VIII-Ⅱ-1. Project Research

Project 6

PR6 Project Research on the New Application 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 T.Morikawa K Mishiba *et al.*)

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

Main Results and Contents

PRS-1 performed the mutation analysis induced by a bystander effect following neutron irradiation. The radiation-induced bystander effects refer to the induction of biological effects in cells that are not directly traversed by a charged particle, but are instead located in close proximity to irradiated cells. They investigated the mutagenic effects of neutron on cells that did not contain boron, but were located near cells that contained ^{10}B. Due to the bystander effect, the frequency of mutations increased in the cells located nearby the ^{10}B-containing cells compared with control cells.

PRS-2 determined the cell survival and focus formation of repair protein of DNA double strand breaks in CHO/K1 and xrs-5 cells after the irradiation of neutron mixed beam of KUR heavy water facility at an operating power of 5 MW. The mixed beam irradiation seemed to be more effective in CHO-K1 cells and slightly effective in xrs5 cells than control Co-60 gamma-ray irradiation. Although the doses were available below 2.6 Gy, extrapolation of the best fit line indicated that the relative biological effectiveness for 10% survival is 3.3 and 1.2 for CHO-K1 and xrs5 cells, respectively.

The aim of PRS-3 is to establish a novel mutagenesis system using boron neutron capture reaction (BNCR) in higher plants. The progeny test is required for getting the homozygous mutants in diploid plant following irradiation. To determine the effectiveness of BNCR for plant mutagenesis at irradiation of dry seed stage, two-row-barley (*Hordeum vulgare*; 2n=2*X*=14) plants were used in the present study because of its early maturity and sensitivity to radiation. Maximum aberration rate (23.3%) was observed at 800μM BPA and after that the rate at 1000μM went down to 21.5%. Linear regression analysis was carried out between chromosome aberration rate (Y %) and BPA concentration (X μM). As the result of the analysis, there was a linear regression at 5 % significant level. The formula was Y=0.12X+11.24. Death rates after 60 days were recorded at fifteen treated groups. The death rate gradually increased and was top at 800μM BPA of 1 and 1.5 hours irradiations. This is the same situation with the chromosome aberration rate in the primary root-tip cells. The growth rate estimated from seedling height for 4 weeks, however, was negatively correlated with the BPA concentrations of 1.5 hours irradiation. The optimum BNCR condition (at 800μM BPA of 1 and 1.5 hours irradiations) for expecting the maximum mutation rate was found out.

PRS-4 previously developed boron tracedrugs UTX-42, UTX-43, and UTX-44, which possess antioxidant potency and UTX-42, UTX-44, UTX-47, UTX-50, and UTX-51 as boron tracedrugs for NDT targeted a model protein BSA. Among boron tracedrugs tested previously, they choose here the boron tracedrug, UTX-51, for our present PK and NDT study to explore their dynamic, beyond chemical, effects when acquired by weak thermal neutron irradiation of low-density lipoprotein (LDL), which is associated with significantly increased stroke mortality, treated with the boron tracedrug UTX-51. As a result, the combination of 4.6 μg LDL with three different concentration of 1, 10, 100 mM of the boron tracedrug UTX-51 showed a decrease in band intensity after neutron irradiation (1.830 Gy for 1 μM; 1.835 Gy for 10 μM; 1.880 Gy for 100μM). In conclusion, all doses of the boron tracedrug UTX-51 caused destructive dynamic damage against LDL during thermal neutron irradiation, suggesting boron tracedrugs could be used as dynamic drugs for NDT targeted LDL as well as a tracedrug for PK.

