

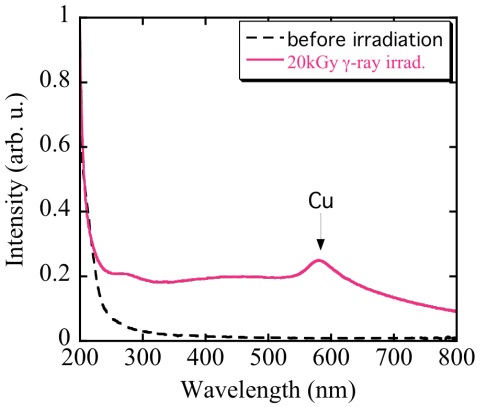

Fig. 1 UV/vis absorption spectra of aqueous solutions including Cu-ions before and after gamma-ray irradiation.

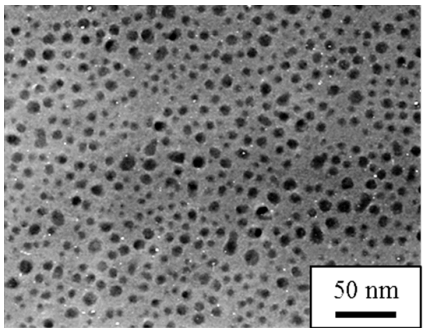

Fig. 2 TEM image of synthesized Cu nanoparticles by gamma-ray irradiation reduction.

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採択課題番号 26010　　ガンマ線照射還元を利用した貴金属ナノ微粒子の創成
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（京大・原子炉）坂本雅昭

CO1-4 The Role of Human Oxidation Resistance 1 (OXR1) in Cellular Response to Radiation

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INTRODUCTION: OXR1 (oxidation resistance 1) is a gene highly conserved in eukaryotes. Previous studies showed that human OXR1 suppressed spontaneous mutation in *E. coli mutH nth* [1]. OXR1 is induced by oxidative stresses such as H_2O_2 and is localized to mitochondria [2]. However, the function of OXR1 remains to be elucidated. Reactive oxygen species (ROS) act as a mediator of ionizing radiation -induced cellular damage. To clarify the protective functions of OXR1 against oxidative damage, we studied the effects of OXR1 on radiation -generated damage. In this study, we used gamma -ray and high LET heavy -ion beams, which are known of stronger cell killing effect than X -rays (3). Our studies showed that OXR1 proteins in HeLa cells were induced by carbon -ion beam, and that OXR1-knockdown HeLa cells were highly sensitive to heavy -ion beams. This year, we found higher micronucleus formation rate in the cells after irradiation. Then, the cells were released from cell cycle check point earlier than control cells. To study the function of OXR1 further, we are now under constructing OXR1-knockout DT40 cells.

EXPERIMENTS:

Irradiation

HeLa cells stably expressing shRNA targeting OXR1 or luciferase were synchronized at G1/S phase, and irradiated with gamma -ray (0.77 Gy/min), carbon -ion (290MeV/nucleon, 87.0keV/um) or iron -ion (500MeV/nucleon, 64.83 mmH_2O) beams generated by the Heavy Ion Mediacl Accelerator in Chiba (HIMAC). The irradiated cells were incubated for about 24 or 34 h, and fixed. The cells were used in the following experiments.

Micronucleus formation

The nuclei were counter -stained with DAPI. The micronuclei were detected with fluorescence microscopy.

Cell cycle analysis

Nuclei were stained with 50 ug/mL PI. The samples were analized with FACS.

Targeted disruption of OXR1 in DT40 cells

We prepared chicken OXR1 disrubtion constructs using puromycin, blasticidin or histidinol resistance gene. The chicken B lymphoma cells DT40 are cultured and transfected with the three gene disruption constructs.

RESULTS: The chicken OXR1 targeting constructs, OXR1-pur, OXR1-bsr and OXR1-his were generated. DT40 cells are now transfected with electroplation method. OXR1 -knockout DT40 cells are not gained.

FUTURE PERSPECTIVE: We will try constructing OXR1 -knockout TD40 cells. After finishing the step, we will study radation –induced ROS, DNA damages and cell death of the construcded cells.

