# VIII-Ⅱ-1. Project Research

# Project 10

# PR10 Project Research on the New Applicant Development Using the Characteristics of the Particles from the Neutron Capture Reaction

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**Objectives and Participating Research Subjects**

In this project, we are intending to develop the new application using the characteristics of the particles from the neutron capture reaction.

PRS-1 Analysis of mutation in the mammalian cells induced by BNCR (boron neutron capture reaction)
( Y. Kinashi *et al.* )

PRS-2 Analysis of double strand breaks in the mammalian cells induced by BNCR
( S.Takahashi *et al.* )

PRS-3 Application of BNCR to plant tissue culture for mutation breeding
( M. Kirihata *et al.* )

PRS-4 Development of pharmacokinetic using boron trace drugs
( H. Hori *et al.* )

**Main Results and Contents**

PRS-1 investigated the radio-sensitivity in DNAPK-deficient mutant, SCID mice following neutron irradiation. The RBE of oral radiation death of SCID mice shows that hyper-sensitivity of SCID mice decreased in acute radiation effect following neutron irradiation. The apoptosis induction of splenocytes was observed in SCID mice 8 days after the neutron irradiation.

PRS-2 determined the survivals of the cultured mammalian cells (CHO/K1 and xrs-5) by colony forming assay. The neutron irradiation was seemed to be more effective than the gamma irradiation with respect to the cell survival. The number of DNA double strand breaks was estimated by counting the gamma-H2AX and 53BP1 foci in the irradiated cells. At present, although it is preliminary, the number of foci did not significantly different between the neutron and gamma irradiation, but the size might be different.

PRS-3 tried to establish a novel mutagenesis system using boron neutron capture reaction (BNCR) in higher plants. Artificial induction of mutation by radiation (e.g. x-ray, gamma irradiation, thermal neutron and ultraviolet light) usually generates recessive mutants; therefore, progeny analysis is required for determining phenotypic and genetic effects of such mutagenesis in diploid plant species. The effectiveness of BNCR for plant mutagenesis in a short period of time was determined using haploid tobacco (*Nicotiana tabacum*) plants. The leaf segments of the haploid and diploid tobacco plants were immersed in different concentrations (0, 200, 400 or 600 μM) of $^{10}$B-enriched *p*-boronophenylalanine (BPA) overnight, and the tissue samples were irradiated for 15 and 30 min. with thermal neutron of KUR. The irradiated tissues were then cultured on a plastic petri-dish containing MS solid medium with kinetin and 2,4-D. The BPA-treated calluses were then transferred to the regeneration medium. Subsequently, many haploid or doubled haploid plants were derived from haploid tissue culture, and diploid plants were obtained from diploid samples. Among them, phenotypic variations, such as chlorophyll deficient (albino), dwarf, abnormal petal number, male sterile, were observed.

PRS-4 invented wholly innovative drugs named “boron trace drug” as “on demand” traceable and next-generation drug model. They presented, as a boron tracedrugs, design, synthesis, and effects of BODIPY- and curcuminoid-boron tracedrugs for neutron dynamic therapy (NDT). In order to explore their dynamic, beyond chemical, effects when acquired by weak thermal neutrons, they performed thermal neutron irradiation of bovine serum albumin (BSA) treated with the boron tracedrugs. Boron tracedrugs including the BODIPY-containing compounds UTX-42, UTX-44, and UTX-47 and the curcuminoid compounds UTX-50 and UTX-51, were designed for neutron dynamic therapy (NDT) based on their molecular orbital calculation. Newly designed UTX-47, UTX-50, and UTX-51 were synthesized. SDS-PAGE was performed to detect the decomposition by thermal neutron irradiation of BSA treated with these five boron tracedrugs. All boron tracedrugs tested caused destructive dynamic damage of BSA during thermal neutron irradiation, suggesting boron tracedrugs could be used as dynamic drugs for NDT.

