I. Project Research

Project 10

PR10 The effect of boron neutron capture therapy on normal tissues

M. Suzuki

Institute for Integrated Radiation and Nuclear Science, Kyoto University

In this research project, five research projects were included. In this summary, two research projects (P10-2 and P10-3) could not be reported due to unexpected or uncontrolled events. Details of each project is referred to the following contents.

P10-1: "The effect of boron neutron capture therapy (BNCT) on normal lung in mice."

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. 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 40-min and 50-min irradiation groups, all the mice survived within an observation period of 40 to 140 days. In 60-min irradiation group, six mice died within an observation period of 12 days, three mice survive within an observation period of 140 to 180 days, and one mouse died at 146 days after the treatment. In 70-min irradiation group, all the three mice died within 10 days.

P10-4: "Study of the influence on normal liver tissue by BNCT."

The purpose of this study is to establish systematically and continuously technique that can analyze the harmful phenomenon in the normal liver tissue of BNCT.

Female C57BL6 mice at 6weeks of age were injected 1000mg/kg p-boronophenylalanine (BPA) solution subcutaneously 2 hours before neutron irradiation. The mice were irradiated for 60 minutes at the 1MW output. One week after irradiation, mice were sacrificed, and the blood and livers were analyzed. Blood and liver boron concentrations 2 hours and 3 hours after the administration of 1000 mg / kg BPA were quantified using Inductively Coupled Plasma Spectrometer (ICP). The livers of the mice 6 months after neutron irradiation were stained with Masson trichrome. HE staining and triglyceride quantification were performed to investigate degree of the steatosis in the mouse normal liver tissue after BNCT. Western blotting was performed to deter- mine the expression level of Sonic Hedgehog protein. In addition, Masson trichrome staining was performed to determine the degree of liver fibrosis six months after neutron irradiation.

P10-5: "The Effect of Boron Neutron Capture Therapy to Normal Bones in Mice."

In this study, the effect of BNCT on normal bone in mice were evaluated regarding to their bone strengths. The tibial bending strength was decreased by reactor-producing radiation including thermal neutron, epithermal neutron, fast neutron, and γ-ray. However, the effect of BPA administration on bone strength was expected to be minimal.

PR10-1 The effect of boron neutron capture therapy (BNCT) on normal lung in mice

M. Suzuki and Y. Tamari
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 on 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.

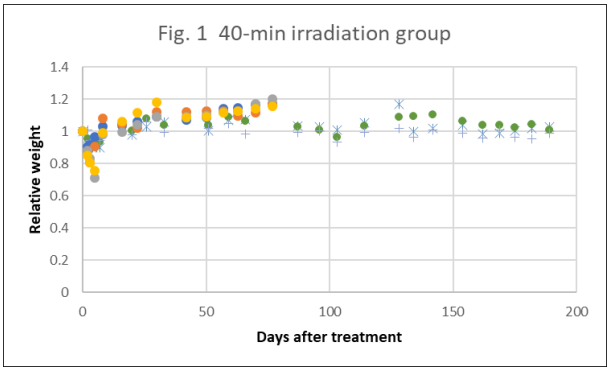
For BNCT to be recognized as effective treatment modality for malignant tumors, to expand indication of BNCT is very important. We have investigated the possibility of BNCT for malignant tumors in body trunk such as liver and lung cancers. In these body trunk tumors, multiple lung metastatic tumor is 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.

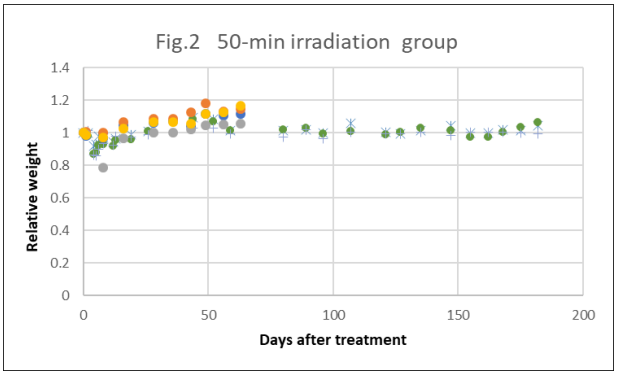
We have investigated the compound biological effectiveness (CBE) factor for normal lung tissues. The CBE factors depend on the biological or clinical endpoint. The CBE factor for normal lung tissue had been reported at 2.3 from the Massachusetts Institute of Technology (MIT) group. In MIT study, the biological endpoint for the CBE factor was the occurrence of lung fibrosis. In our study, the clinical endpoint for the CBE was the death.

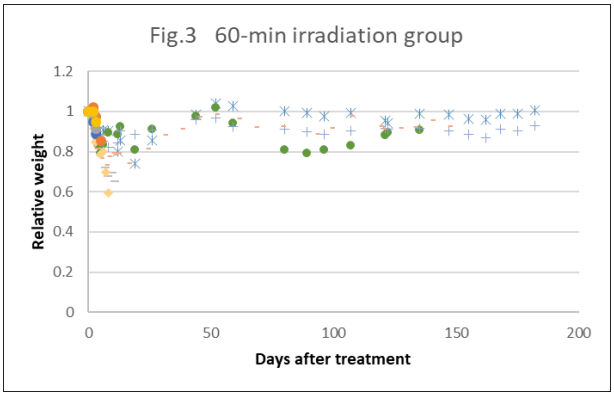
We have already reported the survival fraction following whole thorax irradiation with X-ray irradiation. In 2020, the effect of thermal neutron beam at KUR on normal lung was investigated.