PR6-1 Mutation Analysis Induced by a Bystander Effect following Neutron Irradiation

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INTRODUCTION: We have described the mutagenicity of thermal neutrons in CHO cells and presented evidence for the increased mutagenicity of thermal neutrons in the presence of boron (1, 2). In those previous experiments, boron was located both inside and outside of the cells. Clinically, the problem with BNCT is the potential mutagenic effects of the therapy on the normal tissue cells that do not take up the boron compounds, but are located near the boron-containing tumor cells. Evidence for the existence radiation-induced bystander effects exposed to alpha particles was first reported by Nagasawa and Little. These radiation-induced bystander effects refer to the induction of biological effects in cells that are not directly traversed by a charged particle, but are instead located in close proximity to irradiated cells. In this study, we investigated the mutagenic effects of neutron on cells that did not contain boron , but were located near cells that contained ^{10}B. And more, we have investigated the mutagenic effects of no-boron cells under the neutron-irradiated medium those were located cells that contained ^{10}B.

MATERIALS & METHODS: The lethality and mutagenicity measured by the frequency of mutations induced in the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus were examined in Chinese hamster ovary (CHO) cells irradiated with neutrons (Kyoto University Research Reactor).

Experiment 1/ Neutron were irradiated to the 1:1 mixtures of cells with and without ^{10}B. The cells that did not contain ^{10}B made up 99.4% of the resulting cell population. The mutant frequency was estimated as a function dose of the neutron.

Experiment 2/ The ^{10}B containing cells and no-boron cells were separately irradiated by KUR neutron. The neutron-irradiated medium located cells that contained ^{10}B was taken and poured to the cells that did not contain ^{10}B. The mutant frequency was estimated of neutron irradiated cells without ^{10}B.

RESULTS and DISCUSSION: Figure 1 shows the mutation frequency in the HPRT locus in CHO cells after neutron irradiation of the experiment 1 and 2.

Due to the bystander effect of the experiment 1, the frequency of mutations increased in the cells located nearby the ^{10}B-containing cells compared with control cells. However, the experiment 2 did not induce the frequency of mutations increased in the cells with the medium located at the ^{10}B-containing cells compared with control cells. These results suggested that in BNCT, the mutations caused by the bystander effect and those caused by the original neutron irradiation are induced by different mechanisms.

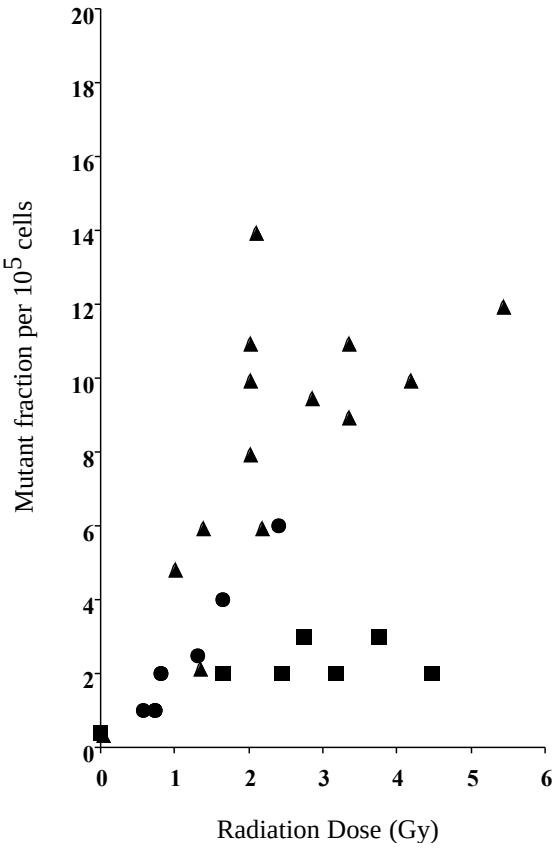

Fig.1 Induction of mutations in the HPRT locus in CHO cells after the neutron irradiation.
Plots of the mutant frequencies obtained with irradiated samples of no-boron cells (●), Exp1-bystander cells (▲), and Exp2-bystander cells (■) are shown.

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1) Kinashi Y *et.al.,* Mutat Res., **337**(1997) 211-215.
2) Kinashi Y *et al.,* Radiat Res., **154**(2000)313-318.

採択課題番号 23P6-1 BNC 反応により誘発される突然変異の解析 プロジェクト
（京大・原子炉）木梨友子、小野公二、高橋千太郎

PR6-2 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 Dr. 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.