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採択課題番号 22P10　硼素中性子捕獲反応(BNCR)誘発粒子線の特性利用の新展開　プロジェクト
（京大・原子炉）木梨　友子

**PR10-1** **Various Measurements of Radio-Sensitivity in DNAPK-Deficient SCID Mice Following Neutron Irradiation**

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**INTRODUCTION:** SCID mice have a mutation in the gene encoding the catalytic subunit of DNA-dependent protein kinase (DNAPKcs) and are defective in end-joining of DNA double strand breaks. We estimated the radio-sensitivity of SCID mice using oral radiation death experiments and apoptosis inductions in the splenocytes following neutron irradiation with 1 MW KUR.

**METHODS:** Oral radiation death / Six-week-old female SCID mice were obtained from Oriental bio. Inc. and acclimated to our laboratory for 4-6 weeks prior to use in experiments. SCID mice were put into the plastic case without anesthesia. The mice heads were irradiated 1-2 Gy with 1 MW of KUR 4 hours after 8 Gy dose of gamma-ray irradiation. Oral mucosal damage caused starvation 10-12days after the irradiation. The survival rate was scored and $LD_{50}$ dose was estimated from the survival curve.

Neutron irradiation induced apoptosis in the splenocytes / To determine apoptosis of splenocytes, mice were sacrificed 8 days after irradiation and their spleens were removed. Single-cell suspensions were eliminated of erythrocytes by incubating at room temperature for 3 min in a solution of Tris-buffered ammonium chloride. After twice washing with PBS, cells were counted and examined for induction of apoptosis. Apoptosis was detected with a sandwich immunoassay system using a cell death detection ELISA kit (Roche Diagnostic Inc.). Apoptosis was measured by following the ELISA protocol. The enrichment factor was the calculated absorbance of each sample divided by the absorbance of corresponding negative control.

**RESULTS and DISCUSSION:** Figure 1 shows the survival fraction of oral radiation death of SCID mice following neutron irradiation. Here, we compared the survival rate following gamma-irradiation. The estimated $LD_{50}$ was 10.3 Gy of neutron irradiation, 11.1 Gy of gamma-irradiation. The RBE of oral radiation death of SCID mice was 11.1-8 / 10.3-8 = 1.4. The RBE of oral radiation death of C3H mice was 2.5. The result shows that hyper-sensitivity of SCID mice decreased in acute radiation effect following neutron irradiation.

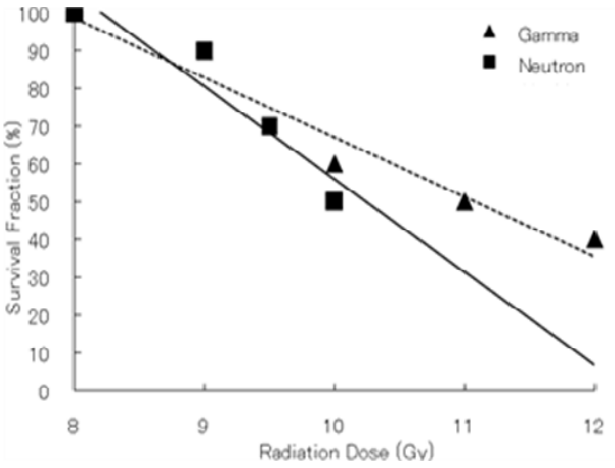


Fig.1. Oral radiation death of SCID mice/▲;Gamma-ray , ■; neutron irradiation.

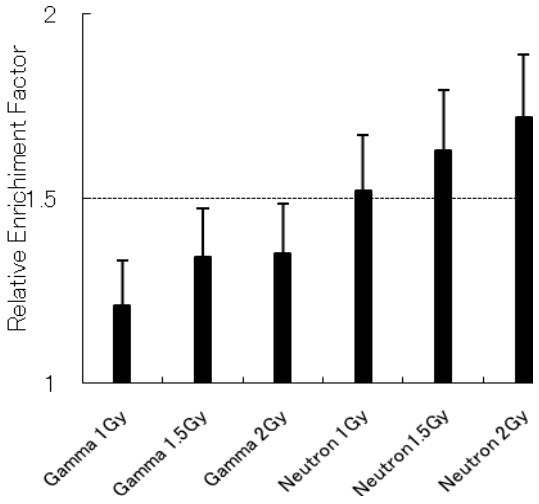


Fig.2 The apoptosis induction of splenocytes observed in SCID mice.

