VIII-Ⅱ-1. Project Research

Project 6

PR6 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
(T.Morikawa *et al.*)

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

MAIN RESULTS AND CONTENTS

PRS-1 investigated dose rate effect of the neutron radiation beam used for BNCT in Kyoto University Research Reactor (KUR) for the mutation induction following neutron irradiation. Neutron irradiation for cells was operated at 0.2Gy/min or at 0.04Gy/min with and without BPA. The frequency of mutations after neutron irradiation at 0.2Gy/min with 10ppm BPA was 1.4-1.9 times and more increased than that at 0.2 Gy/min than 0.04 Gy/min with 10ppm BPA in the function dose over the 1.8Gy. These results suggested that dose rate effect is existence concerning mutation induction following the neutron irradiation in BNCT.

PRS-2 investigated the relationship between the neutron energy profiles and induction of DNA double strand breaks (DNA-dsb). Two types of neutron beams were used. One is a neutron beam provided by the Heavy Water Facility of Kyoto University Research Reactor (KUR-HWF). This is a mixed neutron beam of thermal, epithermal and fast neutrons, and gamma-rays, and typical one used for the BNCT. Another is a middle energy neutron beam with average energy at around 2 MeV, designed for biological researches, and named as the Neutron Accelerator System for Biological Effect Experiments (NASBEE). This is a typical middle-speed neutron beam generated by accelerator.

The focus assay used for detecting the DNA double strand breaks showed that the number of 53BP1 foci was similar between the NASBEE and KUR-HWF neutron beams at the dose range used here at 1hr post-irradiation. The disappearance of the focus was a little faster in the cells irradiated with KUR-HWF than NASBEE, although there were no statistically significant differences. As expected, the disappearance of focus delayed in the xrs5 cells, indicating the impaired DNA damage repair in these cells. These results agree well with the high sensitivity of xrs5 cells for the mixed neutron beam.

PRS-3 compared the different damage effects on plants between BNCR and ^{60}Coγ-ray. To determine the effectiveness of BNCR for plant mutagenesis at irradiation of dry seeds, two-row-barley was easily used because of its compact size of the seeds and higher mutation rate. The dry seeds of two-row-barley plants (*Hordeumvulgare* cv.Hayadori) were immersed in different concentrations (0, 200, 400, 600, 800and1000 μM) of ^{10}B-enriched *p*-boronophenylalanine (BPA) for 24 hours, and all the materials were irradiated with thermal neutron for 120 minutes in the Kyoto University Research Reactor (KUR).^{60}Coγ-ray irradiations were also carried out on the 'Hayadori' dry seeds in the different doses of 0.1, 0.2, 0.3, 0.4 and 0.5 KGy/h by using the Kyoto University's ^{60}Coγ-ray irradiation facility. We have found out that the semi-lethality dose (LD_{50}) in the lethal rate was 469 μM BPA, and RD_{50}in the reduced rate of 4-week-seedlingswas 74.2μM BPA. The 469μM value was transformed to the total physical doses as17.98 Gy, and the 74.2μM as 11.2Gy by using the transformation equation.

PRS-4 developed boron tracedrugs with their "on demand" traceability and their physical force for neutron dynamic therapy (NDT). The boron tracedrug, UTX-51 was studied that their dynamic, beyond chemical, effects when acquired by weak thermal neutron irradiation of glycated BSA as a model of advanced glycation end-products (AGEs), which is linked to diabetes and aged diseases. They found that all doses of the boron tracedrug UTX-51 caused destructive dynamic damage against Gly-BSA during thermal neutron irradiation, suggesting boron tracedrugs could be used as dynamic drugs for NDT targeted glycated proteins, such as Gly-BSA, for serum protein-quality-control treatmet of AGEs-related diseases.

採択課題番号 25P6　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

PR6-1 Dose Rate Effect for Mutation Induction was Observed Following BNCR

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INTRODUCTION: We have described the mutation frequency of CHO cells after neutron irradiation and presented evidence for the increased mutagenicity of thermal neutrons. Dose rate effect for mutation induction after neutron irradiation has not been studied until now. Clinically, the mutagenic effects of BNCT on the normal tissue cells that do not take up the boron compounds may cause the genetic instability and second cancer decade years after BNCT. In this study, we investigated dose rate effect of the neutron radiation beam used for BNCT in Kyoto University Research Reactor (KUR) for the mutation induction following neutron irradiation.