Thermal (<0.5eV)	Epi-thermal (0.5eV -10keV)	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 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.

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[1] Okayasu, R., Okada, M., Okabe, A., Noguchi, M., Takakura, K. and Takahashi, S.: 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, 59-67, 2006.

採択課題番号 23P6-2 BNC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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（放射線医学総合研究所）久保田善久、岡安隆一

PR6-3 Application of Boron Neutron Capture Reaction (BNCR) to Barley Seeds 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. Radiation (e.g. x-ray, γ -ray and thermal neutron) usually knocks out one pair of alleles at a target locus and generates loss-of-function mutations at heterozygote[1]. Therefore, the progeny test is required for getting the homozygous mutants in diploid plant. To determine the effectiveness of BNCR for plant mutagenesis at irradiation of dry seed stage, two-row-barley (*Hordeum vulgare*; 2n=2*X*=14) plants were used in the present study because of its early maturity and sensitivity to radiation.

EXPERIMENTS: The dry seeds of barley plants were immersed in different concentrations (0, 400, 600, 800 and 1000 μM) of ^{10}B-enriched *p*-boronophenylalanine (BPA) for 24hours, and the seeds were irradiated with thermal neutron for 0, 1 and 1.5 hours in the Kyoto University Research Reactor (KUR). The irradiated seeds were kept at 4 ℃ for overnight, and then were germinated on a plastic petri-dish containing moisture filter paper at 25 ℃.

RESULTS: Germination rate of the irradiated seeds were measured. The seeds were germinated normally at all fifteen treated groups (five BPA concentrations × three irradiation times) at the range of from 60% to 100%. There was no relationship between germination rate and dose effect[2]. Chromosome aberrations at primary root-tip cells of barley can easily examine and determine the effect of BNCR to plant-cell divisions. The anaphase cells with chromosome aberrations at 1.5 hours irradiated groups were counted. The frequency of chromosome aberrations was positively correlated with the concentration of BPA. Maximum aberration rate (23.3%) was observed at 800μM BPA and after that the rate at 1000μM went down to 21.5%. Linear regression analysis was carried out between chromosome aberration rate (Y %) and BPA concentration (X μM). As the result of the analysis, there was a linear regression at 5 % significant level. The formula was Y=0.12X+11.24. Death rates after 60 days were recorded at fifteen treated groups. The death rate gradually increased and was top at 800μM BPA of 1 and 1.5 hours irradiations. This is the same situation with the chromosome aberration rate in the primary root-tip cells. The growth rate estimated from seedling height for 4 weeks, however, was negatively correlated with the BPA concentrations of 1.5 hours irradiation.

We have found out the optimum BNCR condition (at 800μM BPA of 1 and 1.5 hours irradiations) for expecting the maximum mutation rate. This result indicates that the dry seeds of two-row-barley plant seem to absorb equal amounts of BPA into the cells depending on the BPA concentrations treated.

REFERENCES:

[1] Y. Ukai and A.Yamashita, Breeding Science 60(2010)121.

[2] Y.Ukai, I.Yamaguchi and H.Takaki, Breeding Science 59(2009)129.

採択課題番号 23P6-3　　硼素中性子捕獲反応(BNCR)の植物育種への応用　　プロジェクト

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PR6-4

Design of Boron Tracedrug for PK and NDT

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INTRODUCTION: The evaluation of pharmacokinetic (PK) properties and ADME-tox 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 life-time. Radiolabeled compounds have been still available with some inherent problems such as their half-life and the specific regulation of experimental facilities.

For the purpose of overcoming these problems and creating traceable drugs without RI forever, we are developing boron tracedrugs with their "on demand" traceability and their physical force for neutron dynamic therapy (NDT).

We previously developed boron tracedrugs UTX-42, UTX-43, and UTX-44, which possess antioxidant potency [1] and UTX-42, UTX-44, UTX-47, UTX-50, and UTX-51 as boron tracedrugs for NDT targeted a model protein BSA [2].