Figure 2 shows the apoptosis induction of splenocytes in SCID mice 8 days after the neutron irradiation. For comparison with gamma-ray, the higher apoptosis was induced following neutron irradiation at the same dose in SCID mice. In case of C3H mice, the apoptosis induction of splenocytes was not observed 8 days after neutron or gamma irradiation. Un-repaired DNA damage still remained 8 days after irradiation in SCID mice, having the defect in end-joining of DNA double strand breaks. These results imply that the hiper-radiosensetive BNCT patients may be able to get the late radiation side effect, especially radiation-induced secondary carcinogenesis and should be considered radio-protection following BNCT.

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採択課題番号 22P10-1　　硼素中性子捕捉反応(BNCR)誘発粒子線の特性利用の新展開　　プロジェクト

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# PR10-2 Analysis of Double Strand Breaks in the Mammalian Cells Induced by BNCR

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**INTRODUCTION:** Boron neutron capture therapy (BNCT) is a useful modality for cancer therapy in some cases with relatively low side effects. But, as similar to the other radiotherapy, normal as well as tumor cells are exposed to a mixed radiation field (thermal, epithermal and fast neutrons, and gamma-rays). However, little is known about the biological effects of such radiation exposures as used for BNCT. It is generally known that high LET radiation may have other biological reaction than ordinal low LET radiation such as X-rays[1)]. Here, the relative biological effectiveness (RBE), and the dose and dose rate effectiveness factor (DDREF) for the mixed irradiation used for BNCT in Kyoto University Research Reactor (KUR), were investigated. In the present study, as the endpoints for biological reaction, DNA double strand breaks were used because of its significance for cancer induction and tissue damages.

**MATERIALS & METHODS:** Commercial cultured cells CHO/K1were used. Their mutant cell line, xrs-5, were given from J.Peggo through R. Okayasu. The cells were irradiated at the KUR irradiation field for BNCT.
The details of radiation field were shown in Table 1. The thermal neutron was approximately one fourth of total physical dose, and half of physical dose was attributable to the gamma ray. The average physical dose rates of thermal (<0.5eV), epithermal (0.5eV-10keV), fast (>10keV) neutrons, and gamma-rays were 10.0, 1.1, 7.4, and 20.5 mGy/min, respectively, when the reactor was operated at 1MW. When operated at 5MW, the dose rates became approximately 5 times higher than those for 1 MW.

Table 1. Physical radiation doses used for the experiments. Values are expressed as mGy/min.

| Neutron Thermal (<0.5eV) | Neutron Epi-thermal (0.5eV -10keV) | Neutron Fast (>10keV) | Gamma | Total |
|---|---|---|---|---|
| 9.0-11 (10.0)* | 1.1-1.2 (1.1) | 6.8-7.6 (7.4) | 19-21 (20.5) | 36-40 (39) |

*Values in parenthesis are averages of 3-6 experiments.

As a reference radiation, Co-60 gamma-ray source was used at the same dose rate as the mixed irradiation. The cells were assayed for conventional colony formation, and DNA double strand breaks (DSBs) were detected by immune-staining using gamma-H2AX and 53BP1 antibodies.

**RESULTS & DISCUSSION:** In this year, we mainly focused to establish an irradiation field for the experiments. As shown in Table 1, relatively high physical doses of thermal neutron could be used for the experiment, but the contamination of gamma ray was significantly large. Thus, we need to use the prolong gamma irradiation as a reference irradiation (control).

At first, cell survival was determined by colony formation in both CHO/K1 and xrs-5 cells, respectively. The experiment was under progress at present, the sensitivity was higher in xrs-5 cells than CHO/K1 cells, indicating the cell survival (cell killing) under these irradiation conditions also attributable to the DNA double strand breaks, because xrs-5 is a cell line that deficient the enzyme to repair DNA double strand breaks. The experiment are being continued to determine relative biological effectiveness (RBE) for this BNCT irradiation condition.

We also tried to detect the DNA double strand breaks with using the immune-chemical staining method. The cells were fixed at scheduled time after the irradiation, and stained with anti-gamma-H2AX and 53BP1 antibody. Although the data are under analysis now, but the number of gamma-H2AX and 53BP1 foci 1hr post-irradiation were similar for the mixed radiation and the reference gamma-ray, and the size of foci seemed to be different. This may indicate that the neutrons may have induced a different type of DNA-DSBs.