MATERIALS & METHODS: The mutagenicity measured by the frequency of mutations induced by neutron irradiation with or without boron compound. The hypoxanthine-guanin-phosphoribosyl-transferase (HPRT) locus was examined in Chinese hamster ovary (CHO) cells irradiated with neutrons of KUR.

A stock solution of 10 B-para-boronophenylanine (BPA) was used for this experiment. Cell suspensions were incubated with BPA at 10 ppm concentration 1 hour before neutron irradiation. High dose rate neutron irradiation was 0.2 Gy/min with 5MW of KUR, and low dose rate neutron irradiation was 0.04 Gy/min with 1MW of KUR. Neutron fluencies were measured by radioactivation of gold foil and gamma-ray dose by TLD. After neutron exposure, to determine mutation frequencies, each treated culture was incubated with non-selective medium for 7-9 days to allow phenotype expression. Then, $2x10^5$ cells were added to each dish containing 6-thioguanine and incubated for 10-14 days, after which time the mutant colonies were counted. The mutation frequency is expressed as the number of resistant colonies divided by the total number of viable cells as determined by cloning efficiency at the time of selection.

RESULTS and DISCUSSION: Figure 1 (the upper part) shows the mutation frequency in the HPRT locus in CHO cells after neutron irradiation at 0.2Gy/min or at 0.04Gy/min with or without BPA. The frequency of mutations after neutron irradiation with 10ppm BPA at 0.2Gy/min was 1.5-1.9 times and more increased than at 0.04Gy/min in the function dose over the 1.8Gy.

These results suggested that dose rate effect is existence concerning mutation induction following neutron irradiation in BNCT. On the other hand, the apparent dose rate effect was not accepted neutron irradiation without BPA. The mutation induction at 0.2Gy/min was slightly higher than 0.04Gy/min after neutron irradiation as same as the gamma-ray study (Fig.1, the lower part).

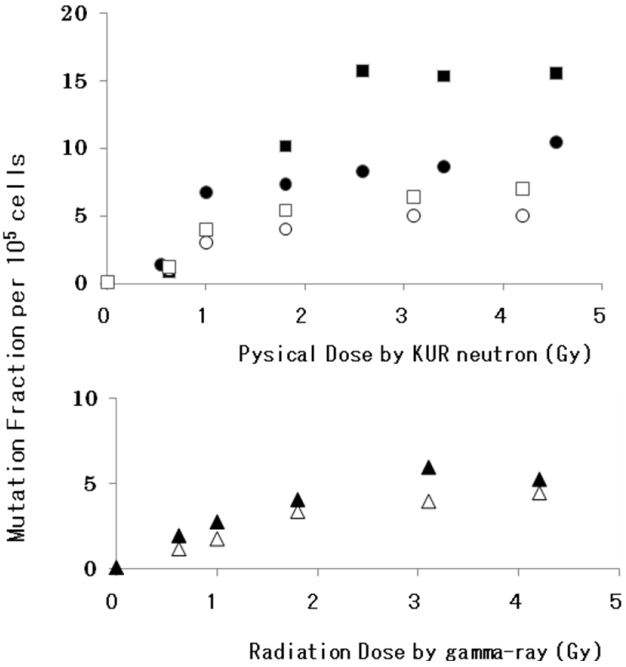

Fig.1 Induction of mutations in the HPRT locus in CHO cells.
Upper figure; Plots of the mean mutant frequencies obtained with irradiated samples following KUR neutron irradiation with 10ppm BPA at 0.2 Gy/min (■) and 0.04Gy/min (●), without BPA at 0.2 Gy/min (□) and 0.04Gy/min (○) are shown.
Lower figure; Plots of the mean mutant frequencies obtained with irradiated samples following gamma-ray irradiation at 0.2 Gy/min (▲) and 0.04Gy/min (△).

In this study, we found dose rate effect of the neutron radiation beam used for BNCT for the mutation induction of normal cells following neutron irradiation.

This dose rate effect of BNCT was mainly due to the nuclear capture reaction that of alpha particles or ^{7}Li nuclei produced by ^{10}B(n,α) ^{7}Li reaction, not due to the reaction with normal tissue hydrogen and nitrogen.

採択課題番号 25P6-1　　BNC 反応により誘発される突然変異の解析　　プロジェクト
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PR6-2 Analysis of DNA Double Strand Breaks in the Mammalian Cells Induced by BNCR

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INTRODUCTION:

Recently, the boron neutron capture therapy (BNCT) is used as unique and effective treatment for cancer, and now becoming a clinical application stage. However, little is known about the biological effects of particle radiation induced by BNC reaction nor thermal- epithermal neutron beams for BNCT. In the projective study, we have investigated the most important biological effects, i.e., DNA damages, after the irradiation with heavy ion particles from BNC reaction and neutron beam. In the FY 2013, the relationship between the neutron energy profiles and induction of DNA double strand breaks (DNA-dsb) was investigated.