Among boron tracedrugs tested previously, we choose the boron tracedrug, UTX-51, for our present NDT study to explore their dynamic, beyond chemical, effects when acquired by weak thermal neutron irradiation of low-density lipprotein (LDL), which is associated with significantly increased stroke mortality, treated with the boron tracedrug UTX-51.

EXPERIMENTS: The curcuminoid boron tracedrugs UTX-51 (it contains boron isotopes, B-10 and B-11 with their natural abundance ratio) was used for neutron dynamic therapy. Thermal neutron irradiation was performed using a reactor neutron beam with a cadmium (Cd) ratio of 9.4. The neutron fluence was measured from the radioactivation of gold foils at the front of the sample tubes, and the average neutron fluence determined from the values measured was used. Contaminating γ-ray doses, including secondary γ-rays, were measured with thermoluminescence dosimeter powder at the front of the sample tubes. The absorbed dose was calculated using the flux-to-dose conversion factor. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to detect the decomposition by thermal neutron irradiation of human LDL and PARP treated with these five boron tracedrugs.

RESULTS: The combination of 4.6 μg LDL with three different concentration of 1, 10, 100 μM of the boron tracedrug UTX-51 showed a decrease in band intensity after neutron irradiation(1.830 Gy for 1 μM; 1.835 Gy for 10 μM; 1.880 Gy for 100μM).

In conclusion, all doses of the boron tracedrug UTX-51 caused destructive dynamic damage against LDL during thermal neutron irradiation, suggesting boron tracedrugs could be used as dynamic drugs for NDT targeted LDL.

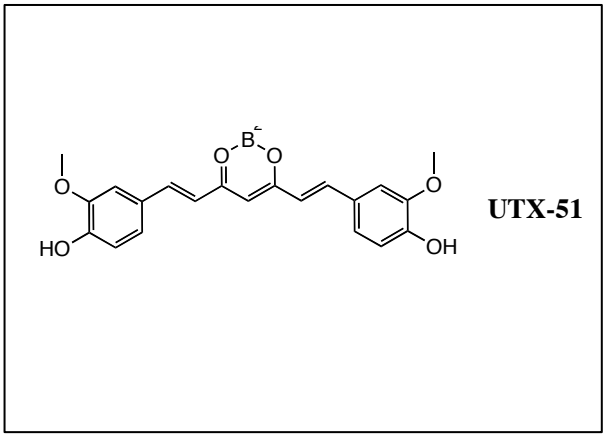

Fig. 1. The curcuminoid boron tracedrug, UTX-51, for NDT.

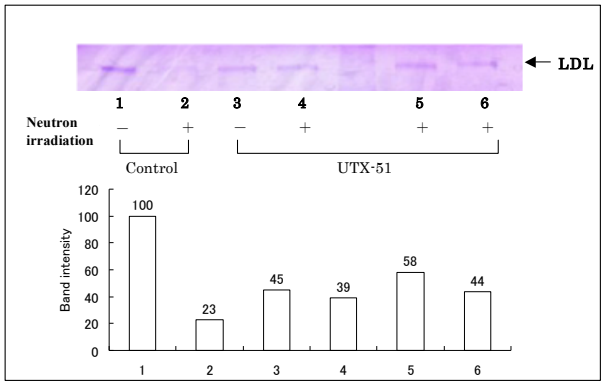

Fig. 2. NDT of LDL using a boron tracedrug UTX-51(lane 4: 1 μM, lane 5: 10 μM, lane 6: 100 μM) .

REFERENCES:

[1] E. Nakata *et al.*, Adv. Exp. Med. Biol., **737** (2012) 301-306.
[2]H. Hori *et al.*, Anticancer Res., **30** (2010) 3233-3242.

採択課題番号 23P6-4　Boron をトレーサとする薬物の動態解析法の開発 ､ ､ ､ ､ ､ ↕ ⌠ Ɒρ x ₦ ⅍
（徳島大学院・ソシオテクノサイエンス研究部）堀　均、那住善治郎、宇都　義浩、（京大・原子炉）高橋　千太郎、小野　公二