I-1. PROJECT RESEARCHES

Project 4

PR4 The effect of boron neutron capture therapy on normal tissues

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In this research project, five research projects were included. One research projects (P4-4 could not be conducted due to delay of begging of KUR by COVID-19).

The effect of normal tissues including lung, liver, brain, and bone were investigated in this study. Details of each project is referred to the following contents.

P4-1: "**The effect of boron neutron capture therapy (BNCT) on normal lung in mice**."

The effect of thermal neutron beam at KUR on normal lung was analyzed with reference to the survival. The mice were sorted into four treatment groups, 40-min, 50-min, 60-min, and 70-min irradiation groups. In each group, six to seven mice were irradiated. In 70-min irradiation group, all the three mice died within 10 days. In 60-min irradiation group, six mice died within an observation period of 12 days, and three mice survive within an observation period of 380 to 540 days. In 50-min irradiation groups, five mice died, and two mice survived within an observation period of 451 to 570 days. In 40-min irradiation groups, two mice died, and five mice survived within an observation period of 465 to 577 days.

P4-2: "**Clarification of the normal cell fractionation as a trigger for radiation-induced liver injury**."

The objective of our study is to clarify the mechanism of radiation-induced liver injury.

We used the following boron cluster-sugar chain-conjugated albumin.

- Asialo-N-glycan-HSA-^{10}B
- Mannose-N-glycan-HSA-^{10}B
- Hybrid-N-glycan-HSA-^{10}B

The sugar chains recognize the normal tissue components, hepatocytes, and non-parenchymal cells. We analyzed boron spatial distribution using autoradiography technique.

P4-3: "**The Effect of Boron Neutron Capture Therapy to Normal Bones in Mice.**"

Cognitive impairment and radiation brain necrosis are late adverse effects after radiation therapy against brain or head and neck tumors. Biological effect of Boron Neutron Capture therapy (BNCT) on central nervous system is unknown. To avoid those late adverse effects,

We investigate the biological effects of BNCT on neuronal cells and brain blood vessels. We used human iPSC-derived Neural stem/progenitor cell (NSPC) lines and blood-brain barrier (BBB) model composed of rat brain capillary endothelial cells, pericytes, and astrocytes.

In neuronal cells, early apoptosis was not detected after 24 hours after BNCT. Frequency of Necrosis occurrence did not increase with increasing BPA concentration.

P4-5: "**The Effect of Boron Neutron Capture Therapy to Normal Bones in Mice.**"

In this project, we have performed X-ray or neutron irradiation to normal tibial bone in mice with and without boronophenylalanine (BPA) and determined the compound biological effectiveness (CBE) factor for BPA as the biological endpoint of the tibial bending strength.

PR4-1 The effect of boron neutron capture therapy (BNCT) on normal lung in mice

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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 in March 2020. Since BNCT will be carried out at the medical institute, the accessibility of BNCT will improve dramatically and much greater patients will be treated with accelerator-BNCT compared with reactor-BNCT. 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. For BNCT to be recognized as effective treatment modality for malignant tumors, to expand indication of BNCT is very important. We have investigated the possibility of BNCT for malignant tumors in body trunk such as liver and lung cancers. In these body trunk tumors, multiple lung metastatic tumor is good candidate for new application. Since the lung contains air, thermal neutron is delivered to the lung tissues in deep portion. In BNCT for multiple lung tumors, whole lung is irradiated with boron thermal neutron capture irradiation. We have investigated the compound biological effectiveness (CBE) factor for normal lung tissues. The CBE factors depend on the biological or clinical endpoint. The CBE factor for normal lung tissue had been reported at 2.3 from the Massachusetts Institute of Technology (MIT) group. In MIT study, the biological endpoint for the CBE factor was the occurrence of lung fibrosis. In our study, the clinical endpoint for the CBE was the death. We have already reported the survival fraction following whole thorax irradiation with X-ray irradiation. In 2020, the effect of thermal neutron beam at KUR on normal lung was investigated.