**REFERRENCES:**

[1] R. Okayasu, M. Okada, A. Okabe, M. Noguchi, K. Takakura and S. Takahashi: Repair of DNA damage induced by accelerated heavy ions in mammalian cells proficient and deficient in the Non-homologous End-joining pathway, Radiation Research, **165** (2006) 59-67.

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採択課題番号 22P10-2　反応に伴う DNA 損傷、特に二重鎖切断とその修復の解析　プロジェクト
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# PR10-3 Application of Boron Neutron Capture Reaction to Plant Tissue Culture for Mutation Breeding

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**INTRODUCTION:**
The aim of the present study is to establish a novel mutagenesis system using boron neutron capture reaction (BNCR) in higher plants. Artificial induction of mutation by radiation (e.g. x-ray, gamma irradiation, thermal neutron and ultraviolet light) usually generates recessive mutants; therefore, progeny analysis is required for determining phenotypic and genetic effects of such mutagenesis in diploid plant species. To effectively determine the effectiveness of BNCR for plant mutagenesis in a short period of time, haploid tobacco (*Nicotiana tabacum*) plants were used in the present study.

**METHODS:**
Haploid tobacco derived from another culture can easily regenerate plants via callus derived from leaf tissues, and the regenerated plants, either diploid (i.e. doubled-haploid) or haploid, are thought to be homogenate. The leaf segments of the haploid and diploid tobacco plants were immersed in different concentrations (0, 200, 400 or 600 μM) of $^{10}$B-enriched *p*-boronophenylalanine (BPA) overnight, and the tissue samples were irradiated with thermal neutron for 15 min and 30 min in the Kyoto University Research Reactor (KUR). The irradiated tissues were then cultured on a plastic petri-dish containing MS solid medium with kinetin and 2,4-D.

**Results and Discussion**
Survival rate and callus induction rate of the haploid cultured tissues were measured. All of the tissues treated with 200 and 400 μM BPA followed by 15 min of thermal neutron irradiation died. Meanwhile, all of the 600 μM BPA treated- and untreated-tissues with 15 min irradiation survived and generated calluses. Regarding the 30 min irradiation treatment, tissues treated with 200 and 400 μM BPA also exhibited low callus induction rates (0 and 28.5%, respectively), whereas many of the tissues treated with 0 and 600 μM BPA survived and induced calluses at much higher frequencies (100 and 33.3%, respectively). Same tendencies of the survival rate and callus induction rate were also observed in diploid samples; higher callus induction rates were observed in the 0 and 600 μM BPA treated tissues and lower survival rates in the 200 and 400 μM BPA treated tissues. The reason of the restoration of the 600 μM BPA-treated tissues is yet unclear.

The BPA-treated calluses were then transferred to the regeneration medium. Subsequently, many haploid or doubled haploid plants were derived from haploid tissue culture, and diploid plants were obtained from diploid samples. Among them, phenotypic variations, such as chlorophyll deficient (albino), dwarf, abnormal petal number, male sterile, were observed.

採択課題番号 22P10-3　硼素中性子捕捉反応(BNCR)誘発粒子線の植物育種への応用　プロジェクト
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服部能英、古川　真、西岡輝美、（京大・原子炉）小野公二、高橋千太郎

**PR10-4** **Development of Pharmacokinetic Methodology Using Designed Boron Tracedrug for NDT**

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**INTRODUCTION:** The evaluation of ADME-tox and pharmacokinetic properties of drug candidates, being under development, and drugs, available on the market, has recently become an increasingly important factor of drug discovery and development, because of increased needs of targeted drugs with less adverse effects. This needs accelerate medicinal chemists to develop drugs with higher traceability, even in their whole lifetime. Radiolabeled compounds have been traditionally studied for their purposes. There are however some inherent problems such as their half-life of much shorter or longer times and the specific regulation of experimental facilities. For the purpose of overcoming these problems and creating drugs with functions required for systems biology and emerging physiology as well as medicinal chemistry, we invented wholly innovative drugs named “boron tracedrug” as “on demand” traceable and next-generation drug model. We present here, as a boron tracedrugs, design, synthesis, and effects of BODIPY- and curcuminoid-boron tracedrugs for neutron dynamic therapy (NDT) [1]. We previously designed the boron tracedrugs UTX-42, UTX-43, and UTX-44, which possess antioxidant potency [2]. In order to explore their dynamic, beyond chemical, effects when acquired by weak thermal neutrons, we performed thermal neutron irradiation of bovine serum albumin (BSA) treated with the boron tracedrugs.