MATERIALS & METHODS:

The cells used were Chinese hamster ovary cell line (CHO/K1) from Riken Cell Bank. Their mutant cell line, xrs-5, were given from P. Jeggo through Dr. Okayasu, NIRS. The cells were irradiated at the KUR irradiation field for BNCT.

Two types of neutron beams were used. One is a neutron beam provided by the Heavy Water Facility of Kyoto University Research Reactor (KUR-HWF). This is a mixed neutron beam of thermal, epithermal and fast neutrons, and gamma-rays, and typical one used for the BNCT. Another is a middle energy neutron beam with average energy at around 2 MeV, designed for biological researches, and named as the Neutron Accelerator System for Biological Effect Experiments (NASBEE). This is a typical middle-speed neutron beam generated by accelerator.

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:

These two neutron beam have continuous energy distribution of neutron, but the energy profiles are much different between them. The beam of KUR-HWF has mean energy of neutron at around 2.3 keV, and that of NASBEE was 2 MeV. The fractions of gamma-rays in the total physical doses are also much different between them. Approximately 40 % of total dose come from the gamma-rays in KUR-HWF, whereas only 13 % in NASBEE.

With respect to the cell survival determined by colony formation assay, the mixed beam of NASBEE was slightly more effective than those of KUR-HWF in both CHO/K1 and xrs-5 cells. As expected, xrs5 cells defective in DNA-dsb repair were more sensitive to both mixed beam irradiation than CHO-K1 cells. This indicates that the Ku-80 may involved in the repair of DNA-dsb induced by these two types of beam as similar to those in gamma-ray irradiation.

The focus assay used for detecting the DNA double strand breaks showed that the number of 53BP1 foci was similar between the NASBEE and KUR-HWF neutron beams at the dose range used here at 1hr post-irradiation. The disappearance of the focus was a little faster in the cells irradiated with KUR-HWF than NASBEE, although there were no statistically significant differences. As expected, the disappearance of focus delayed in the xrs5 cells, indicating the impaired DNA damage repair in these cells. These results agree well with the high sensitivity of xrs5 cells for the mixed neutron beam. At present, we did not find any difference in the characteristics and kinetics of focus between these two types of the mixed neutron beams.

PUBLICATION:

Y. Kinashi, K. Okumura, Y. Kubota, E. Kitajima, R. Okayasu, K. Ono and S. Takahashi; Dose-Rate Effect Was Observed in T98G Glioma Cells Following BNCT. Appl. Radiat. Iso. 88: 81-85, 2014.

Acknowledgements :

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課題採択番号 25 P6-2 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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（放医研）久保田善久、岡安隆一

PR6-3 Estimation of Relative Biological Effectiveness (RBE) of Boron Neutron Capture Reaction (BNCR) to ^{60}Coγ-ray in Barley Plants for Mutation Breeding

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INTRODUCTION: The present study was carried out 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. This time, we try to compare the different damage effects on plants between BNCR and ^{60}Coγ-ray. To determine the effectiveness of BNCR for plant mutagenesis at irradiation of dry seeds, two-row-barley was easily used because of its compact size of the seeds and higher mutation rate.

EXPERIMENTS: The dry seeds of two-row-barley plants (Hordeum vulgare cv. Hayadori) were immersed in different concentrations (0, 200, 400, 600, 800 and 1000 μM) of ^{10}B-enriched p-boronophenylalanine (BPA) for 24 hours, and all the materials were irradiated with thermal neutron for 120 minutes in the Kyoto University Research Reactor (KUR). ^{60}Coγ-ray irradiations were also carried out on the 'Hayadori' dry seeds in the different doses of 0.1, 0.2, 0.3, 0.4 and 0.5 KGy/h by using the Kyoto University's ^{60}Coγ-ray irradiation facility. The both irradiated seeds were kept at 4 ℃ for two days, and then were germinated on a plastic petri-dish containing moisture filter paper at 25 ℃. After germination the plantlets were transplanted to the pots filled with mixtures of soil and compost. To estimate relative biological effectiveness (RBE) of BPA-BNCR to ^{60}Coγ-ray in barley seeds, frequency of chromosome aberration, lethal rate and reduced rate of the both seedlings were examined in the M1. Total irradiation dosage of BPA-BNCR (Gy) is estimated from the equation (1) of neutron physical dosage (1.4Gy) + γphysical dosage (1.7Gy) + αphysical dosage (0.42×^{10}B concentration(Gy)). The ^{10}B concentration of each BPA treated seed is analyzed by ICP-MS.