EXPERIMENTS: Ten- to twelve-week-old female C3H/He mice were used. Since, in this experiment, a large amount of thermal neutrons was needed to cause equivalent biological effect with X-ray irradiation, the irradiations were carried out at the 5MW reactor power. The mice were anesthetized by intraperitoneal injection of three types of mixed anesthesia (MMB), consists of medetomidine, midazolam, and butorphanol. The three or four mice were fixed in the acryl box and the body except for the thorax were shielded with LiF plate. The mice were sorted into four treatment groups, 40-min, 50-min, 60 min, and 70-min irradiation groups. In 40-min, 50-min, and 60-min irradiation group, seven to ten mice were treated. In 70-min irradiation group, three mice were irradiated. The acryl box containing mice were irradiated with thermal neutron beam at the thermal neutron flux of 7.5E+09 n/cm^2/s which was measured by analysis of activation of gold foil attached to the surface of the box. Survival curves have been investigated for each treatment groups.

RESULTS: In 60-min and 70-min irradiation group, six mice (40%) and three mice (100%) died within an observation period of 12 days due to acute side effects. Figure 1 shows the survival curve of each group treated with thermal neutron beam irradiation.

The physical dose by 70-min thermal neutron beam irradiation was 11.0 Gy which consists of hydrogen and nitrogen dose (5.9 Gy) and gamma-ray dose (4.9 Gy).

Fig 1.

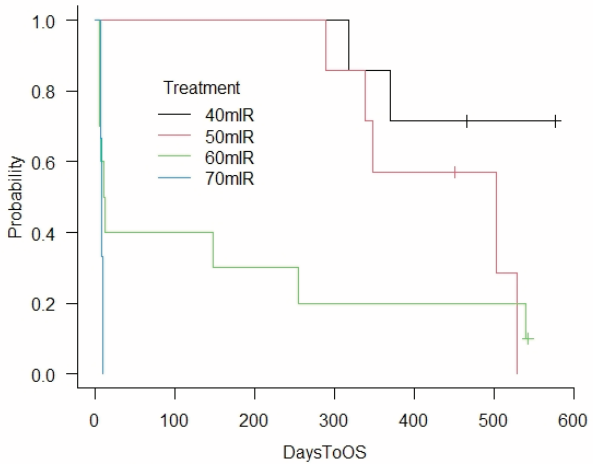
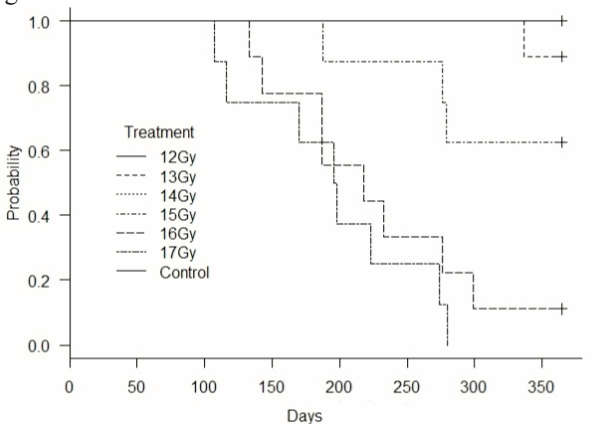


Fig 2.



Discussion: Figure.2 shows the survival for X-ray treatment groups which was already reported. In this experiment, the physical dose causing acute fatal side effect by thermal neutron beam irradiation was revealed to be 11.0 Gy. The relative biological effectiveness (RBE) of high linear energy transfer (LET) component in thermal neutron beam is estimated to be greater than 2.0 as the endpoint of acute fatal effect in whole thoracic irradiation.

To investigate the acute side effects following whole thoracic irradiation, we plan to conduct histopathologic studies.

PR4-2 Clarification of the normal cell fractionation as a trigger for radiation-induced liver injury

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INTRODUCTION:

Even though radiation-induced liver injury is one of the fatal adverse events in radiation therapy, normal cell fractionation, which causes radiation-induced liver injury, is still not clear. We have developed a boron compound that is distributed in different cell fractions of the liver [1,2]. By distributing the drug in each cell fraction and irradiating it with neutrons, it is possible to destroy the cell fraction specifically. The objective of our study is to clarify the mechanism of radiation-induced liver injury using the drugs.

