PR3 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 inspected whether ascorbic acid was effective in protection of the mutation induction of neutron radiation beam used for BNCT in Kyoto University Research Reactor (KUR). The mutagenicity measured by the frequency of mutations induced by neutron irradiation with or without boron compound. The HPRT locus was examined in Chinese hamster ovary (CHO) cells irradiated with neutrons of KUR. High dose rate neutron irradiation was 0.2Gy/min with 5MW of KUR, and low dose rate neutron irradiation was 0.04Gy/min with 1MW of KUR. They investigated that dose rate effect is exist neutron irradiation in BNCT. Ascorbic acid treatment reduced mutation induction following neutron radiation. In neutron irradiation at high dose rate with BPA, the mutation induction was controlled most effectively. This result suggests that the ascorbate may protect the mutagenic effects of BNCT on the normal tissue cells that take up the low dose of boron compounds. They confirmed that the ascorbic acid was effective in protection of the mutation induction of neutron radiation beam used for BNCT.

PRS-2 investigated the most important biological effects, i.e., DNA damages, after the irradiation with heavy ion particles from BNCR and neutron beam. They advanced research related to the status of p53 and biological effects (cell killing and induction of DNA double strand breaks (DNA-dsb) using human glioblastoma cells, A172 and T98G. A172 are wild type of p53, and T98G cells are mutant type.
The results showed that difference of radiation sensitivity between A172 and T98G was decreased by BPA addition irradiation of neutron beam. These results indicate that the difference between the radiation sensitivity was observed between the T98G cells and A172 cells boron neutron capture reaction. Interestingly, it was reduced with BPA where the irradiation mainly depends on the particle radiation emitted by BNCR (Boron Neutron Capture Reaction). This result suggests that BNCT is an effective treatment with glioblastoma that can reduce the difference of radio sensitivity by p53 functional status.

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 *Hordeum-vulgare* cv.Hayadori were immersed in different concentrations of ^{10}B-enriched *p*-boronophenylalanine (BPA) for 24hours, 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 using the Kyoto University's ^{60}Coγ-ray irradiation facility. They found out that the semi-lethality dose (LD_{50}) 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 26P3　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

PR3-1 The effect of ascorbic acid on the HPRT mutation induction after the neutron irradiation

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INTRODUCTION: We have previously reported the increased mutagenicity of thermal neutrons and the dose rate effect of the neutron radiation beam [1]. 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 inspected whether ascorbic acid was effective in protection of the mutation induction of neutron radiation beam used for BNCT in Kyoto University Research Reactor (KUR).

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 10B-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 0.2Gy/min with 5MW of KUR, and low dose rate neutron irradiation was 0.04Gy/min with 1MW of KUR. Neutron fluencies were measured by radioactivation of gold foil and gamma-ray dose by TLD. After neutron exposure, L-ascorbic acid was added to cells at a final concentration of 5mmol/L and removed after 150 min of neutron irradiation. 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 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

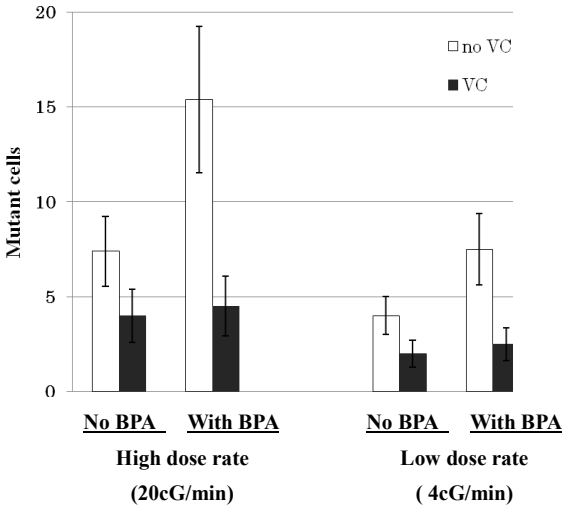

Fig.1 Induction of mutations in the HPRT locus in CHO cells with or without BPA under the ascorbic acid treatment. VC means Vitamin C which is an ascorbate popular name.

0.2Gy/m in 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 exist neutron irradiation in BNCT. Ascorbic acid treatment reduced mutation induction following neutron radiation. This protective effect of mutation induction was more effective with BPA than without BPA. This result shows that an ascorbate scavenged long-lived radicals 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. In neutron irradiation at high dose rate (0.2Gy/min) with BPA, the mutation induction was controlled most effectively. This result suggests that the ascorbate may protect the mutagenic effects of BNCT on the normal tissue cells that take up the low dose of boron compounds, for example the peri-tumoral stroma cells after BNCT.

