I-1. PROJECT RESEARCHES

Project 3

PR3

The effect of BNCT on normal tissues

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In this research project, four research projects were included. One research was not performed. Details of three projects are referred to each progress report.

P3-1. The investigation of early and late effects of Boron Neutron Capture Therapy (BNCT) on a mouse pelvis

In previous study, anti-tumor effect of Boron Neutron Capture Therapy (BNCT) for a mouse mod-el of pelvic recurrence of colorectal cancer (CRC) was reported. In this study, early and late adverse effects in pelvic BNCT was investigated. In BNCT study, animals were divided into three groups (6-7 animals per group); the cold control (no treatment, no neutron irradiation), hot control (neutron irradiation only), and BNCT (intraperitoneal BPA administration and neutron irradiation) groups in early stage (about 1 month) and late stage (about 6 month). All mice did not have symp-toms such as diarrhea and survived until the endpoint. Although remarkable weight loss was not observed in three groups at the endpoint in early stage, significant weight loss was observed in BNCT group of late stage compared with cold control.

P3-3. Effect of BNCT on trans-endothelial electric resistance in blood brain barrier kits

Radiation necrosis is accompanied by chronic inflammation, which is an irreversible late effect, and there is no effective treatment. Assuming that the damage on blood vessel by BNCT plays im-portant role, in this study, the blood brain barrier sensitivity in BNCT was investigated in this study. Measurement of trans-endothelial electric resistance (TEER) was measured by Millicell-ERS2 on 2 and 10 days after irradiation. TEER did not make significant changes at 2 days after irradiation in all groups compared with the non-irradiated control. And at 7 days after irradiation, in the higher dose irradiated group (8-17 Gy), the TEER decreased compared with the non-irradiated control.

P3-4. The effect of boron neutron capture therapy (BNCT) on stomach in mice.

For accelerated BNCT to be recognized as a new radiotherapy, the indication of BNCT should be extended to many malignancies. In case of the treatment with multiple liver tumors by BNCT, stomach should be dealt with one of the organs at risk. The aim of this study is to investigate the effects of BNCT on the stomach.

Thermal neutron was irradiated to upper abdomen including stomach of C3H mouse after admin-istration of BPA at the dose of 500 mg/kg. At the 7days after boron neutron capture irradiation, the stomach was extracted and processed for preparation of the section and hematoxylin-eosin (HE) staining. The histopathological studies demonstrated the gastric mucosa of 15-,20-minutte irradiated mice were severely damaged compared with that of control, 5-, 10- minutes irradiated mice.

PR3-1

The investigation of early and late effects of Boron Neutron Capture Therapy (BNCT) on a mouse pelvis

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INTRODUCTION: Previously, we reported the anti-tumor effect of Boron Neutron Capture Therapy (BNCT) for a mouse model of pelvic recurrence of Colorectal cancer (CRC). On the other hand, we could not fully evaluate side effects in terms of immune response or the later complications, because we examined the effectiveness of BNCT in the nude mouse model only a month after treatment. This study investigates early and late effect of pelvic BNCT. **********

EXPERIMENTS: We used Boronophenylalanine (BPA) as a boron compound. Also, we used seven-week-old female BALB/c mouse. The boron concentrations in blood, skin and various pelvic organs (rectum, bladder, uterus, iliopsoas, vagina and cecum) at 2h, 4h, 6h after 50 mg 10B/kg BPA administration intraperitoneally. (**Figure.1**) According to this result, we decided to inject BPA intraperitoneally at 4h before irradiation. In BNCT study, animals were divided into three groups (6-7 animals per group); the cold control (no treatment, no neutron irradiation), hot control (neutron irradiation only), and BNCT (intraperitoneal BPA administration and neutron irradiation) groups in early stage (about 1 month) and late stage (about 6 month).
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RESULTS: All mice did not have symptoms such as diarrhea and survived until the endpoint. Although remarkable weight loss was not observed in three groups at the endpoint in early stage, significant weight loss was observed in BNCT group of late stage compared with cold control. (**Figure.2,3)**
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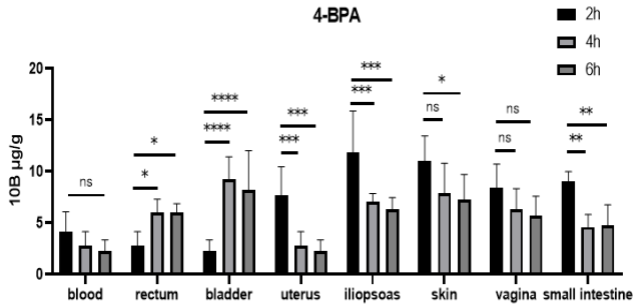