EXPERIMENTS:

In this experiment, we examined the distribution of the drugs in liver using autoradiography. We used the following drugs:

- Asialo-N-glycan-HSA-10B (distributes in hepatocytes)
- Mannose-N-glycan-HSA-10B (distributes in sinusoids)
- Hybrid-N-glycan-HSA-10B

We applied each drug to C57BL or BALB/c mice Intravenously, and sacrificed and resected livers from them 1.5 hour later. Liver tissue sections was put into CR-39 (solid state nuclear track detector) and irradiated with thermal neutrons. Then we analyzed boron spatial distribution using autoradiography technique described in our previous study [3].

RESULTS:

The distribution of each boron compound in liver is shown in Fig. 1. According to this result, the drug distribution of Asialo-N-glycan-HSA-10B in liver tissue seems to show hepatocyte predominance trend. However, the distributions of other drugs were not clear due to the lack of fluence in neutron irradiation (because this is simply a preliminary study). To clarify the drug distribution trend of all the drugs, we need to conduct re-examination with enough fluence.

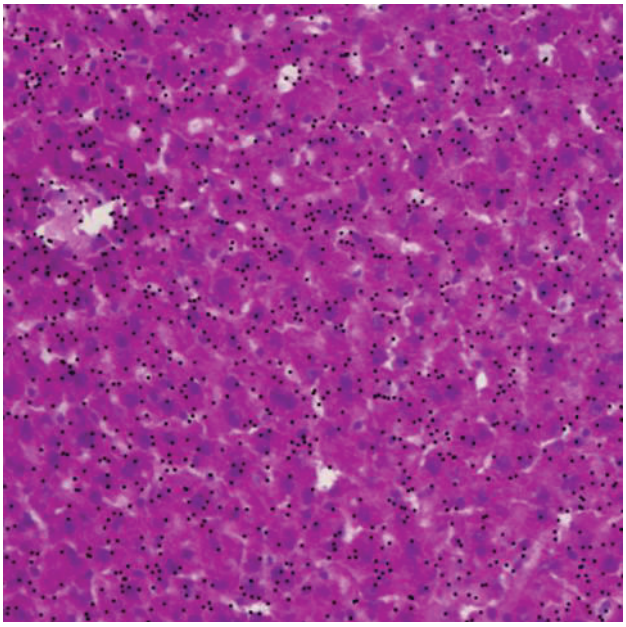

Fig. 1 Micro-distribution of Asialo-N-glycan-HAS-10B in liver tissue section.

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PR4-3 The biological effect on neurons and brain blood vessels induced by Boron Neutron Capture Therapy

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INTRODUCTION: Cognitive impairment and radiation brain necrosis are late adverse effects after radiation therapy against brain or head and neck tumors. Biological effect of Boron Neutron Capture therapy (BNCT) on central nervous system is unknown. To avoid those late adverse effects, we, first of all, investigate the biological effects of BNCT on neuronal cells and brain blood vessels.

EXPERIMENTS:

Cell culture

We used human iPSC-derived Neural stem/progenitor cell (NSPC) lines [1]. We differentiated these iPSC derived NSPCs and seeded into laminin-coated 8 well chambers and T25 flasks. Cells were sealed and transferred from the Osaka national hospital to KURNS the day before irradiation kept at 24 °C in the box and cultured at 37 °C CO_2 incubator after transfer.

We used blood-brain barrier (BBB) model [2] composed of rat brain capillary endothelial cells, pericytes, and astrocytes. This model can form tight junctions between endothelial cells which is the feature of BBB function.

Treatment of L-p-Boronophenylalanine (BPA)

We treated cells with medium containing BPA at the concentration of 5-45 ppm for 2 h. The BPA was formulated and its concentration was measured as previously described [3].