EXPERIMENTS: Ten to twelve-week old female C3H/He mice were used. Since, in this experiment, a large amount of thermal neutrons were 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 intraperitonial injection of pentobarbital solution (5 mg/ml in saline) at the dose of 10 μl/g. The three or four mice were fixed in the paper 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 each group, six to seven mice were treated. 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 and change of weight have been observed twice or three times a week in the first one month and once a week after the second month.

RESULTS: In 40-min and 50-min irradiation groups, all the mice survive within an observation period of 40 to 140 days. In 60-min irradiation group, six mice died within an observation period of 12 days, three mice survive within an observation period of 140 to 180 days, and one mouse died at 146 days after the treatment. In 70-min irradiation group, all the three mice died within 10 days. Figures.1-3 show the weight change of the 40-min, 50-min, and 60-min treatment groups. In all the groups, 20 to 40% weight reduction was observed within two weeks.







DISCUSSION: In X-ray irradiation group, the mice treated with 16-17 Gy died within an observation period of 120 to 300 days. The survival of mice irradiated with thermal neutron beam will be compared with that of mice treated with X-ray irradiation in 2020.

PR10-2 Study of the influence on normal liver tissue by BNCT

Y. Tamari[1], S. Takeno, T. Takata, M. Suzuki

Institute for Integrated Radiation and Nuclear Science, Kyoto University
[1] Department of Radiology, Kyoto Prefectural University of Medicine

INTRODUCTION: Boron neutron capture therapy (BNCT) for liver tumor, which has been conducted up to the present, has used the compound effectiveness factor (CBE) determined by using genotoxicity for hepatocytes as an indicator, which has been clarified by Suzuki et al [1]. But there is a problem whether it is appropriate as a real clinical endpoint. Fundamental researches of liver fibrosis that are the late effect of radiation therapy are necessary. It is necessary to do basic research that uses liver fibrosis, which is a late radiation injury to the liver, as an evaluation index. The hedgehog signaling pathway is one of the important processes involved in animal development, and has been implicated in the maintenance and regeneration of adult tissues. The hedgehog signaling pathway is activated in the damaged liver and affects tissue remodeling. It has also been reported that cell proliferation is promoted and epithelial-mesenchymal transition leading to fibrosis is induced [2][3]. A purpose of this study is to establish systematically and continuously technique that can analyze the harmful phenomenon in the normal liver tissue of BNCT.

EXPERIMENTS: Female C57BL6 mice at 6weeks of age were injected 1000mg/kg p-boronophenylalanine (BPA) solution Subcutaneously 2 hours before neutron irradiation. The mice were irradiated for 60 minutes at the 1MW output. One week after irradiation, mice were sacrificed and the blood and livers were analyzed. Blood and liver boron concentrations 2 hours and 3 hours after the administration of 1000 mg / kg BPA were quantified using Inductively Coupled Plasma Spectrometer (ICP). The livers of the mice 6 months after neutron irradiation were stained with Masson trichrome. HE staining and triglyceride quantification were performed to investigate degree of the steatosis in the mouse normal liver tissue after BNCT. Western blotting was performed to determine the expression level of Sonic Hedgehog protein. In addition, Masson trichrome staining was performed to determine the degree of liver fibrosis six months after neutron irradiation.

RESULTS: Two hours after the administration of BPA, the liver boron concentration was about 8.1 μg/g, and the blood boron concentration was about 9.2 μg/g. Three hours after BPA administration, the liver boron concentration was about 4.1 μg/g, and the blood boron concentration was about 4.4 μg/g. Masson trichrome staining showed a tendency for increased liver fibrosis in the neutron-irradiated group receiving BPA (BNCT group). The result of HE staining demonstrated that the steatosis of the BNCT group was increased. Furthermore, quantification of triglyceride was performed to determine the degree of steatosis of normal mouse liver tissue after BNCT. Triglycerides in mouse normal liver tissue after BNCT tended to be increased compared to control. Furthermore, as a result of Western blotting, the expression of sonic hedgehog protein in the BNCT group was higher than in the group only irradiated with neutrons.