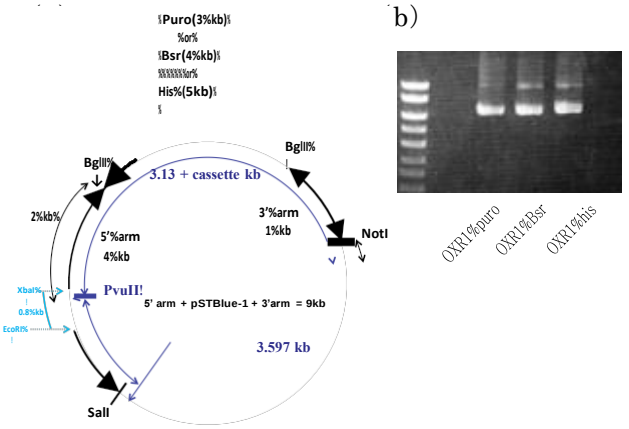

Fig. 1. (a) Schematic representation of the OXR1 disruption constructs. (b) Electrophoresis of three constructs.

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採択課題番号 26018　　酸化ストレス誘導性タンパク質 OXR1 の機能解析　　通常採択
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CO1-5 Development of an Advanced Optical Fiber Type Neutron Detector

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INTRODUCTION: The Boron Neutron Capture Therapy (BNCT) has been developed as one of the promising radiotherapies. In this radiotherapy, the neutron dose evaluation is quite important. Therefore, we are developing a novel small neutron flux monitor using an optical fiber. A small size optical fiber type neutron detector is one of the promising candidates of the on-line small neutron flux monitors in BNCT. The conventional optical fiber neutron detectors, however, show no peak shape corresponding to the neutron absorption reactions, in a pulse height spectrum due to the non-uniform and/or poor light collection [1-3]. These conventional detectors used ZnS-LiF, Li glass or plastic scintillators. On the other hand, we apply a new neutron scintillator Eu:$LiCaAlF_6$. This scintillator has some excellent properties, such as high light yield, high lithium content, transparency and chemical stability. The light yield of this scintillator is approximately 5 times higher than that of the Li glass.

Figure 1 shows the conceptual drawing of the small size optical fiber type neutron detector. Features of our detector are use of a bright scintillator and a quite small scintillator size. The scintillator size is controlled larger than ranges of ^{6}Li(n,t) reaction products and smaller than ranges of fast electrons induced by gamma rays to suppress the signal pulse height only for gamma-ray induced events.

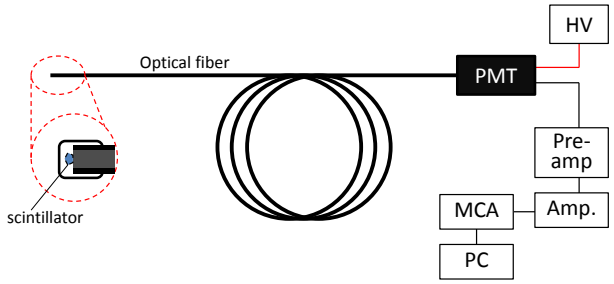

Fig. 1 Conceptual drawing of the optical fiber type neutron detector.

So far, we evaluated dynamic range and long term stability of our detector. Our detector was confirmed to show good linearity up to 10^9 n/cm^2/s. However, peak pulse height decreases due to neutron irradiation. In this report, we experimentally evaluate neutron radiation hardness of the developed detector.

RADIATION HARDNESS TEST: We fabricated the optical fiber type neutron detector using a small Eu:$LiCaAlF_6$ scintillator and experimentally evaluated its radiation hardness at the Heavy Water Neutron Irradiation Facility (HWNIF) of Kyoto University Research Reactor (KUR). One of the reasons of decrease of the peak pulse height is expected to be reduction of the transmittance of the optical fiber. We, therefore, irradiated neutrons to the optical fiber used in our detector. The neutron flux was 10^9 n/cm^2/s. We continuously measured the transmittance of the optical fiber by using a spectrometer during the neutron irradiation. The transmittance of the fiber $T(t)$ decreases as;

$$T(t) = T_0 \exp(-t/\tau)$$

where T_0; initial transmittance, τ; life time of the optical fiber. We can determine the lifetime by fitting this equation to the experimental data of decrease of the optical fiber transmittance. Figure 2 shows the wavelength dependence of the lifetime of the optical fiber under neutron irradiation with 10^9 n/cm^2/s. The lifetime in infrared region is confirmed to be longer than that in ultraviolet and visible region. The emission wavelength of the Eu:$LiCaAlF_6$ scintillator is 380 nm, where the lifetime of the transmittance is not so long. From these results, one of the reasons of decrease of the peak pulse height is confirmed to be reduction of the transmittance of the optical fiber.