Thermal Neutron Irradiation

We irradiated thermal neutron irradiation to 1) the neuronal cells with sealing for 30 minutes with 1 MW power or 2) the blood cells (endothelial cells and pericytes) for 10 min with 5 MW power at room temperature by D_2O facility.

Cell death assay

We stained the neuronal cells in 8 well chamber 24 hour after irradiation with Hoechst 33342, Annexin V-FITC and Propidium Iodide Solution following the manufacture's protocol (nacalai tesque, Japan) and captured them by the microscope [Keyence BZ9000].

Measurement of transendothelial electrical resistance (TEER)

TEER, which reflects in culture conditions the flux of mainly sodium ions through cell layers, was measured by Epithelial-volt-ohm meter (Millipore) and Endohm-6 chamber electrodes (World Precision Instruments, USA). TEER of coated, but cell-free filters was subtracted from measured TEER values of the models shown as Ohm x 0.33 cm^2.

RESULTS:

As shown in table 1, in neuronal cells, early apoptosis was not detected after 24 hours after BNCT. Frequency of Necrosis occurrence didn't increase with increasing BPA concentration.

BNCT	control	0 ppm	5 ppm	20 ppm	45 ppm
Early apoptosis(%)	0.00	0.00	0.00	0.00	0.00
Necrosis(%)	0.54	1.20	2.09	1.62	1.30

Table. 1. Occurrence frequency (%) of apoptosis and necrosis 24 hours after BNCT in neuronal cells.

And as shown in figure 2, TEER decreased with increasing BPA concentration 10 days after BNCT.

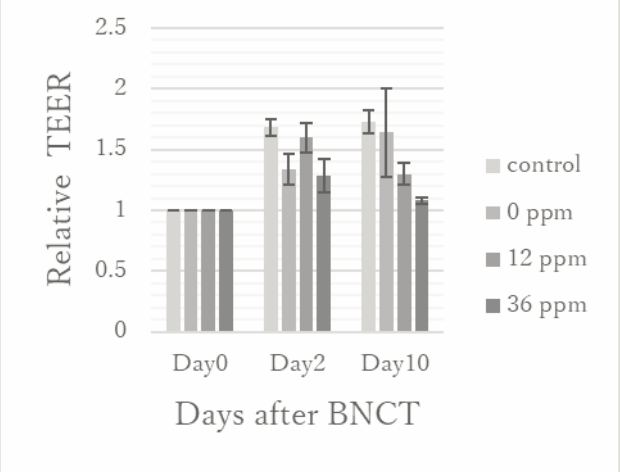

Fig. 2. Change of TEER after BNCT in BBB model

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[2]S.Nakagawa *et al*. Cell Mol Neurobiol 27(2007) 687–694. DOI 10.1007/s10571-007-9195-4

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PR4-4 The Effect of Boron Neutron Capture Therapy to Normal Bones in Mice

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INTRODUCTION: Primary malignant bone tumors have been mainly treated with preoperative chemotherapy followed by surgery. Wide or radical margins including limb amputation are required for local control. Although surgical techniques named limb-salvage therapy become a mainstay of treatment to avoid the limb amputation, complications such as postoperative infection, fracture, or local recurrence often occurred.

Although primary bone tumors have been generally considered as radio-resistant, radiation therapy has been used for the purpose of the functional and cosmetic status of patients. When a large single dose of photon radiation therapy is delivered to achieve the effective tumor control, clinically relevant late effects in the surrounding normal tissues include skin ulceration, neuropathy, and fracture.

Boron neutron capture therapy (BNCT), a tumor cell - selective particle radiation therapy, is considered to be effective for the tumors without any late effects to the normal bone. However, an appropriate BNCT dose irradiated safely to the normal bone, that is evaluated using experiment animals, is not determined. To elucidate the compound biological effectiveness (CBE) factor in normal bone is necessary to calculate the accurate radiation dose for performing BNCT.

In this project, we have performed X-ray or neutron irradiation to normal bone in mice and determined the CBE factor by evaluating the influence on their bone strengths.