Fig.1. At 2 hours after BPA administration,
high peaks of boron concentration were observed in several organs,
including the uterus, iliopsoas, and vagina.
(****P<0.0001, ***P<0.001, **P<0.01, *P<0.05)

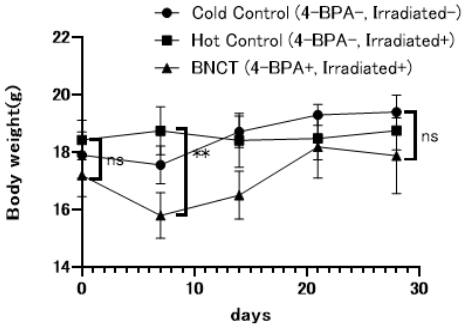

Fig.2. Body weight loss were observed a week after irradiation in BNCT group.
There were not a significant difference in body weight four weeks after irradiation. (**P<0.01) .

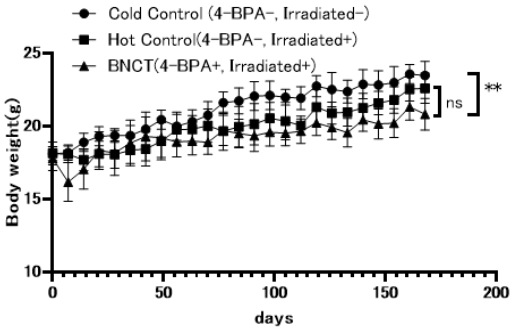

Fig.3. Body weight loss was observed six months after irradiation in BNCT group compared with Control group. (**P<0.01) .

Ongoing study: Using HE staining, western blot analysis, enzyme-linked immunosorbent assay (ELISA) and RNA-sequence, we intend to investigate characterization of early and late effect of pelvic organs.
We will continue this study and the results will be published in the future.

REFERENCES:
[1] J. Arima *et al.,* Biomed. Pharmacother., **154** (2022) (doi) 10.1016/j.biopha.2022.113632.

PR3-2

Effect of BNCT on trans-endothelial electric resistance in blood brain barrier kits

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INTRODUCTION:

The effects on the reactions and functions that occur in normal cranial nerve tissues after BNCT are often unknown. Radiation brain necrosis (RN) often occurs after BNCT for recurrent malignant brain tumors. RN is accompanied by chronic inflammation, which is an irreversible late effect, and there is no effective treatment (medication approved by insurance. RN accompanies with edema and micro-bleedings in pathology. We assume that the damage on blood vessel by BNCT plays im-portant role. In order to perform safer BNCT on cranial nerve tissues, we will investigate the blood brain barrier sensitivity in this study.

EXPERIMENTS:

Cells:

We cultured the brain-blood barrier kit (vascular endothelial cells, pericytes, and astrocytes) in DMEM in a 5 % CO_2 incubator.

Boronophenylalanine (BPA) Treatment:

A stock solution of the *p*-^{10}B-para-boronophenylalanine (BPA)- fructose complex was used. The ^{10}B concentrations was approximately 1000 ± 4.55 ppm. BPA was dissolved in the cell culture medium at a concentration of 25 ppm 2 hours before irradiation.