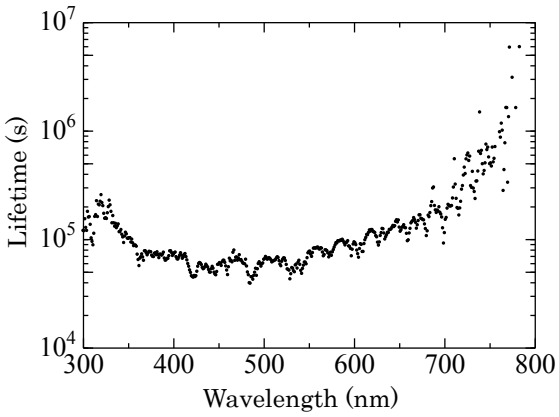

Fig. 2 Wavelength dependence of the lifetime under neutron irradiation.

In infrared region, the neutron radiation hardness is quite high. If we can use a neutron scintillator with infrared emission, the radiation hardness of the optical fiber type neutron detector can be improved.

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採択課題番号 26021 極小検出器を用いた中性子計測手法の高度化に関する研究 通常採択
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CO1-6 Focusing Test of an Ellipsoidal Neutron Mirror with a Metal Substrate

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INTRODUCTION: Progress of neutron optical devices is significant, however, it is still very difficult for neutron aspherical focusing mirror. Because the required surface roughness is smaller than 0.5 nm even for *m*=3 supermirror coating and the mirror size is large. In order to realize large ellipsoid neutron focusing mirror, we are doing several trials. In this study, we show the neutron focusing experimental result of ellipsoid mirror with a metal substrate. In general it is very difficult to make a smooth surface on metal due to the grain structure. By using electroless nickel-phosphorus (NiP) plated material, we can overcome this problem. Electroless NiP has great advantages for realizing neutron mirror because of its amorphous structure, good machinability and relatively large critical angle of total reflection for neutrons without supermirror coating.

EXPERIMENTS: The design, manufacturing and polishing of the mirror were carried out by RIKEN [1]. The form accuracy of the mirror was estimated to be 5.3 μm Peak-to-Valley (P-V) and 0.8 μm P-V for the minor-axis and major-axis direction respectively, while the surface roughness was reduced to 0.2 nm rms. The neutron focusing performance of the mirror was evaluated using CN3 beam line at Kyoto University Research Reactor (KUR-CN3).

RESULTS: As shown in Fig.1, the two-dimensional (2D) image of neutron beam reflected by an ellipsoid focusing mirror was clearly changed as a function of distance between the mirror center and detector. Cadmium (Cd) pinhole with an aperture size of 1 mm in diameter to choose incident beams from the CN3 guide a Cd U-shape slit to remove unnecessary neutron beams affecting the focusing. The circular focusing spot at the focal point was almost equivalent to that of the pinhole.

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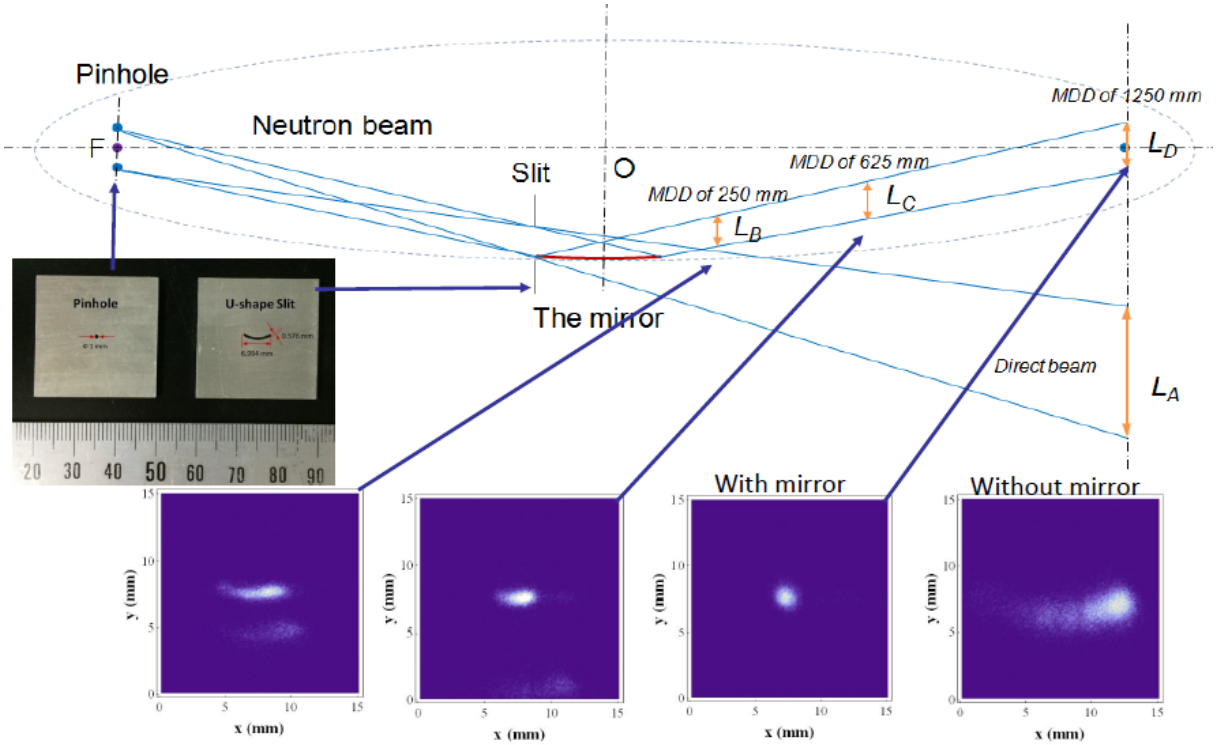