EXPERIMENTS: Female eight-week-old C3H/He mice were used for the study (n = 6 in each group). As boron compound, p-boronophenylalanine (BPA) was prepared at a dose of 25 mg/ml. Irradiation was performed using X-ray and neutron at Gifu University and Kyoto University Reactor, respectively.

X-ray irradiation On the next day after X-ray irradiation to the right hind limb at a top-up dose of 24 Gy, mice were additionally irradiated at a dose of 4, 8, 12, 16, and 20 Gy.

Neutron irradiation On the next day after X-ray irradiation of 24 Gy, neutron irradiation was carried out as follows; neutron beam only (for 60, 90, and 120 min), neutron beam for 60 min after subcutaneously injected into mice at doses of 125, 250, and 500 mg/kg of BPA. Based on a preliminary study of the biodistribution of BPA, irradiation was performed between 30 and 90 min after the injection.

Bone strength analyses Tibias were collected at 12 weeks post-irradiation. Subsequently, they were mechanically tested by three-point bending to determine the bone strength. Tests were performed at HAMRI CO., LTD.

RESULTS: Figures 1−3 show the changes of the tibial bending strength after 12 weeks from the irradiation. The value of the horizontal axis in each figure represents the irradiation dose except for the top-up dose, based on the tissue ^{10}B concentration obtained from our previous study.

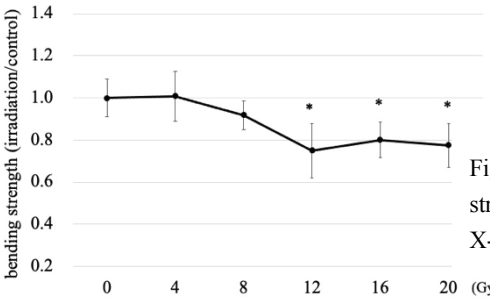

Fig.1. Tibial strength change by X-ray irradiation.

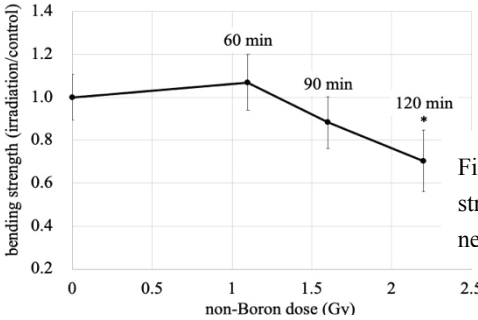

Fig. 2. Tibial strength change by neutron irradiation.

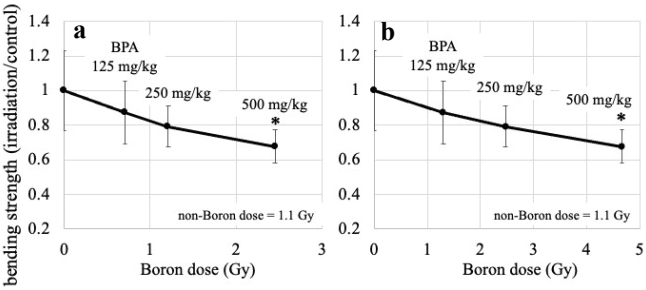

Fig. 3. Tibial strength changes by 60 min-neutron irradiation with BPA administration. Boron doses are calculated based on the ^{10}B concentration in bone (a) and blood (b), respectively.

According to the two inclinations of the straight line, that is, bone strength-X-ray dose line (Fig. 1) and bone strength-boron dose line (Fig. 3), the CBE factor for normal bone using BPA was determined as follows:

CBE factor	notes
4.09	using ^{10}B concentration in whole bone
2.15	using ^{10}B concentration in blood distributed in bone

CONCLUSION: The CBE factor for normal bone was elucidated when decrease in bone strength was set as the biological endpoint by analyzing the tibial bending strength. Further investigation such as identifying the ^{10}B distribution in normal bone using the alpha autoradiography or analyzing the changes of bone construction using the micro-CT will contribute the understanding the influence of BNCT on normal bone.