**EXPERIMENTS:** Boron tracedrugs including Boron tracedrugs, including the BODIPY-containing compounds UTX-42, UTX-44, and UTX-47 and the curcuminoid compounds UTX-50 and UTX-51, were designed for neutron dynamic therapy based on their molecular orbital calculation. Newly designed UTX-47, UTX-50, and UTX-51 were synthesized. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to detect the decomposition by thermal neutron irradiation of BSA treated with these five boron tracedrugs.

**RESULTS:** The combination of 1.0 μM BSA with 100 μM of each of the boron tracedrugs showed a decrease in band intensity after irradiation. In conclusion, all boron tracedrugs tested caused destructive dynamic damage of BSA during thermal neutron irradiation, suggesting boron tracedrugs could be used as dynamic drugs for NDT.

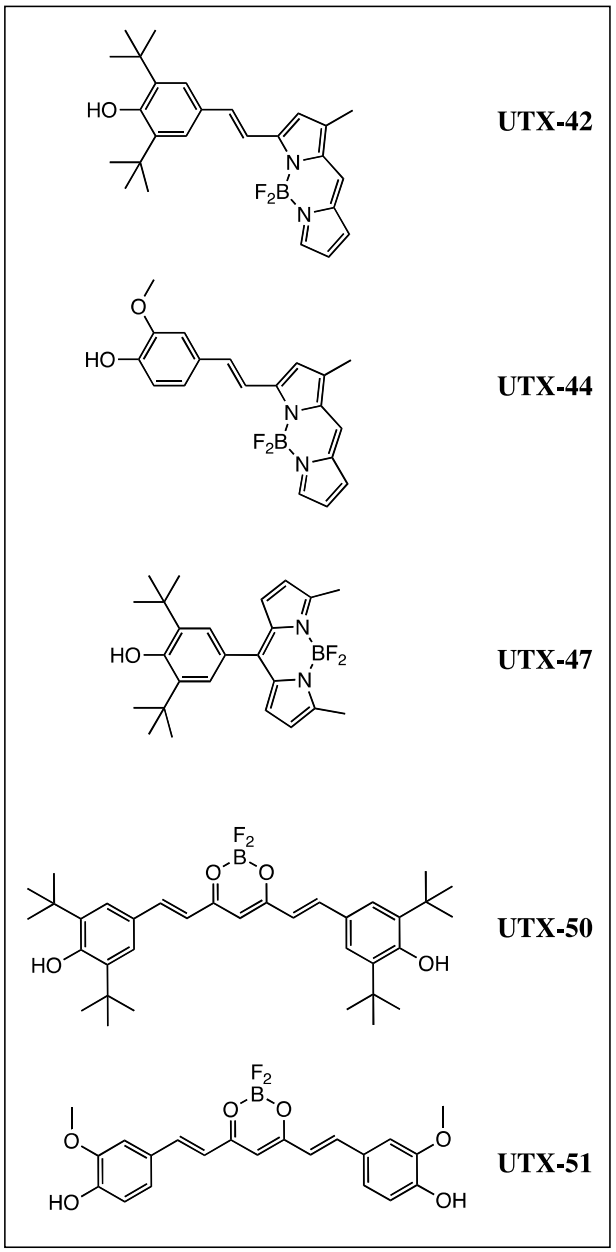


Fig. 1. Boron tracedrugs: BODIPY drugs, UTX-42, UTX-44, UTX-47 and curcuminoid drugs, UTX-50 and UTX-51 for NDT.

**REFERENCES:**

[1] H. Hori *et al.*, Anticancer Res., **30** (2010) 3233-3242.
[2] E. Nakata *et al.*, Adv. Exp. Med. Biol. in press (2011).

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採択課題番号 22P10-4　　Boron をトレーサとする薬物の動態解析法の開発　　プロジェクト
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