Fig.1 Schematic layout of neutron focusing experiment at KUR-CN3 and the 2D images of beam shapes as a function of the mirror center to detector distance (MDD), and the direct beam shape without the focusing mirror at the focal point.

採択課題番号 26065　次期中性子ビーム科学施設のための中性子光学デバイス開発　通常採択
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CO1-7 Examination of the Usefulness as the New Boron Compound of KA-BSH for Born Neutron Capture Therapy

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INTRODUCTION:
Boron neutron capture therapy (BNCT) is an attractive technique for malignant brain tumor treatment. [1] It is important to have a significant differential uptake of ^{10}B between tumor cells and normal cells to achieve potent tumor-selective antitumor effects. Today, clinically used dodecaboranethiol (BSH) is transferred to brain tumors only through the disrupted blood-brain barrier (BBB), so it is difficult for BSH to reach regions that tumor cells invade microscopically where the BBB seems to be normal. On the other hand, boronophenylalanine (BPA), which transfers boron via L-type amino acid transporter, can deliver ^{10}B even in the infiltrating tumor cell population where the BBB is normal. However, some amounts of ^{10}B are inevitably taken into the normal cells by BPA systemic administration. A wide variety of boron delivery agents have been synthesized to solve these problems. [1] Kojic acid (KA) was reported as the agent which showed intense uptake in malignamt tumor. Therefore, we designed and synthesized KA-BSH and evaluated therapeutic effect of KA-BSH as boron delivery agents for BNCT in F98 glioma cell bearing rat brain tumor model.

MATERIALS AND METHODS:
BNCT was performed 14 days following stereotactic implantation of 10^3 F98 glioma cells implanted rat brain tumor model. Rats were transported to the Nuclear Reactor Laboratory at Kyoto University Research Reactor Institute.
Based on the results of the biodistribution study, we prepared 4 groups. The rats were then randomized on the basis of weight into experimental groups of 6-8 animals each as follows: Group 1, KA-BSH administered by intravenous administration (iv) and neutron irradiated; Group 2, KA-BSH administered by convection enhanced delivery (CED) by using Alzet pump, which can deliver solutions directly into the solid tissue with continuous pressure gradient distribution [2, 3], and neutron irradiated; Group 3, neutron irradiation; Group 4, untreated controls. BNCT was initiated 1 h after termination of Alzet pump infusion or i.v. administration. All irradiated rats were anesthetized with a pentobarbital sodium.
The rats were irradiated for 60 min with 1MW. The antitumor effects of BNCT were evaluated in the mean survival times (MSTs) of the rat.

RESULTS:
The estimated physical radiation doses delivered to tumor, brain and blood were calculated according to boron concentrations. The physical radiation doses delivered to the tumor were 1.1Gy for KA-BSH administered by iv. The corresponding normal brain doses were 1.0Gy. The survival data of Group 1, 2, 3 and 4 following BNCT showed that the MSTs were 35.4±8.0 days, 29.8±3.3 days, 30.2±2.2 days and 28.5±3.1 days, respectively.
We accepted significantly extend the duration of survival time of the rats group using KA-BSH administered by iv (Group 1), compared with the neutron irradiation only (Group 3) ($p<0.005$).

CONCLUSION:
Survival times of group1 were significantly prolonged even compared with irradiation group. The therapeutic effect of KA-BSH administered by iv was higher than that we expected from these tissue boron concentrations and physical radiation doses. This study suggested that KA-BSH might have extremely high compound biological effectiveness (CBE), and KA-BSH might be the drug to add therapeutic effect of clinical BNCT using BPA or in combination with BPA / BSH to.

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