RESULTS: The irradiated barley seeds were germinated normally at all six BPA concentration groups at the range of from 80% to 90%. There was a positive relationship between the frequency (%) of chromosome aberration in root-tip cells and the concentration of BPA (μM). The lethal rate of the seedling(Y) was also positively related with the concentration of BPA (X μM). The regression equation was Y=20.614+0.056X. There was also a positive relationship between the reduced rate of the seedling and the concentration of BPA (X μM) in the M1. Barley seeds seem to be highly sensitive to the BNCR treatments.

We have found out that the semi-lethality dose (LD_{50}) in the lethal rate was 469 μM BPA, and RD_{50} in the reduced rate of 4-week-seedlings was 74.2μM BPA. The 469μM value was transformed to the total physical doses as17.98 Gy, and the 74.2 μM as 11.2 Gy by using the transformation equation (1).

In the next steps, we estimated the lethal rate and the reduced rate of 4-week-seedlings irradiated by ^{60}Coγ-ray in barley seeds. The LD_{50} and RD_{50} of ^{60}Coγ-ray irradiations were 193.9 Gy and 237.4 Gy, respectively. Finally, the RBEs of BPA-BNCR to ^{60}Coγ-ray were thought to be 193.9/17.968=10.80 times in LD_{50} and 237.4/11.2=21.20 times in RD_{50}, respectively. Accordingly, it is possible to say that application of the BAP-BNCR on barley seeds is much more effective 10.8 or 21.20 times than ^{60}Coγ-ray irradiation in the inhibition of growth.

採択課題番号 25P6-3　　硼素中性子捕捉反応(BNCR)によるオオムギとタバコの　プロジェクト
突然変異誘発

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（環境農林水産総合研）谷本秀夫、高井雄一朗（京大・原子炉）小野公二、高橋千太郎、木梨友子

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 lifetime. 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] and UTX-51 for LDL [3].

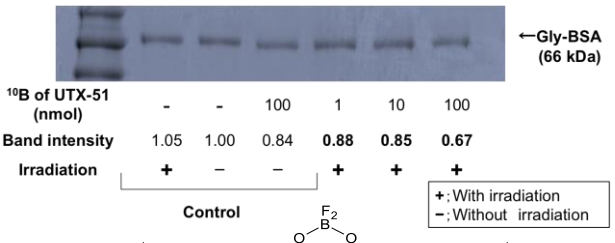
Among boron tracedrugs tested previously, we choose the boron tracedrug, UTX-51, for our present NDT study to explore their dynamic, beyond chemical, effects when acquired by weak thermal neutron irradiation of glycated BSA as a model of advanced glycation end-products (AGEs), which is linked to diabetes and aged diseases, 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. A Gly-BSA was prepared in our laboratory. Thermal neutron irradiation was performed using a reactor neutron beam. 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 Gly-BSA treated with the boron tracedrug UTX-51.

RESULTS: The combination of Gly-BSA BSA (66 μg/100 μl SPB buffer) including UTX-51 with its stoichiometrically ratios (5, 50, 500 nmol) irradiated with thermal neutrons in Kyoto university research reactor institute (KUR)(Irradiation condition; Time: 45 min, Absorbance dose:0.31 Gy at present of B-10 100 nmol) showed a decrease in band intensity of Gly-BSA after neutron irradiation.

In conclusion, all doses of the boron tracedrug UTX-51 caused destructive dynamic damage against Gly-BSA during neutron irradiation, thermal irradiation, suggesting boron tracedrugs could be used as dynamic drugs for NDT targeted glycated proteins, such as Gly-BSA, for serum protein-quality-control treatmet of AGEs-related diseases.

Figure 3.



Scheme 1.

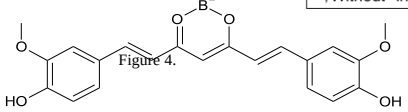


Figure 4.

Fig. 1. A boron tracedrug, UTX-51.

Fig. 2. SDS-PAGE analysis of Gly-BSA samples treated by NDT with UTX-51 under neutron irradiation.

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
[3] H. Hori *et al.*, Adv. Exp. Med. Biol., **789** (2013) 385-389.

採択課題番号 25P6-4　　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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