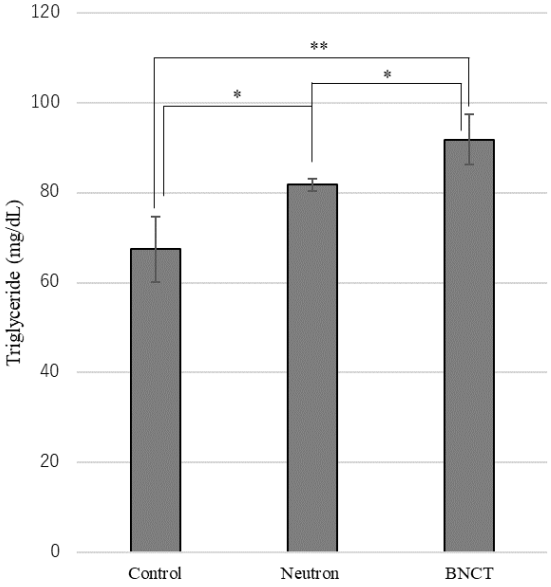


Fig.1 Triglyceride concentration of mouse liver. A significant difference was observed comparing the control and BNCT groups. (**). Error bars indicate standard deviations.

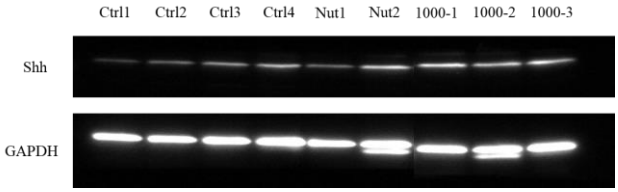


Fig.2 Shh expression level after neutron irradiation. Upper bar is Shh and Under bar is GAPDH of Ctrl (Control) and Nut (Neutron alone), 1000 (BNCT group).

REFERENCES:

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[2] Y. jung *et al.*, Gut, **59** (2010) 655-665.
[3] F. Rangwala *et al.*, J. Pathol., **224** (2011) 401-410.

PR10-3 The Effect of Boron Neutron Capture Therapy to Normal Bones in Mice

R. Iwasaki, R. Yoshikawa[1], T. Mori, Y. Tamari[2], Y. Sakurai[2], M. Suzuki[2] and K. Ono[3]

Faculty of Applied Biological Sciences, Gifu University
[1]United Graduate School of Veterinary Sciences, Gifu University
[2]Institute for Integrated Radiation and Nuclear Science, Kyoto University
[3]Kansai BNCT Medical Center, Osaka Medical College

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 tumors, 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.

In this study, we performed BNCT to normal bone in mice, and evaluated the influence on their bone strengths.

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

X-ray irradiation Mice were irradiated to their right hind limb at a single dose of 24 Gy, which was the dose that did not affect the bone strength, based on our previous study. Five mice were used for each group.

Neutron irradiation On the next day after X-ray irradiation of 24 Gy, mice were irradiated with a reactor neutron beam at a power of 1 MW. Irradiation was carried out as follows; neutron beam only (for 60, 90, and 120 min in each group), neutron beams 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. Five mice were used for each group.

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: As shown in Figure 1, additional neutron irradiation reduced tibial bending strength 12 weeks after irradiation compared to X-ray irradiation alone. Although the tibial bending strength reduced irradiation-time dependently, the strength of the tibia irradiated for 120 min was not different from that of the tibia irradiated for 90 min.

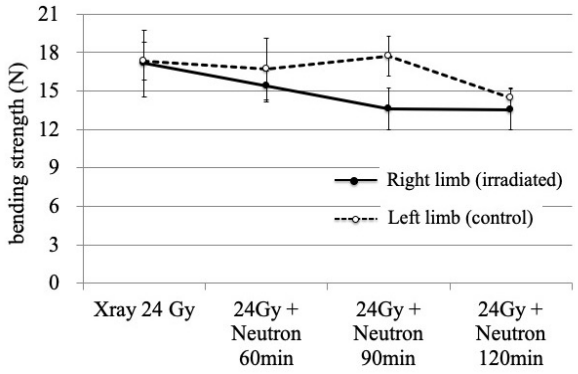

Fig. 1. Tibial strength at 12 weeks after neutron irradiation.

Subsequently, X-ray irradiation followed by neutron irradiation for 60 min with BPA administration was performed (Figure 2). The results showed that neutron irradiation with any dose of BPA administration hardly decreased the tibial strength compared with neutron irradiation alone.

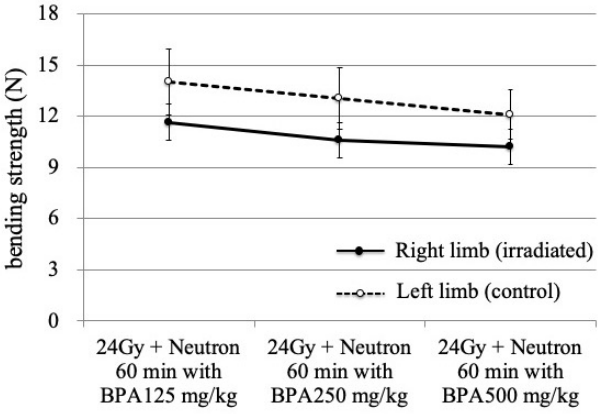

Fig. 2. Bone strengths at 12 weeks after X-ray irradiation followed by neutron irradiation with BPA administration.

CONCLUSION: The tibial bending strength was decreased by reactor-producing radiation including thermal neutron, epithermal neutron, fast neutron, and γ-ray. However, the effect of BPA administration on bone strength was expected to be minimal. Further investigation will be able to elucidate the Compound Biological Effectiveness (CBE) factor in normal bone.