In this study, we found that the ascorbic acid was effective in protection of the mutation induction of neutron radiation beam used for BNCT dose rate effect of the neutron radiation beam used for BNCT.

REFERENCES:

[1] K. Kinashi *et al.*, Appl. Radiat. Isot. , **88** (2014) 153-156.

採択課題番号 26P3-1　　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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PR3-2 Analysis of DNA Double Strand Breaks in the Mammalian Cells Induced by BNCR

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a 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 (BNCR) nor thermal-epithermal neutron beams for BNCT. In the present study, we have investigated the most important biological effects, i.e., DNA damages, after the irradiation with heavy ion particles from BNCR and neutron beam. In the FY 2014, the relationship between the status of p53 and biological effects (cell killing and induction of DNA double strand breaks (DNA-dsb) was investigated.

EXPERIMENTS: Two types of human glioblastoma cells, A172 and T98G were cultured. Both cells were purchased from Riken BRC Cell Bank. A172 are wild type of p53, and T98G cells are mutant type. The cells were cultured in MEMα medium (Invitrogen) supplemented with 10% heat-inactivated FBS (Biowest) and maintained at 37℃ in a humidified atmosphere with 5.0% CO2. The cells were irradiated at the KUR irradiation field for BNCT, with/without BPA. 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 53BP1 antibodies.

RESULTS & DISCUSSION: The data on the cell survival are shown in Fig. 1 for the cell lines of A172 and T98G. In the case of not BPA added cells, A172 got the steep slope graph of survival fraction. This shows T98G cells have lower sensitivity to neutron mixed beam irradiation than A172. On the other hand, in the case of BPA added cells, survival fraction curves of both cells were similar. This shows difference of radiation sensitivity which confirmed the case of not added BPA cells declined by addition of BPA. When compared at 10% survival, in the case of not added BPA cells, A172 cells were required 1.663Gy radiation dose to cause 90% cell death, but T98G required 5.245Gy. This shows T98G were required the dose of 3 times more than the A172 to cause 90% cell death in the case of not added BPA, neutron mixed beam irradiation alone cases. On the other hand, in the case of added BPA cells, D10 of A172 indicated 0.821 Gy, and T98G indicated 1.076 Gy. This data indicated that difference of radiation sensitivity between A172 and T98G was decreased by BPA addition irradiation of neutron mixed beam.

These results indicate that the difference between the radiation sensitivity was observed between the T98G cells and A172 cells boron neutron capture reaction. Interestingly, it was reduced with BPA where the irradiation mainly depends on the particle radiation emitted by BNCR (Boron Neutron Capture Reaction). This result suggests that BNCT is an effective treatment with glioblastoma that can reduce the difference of radiosensitivity by p53 functional status.

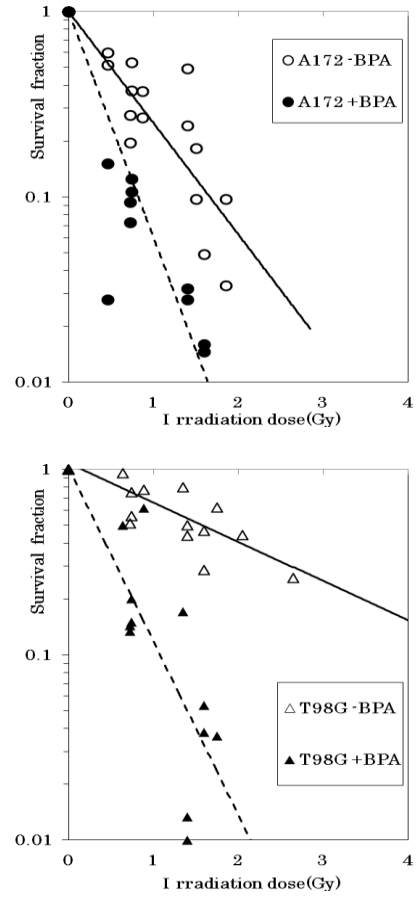

Fig. 1. Survival of glioblastoma cells (A172 and T98G) irradiated with thermal- to epithermal neutron beam of KUR with/without BPA.

PUBLICATION:

[1] K. Seki, Y. Kinashi, S. Takahashi:
The influence of p53 status in glioblastoma on the effects of boron neutron capture therapy. Anticancer Research, 35: 169～174, 2015.

課題採択番号26P3-2 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
（京大原子炉）高橋千太郎、木梨友子、関敬子、横溝夏弥、井川智之、小野公二
（放医研）久保田善久、岡安隆一