Thermal Neutron Irradiation:

The vascular endothelial cells and pericytes on the both sides of inserts in the kits were irradiated using a neutron beam at the Heavy Water Neutron Irradiation Facility installed in the Kyoto University Reactor (KUR-HWNIF). The operating power of the reactor was 1 MW. After irradiation, the cells were placed in the original wells in the kit.

Measurement of trans-endothelial electric resistance (TEER):

TEER was measured by Millicell-ERS2 on 2 and 10 days after irradiation.

RESULTS:

TEER did not make significant changes at 2 days after irradiation in all groups compared with the non-irradiated control. And at 7 days after irradiation, in the higher dose irradiated group (8-17 Gy), the TEER decreased compared with the non-irradiated control.

PR3-3

The effect of boron neutron capture therapy (BNCT) on stomach in mice

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INTRODUCTION: Boron neutron capture therapy (BNCT) has been applied to head and neck cancer as an insured therapy at the medical institutes where accelerator-based BNCT system has been installed. Only head and neck cancer is approved as an insured therapy. My laboratory has been engaged in the basic study for applying BNCT to malignancies in body trunk such as liver or lung cancers [1-3]. For accelerated BNCT to be recognized as a new radiotherapy, the indication of BNCT should be extended to many malignancies. In case of the treatment with multiple liver tumors by BNCT, stomach should be dealt with one of the organs at risk. The aim of the present study is to investigate the effects of BNCT on the stomach.

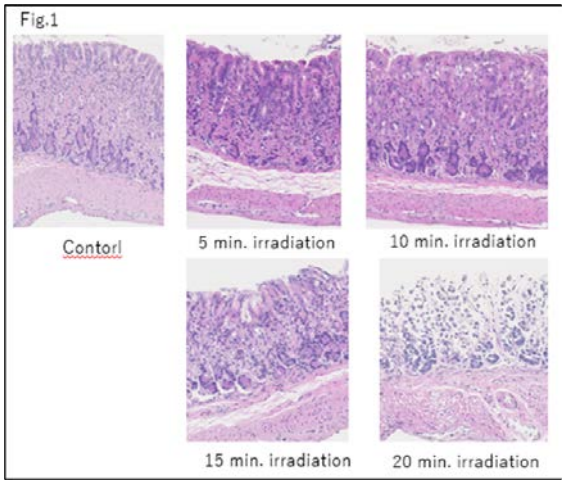
EXPERIMENTS:

Mice: Ten- to twelve-week-old female C3H/He mice were used. The mice were purchased from Japan SLC, Inc.

BNCT and measurement of thermal neutron and γ-ray: In this study, BPA was administered subcutaneously at the dose of 500 mg/kg before the upper thorax irradiation. At the each BNCR, three mice were held within a specially designed acrylic box. LiF plates (5-mm thick) were used to shield the whole body except for chest. Neutron fluences were measured by radio activation of gold foils (3mm diameter; 0.05 mm thick) on the anterior and dorsal surface of the mice. Chemi-luminescent dosimeters were used for γ-ray dosimetry.

Histopathological evaluation of gastric mucosa: The stomachs were extracted at the 7 days after BNCT. The extracted stomachs were fixed in the 10% neutral formaldehyde. The fixed samples were processed for preparation of the section and hematoxylin-eosin (HE) staining.

RESULTS: Figure 1 shows the HE stained histopathological specimen including control, 5-, 10-, 15- and 20-minutes irradiation. The specimen of 15-,20-minutte irradiation were severely damaged compared with control, 5-, 10- minutes irradiation.



Further studies will be performed to compare the effects of thermal neutron irradiation without BPA and X-ray irradiation.

REFERENCES:

[1] M. Suzuki *et al.*, Int. Cancer. Conference J., **1** (2012) 235-238.
[2] M. Suzuki *et al.*, Radiotherapy and Oncology, **92** (2009) 89-95.
[3] M. Suzuki *et al.*, Radiotherapy and Oncology, **88** (2009) 192-195.